Published in final edited form as:

Immunol Rev. 2012 March ; 246(1): 359–378. doi:10.1111/j.1600-065X.2012.01105.x.

Pathogenetic Importance and Therapeutic Implications of NF- κ B in Lymphoid Malignancies

Kian-Huat Lim, Yibin Yang, and Louis M. Staudt

Metabolism Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Summary

Derangement of the nuclear factor κ B (NF- κ B) pathway initiates and/or sustains many types of human cancer. B-cell malignancies are particularly affected by oncogenic mutations, translocations, and copy number alterations affecting key components the NF- κ B pathway, most likely owing to the pervasive role of this pathway in normal B cells. These genetic aberrations cause tumors to be 'addicted' to NF- κ B, which can be exploited therapeutically. Since each subtype of lymphoid cancer utilizes different mechanisms to activate NF- κ B, several different therapeutic strategies are needed to address this pathogenetic heterogeneity. Fortunately, a number of drugs that block signaling cascades leading to NF- κ B are in early phase clinical trials, several of which are already showing activity in lymphoid malignancies.

Keywords

lymphoma; multiple myeloma; ABC DLBCL; CARD11; MyD88; B-cell receptor

Introduction

The nuclear factor- κ B (NF- κ B) family of transcription factors is constructed using five structurally related genes: *NFKB1* (encoding p105 and p50), *NFKB2* (encoding p100 and p52), *RELA* (encoding p65), *RELB* (encoding RelB), and *REL* (encoding c-Rel) (Reviewed in Ghosh *et al.*, this volume). Homo- and hetero-dimerization of these subunits generates a set of transcription factors that have overlapping but not identical function. Genetic ablation of different NF- κ B members or key signaling molecules that activate NF- κ B causes differentiation arrest and prevents activation at various stages of normal B-cell development. Perhaps as a consequence, B-cell malignancies frequently acquire genetic lesions that deregulate NF- κ B.

In quiescent cells, the NF- κ B transcription factors are retained in the cytoplasm by the inhibitor of NF- κ B α (I κ B α), but when cells are stimulated through a variety of surface receptors or by intracellular cues, the NF- κ B factors are released from I κ B α and translocate

Address for correspondence: Louis M. Staudt, 9000 Rockville Pike, Building 10, Room 4N114, Bethesda, MD 20892, Tel.: +1 301 402 1892, Fax: +1 301 496 9956, lstaudt@mail.nih.gov.

The authors declare no conflicts of interest.

into the nucleus (Reviewed in Hinz *et al.*, this volume). NF- κ B activation is achieved through either the canonical or noncanonical pathways. Certain receptors preferentially engage the classical pathway, including the B-cell receptor (BCR), Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors, and tumor necrosis factor (TNF) family receptors. Engagement of these receptors by their ligands leads to activation of a heterotrimeric I κ B kinase (IKK) complex, which consists of α , β , and γ (NEMO) subunits (Reviewed in Liu *et al.*, this volume). Activated IKK directly phosphorylates I κ B α , poising it for recognition and polyubiquitination by the β TrCP ubiquitin ligase followed by proteosomal degradation and release of NF- κ B to the nucleus (Reviewed in Kanarek & Ben-Neriah, this volume). The noncanonical NF- κ B pathway is preferentially initiated by stimulation of different receptors, including the B-cell activating factor belonging to the TNF family (BAFF) receptor and CD40. These receptors activate the NF- κ B-inducing kinase (NIK), which in turn phosphorylates a distinct inhibitor of NF- κ B (I κ B) kinase (IKK) complex comprised of two IKK α subunits. This form of IKK phosphorylates p100, leading to its proteolytic processing into the NF- κ B subunit p52, allowing the heterodimer between p52 and RelB to translocate to the nucleus (Reviewed in Sun, this volume). The common outcome of these two pathways is transcriptional activation of genes that reprogram cells to favor cell cycle progression, survival, cytokine secretion, and inflammation. In normal cells, activation of these pathways is transient and stimulus dependent. Further, various negative feedback mechanisms terminate NF- κ B signaling, including re-accumulation of the I κ B α and induction of A20, a ubiquitin-editing enzyme. This homeostatic regulation of NF- κ B signaling is circumvented by a host of genetic aberrations in lymphoid malignancies.

Historical overview of NF- κ B in cancer

Given the profound influence of the NF- κ B pathway on survival, proliferation, and differentiation of normal cells, it is not surprising that it is frequently deregulated in cancer. The first hint that NF- κ B might have oncogenic potential was the discovery that NF- κ B p50 is homologous to *v-rel*, a highly transforming oncogene carried within the genome of an avian reticuloendotheliosis virus (1, 2).

An early indication that the NF- κ B pathway might be oncogenically subverted in human cancer came from an analysis of Hodgkin lymphoma. Constitutive accumulation of NF- κ B heterodimers in the nucleus was discovered in several Hodgkin lymphoma cell lines and the malignant Hodgkin/Reed-Sternberg (HRS) cells of Hodgkin lymphoma tumors (3). Moreover, inhibition of NF- κ B was shown to reduce the proliferation and survival of Hodgkin lymphoma cell lines (4). Subsequent investigations revealed truncating mutations in I κ B α in Hodgkin lymphoma cell lines and primary HRS cells (5–7). However, removal of I κ B α is only part of the story in Hodgkin lymphoma, since other cell lines and primary HRS cells accumulate NF- κ B in the nucleus as a result of constitutively active IKK (8).

A second strong clue regarding the importance of NF- κ B in B-cell malignancies came from the characteristic translocations in gastric mucosa-associated lymphoid tissue (MALT) lymphoma involving MALT1 and BCL10 (9–12). Shortly thereafter, it was shown that both MALT1 and BCL10 participate in a signaling complex that activates NF- κ B (13), which eventually led to the discovery of CARD11 as an essential signaling adapter in this complex

(14–16). These findings in lymphoma preceded the appreciation that the CARD11-BCL10-MALT1 (CBM) complex is essential for signaling from the B and T-cell antigen receptors to the NF- κ B pathway (17–24, reviewed in Jiang & Lin, this volume).

As discussed in detail in this review, a host of molecular abnormalities affecting the NF- κ B pathway are present in diffuse large B-cell lymphoma (DLBCL). DLBCL is a clinically heterogeneous disease, with some individuals cured by the combination of chemotherapy plus the anti-CD20 antibody rituximab, while others ultimately succumb to the disease. This clinical heterogeneity prompted us to inquire into the molecular phenotype of DLBCL tumors using the nascent gene expression profiling technology (25). The custom cDNA microarray that was initially used, termed the Lymphochip, was enriched for genes that are differentially expressed during B-cell differentiation and activation (26). Because of this, the relationship of certain DLBCL tumors to normal B cells at particular stages of differentiation and activation became evident. One DLBCL subtype resembled normal germinal center B cells and hence was termed the germinal center B cell-like (GCB) DLBCL. The other common subtype lacked the germinal center B-cell gene expression signature but instead expressed genes that are induced upon activation of normal blood B cells through the BCR. Hence, this subtype was christened activated B cell-like (ABC) DLBCL, a label that has proven increasingly apt as it has become clear that these tumors subvert signaling pathways that are used during normal B-cell activation, many of which converge on NF- κ B.

NF- κ B has emerged more recently as critical to the pathogenesis of multiple myeloma, revealing a new upstream pathway that activates NF- κ B (27, 28). Further emerging evidence ties NF- κ B to the pathogenesis of other lymphoid malignancies, such as chronic lymphocytic leukemia (CLL) (29) and T-cell acute lymphoblastic leukemia (30, 31), providing added impetus to the development of drugs that can effectively and safely inhibit NF- κ B, as we highlight at the end of this review.

DLBCL

DLBCL is the most prevalent subtype of non-Hodgkin's lymphoma, comprising roughly 40% of all adult cases. In all cases, it is a clinically aggressive malignancy, but it has long been appreciated that patients with DLBCL differ greatly in response to standard chemotherapy, implying the existence of biologically different entities within this histological subtype. Patients with the ABC DLBCL subtype (see above) have an inferior overall survival compared to patients with the GCB DLBCL subtype (25, 32, 33). A third type of DLBCL, termed primary mediastinal B-cell lymphoma (PMBL), has a gene expression signature that distinguishes it from both ABC and GCB DLBCL but bears an unexpected resemblance to Hodgkin lymphoma by gene expression and by oncogenic mechanisms (34, 35). Clinically, PMBL has a relatively favorable overall survival rate, roughly comparable to that of GCB DLBCL (34). However, patients with PMBL are much younger in age, on average, than patients with ABC and GCB DLBCL, and the anatomical location of the cancer is primarily in the mediastinal region, with infrequent nodal involvement (34). A distinguishing feature among these DLBCL subtypes is a signature of

genes that are induced by NF- κ B, which is upregulated in ABC DLBCL (36) and PMBL (34, 35) but not GCB DLBCL.

Gene expression profiling revealed that the ABC DLBCL tumors are similar to antigen-stimulated normal B cells (25) but also express genes that are characteristic of plasma cells (37). In normal B cells, antigenic stimulation activates the NF- κ B pathway, which reprograms them for both clonal expansion and plasmacytic differentiation. The latter phenotype is due to upregulation of the transcription factor IRF4, which is an NF- κ B target gene. IRF4 in turn transactivates *PRDM1*, encoding Blimp-1, a master transcriptional regulator that propels B cells towards plasma cell differentiation (38, 39). Essentially all ABC DLBCL tumors express IRF4 highly as a consequence of their constitutive NF- κ B activity (25, 36, 37) and as a result upregulate *PRDM1* mRNA. However, in a majority of ABC DLBCL tumors, Blimp-1 protein is not highly expressed due to inactivating point mutations and deletions, epigenetic silencing, or transcriptional repression by Bcl-6 and Spi-B (40–45). As a consequence, ABC DLBCL tumors have initiated plasmacytic differentiation but appear to be arrested at the plasmablast stage because they lack Blimp-1 (37). Thus, a simple formula for ABC DLBCL pathogenesis has emerged, namely constitutive NF- κ B activity plus Blimp-1 inactivation. This model has now garnered experimental support: a genetic cross between mice with conditional inactivation of *PRDM1* and mice with a constitutively active IKK β allele yields lymphomas with an ABC DLBCL phenotype (46).

ABC DLBCL, which comprises ~40% of all DLBCL, is clinically more aggressive and carries a 3 year progression-free survival rate of 40% compared to 75% in GCB DLBCL (33). It is likely that the refractory nature of ABC DLBCL tumors stems from the anti-apoptotic action of NF- κ B. Indeed, NF- κ B can potently block the apoptotic action of cytotoxic chemotherapy (47). The canonical NF- κ B pathway is engaged in ABC DLBCL by sustained activity of IKK β , leading to nuclear translocation of p50/RelA heterodimers and, to a lesser extent, p50/c-Rel heterodimers (36). Importantly, ABC DLBCL cells lines are killed when the NF- κ B pathway is suppressed using a non-degradable form of I κ B α or by treatment with a small molecule IKK β inhibitor (36, 48). These studies suggest that the ABC DLBCL cells are oncogenically ‘addicted’ to high NF- κ B activity for survival and proliferation, justifying therapeutic strategies targeting this pathway.

Sustained nuclear accumulation of the NF- κ B heterodimers dysregulates transcription of a broad array of genes that contribute to the ABC DLBCL phenotype, including several that encode pro-survival proteins (e.g. A1, BCL-XL, c-IAP1, c-IAP2, and c-FLIP) (48). Both IL-6 and IL-10 are NF- κ B targets in ABC DLBCL, and secretion of these cytokines provides an additional means to promote survival of ABC DLBCLs (49). Autocrine stimulation of IL-6 or IL-10 receptors activates JAK family kinases, which in turn phosphorylate the transcription factor STAT3, causing its nuclear translocation. Development of a gene expression signature of STAT3 activity allowed ABC DLBCLs to be dichotomized into STAT3-high or STAT3-low subtypes (49). STAT3-high ABC DLBCLs have higher NF- κ B activity than STAT3-low ABC DLBCLs, potentially because STAT3 can physically interact with NF- κ B heterodimers, thereby increasing their transactivation

potential. Treatment of ABC DLBCL cell lines with both a JAK kinase inhibitor and an IKK β inhibitor yields synergistic cytotoxicity (49).

Genomic-scale RNA interference (RNAi) screens have been instrumental in the identification of upstream signaling pathways that constitutively activate NF- κ B in ABC DLBCL (50). So-called 'Achilles heel' RNAi screens can identify genes that are essential for the proliferation and survival of cancer cells. A complementary technology is high-throughput resequencing of RNA or DNA from cancer cells. Often, cancer gene resequencing reveals mutations in genes encoding components of essential pathways discovered in RNAi screens. Together, these technologies identify the addictions of cancer cells that can be exploited therapeutically.

Chronic active BCR signaling

Tonic signaling from the BCR is essential for survival of B cells throughout their lifespan (51, 52). This mode of BCR signaling is apparently antigen-independent and promotes survival by engaging the phosphoinositide 3-kinase (PI3K) pathway (53). Antigenic stimulation and engagement of NF- κ B via an adapter complex involving CARD11, BCL10 and MALT1 (the CBM complex) is essential for the differentiation and/or maintenance of certain subpopulations of B cells, notably marginal zone and B1 B cells (54). In principle, both pathways could contribute to the survival of malignant lymphoma cells.

The BCR consists of the antigen-binding immunoglobulin heavy (IgH) and light(IgL) chains coupled to a disulphide-linked heterodimer of CD79A (Ig α) and CD79B (Ig β). CD79A and CD79B mediate signaling by the BCR to downstream pathways using the immunoreceptor tyrosine-based activation motif (ITAM) within their cytoplasmic tails. Upon antigen encounter, BCRs aggregate to form microclusters on the cell membrane (55–58). These microclusters serve as a signaling platform that allows a highly orchestrated recruitment of multiple intracellular adapters and enzymes, including Lyn, Syk, BLNK, PLC γ 2, BTK, Vav, and PKC β , thereby forming a 'microsignolosome' that engages downstream signaling (59–61).

Upon antigen encounter, BCR signaling is initialized by recruitment of Src family tyrosine kinases (Lyn, Fyn, Blk), which phosphorylate two invariant tyrosine residues within the ITAMs of CD79A and CD79B. The dually phosphorylated ITAMs serve as docking sites for recruitment of the tyrosine kinase Syk. BLNK is also recruited to CD79A and is subsequently phosphorylated by Syk, thereby engaging Bruton's tyrosine kinase (BTK), which is phosphorylated and activated by the neighboring Lyn and Syk kinases (62). Other evidence suggests that BTK is recruited to the plasma membrane by binding phosphatidylinositol (3,4,5)-triphosphate (PIP3) generated as a consequence of BCR-dependent PI3K activation (62). BTK phosphorylates and activates PLC γ 2, which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) into the secondary messengers diacylglycerol (DAG) and inositol triphosphate (IP3). The surge in IP3 causes the endoplasmic reticulum to release its calcium stores, prompting the opening of the CRAC calcium channels on the plasma membrane. Together, calcium and DAG activate PKC β to

phosphorylate CARD11, promoting formation of the CBM complex and activation of NF- κ B (62, 63, reviewed in Kaileh & Sen, this volume).

The survival of most ABC DLBCL cell lines depends upon signals emanating from the BCR. Knockdown of BCR subunits (IgH, IgL, CD79A, CD79B) or downstream components of the BCR pathway (BTK, SYK, BLNK, PLC γ 2, PI3K δ , PKC β , CARD11, BCL10, MALT1) is lethal to these ABC DLBCL lines but not to GCB DLBCL lines (50, 64). BCR signaling in ABC DLBCL contributes to their constitutive NF- κ B activity as well as to PI3K signaling, both of which promote ABC DLBCL survival (64). Total internal reflection microscopy revealed that BCRs in ABC DLBCL cells form prominent, immobile clusters in the plasma membrane (64), akin to those observed in antigen-stimulated normal B cells (61). These microclusters coincide with phosphotyrosine staining, indicating that they are functioning as BCR signalosomes. By contrast, the BCRs in GCB DLBCL cell lines are diffusely scattered on the plasma membrane (64), as is observed in resting normal B cells (61).

This constitutive form of BCR signaling in ABC DLBCLs was dubbed 'chronic active' BCR signaling to deliberately distinguish it from tonic BCR signaling (64). As mentioned above, tonic BCR signaling is likely to be CARD11-independent whereas chronic active BCR signaling is CARD11-dependent. The clustering of the BCR in chronic active BCR signaling is reminiscent of antigen-stimulated B cells and not resting B cells, which are dependent on tonic BCR signaling. Finally, chronic active BCR signaling elicits NF- κ B activity, which sustains ABC DLBCL survival, whereas survival cues generated by tonic BCR signaling are predominantly derived from PI3K signaling.

Mechanisms contributing to chronic active BCR signaling in the ABC DLBCL have been partly revealed by cancer gene resequencing. Somatic mutations in *CD79A* and *CD79B* are present in ABC DLBCL cell lines and patient samples, all of which affect the ITAM signaling motif (64). These mutations are present in over 20% of ABC DLBCL tumors but are rare or absent in other lymphoma subtypes. Remarkably, 18% of ABC DLBCL tumors have point mutations that change the N-terminal tyrosine of the CD79B ITAM to a different amino acid. In contrast, the second CD79B ITAM tyrosine is never altered by such point mutations, pointing to an unexpected asymmetry in the function of the CD79B tyrosines. It is also important to emphasize that ABC DLBCL tumors always retain either an intact CD79A or CD79B ITAM region. This implies selection for SYK recruitment and activation, which requires an intact ITAM in either CD79A or CD79B.

Mutated CD79A or CD79B in ABC DLBCL renders their BCRs resistant to negative regulation by Lyn kinase (64). During BCR activation, Lyn kinase phosphorylates the ITAM tyrosines of CD79A and CD79B to initiate downstream signaling. However, Lyn kinase also plays an essential role in turning off BCR signaling. Upon binding to the ITAMs, Lyn kinase is activated (65), allowing it to phosphorylate the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) on Fc γ RIIb or CD22 (66). The phosphorylated ITIM recruits the phosphatase SHP1, which can dephosphorylate and inactivate the ITAM motifs of CD79A and CD79B, thereby quenching BCR signaling (67). Mice deficient in Lyn kinase have an expanded population of B lymphoblasts that proliferate abnormally well to BCR stimulation, and

consequently develop an antibody-mediated autoimmune disease (68). A second, related phenotype conferred by CD79A and CD79B mutations is enhanced surface BCR expression due to diminished BCR internalization. Similarly, mice defective with CD79A or CD79B ITAM mutations have higher BCR levels and prolonged BCR-dependent signaling upon antigen stimulation (69–71). Thus, the selective advantage for ABC DLBCL tumors of the CD79A and CD79B mutations appears to be enhancement of BCR signaling.

Although the CD79A and CD79B mutations in ABC DLBCL are obviously ‘driver’ mutations that promote the malignant phenotype, they are not strongly oncogenic on their own, in that their introduction into GCB DLBCL cell lines does not promote BCR clustering (64). This suggests that other attributes of ABC DLBCL contribute to chronic active BCR signaling. Two general models may explain the BCR clustering in ABC DLBCLs. First, ABC DLBCLs may be defective in the regulation of BCR cluster formation. In normal B cells, CD19 regulates the BCR clustering response to membrane bound antigens (55). Additionally, the IgM heavy chain itself appears to regulate BCR clustering since deletions within this domain can result in spontaneous BCR clustering (56). Either of these regulatory mechanisms could be impaired in ABC DLBCL. A second general model postulates that the BCRs in ABC DLBCL are reacting with an antigen, resulting in the observed BCR clustering. In this regard, it is notable that a substantial fraction of the germ line-encoded Ig variable regions are autoreactive (72). Normal B cells expressing autoreactive BCRs are either deleted or are rendered anergic. Anergic B cells are refractory to further BCR stimulation, apparently because their BCRs are associated with high levels of activated Lyn kinase (67, 73). Thus, ABC DLBCLs might originate from such an anergic B cell, and escape anergy by acquiring mutations in CD79A or CD79B that quench Lyn activity.

Oncogenic CARD11 mutations

In some ABC DLBCL lines, blockade of BCR signaling has no effect on NF- κ B activity, but knockdown of CARD11 is nonetheless toxic, leading to the discovery of oncogenic mutations in CARD11 in ABC DLBCL (64, 74). CARD11 consists of an N-terminal CARD motif, a coiled-coil domain, a linker domain, and a C-terminal MAGUK domain that contains multiple protein-protein interaction sub-domains (Reviewed in Jiang & Lin, this volume) (Fig. 1). In resting lymphocytes, CARD11 is predicted to exist in a closed conformation in the cytoplasm in which the linker domain is folded against the CARD and coiled-coil domains, presumably blocking CARD11 multimerization (75). Upon antigenic stimulation of B cells, CARD11 is phosphorylated within the linker domain by PKC β and IKK β (76–79), presumably resulting in an open conformation that allows CARD11 to interact with BCL10 and MALT1 to form a stable CBM complex (77). An additional component of the CBM complex is casein kinase-1- α (CK1 α), which was shown to be required for the survival of ABC DLBCL cells (80). Interestingly, CK1 α performs two opposing functions: it promotes CBM assembly in a kinase-independent fashion but also terminates CARD11 activity by phosphorylating CARD11 in the linker domain at sites that are distinct from those phosphorylated by PKC β .

Resequencing of CARD11 in DLBCL tumors revealed a diverse set of somatic mutations within the coiled-coil domain (74) (Fig. 1). These mutations are present in ~10% of ABC

and in ~3% of GCB DLBCL, but are never present in gastric MALT lymphomas, despite the importance of the CBM pathway in this lymphoma subtype (74) (see below). While ABC DLBCL tumors have a universally high degree of NF- κ B activation, irrespective of CARD11 mutational status, GCB DLBCL tumors with mutant CARD11 have higher NF- κ B activity than those with wildtype CARD11 (74). Ectopic expression of CARD11 mutants, but not wildtype CARD11, upregulates NF- κ B, demonstrating their gain-of-function nature. In transduced T cells, these CARD11 mutants render the CBM hyperresponsive to signals derived from the T-cell antigen receptor (74). This finding suggests that some weaker CARD11 mutants might augment chronic active BCR signaling in ABC DLBCL. Indeed, some CARD11 mutations coexist with CD79B mutations in the same ABC DLBCL tumors, implying functional cooperation (81).

The mechanism whereby these CARD11 mutants activate NF- κ B appears to relate to their propensity to form large cytoplasmic aggregates (74). Each different CARD11 mutant differs in its ability to form these aggregates, a phenotype that is directly proportional to their ability to engage NF- κ B. These cytoplasmic aggregates colocalize with MALT, CK1 α , and IKK β , further suggesting that they are the sites of NF- κ B activation in these lymphomas. The CARD11 coiled-coil domain mutations are likely to promote aggregation by disrupting the inhibitory interaction between the linker domain and the coiled-coil domain. Indeed, *in vitro* experiments revealed that these mutant coiled-coil domains have decreased ability to bind to the linker domain and also have increased ability to bind to BCL10 (82).

Interestingly, the coiled-coil domain mutations in DLBCL are decidedly non-random in their distribution within this domain. Fig. 1 displays a compilation of CARD11 mutants occurring in nodal ABC and GCB DLBCL (74, 83, 84), extranodal DLBCLs [primary gastric DLBCL (85) and primary central nervous system DLBCL (86)], as well as in follicular lymphoma (84). Of note in this regard is that primary central nervous system DLBCL appears to be a form of ABC DLBCL that arises initially within the central nervous system rather than the lymph nodes (87). Certain hot spots in the CARD11 coiled-coil domain are recurrently mutated while other regions are relatively devoid of mutations, irrespective of the DLBCL subtype. Several of the mutations cause the deletion or insertion of amino acids, which would likely perturb coiled-coil interactions (88). Others affect conserved charged residues that can be instrumental in nucleating and positioning coiled-coil interactions (88). As mentioned above, it is possible that some of the mutated amino acids make functionally critical contacts with the linker domain, and therefore their substitution might allow the coiled-coil domain to dimerize, multimerize, and/or interact with other proteins to form the CBM complex. The CARD11 coiled-coil appears to be a multipart module, with two separate regions with coiled-coil potential separated by a loop (Fig. 1). Both modules are functionally important, controlling both dimerization and recruitment of CARD11 to the plasma membrane (89), and both modules sustain mutations in ABC DLBCL. There is a hot-spot of mutational activity just N-terminal to the first coiled-coil subdomain, suggesting that this region may serve as a 'trigger site' that regulates the coiled-coil formation (88). Clearly, a detailed structural analysis of the coiled-coil domain will be needed to fully understand these mutants, but these considerations suggest that the CARD11 coiled-coil

domain is a complicated molecular machine that can be regulated in its ability to form homotypic interactions and bind other signaling components.

Enzymatic function of MALT1

Besides serving as an adaptor within the CBM complex, MALT1 contains a proteolytic activity that is constitutively active in ABC DLBCL and is required for full NF- κ B induction and cell survival in this setting (90, 91). In normal lymphocytes, the defined substrates of MALT1 include A20, BCL10, and RelB (92–94). Inhibition of MALT1 proteolytic activity decreases A20 cleavage in ABC DLBCL, presumably allowing the full-length A20 to inhibit NF- κ B (90, 91). Unexpectedly, the cleavage of RelB by MALT1 enhances activation of certain NF- κ B target genes in ABC DLBCL (94). Overexpression of RelB inhibits the DNA binding ability of NF- κ B heterodimers containing p65 or c-rel, suggesting that MALT1-mediated cleavage may promote ABC DLBCL survival by enhancing canonical NF- κ B pathway activation. Hence, MALT1 protease activity appears to be an attractive target for therapeutic development in ABC DLBCL. Indeed, the irreversible MALT1 inhibitor z-VRPR-fmk downregulates NF- κ B target genes and curbs survival and proliferation of ABC DLBCLs (90, 91).

Oncogenic *MyD88* mutations

In addition to chronic active BCR signaling, aberrant activation of innate immune signaling cascades promotes NF- κ B activity and cell survival in ABC DLBCL (81). An RNAi screen demonstrated that the survival of ABC DLBCL cells depends upon MyD88 and IRAK1 kinase, two crucial components of the Toll-like receptor (TLR) signaling pathway (81). By contrast, this pathway is not required by either GCB DLBCL or multiple myeloma cell lines. TLRs are widely expressed pattern recognition receptors that sense a wide variety of pathogen-associated molecular patterns (PAMPs) derived from bacteria, viruses, and fungi. At least ten TLR family members are encoded in the human genome, each recognizing distinct PAMPs, including lipoproteins, single- and double-stranded RNA, flagellin, and double-stranded DNA. Upon ligand binding, TLRs aggregate and initiate intracellular signaling by engaging various cytosolic adapters, including MyD88, TIRAP/Mal, TRIF/TICAM1, TRAM/TICAM2, and SARM. These TLRs use the TIR domain in their cytoplasmic portion to interact with TIR domains within these adapters, with each TLR able to interact with some but not all adapters. MyD88 binding is shared by all TLRs except TLR3. The MyD88 protein consists of an N-terminal death domain, a linker region and a C-terminal TIR domain. Activation and dimerization of TLRs presumably induces a conformational change in their cytoplasmic TIR domain, leading to MyD88 recruitment (95–98). The IRAK kinases (IRAK1, IRAK2, IRAK4) are subsequently bound to MyD88 through homophilic interactions involving their death domains, forming a helical protein complex (99). Within this complex, IRAK4 phosphorylates IRAK1, which in turn binds the ubiquitin ligase TRAF6. TRAF6 catalyzes lysine 63-linked polyubiquitination of the kinase TAK1, which then complexes with two zinc finger proteins, TAB2 and TAB3, to become enzymatically active. TAK1 phosphorylates IKK β as well as mitogen-activated protein kinases (MAPKs), which respectively trigger the NF- κ B and JNK/p38 MAPK signaling pathways, leading to production of inflammatory cytokines and growth factors (100).

The constitutive activity of the MyD88 signaling pathway in ABC DLBCL contributes to NF- κ B activation, which together with MAPK activation initiates IL-6 and IL-10 transcription (81). These cytokines bind to their receptors in an autocrine fashion and activate JAK family kinases to phosphorylate the transcription factor STAT3, leading to its nuclear transit. Gene expression profiling revealed that roughly 50% of ABC DLBCLs express a set of STAT3-dependent target genes in association with high IL-6 expression and STAT3 phosphorylation (49). Of note, this subset of ABC DLBCL tumors also has higher expression an NF- κ B target gene signature. This may be due to the ability of STAT3 to physically interact with NF- κ B heterodimers, enhancing transactivation of NF- κ B target genes (101). Both JAK kinases and NF- κ B deliver survival signals in ABC DLBCL since a JAK kinase inhibitor synergizes with an IKK β inhibitor in killing ABC DLBCL cell lines (49).

MyD88 signaling in ABC DLBCL also promotes the secretion of interferon- β , which induces the expression of type I interferon target genes (81). The selective advantage of this aspect of MyD88 signaling is unclear. Type I interferon is potentially immunosuppressive, suggesting that ABC DLBCL malignant cells may use this mechanism to escape immune surveillance. However, it is also possible that type I interferon secretion is merely the 'price of doing business', an unselected phenotype that coincides with the pro-survival phenotypes of NF- κ B and JAK/STAT3 signaling.

Cancer gene resequencing revealed somatic mutations in the MyD88 TIR domain in ABC DLBCL (81). Although many different MyD88 mutations are recurrent, by far the most prevalent mutation substitutes a proline residue for a leucine residue at position 265 in the protein. This L265P mutation is present in most ABC DLBCL cell lines and in 29% of ABC DLBCL biopsies but is rarely if ever observed in GCB DLBCL, Burkitt lymphoma, or PMBL biopsies. Interestingly, this mutant is present in ~9% of gastric MALT lymphoma biopsies (81) and in 3% of CLL samples (102), indicating that MyD88 can be oncogenic in other lymphoid malignancies. Indeed, CLL cells bearing the L265P MyD88 mutant have activated NF- κ B and STAT3 and, in response to TLR ligands, secrete greater amounts of cytokines and chemokines than CLL cells with wildtype MyD88 (102). CLL with the MyD88 L265P mutation occurs in patients who are ~20 years younger than average for CLL, and the leukemia is at an advance clinical stage at diagnosis. Other MyD88 TIR domain mutations besides L265P are present in an additional 10% of ABC DLBCL cases and occur at a comparable frequency in GCB DLBCL, but not in other lymphoma subtypes. Overall, 39% of ABC DLBCL tumors harbor MyD88 mutations, making MDy88 the most frequently mutated oncogene in this lymphoma subtype.

Mutations of MyD88 in the ABC DLBCL are both gain-of-function and oncogenic. Ectopic expression of mutant but not wildtype MyD88 isoforms in GCB DLBCL lines induces high NF- κ B activity. The mutations differ in their potency, however, with MyD88 L265P being one of the most active mutants. However, other MyD88 mutants are equally able to induce NF- κ B, suggesting that the reason that L265P is the most prevalent mutant is due to some other phenotypic attribute of this mutant isoform. Importantly, ABC DLBCL cell lines with the L265P mutation can be rescued from the toxicity of MyD88 knockdown by ectopic re-

expression of MyD88 L265P but not wildtype MyD88, indicating that these cell lines are addicted to the oncogenic action of MyD88 L265P.

The biochemical basis for the constitutive activation of NF- κ B by the MyD88 L265P mutant appears to be its ability to spontaneously nucleate a signaling complex involving IRAK4 and IRAK1 (81). IRAK1 in this complex is hyperphosphorylated due to the action of IRAK4. Accordingly, small molecule inhibitors of IRAK4 kinase extinguish NF- κ B activation by MyD88 L265P, indicating that this complex is likely to be responsible for constitutive MyD88 signaling in ABC DLBCL. However, the biochemical basis for NF- κ B activation by the less common MyD88 mutants is currently unclear.

The location of the MyD88 mutations within the tertiary structure of the MyD88 TIR domain provides hints regarding their function (81) (Fig. 2). MyD88 L265P is nestled deep within the hydrophobic core of the TIR domain, which is formed by five parallel β -sheets. Interestingly, another recurrent mutant, M232T, alters a methionine that is adjacent to L265, suggesting that these two mutants may have related function. Other MyD88 mutations are clustered within the so-called 'BB loop' of the TIR domain, a region of MyD88 that likely affects its interaction with other TIR-containing proteins such as the TLRs (103). Thus, a working model would imagine that the MyD88 L265P mutation changes the structure of the TIR domain so as to fix it in a normally transient state that allows spontaneous interaction with IRAK4 and IRAK1. The BB loop mutations could make more local changes in the BB loop conformation that might favor interactions with other TIR domain proteins.

Among ABC DLBCL tumors, MyD88 L265P mutations coexist with CD79B mutations more often than expected by chance (81). This implies a functional interaction between the BCR and MyD88 signaling pathways. Indeed, knockdown of both CD79A and MyD88 is more toxic to ABC DLBCL cell lines than knockdown of either one alone. This signaling cross-talk may relate to the interrelationship of the BCR and TLR signaling pathways in normal immune responses and in autoimmune disease. MyD88 is required for an optimal germinal center response to a retroviral antigen, presumably because the retroviral antigens need to be recognized both by TLR7 and by the BCR to achieve clonal expansion (104). In mouse models of systemic lupus erythematosus, MyD88, acting downstream of TLR7 and TLR9, is necessary to mount an autoimmune antibody response to self antigens that contain both protein and nucleic acids (105). It is therefore conceivable that the MyD88 mutations that occur in lymphoma may allow malignant B cells to respond more readily to self antigens that require TLR engagement.

Inactivation of A20

In normal lymphocytes, multiple negative feedback mechanisms are triggered following BCR and MyD88 signaling to turn off NF- κ B activity. In the face of stimuli that activate NF- κ B, failure of these negative feedback mechanisms may sustain NF- κ B activation, favoring cell survival and ultimately malignancy. A clear example is the tumor suppressor gene *TNFAIP3*, which encodes A20 (Reviewed in Harhaj & Dixit, this volume). A20 possesses dual functions as a deubiquitinating enzyme that removes activating K63-linked ubiquitin marks and as a E3 ubiquitin ligase that adds K48-linked polyubiquitin chains,

thereby promoting proteasomal degradation (106). *TNFAIP3* is transcriptionally activated by NF- κ B, allowing A20 to attenuate NF- κ B signaling by deubiquitinating and hence inactivating TRAF6, MALT1, and IKK γ , among other substrates (106–108). Consequently, mice lacking A20 develop a fatal inflammatory disease linked to NF- κ B-dependent cytokine expression (109). In ABC DLBCL, expression or function of *TNFAIP3* is frequently lost in association with biallelic gene deletion, inactivating missense or nonsense mutations, or promoter hypermethylation (110, 111). Reintroduction of A20 into A20-null lymphoma cell lines results in growth arrest and cell death (110, 111). A20 mutations coexist with both CD79B and MyD88 mutations in ABC DLBCL tumors, suggesting that A20 can enhance chronic active BCR signaling as well as MyD88 signaling (81). However, some ABC DLBCL tumors inactivate A20 but do not have mutations in CD79A/B, MyD88, or CARD11. This may imply that some ABC DLBCL tumors activate NF- κ B by simply removing negative regulation by A20. This seems somewhat unlikely given that the inflammatory disease in A20-deficient mice relies on NF- κ B-inducing signals from MyD88, presumably in response to commensal bacterial flora (112). Hence, it is probable that ABC DLBCL tumors utilize additional mechanisms beyond the prevalent mutations in CD79A/B, MyD88, and CARD11 to positively engage NF- κ B signaling. There may well be activating mutations in other NF- κ B regulators that have not yet been discovered. Alternatively, the BCR and/or MyD88 pathways may be able to signal to NF- κ B in the absence of somatic mutations affecting these pathways.

PMBL

PMBL represents a distinct subtype of DLBCL, both from clinical manifestations and gene expression analysis (34, 35). PMBL is similar to Hodgkin lymphoma in gene expression profile, including high expression of NF- κ B target genes. The molecular mechanisms underlying NF- κ B activation in this lymphoma are beginning to unravel. Loss of A20 occurs in about one third of PMBL cases (113). In addition, up to 75% of PMBL tumors harbor amplification of the *REL* locus, which correlates with increased *REL* mRNA, nuclear c-Rel, and NF- κ B activity (114). Inhibition of NF- κ B signaling in PMBL cell lines using a small molecule inhibitor of IKK β or using a dominant negative I κ B α super-repressor leads to rapid cell death (48). The mechanisms that activate IKK in this lymphoma subtype have yet to be fully delineated, however.

Hodgkin lymphoma

Based on histological and immunohistochemical features, Hodgkin lymphoma can be divided into several subtypes, including classical Hodgkin lymphoma (cHL) and nodular lymphocyte predominant Hodgkin lymphoma (LPHL). Both malignancies are likely to be derived from a germinal center B cells, but cHL typically loses many of the characteristic features of these normal cells whereas LPHL retains many germinal center B-cell markers. Aberrant activation of the NF- κ B pathway is an oncogenic mechanism in both of these Hodgkin lymphoma subtypes and can be driven by both cell-autonomous processes within the malignant cells as well as by signaling inputs from the tumor microenvironment.

The malignant component of cHL tumors, the HRS cell, represents a minority of the cells within these tumors, with the bulk of the tumor composed of various inflammatory cell types, including B cells, T cells, macrophages and granulocytes. This unique inflammatory milieu provides abundant cytokines that can activate NF- κ B. HRS cells have high surface expression of several TNF receptor family members, including CD30 (115), CD40 (116), RANK (117), TACI, and BCMA (118), each of which can be chronically stimulated by ligands produced by the microenvironment or by the HRS cells themselves. Stimulation of these TNF receptors leads to oligomerization of their cytoplasmic domains, allowing recruitment of adaptor proteins such as TRAF2, TRAF5, and TRAF6, culminating in IKK activation of NF- κ B.

Cell-autonomous activation of the NF- κ B pathway in Hodgkin lymphoma can be acquired through somatic genetic alterations or through infection by the Epstein-Barr virus (EBV). Up to 40% of Hodgkin lymphomas are latently infected with EBV, which can promote lymphomagenesis through expression of viral oncoproteins, including latent membrane protein 1 and 2A (LMP1 and LMP2A) (119). LMP1 protein shares high homology to the cytoplasmic domain of CD40 and can spontaneously form ligand-independent signaling aggregates that recruit TRAF proteins and activate IKK (120). Expression of LMP1 can transform cultured rodent fibroblasts and B cells, and transgenic mice with B cell-restricted LMP1 expression spontaneously develop lymphomas with age (121, 122). Likewise, transgenic expression of a constitutively active LMP1/CD40 chimeric receptor in B cells produces B-cell lymphomas at a high frequency (123). LMP2A was initially shown to have a dominant-negative effect on BCR signaling by binding and sequestering the tyrosine kinases Lyn and Syk (124, 125). However, transgenic expression of LMP2A promotes the abnormal survival and proliferation B cells, even in the absence of the BCR (126–128). Thus, LMP2A actually functions as a BCR mimic to provide tonic BCR signaling essential for B-cell survival, supplanting normal BCR signaling. Although NF- κ B is normally activated by BCR signaling, it has not been formally investigated whether LMP2A has the same function or whether it instead promotes survival through the PI3K pathway.

Genetic or epigenetic mechanisms that cause constitutive activation of the NF- κ B pathway in Hodgkin's lymphoma are emerging. Amplification of the *REL* locus is present in more than 50% of cHL cases (129–131). cHL with this amplicon have strong nuclear c-Rel staining, a surrogate marker for NF- κ B activity. Frameshift or nonsense mutations in *NFKBIA*, which encodes I κ B α , produce inactive, truncated I κ B α isoforms and occur in ~5–20% of Hodgkin lymphoma cases (5, 6, 8, 132). Additionally, loss-of-function mutations occur in *NFKBIE*, encoding I κ B α , albeit at a lower frequency (133). Inactivation of *TNFAIP3* by biallelic deletions or frameshift/nonsense mutations occurs in 30–40% of Hodgkin lymphoma cases (113, 134). Ectopic provision of A20 into A20-null Hodgkin's lymphoma cell lines extinguishes NF- κ B activity and produces growth arrest and apoptosis. Loss of *NFKBIA* and *TNFAIP3* occurs preferentially in EBV-negative Hodgkin lymphoma, supporting the view that NF- κ B is a central oncogenic pathway in Hodgkin lymphoma that can be invoked by different mechanisms.

The much less prevalent form of Hodgkin lymphoma, LPHL, can be distinguished from cHL not only by its histopathologic features but also by its more indolent course and favorable

prognosis. Nonetheless, the malignant cell in LPHL, the lymphocytic and histiocytic (L & H) cell, has high NF- κ B activity, comparable to that of the HRS cells in cHL (135). The underlying mechanism for constitutive NF- κ B activity LPHL is unclear, but it appears not to involve EBV infection, amplification of *REL*, loss of *NFKBIA*, or *TNFAIP3* inactivation (136).

Marginal zone lymphoma

As the name implies, marginal zone lymphoma (MZL) arises from normal marginal zone B cells and encompasses extranodal MALT lymphomas, nodal MZL, or primary splenic MZL. Development of MZL is often related to inflammation secondary to chronic infections or autoimmune diseases. The autoimmune diseases Hashimoto's thyroiditis, Sjogren's syndrome, and systemic lupus erythematosus are strong risk factors for development of MALT lymphoma (137). Moreover, up to 90% of patients with gastric MALT lymphoma have chronic infection with *Helicobacter pylori* (138). Other pathogens, including hepatitis C virus, *Campylobacter jejuni*, *Borrelia burgdorferi*, and *Chlamyophila psittaci* have also been implicated in development of MZL in the spleen, small intestine, skin and ocular adnexa, respectively (139–142). Remarkably, MZLs can be often by successfully treated by eradication of the associated pathogens, especially during early stages of the disease, suggests that these lymphomas rely on an antigen-driven process. MZLs have been reported to express BCRs that possess rheumatoid factor activity, allowing them to bind to immunoglobulin constant regions (143). This observation led to a proposal that MZL cells may be activated by immune complexes containing antibodies to *H. pylori* antigens produced by reactive, non-malignant B cells. However, another report provided evidence that the BCRs of MZLs are polyreactive, potentially allowing direct activation by environmental antigens. The uncontrolled growth of MZLs may be further stimulated by T helper cells since the proliferative response of cultured MZL cells to *H. pylori* antigens requires co-culture with tumor-infiltrating T cells, which provide CD40 ligand and cytokines such as IL-4 and IL-10 (144, 145).

Several chromosomal translocations are uniquely acquired by MALT lymphomas, whose oncogenic gene products activate the NF- κ B pathway. These include the t(11;18) translocation that produces an c-IAP2-MALT1 fusion protein as well as the t(14;18) and t(1;14) translocations that link the IgH locus enhancers to *MALT1* (146, 147) and *BCL10* (11, 12), respectively. The t(11;18) translocation is present in ~20–30% of gastric MALT lymphoma biopsies (9, 10, 148–150). MALT lymphomas bearing this lesion are not responsive to *H. pylori* eradication and do not express BCRs with rheumatoid factor activity, suggesting that there may be two separate oncogenic routes to MALT lymphoma, one antigen-dependent and the other antigen-independent (143, 151). Overexpression of the c-IAP2-MALT1 fusion protein potentially activates NF- κ B (13, 152, 153). Moreover, transgenic mice expressing c-IAP2-MALT1 have an expanded splenic marginal zone B-cell compartment with high NF- κ B activity and develop lymphomas as they age (154). The c-IAP2-MALT1 fusion protein consists of the N-terminal region of c-IAP2 (API2; BIRC3), which is comprised of three BIR domains, and the C-terminal region of MALT1, containing its paracaspase domain. The BIR domains of the c-IAP2 moiety foster c-IAP2-MALT1 oligomerization through heterotypic interaction with the C-terminal MALT1 moiety (155).

The resulting c-IAP2-MALT1 aggregate recruits and activates the E3 ligase activity of TRAF6, which polyubiquitinates and activates the IKK complex (156, 157). While BCL10 is a crucial component of the CBM complex, its role in c-IAP2-MALT1 signaling functions remains controversial (153, 157, 158).

An unexpected recent finding is the ability of the c-IAP2-MALT1 fusion protein to activate the noncanonical NF- κ B pathway. The c-IAP2 component of this fusion protein interacts with NIK, allowing the MALT1 paracaspase domain to cleave this kinase. The resultant proteolytic product lacks the N-terminal 325 amino acids of NIK, which includes the region that interacts with TRAF3. Since TRAF3 normally polyubiquitinates NIK and targets it for proteasomal degradation, the MALT1 cleavage product is stable and constitutively active as a kinase (159). The consequence is activation of the noncanonical NF- κ B pathway, in which p52/RelB translocates to the nucleus and activates target genes such as Pim-2, cyclin D1, CXCR4 and RANKL (159).

As mentioned above, the MyD88 L265P mutation is present in 9% of gastric MALT lymphoma biopsies (81), presumably activating NF- κ B. Given that MyD88 L265P renders CLL cells more responsive to TLR ligands (102), it is likely that this mutant isoform amplifies signaling by binding to TLRs. In the case of MALT lymphoma, TLR5 is a likely candidate because it recognizes the bacterial flagellar antigen of *H. pylori*. Alternatively, MyD88 L265P may allow MALT lymphomas to proliferate in response to other TLR ligands present within the tumor microenvironment. Whether or not MALT lymphomas with MyD88 L265P mutation are dependent on *H. pylori* is currently unknown.

Inactivation of *TNFAIP3* by chromosomal deletions, inactivating mutations, or promoter methylation occurs in ~20% of MZLs, particularly in ocular adnexal lymphomas (160–164). MZL tumors with loss of A20 concurrently acquire copy number gains of a region encompassing the *TNF* locus, suggesting that TNF α signaling may activate NF- κ B in this setting (160). These MZLs lack the recurrent translocations of MZLs and are associated with more aggressive disease and poorer outcome.

Multiple myeloma

Multiple myeloma is a B-cell malignancy that phenotypically resembles terminally differentiated plasma cells. However, multiple myeloma is likely to be initiated within the germinal center, since several recurrent translocations in this cancer are created by DNA breaks caused by AID, an enzyme present in germinal center B cells but not plasma cells. After acquiring these initiating genetic lesions, the pre-malignant plasma cell can migrate to the bone marrow and establish a benign condition known as monoclonal gammopathy of undetermined significance (MGUS), which progresses to myeloma at a rate of 1% per year. Despite recent advances in therapy, multiple myeloma remains incurable in most cases. Therapy resistance may derive from interactions between multiple myeloma cells and non-malignant cells in the bone marrow microenvironment (165, 166).

One of the characteristic features of multiple myeloma cells that may contribute to therapy resistance is NF- κ B pathway activation (27, 28). Over 80% of primary samples from patients with multiple myeloma have strong expression of a set of NF- κ B target genes,

which is directly correlated with nuclear translocation of NF- κ B p65 in these cases (28). Thus, a significant fraction of multiple myeloma tumors activate the canonical NF- κ B pathway. Multiple myeloma cell lines that express NF- κ B signature genes are sensitive to IKK β inhibitors, again indicating activation of the canonical NF- κ B pathway (28, 167, 168). In addition, however, multiple myeloma cells with NF- κ B target gene expression have nuclear p52 and RelB, indicating noncanonical NF- κ B pathway activation (27, 28).

NF- κ B activation in multiple myeloma can be accounted for by two alternative but non-exclusive mechanisms. First, NF- κ B may be activated in response to signals from the bone marrow microenvironment. Two TNF family ligands in the marrow microenvironment, BAFF and APRIL, can induce NF- κ B activation in multiple myeloma cells (169–172). Furthermore, TACI and BCMA, both receptors for BAFF and APRIL, are highly expressed in normal plasma cells and multiple myeloma. Indeed, among normal B cell subsets, NF- κ B signature gene expression is highest in plasma cells, presumably due to the influence of BAFF and APRIL in the bone marrow microenvironment (28). Second, multiple myeloma cells often have mutations in genes encoding positive and negative regulators of NF- κ B signaling, which are responsible for both the canonical and noncanonical NF- κ B activation (27, 28). Aberrations in eleven genes involved in the regulation of NF- κ B activation have been identified in 42% of multiple myeloma cell lines and in at least 17% of primary multiple myeloma samples (27, 28, 173). Myeloma cases with mutations in the NF- κ B pathway are associated with higher NF- κ B target gene expression than cases without identified mutations affecting this pathway (28).

Gain-of-function mutations that activate NF- κ B pathway in multiple myeloma include various translocations and amplifications that upregulate the genes encoding NIK, NFKB1 (p105/p50), and three receptors known to activate NF- κ B (CD40, TACI, LTBR). Overexpression of these TNF receptor family members may be sufficient to activate the NF- κ B pathway and/or may make the myeloma cells more sensitive to cytokines in the tumor microenvironment. Substantial overexpression of p50 could directly activate the canonical NF- κ B pathway by overwhelming the ability of I κ B α to block nuclear import of NF- κ B heterodimers. The kinase NIK can activate both the canonical and noncanonical NF- κ B pathways (174–177). In some myeloma cases, the genomic locus encoding NIK is highly amplified, resulting in NIK protein overexpression and constitutive activation of NF- κ B. Additionally, NIK can be overexpressed by translocation. In one instance, the translocation created a fusion protein that lacks the N-terminal 86 amino acids of NIK (28), which are required for TRAF3-mediated NIK degradation (178). The resultant fusion protein is highly stable, suggesting that TRAF3 mediates NIK turnover in myeloma cells.

Constitutive NF- κ B activation can also be initiated in multiple myeloma by loss-of-function mutations in negative regulators of NF- κ B signaling. Deletions, mutations, or transcriptional silencing can inactivate six negative regulators of the canonical and/or noncanonical NF- κ B pathways, including the genes encoding CYLD, NFKB2 (p100/p52), c-IAP1, c-IAP2, TRAF3, and TRAF2 (173). CYLD negatively regulates NF- κ B signaling by deubiquitinating several targets, including the NEMO/IKK γ , TRAF2, TRAF6, and BCL3 (179–183). The functionally relevant target of CYLD in multiple myeloma is not known. In the case of NFKB2, the mutations remove the I κ B-like domain in the p100 isoform, which

promotes processing of p100 to p52 and activation of the noncanonical NF- κ B pathway (27, 28, 184). The removal of the C-terminal domain of p100 presumably allows the truncated isoforms to pair with RelB and function as an NF- κ B transcriptional activator. The most prevalent genetic aberrations that activate NF- κ B in multiple myeloma are various mutations and deletions that inactivate TRAF3, which occur in more than 10% of cases (27, 28). In myeloma cells with TRAF3 mutations, NIK protein levels are elevated, indicating that TRAF3 inactivation stabilizes NIK protein, leading to chronic NF- κ B activation. At a lower frequency, some multiple myeloma cases acquire genomic deletions of the genomic locus encoding the ubiquitin ligases c-IAP1 and c-IAP2 (27, 28). Myeloma cell lines with this deletion also have high protein expression of NIK and TRAF3, providing the first clue that c-IAP1 and c-IAP2 are linked in a biochemical pathway with NIK and TRAF3. Some multiple myeloma tumors samples and cell lines have homozygous deletions of TRAF2 (27, 28), which also increase NIK protein levels, thereby implicating TRAF2 in NIK protein stability.

Subsequent biochemical studies established a signaling pathway that regulates NIK protein stability, involving TRAF2, TRAF3, and c-IAP1/2 (185–187). In normal cells, the regulation of NIK protein levels is mediated by a TRAF2/c-IAP1/2 ubiquitin ligase complex, which adds lysine 48-linked polyubiquitin chains to NIK and marks it for rapid proteasomal degradation. TRAF3 works as a molecular bridge to bring the TRAF2/c-IAP1/2 complex to NIK through a TRAF2-TRAF3 interaction. The activation of certain TNF receptor family members, notably CD40 and BAFFR, results in the recruitment of the TRAF2/c-IAP1/2 complex to the receptor, which stimulates polyubiquitination of TRAF3 by c-IAP1/2, causing its proteasomal degradation. As a result, NIK is stabilized since TRAF3 depletion dissociates c-IAP1/2 from NIK. NIK is a constitutively active kinase that can subsequently activate the noncanonical NF- κ B pathway through IKK α activation (188, 189).

Both the canonical and noncanonical NF- κ B pathways appear to be activated in multiple myeloma. Some genetic aberrations mainly affect the canonical pathway, such as those involving *NFKB1* and *CYLD*, whereas *NFKB2* aberrations directly activate the noncanonical pathway. However, the most prevalent abnormalities in myeloma cause prolonged, high level expression of NIK protein, which activates both the canonical and noncanonical NF- κ B pathways (28, 173). A small molecule inhibitor of IKK β , MLN120B, that does not inhibit IKK α (190) is toxic to the multiple myeloma cells with NIK overexpression (28, 167, 168). This demonstrates that the canonical pathway performs a non-redundant role in sustaining myeloma cell survival. By contrast, a near complete knockdown of IKK α by RNA interference is not toxic to myeloma cells with mutations that stabilize NIK (28). Although residual IKK α present following knockdown might be sufficient to maintain noncanonical NF- κ B signaling, this experiment suggests that the noncanonical NF- κ B pathway plays a subsidiary role in maintaining myeloma cell survival.

In summary, both cell-intrinsic and cell-extrinsic mechanisms account for NF- κ B activation in multiple myeloma cells. These two mechanisms are not mutually exclusive since overexpression of CD40, LTBR, and TAC1 may enhance the sensitivity of myeloma cells to NF- κ B stimulators in the bone marrow microenvironment. Compared with primary bone

marrow samples of multiple myeloma, the frequency of NF- κ B mutations is greater in myeloma cell lines, which are typically derived from late-stage disease that is growing in an extramedullary fashion outside of the bone marrow (28). Conceivably, NF- κ B-activating mutations could decrease the dependence of myeloma cells on the bone marrow microenvironment for NF- κ B signals, allowing the malignant cell to progress and grow in an extramedullary fashion.

Therapeutic targeting of NF- κ B in lymphoid malignancies

The NF- κ B pathway is a prime therapeutic target in many lymphoid malignancies, especially in ABC DLBCL (Fig. 3). Genetic abnormalities affecting NF- κ B regulators cause tumor cells to be addicted to the NF- κ B pathway. This may provide a therapeutic window, allowing NF- κ B-targeted therapies to have a preferential effect on the malignant cells relative to normal immune cells. Long-term administration of an NF- κ B inhibitor would be predicted to impair immune function, but it is entirely conceivable that short-term treatment of cancer patients with such agents might be manageable. These considerations imply that tumors that are truly addicted to the NF- κ B pathway must be identified, which might be accomplished by gene expression profiling of NF- κ B target genes along with cancer gene resequencing/copy number analysis to identify relevant genetic lesions.

As with any cancer targets, the successful end-game will be to find rational combination therapies. Combining NF- κ B inhibitors with cytotoxic chemotherapeutic drugs is logical given the ability of NF- κ B to block the apoptotic response to these agents (47). In a phase II study, patients with relapsed or refractory DLBCL were treated with a combination of cytotoxic chemotherapy and bortezomib, a proteasome inhibitor that suppresses NF- κ B by preventing the degradation of phospho-I κ B α (191). Patients with ABC DLBCL had a much higher complete response rate as well as longer median and overall survival than patients with GCB DLBCL. This apparent synergism between bortezomib and chemotherapy in ABC DLBCL has been replicated in another cohort of previously untreated patients with DLBCL (192). By contrast, treatment with cytotoxic chemotherapy alone yields consistently inferior results in patients with ABC DLBCL (25, 33, 193). Therefore, while bortezomib is ineffective by itself in DLBCL (191), its combination with chemotherapy may be particularly effective in tumors with constitutive NF- κ B activity.

The development of IKK inhibitors remains an enticing prospect despite some concerns regarding side effects (Reviewed in Liu *et al.*, this volume). In particular, IKK β inhibition may induce the secretion of IL-1 β , which could cause fevers and systemic toxicity (194). While these side effects might limit the utility of IKK β inhibitors in chronic inflammatory and autoimmune diseases, they may be manageable in the setting of cancer therapy. Another concern with IKK β inhibitors is that compensatory mechanisms may circumvent their effect. Specifically, treatment of ABC DLBCL cells with an IKK β inhibitor results in compensatory utilization of IKK β to phosphorylate I κ B α (190). An alternative strategy might be to target HSP90, a chaperone that is an integral component of the IKK complex and prevents the proteasomal degradation of both IKK α and IKK β (195). Several HSP90 inhibitors are now in clinical trials and promising results have been reported (196).

However, numerous other client proteins critically depend on HSP90 for proper function, making it difficult to rationally combine HSP90 inhibitors with other drugs.

A new approach to NF- κ B inhibition is to prevent polyubiquitination of I κ B α by its ubiquitin ligase, β TrCP. Since β TrCP requires neddylation for its activity, inhibition of the NEDD8-activating enzyme (NAE) would stabilize I κ B α . MLN4924, a small molecule NAE inhibitor, can effectively block degradation of phospho-I κ B α thereby blocking NF- κ B and causing the regression of ABC DLBCL tumors in a xenograft model (197). Neddylation is required for the proper degradation of other substrates, such as the DNA replication licensing factor CDT1, indicating that MLN4924 may prove useful in many other cancer types (198).

An attractive notion is to target upstream signaling pathways that feed into NF- κ B since other survival pathways may be simultaneously inhibited. Chronic active BCR signaling in ABC DLBCL offers many interesting therapeutic targets— including BTK, SYK, SRC-family kinases, PKC β , and PI3K δ (64) – that are targeted by drugs in early phase clinical trials. A highly selective BTK inhibitor, PCI-32765, is toxic to ABC DLBCL and CLL cells at low nanomolar concentrations (64, 199, 200) and is currently showing promising results in phase I/II trials in relapsed or refractory DLBCL, mantle cell lymphoma, and CLL. The attractiveness of this inhibitor is that it covalently modifies BTK using a cysteine residue in the active site of only 10 kinases in the human genome. This feature insures relative selectivity for BTK and endows the drug with favorable pharmacodynamic properties. A Syk kinase inhibitor, fostamatinib, has also shown activity as a single agent in phase I/II trials involving a variety of B-cell lymphomas. In CLL and its lymphomatous counterpart, small lymphocytic lymphoma, fostamatinib produced complete and partial responses in 55% of relapsed/refractory patients (201). Sotrastaurin, an inhibitor of PKC β , is selectively toxic towards ABC DLBCLs with chronic active BCR signaling *in vitro*, supporting clinical trials with this agent in this setting (202).

The anti-cancer potential of agents targeting the PI3K/AKT pathway has long been appreciated. However, many PI3K inhibitors target the p110 α catalytic isoform, which may limit their utility owing to the role of this pathway in many essential organismal functions. CAL-101 is a specific inhibitor of PI3K δ , which has a lymphoid-restricted expression pattern. Treatment of CLL, DLBCL and multiple myeloma cells with CAL-101 potently decreases phosphorylation of AKT and induces cell death (203–205). CAL-101 treatment of patients with CLL causes an egress of CLL cells from lymph nodes into the circulation, which is correlated with a decline in plasma levels of the chemokines CCL3, CCL4 and CXCL13, consistent with inhibition of BCR signaling (206). CAL-101 sensitizes CLL cells to common cytotoxic chemotherapeutic agents such as bendamustine, fludarabine, and dexamethasone *in vitro* (206). CAL-101 may be useful when administered prior to cytotoxic chemotherapy since it would mobilize CLL cells from the protective lymph node microenvironment into the circulation, allowing them to be killed by cytotoxic agents. In ABC DLBCL, PI3K α is required for survival, potentially by two mechanisms. First, PI3K activates AKT and mTOR, rendering these lymphomas sensitive to rapamycin, especially in combination with an IKK β inhibitor (64). Second, PI3K potentiates NF- κ B signaling in

ABC DLBCL, apparently through the kinase PDK1 (207). These considerations support the development of CAL-101 in ABC DLBCL.

The discovery of recurrent oncogenic MyD88 mutations in ABC DLBCL, MALT lymphoma, and CLL opens up new avenues for targeted therapies. Inhibition of IRAK4 downstream of MyD88 effectively blocks NF- κ B activation and survival of ABC DLBCL cells (81). While many inhibitors of IRAK4 are currently being developed as anti-inflammatory agents (208), they should also be evaluated for the treatment of lymphomas. Patients with congenital IRAK4 deficiency develop recurrent pyogenic infections early in life but invariably are healthy by adolescence (209, 210), indicating that IRAK4 inhibitors can most likely be administered safely as long as patients are given proper antibacterial prophylaxis. MyD88 signaling also promotes IL-6 and IL-10 secretion, resulting in autocrine stimulation of JAK kinase and activation of STAT3 (81). Small molecule inhibitors of JAK kinase synergize with IKK β inhibitors in killing ABC DLBCL cells (49), supporting the development of such agents for this indication. Certain ABC DLBCLs activate NF- κ B through both the BCR and MyD88 pathways (81). Consequently, concomitant inhibition of both pathways is synergistically toxic for these lymphomas (81), supporting the development of combination regimens targeting both pathways.

The cell-intrinsic and cell-extrinsic mechanisms that activate NF- κ B in multiple myeloma suggest several therapeutic strategies. Early phase clinical trials have been initiated in multiple myeloma with atacicept, a fusion protein containing the ligand binding domain of TACI that can inhibit both BAFF and APRIL, potentially preventing NF- κ B activation by these cytokines within the bone marrow microenvironment (211). Given the requirement for the canonical NF- κ B pathway in multiple myeloma (28), the aforementioned strategies to inhibit this pathway should be evaluated in multiple myeloma (28, 167, 168, 211). Small molecule inhibitors of NIK might be especially worthwhile given the prevalence of mutations that stabilize this kinase in myeloma. Such inhibitors might also be useful in gastric MALT lymphomas expressing the API2-MALT1 fusion oncoprotein since it proteolytically clips NIK, relieving NIK from TRAF3-dependent degradation (159).

Clearly, NF- κ B is an Achilles heel of many lymphoid malignancies, making us optimistic that therapies targeting this pathway will find their way into the routine therapy of these diseases. While some of these cancers can be cured by cytotoxic chemotherapy, there is considerable toxicity associated with this approach. Many agents targeting signal transduction have considerably less toxicity, at least acutely, although long-term administration of NF- κ B inhibitors might lead to overwhelming infections. Hence, the goal in lymphoid malignancies should be to combine NF- κ B inhibitors rationally with other targeted agents to simultaneously block several pathways that sustain malignant cell survival, hopefully achieving more cures with less toxicity.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

References

1. Ghosh S, Gifford AM, Riviere LR, Tempst P, Nolan GP, Baltimore D. Cloning of the p50 DNA binding subunit of NF-kappa B: homology to rel and dorsal. *Cell*. 1990; 62:1019–1029. [PubMed: 2203532]
2. Kieran M, et al. The DNA binding subunit of NF-kappa B is identical to factor KBF1 and homologous to the rel oncogene product. *Cell*. 1990; 62:1007–1018. [PubMed: 2203531]
3. Bargou RC, et al. High-level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood*. 1996; 87:4340–4347. [PubMed: 8639794]
4. Bargou RC, et al. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. *J Clin Invest*. 1997; 100:2961–2969. [PubMed: 9399941]
5. Emmerich F, et al. Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in reed-sternberg cells. *Blood*. 1999; 94:3129–3134. [PubMed: 10556199]
6. Cabannes E, Khan G, Aillet F, Jarrett RF, Hay RT. Mutations in the IkBa gene in Hodgkin's disease suggest a tumour suppressor role for IkappaBalpha. *Oncogene*. 1999; 18:3063–3070. [PubMed: 10340377]
7. Jungnickel B, et al. Clonal deleterious mutations in the IkappaBalpha gene in the malignant cells in Hodgkin's lymphoma. *J Exp Med*. 2000; 191:395–402. [PubMed: 10637284]
8. Krappmann D, Emmerich F, Kordes U, Scharschmidt E, Dorken B, Scheidereit C. Molecular mechanisms of constitutive NF-kappaB/Rel activation in Hodgkin/Reed-Sternberg cells. *Oncogene*. 1999; 18:943–953. [PubMed: 10023670]
9. Akagi T, et al. A novel gene, MALT1 at 18q21, is involved in t(11;18) (q21;q21) found in low-grade B-cell lymphoma of mucosa-associated lymphoid tissue. *Oncogene*. 1999; 18:5785–5794. [PubMed: 10523859]
10. Morgan JA, et al. Breakpoints of the t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma lie within or near the previously undescribed gene MALT1 in chromosome 18. *Cancer Res*. 1999; 59:6205–6213. [PubMed: 10626814]
11. Willis TG, et al. Bcl10 is involved in t(1;14)(p22;q32) of MALT B cell lymphoma and mutated in multiple tumor types. *Cell*. 1999; 96:35–45. [PubMed: 9989495]
12. Zhang Q, et al. Inactivating mutations and overexpression of BCL10, a caspase recruitment domain-containing gene, in MALT lymphoma with t(1;14)(p22;q32). *Nat Genet*. 1999; 22:63–68. [PubMed: 10319863]
13. Uren AG, et al. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol Cell*. 2000; 6:961–967. [PubMed: 11090634]
14. Bertin J, et al. CARD11 and CARD14 are novel caspase recruitment domain (CARD)/membrane-associated guanylate kinase (MAGUK) family members that interact with BCL10 and activate NF-kappa B. *J Biol Chem*. 2001; 276:11877–11882. [PubMed: 11278692]
15. Gaide O, Martinon F, Micheau O, Bonnet D, Thome M, Tschopp J. Carma1, a CARD-containing binding partner of Bcl10, induces Bcl10 phosphorylation and NF-kappaB activation. *FEBS Lett*. 2001; 496:121–127. [PubMed: 11356195]
16. McAllister-Lucas LM, et al. Biml, a MAGUK family member linking protein kinase C activation to Bcl10-mediated NF-kappaB induction. *J Biol Chem*. 2001; 276:30589–30597. [PubMed: 11387339]
17. Gaide O, et al. CARMA1 is a critical lipid raft-associated regulator of TCR-induced NF-kappa B activation. *Nat Immunol*. 2002; 3:836–843. [PubMed: 12154360]
18. Pomerantz JL, Denny EM, Baltimore D. CARD11 mediates factor-specific activation of NF-kappaB by the T cell receptor complex. *Embo J*. 2002; 21:5184–5194. [PubMed: 12356734]
19. Wang D, et al. A requirement for CARMA1 in TCR-induced NF-kappa B activation. *Nat Immunol*. 2002; 3:830–835. [PubMed: 12154356]
20. Egawa T, et al. Requirement for CARMA1 in antigen receptor-induced NF-kappa B activation and lymphocyte proliferation. *Curr Biol*. 2003; 13:1252–1258. [PubMed: 12867038]

21. Hara H, et al. The MAGUK family protein CARD11 is essential for lymphocyte activation. *Immunity*. 2003; 18:763–775. [PubMed: 12818158]
22. Jun JE, et al. Identifying the MAGUK protein Carma-1 as a central regulator of humoral immune responses and atopy by genome-wide mouse mutagenesis. *Immunity*. 2003; 18:751–762. [PubMed: 12818157]
23. Newton K, Dixit VM. Mice lacking the CARD of CARMA1 exhibit defective B lymphocyte development and impaired proliferation of their B and T lymphocytes. *Curr Biol*. 2003; 13:1247–1251. [PubMed: 12867037]
24. Ruefli-Brasse AA, French DM, Dixit VM. Regulation of NF-kappaB-dependent lymphocyte activation and development by paracaspase. *Science*. 2003; 302:1581–1584. [PubMed: 14576442]
25. Alizadeh AA, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000; 403:503–511. [PubMed: 10676951]
26. Alizadeh A, Eisen M, Botstein D, Brown PO, Staudt LM. Probing lymphocyte biology by genomic-scale gene expression analysis. *J Clin Immunol*. 1998; 18:373–379. [PubMed: 9857281]
27. Keats JJ, et al. Promiscuous mutations activate the noncanonical NF-kappaB pathway in multiple myeloma. *Cancer Cell*. 2007; 12:131–144. [PubMed: 17692805]
28. Annunziata CM, et al. Frequent engagement of the classical and alternative NF-kappaB pathways by diverse genetic abnormalities in multiple myeloma. *Cancer Cell*. 2007; 12:115–130. [PubMed: 17692804]
29. Herishanu Y, et al. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chronic lymphocytic leukemia. *Blood*. 2011; 117:563–574. [PubMed: 20940416]
30. Espinosa L, et al. The Notch/Hes1 pathway sustains NF-kappaB activation through CYLD repression in T cell leukemia. *Cancer Cell*. 2010; 18:268–281. [PubMed: 20832754]
31. Vilimas T, et al. Targeting the NF-kappaB signaling pathway in Notch1-induced T-cell leukemia. *Nat Med*. 2007; 13:70–77. [PubMed: 17173050]
32. Rosenwald A, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002; 346:1937–1947. [PubMed: 12075054]
33. Lenz G, et al. Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med*. 2008; 359:2313–2323. [PubMed: 19038878]
34. Rosenwald A, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med*. 2003; 198:851–862. [PubMed: 12975453]
35. Savage KJ, et al. The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. *Blood*. 2003; 102:3871–3879. [PubMed: 12933571]
36. Davis RE, Brown KD, Siebenlist U, Staudt LM. Constitutive nuclear factor kappa B activity is required for survival of activated B Cell-like diffuse large B cell lymphoma cells. *J Exp Med*. 2001; 194:1861–1874. [PubMed: 11748286]
37. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. *Proc Natl Acad Sci U S A*. 2003; 100:9991–9996. [PubMed: 12900505]
38. Klein U, et al. Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination. *Nat Immunol*. 2006; 7:773–782. [PubMed: 16767092]
39. Shaffer AL, et al. IRF4 addiction in multiple myeloma. *Nature*. 2008; 454:226–231. [PubMed: 18568025]
40. Iqbal J, et al. Distinctive patterns of BCL6 molecular alterations and their functional consequences in different subgroups of diffuse large B-cell lymphoma. *Leukemia*. 2007; 21:2332–2343. [PubMed: 17625604]
41. Mandelbaum J, et al. BLIMP1 is a tumor suppressor gene frequently disrupted in activated B cell-like diffuse large B cell lymphoma. *Cancer Cell*. 2010; 18:568–579. [PubMed: 21156281]
42. Pasqualucci L, et al. Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. *J Exp Med*. 2006; 203:311–317. [PubMed: 16492805]

43. Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, Staudt LM. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity*. 2000; 13:199–212. [PubMed: 10981963]
44. Tam W, Gomez M, Chadburn A, Lee JW, Chan WC, Knowles DM. Mutational analysis of PRDM1 indicates a tumor-suppressor role in diffuse large B-cell lymphomas. *Blood*. 2006; 107:4090–4100. [PubMed: 16424392]
45. Schmidlin H, et al. Spi-B inhibits human plasma cell differentiation by repressing BLIMP1 and XBP-1 expression. *Blood*. 2008; 112:1804–1812. [PubMed: 18552212]
46. Calado DP, et al. Constitutive canonical NF-kappaB activation cooperates with disruption of BLIMP1 in the pathogenesis of activated B cell-like diffuse large cell lymphoma. *Cancer Cell*. 2010; 18:580–589. [PubMed: 21156282]
47. Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. *J Clin Invest*. 2001; 107:241–246. [PubMed: 11160144]
48. Lam LT, et al. Small molecule inhibitors of IkappaB kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. *Clin Cancer Res*. 2005; 11:28–40. [PubMed: 15671525]
49. Lam LT, et al. Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor- κ B pathways in subtypes of diffuse large B-cell lymphoma. *Blood*. 2008; 111:3701–3713. [PubMed: 18160665]
50. Ngo VN, et al. A loss-of-function RNA interference screen for molecular targets in cancer. *Nature*. 2006; 441:106–110. [PubMed: 16572121]
51. Lam KP, Kuhn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell*. 1997; 90:1073–1083. [PubMed: 9323135]
52. Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K. Survival of resting mature B lymphocytes depends on BCR signaling via the Igalpha/beta heterodimer. *Cell*. 2004; 117:787–800. [PubMed: 15186779]
53. Srinivasan L, et al. PI3 kinase signals BCR-dependent mature B cell survival. *Cell*. 2009; 139:573–586. [PubMed: 19879843]
54. Thome M. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol*. 2004; 4:348–359. [PubMed: 15122200]
55. Depoil D, et al. CD19 is essential for B cell activation by promoting B cell receptor-antigen microcluster formation in response to membrane-bound ligand. *Nat Immunol*. 2008; 9:63–72. [PubMed: 18059271]
56. Tolar P, Hanna J, Krueger PD, Pierce SK. The constant region of the membrane immunoglobulin mediates B cell-receptor clustering and signaling in response to membrane antigens. *Immunity*. 2009; 30:44–55. [PubMed: 19135393]
57. Fleire SJ, Goldman JP, Carrasco YR, Weber M, Bray D, Batista FD. B cell ligand discrimination through a spreading and contraction response. *Science*. 2006; 312:738–741. [PubMed: 16675699]
58. Weber M, et al. Phospholipase C-gamma2 and Vav cooperate within signaling microclusters to propagate B cell spreading in response to membrane-bound antigen. *J Exp Med*. 2008; 205:853–868. [PubMed: 18362175]
59. Harwood NE, Batista FD. Early events in B cell activation. *Annu Rev Immunol*. 2010; 28:185–210. [PubMed: 20192804]
60. Harwood NE, Batista FD. The cytoskeleton coordinates the early events of B-cell activation. *Cold Spring Harb Perspect Biol*. 2011;3.
61. Tolar P, Sohn HW, Liu W, Pierce SK. The molecular assembly and organization of signaling active B-cell receptor oligomers. *Immunol Rev*. 2009; 232:34–41. [PubMed: 19909354]
62. Kurosaki T, Hikida M. Tyrosine kinases and their substrates in B lymphocytes. *Immunol Rev*. 2009; 228:132–148. [PubMed: 19290925]
63. Kurosaki T, Shinohara H, Baba Y. B cell signaling and fate decision. *Annu Rev Immunol*. 28:21–55. [PubMed: 19827951]
64. Davis RE, et al. Chronic active B-cell-receptor signalling in diffuse large B-cell lymphoma. *Nature*. 2010; 463:88–92. [PubMed: 20054396]

65. Johnson SA, Pleiman CM, Pao L, Schneringer J, Hippen K, Cambier JC. Phosphorylated immunoreceptor signaling motifs (ITAMs) exhibit unique abilities to bind and activate Lyn and Syk tyrosine kinases. *J Immunol.* 1995; 155:4596–4603. [PubMed: 7594458]
66. Gauld SB, Cambier JC. Src-family kinases in B-cell development and signaling. *Oncogene.* 2004; 23:8001–8006. [PubMed: 15489917]
67. Cornall RJ, et al. Polygenic autoimmune traits: Lyn, CD22, and SHP-1 are limiting elements of a biochemical pathway regulating BCR signaling and selection. *Immunity.* 1998; 8:497–508. [PubMed: 9586639]
68. Chan VW, Meng F, Soriano P, DeFranco AL, Lowell CA. Characterization of the B lymphocyte populations in Lyn-deficient mice and the role of Lyn in signal initiation and down-regulation. *Immunity.* 1997; 7:69–81. [PubMed: 9252121]
69. Torres RM, Hafen K. A negative regulatory role for Ig-alpha during B cell development. *Immunity.* 1999; 11:527–536. [PubMed: 10591178]
70. Kraus M, Saijo K, Torres RM, Rajewsky K. Ig-alpha cytoplasmic truncation renders immature B cells more sensitive to antigen contact. *Immunity.* 1999; 11:537–545. [PubMed: 10591179]
71. Gazumyan A, Reichlin A, Nussenzweig MC. Ig beta tyrosine residues contribute to the control of B cell receptor signaling by regulating receptor internalization. *J Exp Med.* 2006; 203:1785–1794. [PubMed: 16818674]
72. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science.* 2003; 301:1374–1377. [PubMed: 12920303]
73. Gross AJ, Lyandres JR, Panigrahi AK, Prak ET, DeFranco AL. Developmental acquisition of the Lyn-CD22-SHP-1 inhibitory pathway promotes B cell tolerance. *J Immunol.* 2009; 182:5382–5392. [PubMed: 19380785]
74. Lenz G, et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. *Science.* 2008; 319:1676–1679. [PubMed: 18323416]
75. Rawlings DJ, Sommer K, Moreno-Garcia ME. The CARMA1 signalosome links the signalling machinery of adaptive and innate immunity in lymphocytes. *Nat Rev Immunol.* 2006; 6:799–812. [PubMed: 17063183]
76. Matsumoto R, et al. Phosphorylation of CARMA1 plays a critical role in T Cell receptor-mediated NF-kappaB activation. *Immunity.* 2005; 23:575–585. [PubMed: 16356856]
77. Sommer K, et al. Phosphorylation of the CARMA1 linker controls NF-kappaB activation. *Immunity.* 2005; 23:561–574. [PubMed: 16356855]
78. Shinohara H, Maeda S, Watarai H, Kurosaki T. IkappaB kinase beta-induced phosphorylation of CARMA1 contributes to CARMA1 Bcl10 MALT1 complex formation in B cells. *J Exp Med.* 2007; 204:3285–3293. [PubMed: 18086859]
79. McCully RR, Pomerantz JL. The protein kinase C-responsive inhibitory domain of CARD11 functions in NF-kappaB activation to regulate the association of multiple signaling cofactors that differentially depend on Bcl10 and MALT1 for association. *Mol Cell Biol.* 2008; 28:5668–5686. [PubMed: 18625728]
80. Bidere N, et al. Casein kinase 1alpha governs antigen-receptor-induced NF-kappaB activation and human lymphoma cell survival. *Nature.* 2009; 458:92–96. [PubMed: 19118383]
81. Ngo VN, et al. Oncogenically active MyD88 mutations in human lymphoma. *Nature.* 2011; 470:115–119. [PubMed: 21179087]
82. Lamason RL, McCully RR, Lew SM, Pomerantz JL. Oncogenic CARD11 mutations induce hyperactive signaling by disrupting autoinhibition by the PKC-responsive inhibitory domain. *Biochemistry.* 2010; 49:8240–8250. [PubMed: 20799731]
83. Pasqualucci L, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat Genet.* 2011
84. Morin RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature.* 2011
85. Dong G, et al. A20, ABIN-1/2, and CARD11 mutations and their prognostic value in gastrointestinal diffuse large B-cell lymphoma. *Clin Cancer Res.* 2011; 17:1440–1451. [PubMed: 21266526]

86. Montesinos-Rongen M, et al. Mutations of CARD11 but not TNFAIP3 may activate the NF-kappaB pathway in primary CNS lymphoma. *Acta Neuropathol.* 2010; 120:529–535. [PubMed: 20544211]
87. Camilleri-Broet S, et al. A uniform activated B-cell-like immunophenotype might explain the poor prognosis of primary central nervous system lymphomas: analysis of 83 cases. *Blood.* 2006; 107:190–196. [PubMed: 16150948]
88. Burkhard P, Stetefeld J, Strelkov SV. Coiled coils: a highly versatile protein folding motif. *Trends Cell Biol.* 2001; 11:82–88. [PubMed: 11166216]
89. Tanner MJ, Hanel W, Gaffen SL, Lin X. CARMA1 coiled-coil domain is involved in the oligomerization and subcellular localization of CARMA1 and is required for T cell receptor-induced NF-kappaB activation. *J Biol Chem.* 2007; 282:17141–17147. [PubMed: 17428801]
90. Ferch U, et al. Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med.* 2009; 206:2313–2320. [PubMed: 19841089]
91. Hailfinger S, et al. Essential role of MALT1 protease activity in activated B cell-like diffuse large B-cell lymphoma. *Proc Natl Acad Sci U S A.* 2009; 106:19946–19951. [PubMed: 19897720]
92. Coornaert B, et al. T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-kappaB inhibitor A20. *Nat Immunol.* 2008; 9:263–271. [PubMed: 18223652]
93. Rebeaud F, et al. The proteolytic activity of the paracaspase MALT1 is key in T cell activation. *Nat Immunol.* 2008; 9:272–281. [PubMed: 18264101]
94. Hailfinger S, et al. Malt1-dependent RelB cleavage promotes canonical NF- κ B activation in lymphocytes and lymphoma cell lines. *Proc Natl Acad Sci U S A.* 2011; 108:14596–14601. [PubMed: 21873235]
95. Wesche H, Henzel WJ, Shillinglaw W, Li S, Cao Z. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity.* 1997; 7:837–847. [PubMed: 9430229]
96. Medzhitov R, et al. MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell.* 1998; 2:253–258. [PubMed: 9734363]
97. Burns K, et al. MyD88, an adapter protein involved in interleukin-1 signaling. *J Biol Chem.* 1998; 273:12203–12209. [PubMed: 9575168]
98. Muzio M, Ni J, Feng P, Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science.* 1997; 278:1612–1615. [PubMed: 9374458]
99. Lin SC, Lo YC, Wu H. Helical assembly in the MyD88-IRAK4-IRAK2 complex in TLR/IL-1R signalling. *Nature.* 2010; 465:885–890. [PubMed: 20485341]
100. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol.* 2010; 11:373–384. [PubMed: 20404851]
101. Yang J, Liao X, Agarwal MK, Barnes L, Auron PE, Stark GR. Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFkappaB. *Genes Dev.* 2007; 21:1396–1408. Epub 2007 May 17. [PubMed: 17510282]
102. Puente XS, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature.* 2011
103. Jiang Z, et al. Details of Toll-like receptor:adapter interaction revealed by germ-line mutagenesis. *Proc Natl Acad Sci U S A.* 2006; 103:10961–10966. [PubMed: 16832055]
104. Hou B, et al. Selective Utilization of Toll-like Receptor and MyD88 Signaling in B Cells for Enhancement of the Antiviral Germinal Center Response. *Immunity.* 2011; 34:375–384. [PubMed: 21353603]
105. Marshak-Rothstein A, Rifkin IR. Immunologically active autoantigens: the role of toll-like receptors in the development of chronic inflammatory disease. *Annu Rev Immunol.* 2007; 25:419–441. [PubMed: 17378763]
106. Wertz IE, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature.* 2004; 430:694–699. [PubMed: 15258597]
107. Lin SC, et al. Molecular basis for the unique deubiquitinating activity of the NF-kappaB inhibitor A20. *J Mol Biol.* 2008; 376:526–540. [PubMed: 18164316]
108. Hymowitz SG, Wertz IE. A20: from ubiquitin editing to tumour suppression. *Nat Rev Cancer.* 2010; 10:332–341. [PubMed: 20383180]

109. Lee EG, et al. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science*. 2000; 289:2350–2354. [PubMed: 11009421]
110. Compagno M, et al. Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. *Nature*. 2009; 459:717–721. [PubMed: 19412164]
111. Honma K, et al. TNFAIP3/A20 functions as a novel tumor suppressor gene in several subtypes of non-Hodgkin lymphomas. *Blood*. 2009; 114:2467–2475. [PubMed: 19608751]
112. Turer EE, et al. Homeostatic MyD88-dependent signals cause lethal inflammation in the absence of A20. *J Exp Med*. 2008; 205:451–464. [PubMed: 18268035]
113. Schmitz R, et al. TNFAIP3 (A20) is a tumor suppressor gene in Hodgkin lymphoma and primary mediastinal B cell lymphoma. *J Exp Med*. 2009; 206:981–989. [PubMed: 19380639]
114. Weniger MA, et al. Gains of REL in primary mediastinal B-cell lymphoma coincide with nuclear accumulation of REL protein. *Genes Chromosomes Cancer*. 2007; 46:406–415. [PubMed: 17243160]
115. Stein H, et al. Identification of Hodgkin and Sternberg-reed cells as a unique cell type derived from a newly-detected small-cell population. *Int J Cancer*. 1982; 30:445–459. [PubMed: 6754630]
116. Gruss HJ, et al. Expression and function of CD40 on Hodgkin and Reed-Sternberg cells and the possible relevance for Hodgkin's disease. *Blood*. 1994; 84:2305–2314. [PubMed: 7522624]
117. Fiumara P, et al. Functional expression of receptor activator of nuclear factor kappaB in Hodgkin disease cell lines. *Blood*. 2001; 98:2784–2790. [PubMed: 11675352]
118. Chiu A, et al. Hodgkin lymphoma cells express TACI and BCMA receptors and generate survival and proliferation signals in response to BAFF and APRIL. *Blood*. 2007; 109:729–739. [PubMed: 16960154]
119. Deacon EM, et al. Epstein-Barr virus and Hodgkin's disease: transcriptional analysis of virus latency in the malignant cells. *J Exp Med*. 1993; 177:339–349. [PubMed: 8381153]
120. Graham JP, Arcipowski KM, Bishop GA. Differential B-lymphocyte regulation by CD40 and its viral mimic, latent membrane protein 1. *Immunol Rev*. 2010; 237:226–248. [PubMed: 20727039]
121. Kulwichit W, Edwards RH, Davenport EM, Baskar JF, Godfrey V, Raab-Traub N. Expression of the Epstein-Barr virus latent membrane protein 1 induces B cell lymphoma in transgenic mice. *Proc Natl Acad Sci U S A*. 1998; 95:11963–11968. [PubMed: 9751773]
122. Uchida J, et al. Mimicry of CD40 signals by Epstein-Barr virus LMP1 in B lymphocyte responses. *Science*. 1999; 286:300–303. [PubMed: 10514374]
123. Homig-Holzel C, et al. Constitutive CD40 signaling in B cells selectively activates the noncanonical NF-kappaB pathway and promotes lymphomagenesis. *J Exp Med*. 2008; 205:1317–1329. [PubMed: 18490492]
124. Fruehling S, et al. Identification of latent membrane protein 2A (LMP2A) domains essential for the LMP2A dominant-negative effect on B-lymphocyte surface immunoglobulin signal transduction. *J Virol*. 1996; 70:6216–6226. [PubMed: 8709248]
125. Fruehling S, Longnecker R. The immunoreceptor tyrosine-based activation motif of Epstein-Barr virus LMP2A is essential for blocking BCR-mediated signal transduction. *Virology*. 1997; 235:241–251. [PubMed: 9281504]
126. Caldwell RG, Wilson JB, Anderson SJ, Longnecker R. Epstein-Barr virus LMP2A drives B cell development and survival in the absence of normal B cell receptor signals. *Immunity*. 1998; 9:405–411. [PubMed: 9768760]
127. Mancao C, Hammerschmidt W. Epstein-Barr virus latent membrane protein 2A is a B-cell receptor mimic and essential for B-cell survival. *Blood*. 2007; 110:3715–3721. [PubMed: 17682125]
128. Casola S, et al. B cell receptor signal strength determines B cell fate. *Nat Immunol*. 2004; 5:317–327. [PubMed: 14758357]
129. Barth TF, et al. Gains of 2p involving the REL locus correlate with nuclear c-Rel protein accumulation in neoplastic cells of classical Hodgkin lymphoma. *Blood*. 2003; 101:3681–3686. [PubMed: 12511414]
130. Martin-Subero JJ, et al. Recurrent involvement of the REL and BCL11A loci in classical Hodgkin lymphoma. *Blood*. 2002; 99:1474–1477. [PubMed: 11830502]

131. Joos S, et al. Classical Hodgkin lymphoma is characterized by recurrent copy number gains of the short arm of chromosome 2. *Blood*. 2002; 99:1381–1387. [PubMed: 11830490]
132. Lake A, et al. Mutations of NFKB1A, encoding IkappaB alpha, are a recurrent finding in classical Hodgkin lymphoma but are not a unifying feature of non-EBV-associated cases. *Int J Cancer*. 2009; 125:1334–1342. [PubMed: 19507254]
133. Emmerich F, et al. Inactivating I kappa B epsilon mutations in Hodgkin/Reed-Sternberg cells. *J Pathol*. 2003; 201:413–420. [PubMed: 14595753]
134. Kato M, et al. Frequent inactivation of A20 in B-cell lymphomas. *Nature*. 2009; 459:712–716. [PubMed: 19412163]
135. Brune V, et al. Origin and pathogenesis of nodular lymphocyte-predominant Hodgkin lymphoma as revealed by global gene expression analysis. *J Exp Med*. 2008; 205:2251–2268. [PubMed: 18794340]
136. Schumacher MA, et al. Mutations in the genes coding for the NF-kappaB regulating factors IkappaBalpha and A20 are uncommon in nodular lymphocyte-predominant Hodgkin's lymphoma. *Haematologica*. 2009; 95:153–157. [PubMed: 19648161]
137. Bende RJ, van Maldegem F, van Noesel CJ. Chronic inflammatory disease, lymphoid tissue neogenesis and extranodal marginal zone B-cell lymphomas. *Haematologica*. 2009; 94:1109–1123. [PubMed: 19608670]
138. Wotherspoon AC, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet*. 1991; 338:1175–1176. [PubMed: 1682595]
139. Roggero E, et al. Eradication of Borrelia burgdorferi infection in primary marginal zone B-cell lymphoma of the skin. *Hum Pathol*. 2000; 31:263–268. [PubMed: 10685647]
140. Lecuit M, et al. Immunoproliferative small intestinal disease associated with Campylobacter jejuni. *N Engl J Med*. 2004; 350:239–248. [PubMed: 14724303]
141. Ferreri AJ, et al. Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. *J Natl Cancer Inst*. 2004; 96:586–594. [PubMed: 15100336]
142. Hermine O, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med*. 2002; 347:89–94. [PubMed: 12110736]
143. Bende RJ, Aarts WM, Riedl RG, de Jong D, Pals ST, van Noesel CJ. Among B cell non-Hodgkin's lymphomas, MALT lymphomas express a unique antibody repertoire with frequent rheumatoid factor reactivity. *J Exp Med*. 2005; 201:1229–1241. [PubMed: 15837810]
144. Hussell T, Isaacson PG, Crabtree JE, Spencer J. Helicobacter pylori-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B cells in low-grade gastric lymphoma of mucosa-associated lymphoid tissue. *J Pathol*. 1996; 178:122–127. [PubMed: 8683376]
145. Greiner A, et al. Low-grade B cell lymphomas of mucosa-associated lymphoid tissue (MALT-type) require CD40-mediated signaling and Th2-type cytokines for in vitro growth and differentiation. *Am J Pathol*. 1997; 150:1583–1593. [PubMed: 9137085]
146. Sanchez-Izquierdo D, et al. MALT1 is deregulated by both chromosomal translocation and amplification in B-cell non-Hodgkin lymphoma. *Blood*. 2003; 101:4539–4546. [PubMed: 12560219]
147. Murga Penas EM, et al. Translocations t(11;18)(q21;q21) and t(14;18)(q32;q21) are the main chromosomal abnormalities involving MLT/MALT1 in MALT lymphomas. *Leukemia*. 2003; 17:2225–2229. [PubMed: 12931213]
148. Auer IA, et al. t(11;18)(q21;q21) is the most common translocation in MALT lymphomas. *Ann Oncol*. 1997; 8:979–985. [PubMed: 9402171]
149. Dierlamm J, et al. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21)p6ssociated with mucosa-associated lymphoid tissue lymphomas. *Blood*. 1999; 93:3601–3609. [PubMed: 10339464]
150. Rosenwald A, et al. Exclusive detection of the t(11;18)(q21;q21) in extranodal marginal zone B cell lymphomas (MZBL) of MALT type in contrast to other MZBL and extranodal large B cell lymphomas. *Am J Pathol*. 1999; 155:1817–1821. [PubMed: 10595910]

151. Au WY, et al. Post-transplantation lymphoproliferative disease in Chinese: the Queen Mary Hospital experience in Hong Kong. *Leuk Lymphoma*. 2002; 43:1403–1407. [PubMed: 12389620]
152. Lucas PC, et al. Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-kappa B signaling pathway. *J Biol Chem*. 2001; 276:19012–19019. [PubMed: 11262391]
153. Ruland J, Duncan GS, Wakeham A, Mak TW. Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity*. 2003; 19:749–758. [PubMed: 14614861]
154. Baens M, et al. Selective expansion of marginal zone B cells in Emicro-API2-MALT1 mice is linked to enhanced IkappaB kinase gamma polyubiquitination. *Cancer Res*. 2006; 66:5270–5277. [PubMed: 16707452]
155. Lucas PC, et al. A dual role for the API2 moiety in API2-MALT1-dependent NF-kappaB activation: heterotypic oligomerization and TRAF2 recruitment. *Oncogene*. 2007; 26:5643–5654. [PubMed: 17334391]
156. Sun L, Deng L, Ea CK, Xia ZP, Chen ZJ. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol Cell*. 2004; 14:289–301. [PubMed: 15125833]
157. Noels H, et al. A Novel TRAF6 binding site in MALT1 defines distinct mechanisms of NF-kappaB activation by API2middle dot MALT1 fusions. *J Biol Chem*. 2007; 282:10180–10189. [PubMed: 17287209]
158. Hu S, et al. cIAP2 is a ubiquitin protein ligase for BCL10 and is dysregulated in mucosa-associated lymphoid tissue lymphomas. *J Clin Invest*. 2006; 116:174–181. [PubMed: 16395405]
159. Rosebeck S, et al. Cleavage of NIK by the API2-MALT1 fusion oncoprotein leads to noncanonical NF-kappaB activation. *Science*. 2011; 331:468–472. [PubMed: 21273489]
160. Chanudet E, et al. A20 is targeted by promoter methylation, deletion and inactivating mutation in MALT lymphoma. *Leukemia*. 2010; 24:483–487. [PubMed: 19907439]
161. Rinaldi A, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. *Blood*. 2011; 117:1595–1604. [PubMed: 21115979]
162. Novak U, et al. The NF- κ B negative regulator TNFAIP3 (A20) is inactivated by somatic mutations and genomic deletions in marginal zone B-cell lymphomas. *Blood*. 2009
163. Kim WS, et al. Genome-wide array-based comparative genomic hybridization of ocular marginal zone B cell lymphoma: comparison with pulmonary and nodal marginal zone B cell lymphoma. *Genes Chromosomes Cancer*. 2007; 46:776–783. [PubMed: 17492759]
164. Honma K, et al. TNFAIP3 is the target gene of chromosome band 6q23.3-q24.1 loss in ocular adnexal marginal zone B cell lymphoma. *Genes Chromosomes Cancer*. 2008; 47:1–7. [PubMed: 17886247]
165. Dalton WS. The tumor microenvironment: focus on myeloma. *Cancer Treat Rev*. 2003; 29(Suppl 1):11–19. [PubMed: 12738239]
166. Hideshima T, Anderson KC. Molecular mechanisms of novel therapeutic approaches for multiple myeloma. *Nat Rev Cancer*. 2002; 2:927–937. [PubMed: 12459731]
167. Hideshima T, et al. MLN120B, a novel IkappaB kinase beta inhibitor, blocks multiple myeloma cell growth in vitro and in vivo. *Clin Cancer Res*. 2006; 12:5887–5894. [PubMed: 17020997]
168. Jourdan M, et al. Targeting NF-kappaB pathway with an IKK2 inhibitor induces inhibition of multiple myeloma cell growth. *Br J Haematol*. 2007; 138:160–168. [PubMed: 17542984]
169. Hideshima T, et al. NF-kappa B as a therapeutic target in multiple myeloma. *J Biol Chem*. 2002; 277:16639–16647. [PubMed: 11872748]
170. Marsters SA, Yan M, Pitti RM, Haas PE, Dixit VM, Ashkenazi A. Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI. *Curr Biol*. 2000; 10:785–788. [PubMed: 10898980]
171. Moreaux J, et al. The level of TACI gene expression in myeloma cells is associated with a signature of microenvironment dependence versus a plasmablastic signature. *Blood*. 2005; 106:1021–1030. [PubMed: 15827134]

172. O'Connor BP, et al. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J Exp Med*. 2004; 199:91–98. [PubMed: 14707116]
173. Demchenko YN, Glebov OK, Zingone A, Keats JJ, Bergsagel PL, Kuehl WM. Classical and/or alternative NF-kappaB pathway activation in multiple myeloma. *Blood*. 2010; 115:3541–3552. [PubMed: 20053756]
174. Claudio E, Brown K, Park S, Wang H, Siebenlist U. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. *Nat Immunol*. 2002; 3:958–965. [PubMed: 12352969]
175. Coope HJ, et al. CD40 regulates the processing of NF-kappaB2 p100 to p52. *Embo J*. 2002; 21:5375–5385. [PubMed: 12374738]
176. Ramakrishnan P, Wang W, Wallach D. Receptor-specific signaling for both the alternative and the canonical NF-kappaB activation pathways by NF-kappaB-inducing kinase. *Immunity*. 2004; 21:477–489. [PubMed: 15485626]
177. Yin L, et al. Defective lymphotoxin-beta receptor-induced NF-kappaB transcriptional activity in NIK-deficient mice. *Science*. 2001; 291:2162–2165. [PubMed: 11251123]
178. Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. *J Biol Chem*. 2004; 279:26243–26250. [PubMed: 15084608]
179. Brummelkamp TR, Nijman SM, Dirac AM, Bernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. *Nature*. 2003; 424:797–801. [PubMed: 12917690]
180. Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. *Nature*. 2003; 424:801–805. [PubMed: 12917691]
181. Massoumi R, Chmielarska K, Hennecke K, Pfeifer A, Fassler R. Cyld inhibits tumor cell proliferation by blocking Bcl-3-dependent NF-kappaB signaling. *Cell*. 2006; 125:665–677. [PubMed: 16713561]
182. Regamey A, et al. The tumor suppressor CYLD interacts with TRIP and regulates negatively nuclear factor kappaB activation by tumor necrosis factor. *J Exp Med*. 2003; 198:1959–1964. [PubMed: 14676304]
183. Trompouki E, Hatzivassiliou E, Tschritzis T, Farmer H, Ashworth A, Mosialos G. CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. *Nature*. 2003; 424:793–796. [PubMed: 12917689]
184. Migliazza A, et al. Heterogeneous chromosomal aberrations generate 3' truncations of the NFKB2/lyt-10 gene in lymphoid malignancies. *Blood*. 1994; 84:3850–3860. [PubMed: 7949142]
185. Matsuzawa A, et al. Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science*. 2008; 321:663–668. [PubMed: 18635759]
186. Vallabhapurapu S, et al. Nonredundant and complementary functions of TRAF2 and TRAF3 in a ubiquitination cascade that activates NIK-dependent alternative NF-kappaB signaling. *Nat Immunol*. 2008; 9:1364–1370. [PubMed: 18997792]
187. Zarnegar BJ, et al. Noncanonical NF-kappaB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nat Immunol*. 2008; 9:1371–1378. [PubMed: 18997794]
188. Xiao G, Harhaj EW, Sun SC. NF-kappaB-inducing kinase regulates the processing of NF-kappaB2 p100. *Mol Cell*. 2001; 7:401–409. [PubMed: 11239468]
189. Senftleben U, et al. Activation by IKKalpha of a second, evolutionary conserved, NF-kappa B signaling pathway. *Science*. 2001; 293:1495–1499. [PubMed: 11520989]
190. Lam LT, et al. Compensatory IKKalpha activation of classical NF-kappaB signaling during IKKbeta inhibition identified by an RNA interference sensitization screen. *Proc Natl Acad Sci U S A*. 2008; 105:20798–20803. [PubMed: 19104039]
191. Dunleavy K, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. *Blood*. 2009; 113:6069–6076. [PubMed: 19380866]
192. Ruan J, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. *J Clin Oncol*. 2010; 29:690–697. [PubMed: 21189393]

193. Rosenwald A, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med.* 2001; 194:1639–1648. [PubMed: 11733578]
194. Greten FR, et al. NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. *Cell.* 2007; 130:918–931. [PubMed: 17803913]
195. Broemer M, Krappmann D, Scheidereit C. Requirement of Hsp90 activity for IkappaB kinase (IKK) biosynthesis and for constitutive and inducible IKK and NF-kappaB activation. *Oncogene.* 2004; 23:5378–5386. [PubMed: 15077173]
196. Trepel J, Mollapour M, Giaccone G, Neckers L. Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer.* 2010; 10:537–549. [PubMed: 20651736]
197. Milhollen MA, et al. MLN4924, a NEDD8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of NF-{kappa}B-dependent lymphoma. *Blood.* 2010; 116:1515–1523. [PubMed: 20525923]
198. Soucy TA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature.* 2009; 458:732–736. [PubMed: 19360080]
199. Honigberg LA, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci U S A.* 2010; 107:13075–13080. [PubMed: 20615965]
200. Herman SE, et al. Bruton tyrosine kinase represents a promising therapeutic target for treatment of chronic lymphocytic leukemia and is effectively targeted by PCI-32765. *Blood.* 2011; 117:6287–6296. [PubMed: 21422473]
201. Friedberg JW, et al. Inhibition of Syk with fostamatinib disodium has significant clinical activity in non Hodgkin's lymphoma and chronic lymphocytic leukemia. *Blood.* 2009
202. Naylor TL, et al. Protein kinase C inhibitor Sotrastaurin selectively inhibits the growth of CD79-mutant diffuse large B-cell lymphomas. *Cancer Res.* 2011
203. Ikeda H, et al. PI3K/p110{delta} is a novel therapeutic target in multiple myeloma. *Blood.* 2010; 116:1460–1468. [PubMed: 20505158]
204. Herman SE, et al. Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. *Blood.* 2010; 116:2078–2088. [PubMed: 20522708]
205. Lannutti BJ, et al. CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. *Blood.* 2010; 117:591–594. [PubMed: 20959606]
206. Hoellenriegel J, et al. The phosphoinositide 3'-kinase delta inhibitor, CAL-101, inhibits B-cell receptor signaling and chemokine networks in chronic lymphocytic leukemia. *Blood.* 2011
207. Kloos B, et al. Critical role of PI3K signaling for NF-kappaB-dependent survival in a subset of activated B-cell-like diffuse large B-cell lymphoma cells. *Proc Natl Acad Sci U S A.* 2011; 108:272–277. [PubMed: 21173233]
208. Wang Z, Wesche H, Stevens T, Walker N, Yeh WC. IRAK-4 inhibitors for inflammation. *Curr Top Med Chem.* 2009; 9:724–737. [PubMed: 19689377]
209. Picard C, et al. Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science.* 2003; 299:2076–2079. [PubMed: 12637671]
210. Ku CL, et al. Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. *J Exp Med.* 2007; 204:2407–2422. [PubMed: 17893200]
211. Rossi JF, et al. Atacicept in relapsed/refractory multiple myeloma or active Waldenstrom's macroglobulinemia: a phase I study. *Br J Cancer.* 2009; 101:1051–1058. [PubMed: 19789533]

Fig. 1. Compendium of CARD11 mutants in lymphoma
The coiled-coil domain of CARD11 is presented with lymphoma-derived mutants indicated. As indicated, mutants are derived from nodal DLBCL classified into the ABC or GCB subtypes (74, 83, 84), unclassified nodal and gastric DLBCL (84, 85), primary central nervous system (CNS) lymphoma (86), and follicular lymphoma (84). A ‘-’ indicates a removal of amino acids, and a lower case ‘i’ followed by a number indicates insertion of amino acids.

Fig. 2. Location of recurrent MyD88 mutants present in ABC DLBCL tumors

A. The MyD88 TIR domain structure with ABC DLBCL mutants indicated. B. Space-filling model of MyD88 TIR domain illustrating that the highly recurrent L265P mutant is buried within the hydrophobic core of the domain. Also note that another recurrent mutant, M232T, is adjacent to L265 and makes Van der Waals interactions with L265.

Fig. 3. Therapeutic targets in ABC DLBCL

The pathogenesis of ABC DLBCL involves constitutive activation of BCR, MyD88 and IL-6/IL-10 signaling pathway, promoting cell survival through NF- κ B, STAT3 and PI3K signaling pathways. Signaling in these pathways is induced or accentuated by recurrent mutations in ABC DLBCL that affect CD79B, MyD88, CARD11 and A20 (denoted with and asterisk). Therapeutic targets in ABC DLBCL and exemplar small molecule inhibitors include: 1. Src-family kinase (Dasatinib) (64); 2. SYK (fostamatinib/R406) (64, 201); 3. PI3K δ (CAL-101) (64, 206, 207); 4. mTOR (rapamycin) (64); 5. BTK (PCI-32765, Dasatinib) (64); 6. PKC β (Sotrastaurin) (64, 202); 7. MALT1 (50, 74); 8. IKK β (MLN120B) (48, 90, 91); 9. HSP90 (geldanamycin) (195, 196); 10. IRAK4 (81); 11. NEDD8-activating enzyme (NAE) (MLN4924); JAK1 (AZD1480) (49, 81); 12. Proteasome (bortezomib) (191).