Published in final edited form as:

Leuk Lymphoma. 2008 May ; 49(5): 874–882. doi:10.1080/10428190801895345.

Therapeutic targeting of the BCL6 oncogene for diffuse large B-cell lymphomas

Samir S Parekh¹, Gilbert G Privé², and Ari Melnick^{3,*}

¹ Department of Medical Oncology. Albert Einstein College of Medicine. 1300 Morris Park Ave, Bronx, NY 10461.

² Department of Biochemistry and Department of Medical Biophysics of the University of Toronto; and Division of Cancer Genomics and Proteomics, Ontario Cancer Institute, 101 College Street, Toronto, Ontario M5G 1L7, Canada

³ Department of Developmental and Molecular Biology. Albert Einstein College of Medicine. 1300 Morris Park Ave, Bronx, NY 10461.

Abstract

BCL6 is a transcriptional repressor often expressed constitutively in diffuse large B-cell lymphomas (DLBCL) due to mutations of its genomic locus. BCL6 mediates aberrant survival, proliferation, genomic instability and differentiation blockade in DLBCL cells. The biochemical study of BCL6 mediated gene repression has provided the basis for design of agents that inhibit BCL6 and kill lymphoma cells. The repressor activity of the BCL6 BTB domain is particularly well defined from the structural standpoint. Design of inhibitors targeting BCL6 BTB domain protein interaction surfaces appears to be an effective approach, which reactivates important BCL6 target genes and readily kills DLBCL cells. Targeting other domains of BCL6 or using histone deacetylase inhibitors to overcome BCL6 mediated repression may also be useful. Recent studies in DLBCL transcriptional signatures have revealed a subset of DLBCLs that are particularly dependent on BCL6 to maintain their survival and these patients could be candidates for clinical trials of BCL6 inhibitors.

Introduction

One of the ultimate goals of experimental therapeutic research in cancer is to develop agents that are truly specifically targeted to the causative molecular lesions of individual types of tumors. Such agents hold the promise of potent anti-tumor effects with favorable toxicity profiles, since often tumors are addicted to aberrant survival pathways that are not essential in normal cells. Very few such agents currently exist for any kind of cancer. The most dramatically successful examples of such agents in hematologic malignancies are alltrans retinoic acid, which targets the PML-RAR α (promyelocytic leukemia-retinoic acid receptor alpha) fusion protein of t(15;17) acute promyelocytic leukemia[1], and imatinib, which targets the BCR-ABL fusion protein of t(9;22) chronic myeloid leukemia[1]. Currently no such targeted therapies exist for B-cell lymphomas. Although immunotherapy approaches such as anti-CD20 antibodies can target B-cells and are clinically useful, they do not target the molecular mechanism of disease in lymphoma and damage a wide variety of normal CD20 expressing B-cells in addition to the tumor. This review will discuss the potential for direct therapeutic

*Corresponding author: Ari Melnick, MD Department of Developmental and Molecular Biology Albert Einstein College of Medicine 1300 Morris Park Ave, Chanin building Room 302A Bronx, NY. 10128 USA Tel: 718-430-4238 Fax: 718-430-8567 amelnick@aecom.yu.edu.

targeting of the BCL6 (**B-cell lymphoma 6**) transcriptional repressor, which is the most commonly involved oncogene in DLBCL.

BCL6 regulates proliferation, genomic stability and differentiation in normal and malignant B-cells (Fig 1)

In normal B-cell biology BCL6 is required for activated B-cells to form germinal centers and undergo antibody affinity maturation[2,3]. Downregulation of BCL6 is required for germinal center cells to undergo further differentiation into memory or plasma cells[4-7]. Due in part to the biological actions of BCL6, GC B-cells acquire the unique and dangerous capability of rapidly proliferating while simultaneously undergoing genetic recombination of their immunoglobulin loci (e.g. as reviewed in[8]). The purpose of the GC reaction is to quickly generate clonal diversity of B-cells in order maximize the chance of forming high affinity antibody against the offending antigen[8]. During this process the immunoglobulin loci are subjected to the processes of class switch recombination and somatic hypermutation, which introduce double strand breaks and point mutations respectively through the actions of the enzyme AID (activation induced cytosine deaminase) (e.g. as reviewed in [9]). GC B-cells are therefore specialized to undergo a form of physiologic genomic instability. An unintended consequence of this process is the mistargeting of AID to other transcriptionally active loci [10]. When AID inadvertently activates cellular proto-oncogenes a survival advantage is conferred that can lead to lymphomagenesis. It is therefore not surprising that majority of B-cell lymphomas show evidence of having transited through the GC reaction[11]. One of the most commonly mis-targeted proto-oncogene loci is that of BCL6, which can become constitutively expressed as a consequence of translocations to heterologous promoters or the introduction of point mutations in promoter negative regulatory elements in approximately 70% of DLBCLs[12]. When such BCL6 translocations are genetically engineered in mice the outcome is development of DLBCL, similar to the human disease[13,14].

BCL6 is a transcriptional repressor and mediates its biological effects through downregulation of over 500 direct target genes mainly involved in control of cell cycle, gene transcription, DNA damage sensing, protein ubiquitylation and chromatin structure[15]. Among these direct targets are genes belonging to a critical pathway involved in DNA damage sensing and checkpoint activation involving the genes *ATR* (ataxia telangiectasia and Rad3 related), *CHEK1* (CHK1 checkpoint homolog (*S. pombe*)), *TP53* (tumor protein p53) and *CDKN1A* (cyclin-dependent kinase inhibitor 1A)[15-18]. ATR is a ubiquitously expressed and essential protein that maintains genomic integrity during replication and senses single and double strand breaks in DNA[19-21]. DNA damage triggers ATR kinase domain dependent phosphorylation of a number of downstream DNA damage response, repair and checkpoint proteins including H2AX (H2A histone family, member X) and CHK1[22,23]. In turn, CHK1 can induce cell cycle arrest through regulation of Cdc25 isoforms, phosphorylation of TP53 and other mechanisms (e.g.[24-26]. Activation of TP53 by CHK1 or directly via ATR triggers its suppressive effects over the cell cycle (in part by induction of CDKN1A) and its pro-apoptotic effects[25,27]. By directly repressing ATR, CHK1, TP53 and CDKN1A, BCL6 can attenuate cellular response to DNA damage, thus allowing activated B-cells to adopt the GC phenotype of proliferation during DNA recombination[16-18]. By the same token BCL6 repression of these genes facilitates proliferation and genomic instability in DLBCL cells[16-18]. Reciprocally, high levels of DNA damage can lead to proteolytic degradation of BCL6 through the ATM (ataxia telangiectasia mutated) pathway[28].

BCL6 also contributes to maintaining the GC phenotype by directly repressing the *PRDM1* (PR domain containing 1, with ZNF domain) gene, which is a mediator of plasma cell differentiation[4,29,30]. Down-regulation of BCL6 is required for B-cells to exit the germinal center reaction and undergo further differentiation[4-7]. In turn, PRDM1, which is also a

transcriptional repressor, can induce downregulation of BCL6[31], further reducing the chance that B-cells could re-acquire GC characteristics once affinity maturation is complete. The primary mechanisms through which BCL6 activity is terminated in GC cells are combinatorial, and may be triggered when B-cells make contact with T-cells and follicular dendritic cells within germinal centers. T-cells express CD40 ligand, which can generate a dual CD40 receptor-dependent inhibitory effect on BCL6. Firstly, CD40 signaling via NFkB (nuclear factor of kappa light polypeptide gene enhancer in B-cells) can disrupt the interaction of BCL6 with some of the corepressor proteins through which BCL6 represses its target genes[16]. This can re-activate expression of ATR and possibly allow B-cells to undergo a quality control step after which damaged cells can be executed by checkpoint pathways[16]. This effect on BCL6 is reversible and may explain in part the reason for the observed transient interactions between GC B and T cells, and why GC T-cells are sometimes observed associated with dead B-cells [32]. More sustained CD40 signaling can upregulate the IRF4 (interferon regulatory factor 4) transcription factor, which irreversibly downregulates BCL6[33]. Point mutations of IRF4 binding elements in the BCL6 locus occur in DLBCL and might contribute to its constitutive expression[33]. STAT5 (signal transducer and activator of transcription 5) can also repress BCL6 expression through elements that are found to be mutated in lymphoma cells [34]. Taken together, the functional contributions of BCL6 to the DLBCL phenotype suggest that it could be an excellent therapeutic target (Fig 1).

Rational design of BCL6 inhibitors

Transcription factors such as BCL6 can be effectively targeted if an in-depth knowledge of their biochemical mechanism of action is available. BCL6 is a member of the BTB/POZ (**bric-à-brac**, **tramtrack**, **broad complex/pox virus zinc finger**) – zinc finger family of proteins (Fig. 2). The 707 amino acid protein can be functionally and structurally divided into three regions: the N-terminal BTB domain (amino acids 5–129), a central unstructured region (residues 129–517), and a set of six C-terminal zinc finger domains (residues 518–681). Residues 1–4 and 682–707 are predicted to be unstructured and have no known function (Fig. 2). The primary function of the zinc finger region is to bind to recognition elements in the regulatory regions of the genes that are under BCL6 control. The central “linker” region is largely unstructured, but contains several functional motifs, such as the RD2 (repression domain 2). This central region can be considered as a flexible tether that connects the BTB and zinc finger regions of the protein. At a molecular level, the best characterized motif in the protein is the BTB domain, which has autonomous repressor activity[35]. X-ray crystallography studies have shown that the BCL6 BTB domain forms a tightly interwound homodimer, and in solution, the BCL6 BTB appears to exist exclusively as a dimer, consistent with a very low dissociation constant[36] (Fig. 3A&B). The BCL6 BTB domain can interact in a mutually exclusive manner with three corepressors: SMRT (silencing mediator of retinoid and thyroid hormone receptors), N-CoR (nuclear receptor corepressor) and BCOR (BCL6 corepressor) [36–38]. All three of these corepressors bind to a common exposed surface at the interface of the two chains of the dimer, which we refer to as the lateral groove[36] (Fig. 3C). N-CoR and SMRT recognize the lateral groove via a 17 residue BBD (BCL6 BTB binding domain)[36]. The BCL6 amino-acid side chains that line the lateral groove are unique to this repressor, and the SMRT and N-CoR BBDs do not interact with the related BTB-ZF repressors PLZF and LRF [39]. Mutations that change the surface of the BCL6 lateral groove without affecting the overall structure of the domain no longer bind to the corepressor BBDs, and these mutations abrogate BCL6 BTB domain repressor activity[36].

Taken together, these features suggest that the BCL6 lateral groove could be an excellent therapeutic target. Agents that bind to the lateral groove and compete for co-repressor binding can, in principle, reverse the repression activities of BCL6. However, the length and complexity of the interface between the BBD and the lateral groove are potential barriers toward developing

effective small molecule inhibitors. Molecules such as BBD peptides, which have molecular weights of nearly 2 kDa and contain many polar and charged amino acids, interact with a large extended surface of the BCL6 BTB dimer, mostly through hydrogen bonds and multiple van der Waals contacts. Molecules large enough to fully occupy the lateral groove would be unlikely to readily penetrate cells. One way to deliver larger molecules such as peptides is the use of protein transduction domains such as pTAT, which have been shown to penetrate virtually all cell types both *in vitro* and *in vivo*[40]. Accordingly, a recombinant TAT-BBD fusion peptide was shown to penetrate B-cells *in vitro* and disrupt the interaction between BCL6 and SMRT[41]. This BCL6 peptide inhibitor (BPI) was able to re-activate BCL6 target genes and induced apoptosis and cell cycle arrest in DLBCL cells [41]. Moreover, *in vivo* administration of BPI abrogated formation of GCs in response to a T-cell dependent antigenic stimulus[41]. However, BPI could not induce differentiation in DLBCL cells[41]. Accordingly, BPI could upregulate ATR and TP53, but not PRDM1[16,41,42]. Although BPI was effective in the high nanomolar and low micromolar range, the peptide had a short half-life *in vivo* [41]. The development of a more stable form of BPI could potentially serve in BCL6 targeted therapy.

Although the contact surface of the co-repressor BBDs to the BCL6 BTB lateral groove is large area that involves many amino acid residues, smaller chemical entities may also prove to be effective inhibitors. Lower molecular weight compounds that bind tightly in small critical pockets within the BTB lateral groove may be able to prevent co-repressor binding. A careful consideration of the specific BBD side chain contacts indicate that certain key co-repressor residues make larger contributions to the interaction. Chemical mimics of these BBD fragments may prove to be a route to smaller, more drug like compounds that can block BCL6 repression activity.

Homodimerization of the BCL6 BTB domain is mediated through an extensive hydrophobic surface [36] (Fig. 3B). Homodimerization is required for proper folding of the BTB domain and generates the surface-exposed lateral grooves [36]. In contrast to the exposed lateral groove, the buried residues at the BCL6 BTB dimer interface are highly conserved in the 43 human BTB-ZF proteins [43]. This surface is even more extensive than the lateral groove, and approximately one quarter of the monomer surface area is buried upon dimer formation [44]. The dimer interface is thus a more challenging target site for molecular intervention. Since BTB domains cannot fold as monomers, it would be difficult for an interfering agent to break apart existing BCL6 dimers. Targeting newly formed monomers might work, although the fact that the dimer interface is highly conserved between BTB domain proteins makes it likely that off-target effects would occur.

An additional surface feature of BCL6 is implicated in transcriptional repression. The “top” portion of the dimers form a charged pocket motif, which is highly conserved across to the BTB domain family[36,44,45] (Fig. 3D). The charged pocket of BCL6 was shown to contribute to transcriptional repression[45]. A recently performed aptamer screening strategy identified a peptide called aptamer48 that can bind near the BCL6 charged pocket, could block BCL6 repressor activity without affecting the interaction with SMRT or N-CoR[46]. Aptamer 48 was also able to inhibit the proliferation of lymphoma cells[46]. These data suggest that Aptamer 48 might also serve as a precursor for BCL6 targeted therapy.

The RD2 region of BCL6 was shown to interact with the C-terminal domain of the MTA3 (metastasis associated 1 family, member 3) corepressor, which in turn can recruit the nucleosome remodeling and histone deacetylase (NuRD) repressor complex to BCL6[47]. MTA3 expression closely parallels that of BCL6 in both during normal germinal center differentiation as well as in DLBCL[29]. Depletion of MTA3 from lymphoma cells induces plasma cell differentiation and ectopic expression of MTA3 in plasma cells, especially in

combination with BCL6 could reprogram cells to express germinal center transcripts[47]. MTA3 was shown to form a complex with BCL6 at the PRDM1 locus and to repress its transcription[29]. In contrast, depletion of MTA3 did not re-activate expression of ATR or p53, nor did it kill lymphoma cells[29,47]. Lateral groove blockade of SMRT and N-CoR by BPI and depletion of MTA3 therefore have completely different biological effects. These data indicate that BCL6 regulates target genes belonging to DNA damage pathways or differentiation through different biochemical mechanisms. From the therapeutic standpoint, disruption of the BCL6 –MTA3 interaction might thus force differentiation of DLBCL cells rather than kill them, more akin to the effect of ATRA in the therapy of acute promyelocytic leukemia. This may be a worthwhile strategy to pursue in targeting BCL6. Even though MTA3 depletion alone could not kill DLBCL cells, its combination with BPI led to greater cell death than BPI alone, suggesting that combined targeting of BCL6 repression functions might provide a superior therapeutic effect[29].

The ETO (eight twenty one) corepressor protein was shown to bind to the C-terminal zinc fingers of BCL6 and contributed to transcriptional repression of the *CCND2* (cyclin D2) gene [48]. An effort to identify additional BCL6 binding partners by mass spectrometry yielded many additional cofactors including HDAC9 (histone deacetylase 9), MLL4 (myeloid/lymphoid or mixed-lineage leukemia 4), SENP7 (SUMO1/sentrin specific peptidase 7)[49]. The structural basis of these interactions remains unknown, but might provide additional opportunities for transcriptional reprogramming and therapeutic targeting of lymphoma cells.

Targeting BCL6 through protein acetylation

Bereschenko et. al. demonstrated that the p300 histone acetyltransferase can acetylate the RD2 domain of BCL6 and diminish its ability to repress target genes[50]. This may be related to the fact that acetylated BCL6 RD2 can no longer bind to MTA3 [47]. The N-CoR and SMRT corepressor complexes contain histone deacetylase (HDAC) activity. [51] Combinations of the class I and II HDAC inhibitor drug trichostatin A (TSA) along with class III HDAC (or sirtuin) inhibitor Nicotinamide Adenine Dinucleotide) led to an additive effect on the accumulation of acetylated BCL6 and de-repression of gene targets[50]. Moreover, HDAC inhibitors (HDIs) have strong anti-lymphoma effects *in vitro*, suggesting that they might be therapeutically useful [52]. More recently, cambinol, a specific small molecule inhibitor of SIR1/SIR2 was also shown to mediate effective tumor growth inhibition in murine xenografts of B cell lymphoma [53]. Inhibition of protein deacetylase activity by HDIs has widespread cellular effects and it is unclear whether the effects of these drugs on BCL6 figure prominently in their anti-lymphoma activity. However, the BCL6 inhibitory activity of these agents should be beneficial in the development of non-chemotherapy based biological targeting of DLBCL.

Targeting BCL6 levels by RNA and protein modulation

Targeting RNA by antisense oligonucleotides (ASO) or RNA interference (RNAi) has been used to eliminate BCL6 from lymphoma cells. Kalota et. al. have reported a reliable mapping technology employing self quenching reporter molecules (SQRM) to identify sites accessible for hybridization by ASO and RNAi on the BCL6 mRNA[54]. An ASO targeting the 1310 SQRM and siRNA targeting the 2241 SQRM were found to produce the greatest depletion of BCL6 protein and mRNA respectively. RNA interference molecules based on the SQRM strategy had anti-lymphoma activity when transduced into lymphoma cells[54].

MicroRNAs (miRNAs) are short (~22 nucleotide) non coding RNAs that bind to target RNAs and prevent translation and promote mRNA degradation[55]. miRNA-127 has been suggested to have a tumor suppressive function by decreasing BCL6 levels and is itself decreased in a Burkitt lymphoma cell line due to epigenetic modification. Induction of miRNA-127 by

phenylbutyrate has been shown to decrease BCL6 levels in Ramos B cell lymphoma cells demonstrating potential for anti-lymphoma activity[55]. In addition to phenylbutyrate (an HDAC inhibitor), 5-azacytidine, a DNA hypomethylating agent, can induce miRNA-127 and similarly effect BCL6 repression. If the continuing efforts to package and deliver RNAi and miRNA are successful, this could be a useful strategy for targeting BCL6. A summary of strategies for targeting BCL6 is shown in Figure 4.

Selecting patients for BCL6 targeted therapy

One of the major pitfalls in clinical testing of targeted therapy is identifying patients in which the therapeutic target plays a critical biological role. Administration of a targeted agent to the wrong patients would underestimate the efficacy of the drug and result in the inappropriate enrollment of patients into a trial unlikely to provide them with any benefit. This could be a particularly challenging problem in DLBCLs. Multiple lines of evidence suggest that expression of BCL6 alone is unlikely to be sensitive as an indicator of BCL6 dependent DLBCL. For example, while the absence of BCL6 always predicted for resistance to BPI in DLBCL cell lines, the presence and expression level of BCL6 did not predict responsiveness [15,41]. DLBCLs have been classified according to their microarray gene expression profile into two different classifications intended to capture the molecular heterogeneity of the disease. In the cell of origin (COO) classification a signature with similarities to normal germinal center B cells (GCB) was shown to have a favorable prognosis compared to patients with a signature reminiscent of activated peripheral blood B-cells (ABC) [56]. GCB tumors more frequently express BCL6, although BCL6 levels and target gene expression were quite variable within this group. ABC tumors less frequently express BCL6 but have more frequent BCL6 translocations[56]. In vitro, both ABC and GCB DLBCL cell lines that expressed BCL6 were responsive to BPI[15,41]. Another DLBCL classification scheme grouped patients into cohorts using multiple clustering methods and comprehensive genetic analyses[57]. This comprehensive consensus clustering (CCC) approach demonstrated the existence of three dominant signatures in DLBCL, featuring expression of B-cell receptor and proliferation genes (BCR), expression of oxidative phosphorylation genes (OxPhos), or a signature dependent mostly on infiltrating host immune response cells (HR) [57]. These signatures did not overlap with the COO and in this case BCL6 expression and translocations were more frequently associated with the BCR type signature. In an effort to identify a genetic footprint of BCL6 activity, Polo et. al. performed high throughput chromatin immunoprecipitation to identify BCL6 target genes[15]. A gene set enrichment analysis (a method that can detect whether a differential regulation of genes is occurring in certain gene expression profiles) demonstrated that BCL6 target genes were coordinately regulated in BCR but not OxPhos DLBCLs[15]. Accordingly BCR DLBCL cell lines uniformly responded to BPI while OxPhos DLBCL cell lines were uniformly resistant[15]. If confirmed in primary DLBCLs, this result might guide future clinical trials of BCL6 inhibitors to those patients with BCL6 positive BCR-type DLBCLs.

How BCL6 targeted therapy could be incorporated into clinical practice

Like most tumors, DLBCL is a complex and multi-hit disease, unlikely to respond to single agent therapy, even targeting a crucial oncogene like BCL6. The experience with ATRA treatment in APL can again serve as a predictive model for BCL6 targeted therapy in DLBCL. ATRA therapy is highly effective in transcriptionally reprogramming APL cells, but is not curative alone, and must be administered with chemotherapy[1]. It is the combination of ATRA plus chemotherapy that has greatly improved the survival of patients with APL. There is a mechanistic basis to predict that BCL6 targeted therapy would enhance the actions of chemotherapy. BPI can induce expression of DNA damage checkpoint genes such as TP53, and TP53 is an important contributor to chemotherapy induced apoptosis (by lymphoma drugs

such as doxorubicin) [42]. It is therefore reasonable to predict that treatment of DLBCL first with BPI followed by R-CHOP or a similar regimen might result in enhanced killing of tumors. Accordingly, *in vitro* data show potent synergy between BCL6 and doxorubicin. Thinking further down the line, it may be possible to combine specific agents targeting multiple lymphoma pathways into a non-chemotherapeutic combinatorial therapies. For example, combinations of agents targeting BCL2 (B-cell lymphoma 2) and BCL6 might together have a potent effect in disrupting the aberrant survival pathways that maintain lymphoma cells. Resveratrol is a polyphenolic compound that has been shown *in vitro* to reduce BCL6 and BCL2 levels and enhance chemotherapy effect on lymphoma cells [58,59]. Adding these drugs to other biological agents such as HDIs, proteasome inhibitors, etc might provide sufficient biological breadth of action to fully eliminate lymphoma cells in the clinical setting.

Summary

Taken together, it is clear that BCL6 plays a central role in mediating the malignant phenotype of many DLBCLs. The mechanistic basis of this is becoming increasingly clear and involves direct and confirmed effects on cell proliferation, survival, genomic stability and differentiation. Biochemical data demonstrate that therapeutic targeting of BCL6 is feasible, and patients with BCL6 dependent DLBCLs could be identified as candidates for clinical trials. Based on these data it seems that BCL6 targeted therapy would be an important therapeutic approach worth testing in the clinical arena.

Acknowledgements

GGP is supported by the National Cancer Institute of Canada/Canadian Cancer Society and the Canadian Institutes of Health Research. AMM is supported by NCI R01 CA104348, the G&P foundation, Chemotherapy foundation, and the Leukemia and lymphoma Society of America. Both GGP and AMM are supported by a program grant from the Samuel Waxman Cancer Research Foundation.

References

1. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood* 1999;93(10):3167–215. [PubMed: 10233871]
2. Dent AL, Shaffer AL, Yu X, Allman D, Staudt LM. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science* 1997;276(5312):589–92. [PubMed: 9110977]
3. Ye BH, Cattoretti G, Shen Q, Zhang J, Hawe N, de Waard R, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. *Nat Genet* 1997;16(2):161–70. [PubMed: 9171827]
4. Tunyaplin C, Shaffer AL, Angelin-Duclos CD, Yu X, Staudt LM, Calame KL. Direct repression of *prdm1* by Bcl-6 inhibits plasmacytic differentiation. *J Immunol* 2004;173(2):1158–65. [PubMed: 15240705]
5. Kuo TC, Shaffer AL, Haddad J Jr. Choi YS, Staudt LM, Calame K. Repression of BCL-6 is required for the formation of human memory B cells *in vitro*. *J Exp Med* 2007;204(4):819–30. [PubMed: 17403935]
6. 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(2):199–212. [PubMed: 10981963]
7. Reljic R, Wagner SD, Peakman LJ, Fearon DT. Suppression of signal transducer and activator of transcription 3-dependent B lymphocyte terminal differentiation by BCL-6. *J Exp Med* 2000;192(12):1841–8. [PubMed: 11120780]
8. Allen CD, Okada T, Cyster JG. Germinal-center organization and cellular dynamics. *Immunity* 2007;27(2):190–202. [PubMed: 17723214]
9. Okazaki IM, Kotani A, Honjo T. Role of AID in tumorigenesis. *Adv Immunol* 2007;94:245–73. [PubMed: 17560277]

10. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Kuppers R, et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature* 2001;412(6844):341–6. [PubMed: 11460166]
11. Spencer JO, Dunn-Walters DK. Somatic hypermutation and B-cell malignancies. *J Pathol* 1999;187(2):158–63. [PubMed: 10365090]
12. Pasqualucci L, Bereschenko O, Niu H, Klein U, Basso K, Guglielmino R, et al. Molecular pathogenesis of non-Hodgkin's lymphoma: the role of Bcl-6. *Leuk Lymphoma* 2003;44(Suppl 3):S5–12. [PubMed: 15202519]
13. Cattoretti G, Pasqualucci L, Ballon G, Tam W, Nandula SV, Shen Q, et al. Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. *Cancer Cell* 2005;7(5):445–55. [PubMed: 15894265]
14. Baron BW, Anastasi J, Montag A, Huo D, Baron RM, Karrison T, et al. The human BCL6 transgene promotes the development of lymphomas in the mouse. *Proc Natl Acad Sci U S A* 2004;101(39):14198–203. [PubMed: 15375218]
15. Polo JM, Juszczynski P, Monti S, Cerchietti L, Ye K, Greally JM, et al. Transcriptional signature with differential expression of BCL6 target genes accurately identifies BCL6-dependent diffuse large B cell lymphomas. *Proc Natl Acad Sci U S A* 2007;104(9):3207–12. [PubMed: 17360630]
16. Ranuncolo SM, Polo JM, Dierov J, Singer M, Kuo T, Greally J, et al. Bcl-6 mediates the germinal center B cell phenotype and lymphomagenesis through transcriptional repression of the DNA-damage sensor ATR. *Nat Immunol* 2007;8(7):705–14. [PubMed: 17558410]
17. Phan RT, Dalla-Favera R. The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. *Nature* 2004;432(7017):635–9. [PubMed: 15577913]
18. Phan RT, Saito M, Basso K, Niu H, Dalla-Favera R. BCL6 interacts with the transcription factor Miz-1 to suppress the cyclin-dependent kinase inhibitor p21 and cell cycle arrest in germinal center B cells. *Nat Immunol* 2005;6(10):1054–60. [PubMed: 16142238]
19. Wright JA, Keegan KS, Herendeen DR, Bentley NJ, Carr AM, Hoekstra MF, et al. Protein kinase mutants of human ATR increase sensitivity to UV and ionizing radiation and abrogate cell cycle checkpoint control. *Proc Natl Acad Sci U S A* 1998;95(13):7445–50. [PubMed: 9636169]
20. Cuadrado M, Martinez-Pastor B, Murga M, Toledo LI, Gutierrez-Martinez P, Lopez E, et al. ATM regulates ATR chromatin loading in response to DNA double-strand breaks. *J Exp Med.* 2006
21. Petermann E, Caldecott KW. Evidence that the ATR/Chk1 pathway maintains normal replication fork progression during unperturbed S phase. *Cell Cycle* 2006;5(19):2203–9. [PubMed: 16969104]
22. Ward IM, Chen J. Histone H2AX is phosphorylated in an ATR-dependent manner in response to replicational stress. *J Biol Chem* 2001;276(51):47759–62. [PubMed: 11673449]
23. Liu Q, Guntuku S, Cui XS, Matsuoka S, Cortez D, Tamai K, et al. Chk1 is an essential kinase that is regulated by Atr and required for the G(2)/M DNA damage checkpoint. *Genes Dev* 2000;14(12):1448–59. [PubMed: 10859164]
24. Mailand N, Falck J, Lukas C, Syljuasen RG, Welcker M, Bartek J, et al. Rapid destruction of human Cdc25A in response to DNA damage. *Science* 2000;288(5470):1425–9. [PubMed: 10827953]
25. Shieh SY, Ahn J, Tamai K, Taya Y, Prives C. The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev* 2000;14(3):289–300. [PubMed: 10673501]
26. Sanchez Y, Wong C, Thoma RS, Richman R, Wu Z, Piwnicka-Worms H, et al. Conservation of the Chk1 checkpoint pathway in mammals: linkage of DNA damage to Cdk regulation through Cdc25. *Science* 1997;277(5331):1497–501. [PubMed: 9278511]
27. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh SY, et al. A role for ATR in the DNA damage-induced phosphorylation of p53. *Genes Dev* 1999;13(2):152–7. [PubMed: 9925639]
28. Phan RT, Saito M, Kitagawa Y, Means AR, Dalla-Favera R. Genotoxic stress regulates expression of the proto-oncogene Bcl6 in germinal center B cells. *Nat Immunol* 2007;8(10):1132–9. [PubMed: 17828269]
29. Parekh S, Polo JM, Shaknovich R, Juszczynski P, Lev P, Ranuncolo SM, et al. BCL6 programs lymphoma cells for survival and differentiation through distinct biochemical mechanisms. *Blood* 2007;110(6):2067–74. [PubMed: 17545502]

30. Vasanwala FH, Kusam S, Toney LM, Dent AL. Repression of AP-1 function: a mechanism for the regulation of Blimp-1 expression and B lymphocyte differentiation by the B cell lymphoma-6 protooncogene. *J Immunol* 2002;169(4):1922–9. [PubMed: 12165517]
31. Shaffer AL, Lin KI, Kuo TC, Yu X, Hurt EM, Rosenwald A, et al. Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program. *Immunity* 2002;17(1):51–62. [PubMed: 12150891]
32. Allen CD, Okada T, Tang HL, Cyster JG. Imaging of germinal center selection events during affinity maturation. *Science* 2007;315(5811):528–31. [PubMed: 17185562]
33. Saito M, Gao J, Basso K, Kitagawa Y, Smith PM, Bhagat G, et al. A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma. *Cancer Cell* 2007;12(3):280–92. [PubMed: 17785208]
34. Walker SR, Nelson EA, Frank DA. STAT5 represses BCL6 expression by binding to a regulatory region frequently mutated in lymphomas. *Oncogene* 2007;26(2):224–33. [PubMed: 16819511]
35. Chang CC, Ye BH, Chaganti RS, Dalla-Favera R. BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. *Proc Natl Acad Sci U S A* 1996;93(14):6947–52. [PubMed: 8692924]
36. Ahmad KF, Melnick A, Lax S, Bouchard D, Liu J, Kiang CL, et al. Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. *Mol Cell* 2003;12(6):1551–64. [PubMed: 14690607]
37. Huynh KD, Bardwell VJ. The BCL-6 POZ domain and other POZ domains interact with the co-repressors N-CoR and SMRT. *Oncogene* 1998;17(19):2473–84. [PubMed: 9824158]
38. Huynh KD, Fischle W, Verdin E, Bardwell VJ. BCoR, a novel corepressor involved in BCL-6 repression. *Genes Dev* 2000;14(14):1810–23. [PubMed: 10898795]
39. Stogios PJ, Chen L, Prive GG. Crystal structure of the BTB domain from the LRF/ZBTB7 transcriptional regulator. *Protein Sci* 2007;16(2):336–42. [PubMed: 17189472]
40. Prive GG, Melnick A. Specific peptides for the therapeutic targeting of oncogenes. *Curr Opin Genet Dev* 2006;16(1):71–7. [PubMed: 16377176]
41. Polo JM, Dell'Oso T, Ranuncolo SM, Cerchietti L, Beck D, Da Silva GF, et al. Specific peptide interference reveals BCL6 transcriptional and oncogenic mechanisms in B-cell lymphoma cells. *Nat Med* 2004;10(12):1329–35. [PubMed: 15531890]
42. Cerchietti L, Polo J, DaSilva G, Dowdy S, Cattoretti G, Shakhovich R, et al. BCL6, p53 and Molecular Targeted Therapy in B-Cell Lymphomas. *Blood* 2005;106(11)
43. Stogios PJ, Downs GS, Jauhal JJ, Nandra SK, Prive GG. Sequence and structural analysis of BTB domain proteins. *Genome Biol* 2005;6(10):R82. [PubMed: 16207353]
44. Ahmad KF, Engel CK, Prive GG. Crystal structure of the BTB domain from PLZF. *Proc Natl Acad Sci U S A* 1998;95(21):12123–8. [PubMed: 9770450]
45. Melnick A, Carlile G, Ahmad KF, Kiang CL, Corcoran C, Bardwell V, et al. Critical residues within the BTB domain of PLZF and Bcl-6 modulate interaction with corepressors. *Mol Cell Biol* 2002;22(6):1804–18. [PubMed: 11865059]
46. Chattopadhyay A, Tate SA, Beswick RW, Wagner SD, Ko Ferrigno P. A peptide aptamer to antagonize BCL-6 function. *Oncogene* 2006;25(15):2223–33. [PubMed: 16331266]
47. Fujita N, Jaye DL, Geigerman C, Akyildiz A, Mooney MR, Boss JM, et al. MTA3 and the Mi-2/NuRD complex regulate cell fate during B lymphocyte differentiation. *Cell* 2004;119(1):75–86. [PubMed: 15454082]
48. Chevallier N, Corcoran CM, Lennon C, Hyjek E, Chadburn A, Bardwell VJ, et al. ETO protein of t(8;21) AML is a corepressor for Bcl-6 B-cell lymphoma oncoprotein. *Blood* 2004;103(4):1454–63. [PubMed: 14551142]
49. Miles RR, Crockett DK, Lim MS, Elenitoba-Johnson KS. Analysis of BCL6-interacting proteins by tandem mass spectrometry. *Mol Cell Proteomics* 2005;4(12):1898–909. [PubMed: 16147992]
50. Bereshchenko OR, Gu W, Dalla-Favera R. Acetylation inactivates the transcriptional repressor BCL6. *Nat Genet* 2002;32(4):606–13. [PubMed: 12402037]
51. Li J, Wang J, Nawaz Z, Liu JM, Qin J, Wong J. Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. *Embo J* 2000;19(16):4342–50. [PubMed: 10944117]

52. Sakajiri S, Kumagai T, Kawamata N, Saitoh T, Said JW, Koeffler HP. Histone deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell lines. *Exp Hematol* 2005;33(1): 53–61. [PubMed: 15661398]
53. Heltweg B, Gathbonton T, Schuler AD, Posakony J, Li H, Goehle S, et al. Antitumor activity of a small-molecule inhibitor of human silent information regulator 2 enzymes. *Cancer Res* 2006;66(8): 4368–77. [PubMed: 16618762]
54. Kalota A, Opalinska JB. Design of antisense oligonucleotides and short interfering RNA duplexes (siRNA) targeted to BCL6 mRNA: towards rational drug development for specific lymphoma subsets. *Blood Cells Mol Dis* 2007;38(3):199–203. [PubMed: 17254814]
55. Saito Y, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* 2006;9(6):435–43. [PubMed: 16766263]
56. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403(6769):503–11. [PubMed: 10676951]
57. Monti S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Mihm M, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. *Blood* 2005;105(5):1851–61. [PubMed: 15550490]
58. Faber AC, Chiles TC. Resveratrol induces apoptosis in transformed follicular lymphoma OCI-LY8 cells: evidence for a novel mechanism involving inhibition of BCL6 signaling. *Int J Oncol* 2006;29(6):1561–6. [PubMed: 17088997]
59. Jazirehi AR, Bonavida B. Resveratrol modifies the expression of apoptotic regulatory proteins and sensitizes non-Hodgkin's lymphoma and multiple myeloma cell lines to paclitaxel-induced apoptosis. *Mol Cancer Ther* 2004;3(1):71–84. [PubMed: 14749477]

Figure 1. The role of BCL6 in normal and malignant B-cells

Panel A: During normal B-cell maturation, activated B-cells form germinal centers (GC) (shaded circle) in order to form high affinity antibodies. Mature B-cells first become centroblasts, highly proliferating cells in which BCL6 expression is induced. These cells form the dark zone of the GC. Pictured below the centroblast cell is a representation of BCL6 and its three domains, the BTB, RD2 and Zinc fingers. BCL6 contributes to the centroblast phenotype by directly repressing the ATR, CHEK1, TP53 and CDKN1A genes through its BTB domain in order to facilitate proliferation and survival during class switch recombination and somatic hypermutation. BCL6 also represses the PRDM1 gene mostly through its second repression domain in order to block further differentiation. Centroblasts eventually migrate to

a more heterogeneous area of the GC called the light zone where they encounter T-cells and follicular dendritic cells (FDC). CD40 signaling by T-cells can block the function of the BCL6 BTB domain by blocking its association with N-CoR and thus de-repress checkpoint genes. This presumably allows B cells damaged during affinity maturation to be weeded out (an apoptotic B-cell is pictured attached to a T-cells). Repression of PRDM1 is independent of the BTB domain, which allows B-cells to sustained blockade of PRDM1 and thus prevents premature differentiation. When CD40 signaling is transient these effects are reversible so that B-cells could maintain the centroblast phenotype and undergo further rounds of affinity maturation. More sustained CD40 signaling can induce IRF4 mediated repression of BCL6 and facilitate terminal differentiation of GC B cells selected by the FDCs into plasma cells or memory cells. GC B cells that have reached this stage in the light zone are called centrocytes. BCL6 can also be downregulated through the ATM pathway via proteolytic degradation when genomic damage reaches a critical level in B-cells. **Panel B:** Translocations or point mutations of the BCL6 locus can cause it to be expression constitutively and contribute to malignant transformation. Exposure of DLBCL cells to BPI can block the repressor effect of the BCL6 BTB domain and induce expression of ATR, CHEK1, TP53 and CDKN1A resulting in cell death. Downregulation of MTA3 can block the repressor effect of the RD2 and induce PRDM1 resulting in differentiation.

2. Domain structure and partner proteins of BCL6

Panel A: Cartoon representation of BCL6. BCL6 forms a homodimer through the N-terminal BTB domain. The middle region of BCL6 acts as an unstructured linker to its C-terminal zinc fingers, which bind to DNA and other proteins. **Panel B:** The BTB domain of BCL6 can directly bind with the SMRT, N-CoR and BCoR corepressors. the second repression domain can directly interact with the MTA3 corepressor and also contains motifs through which BCL6 function and stability are regulated by acetylation and phosphorylation. The zinc fingers of BCL6 can interact with the ETO corepressor.

Figure 3. Structural features of the BCL6 BTB domain

Panel A: Ribbon representation of the BCL6 BTB dimer, with one chain in red and one in blue. The C and N termini of each chain are indicated. The linker region in full-length BCL6 continues from the C terminus of each BTB chain. **Panel B:** The buried interface surface in the BCL6 BTB dimer. The red chain in (A) is shown in surface representation, and the monomer surface that is buried upon dimer formation is colored dark red. An equivalent surface is buried in the blue chain. **Panel C:** Both BCL6 BTB chains are shown in surface representation, and the SMRT BBD peptide is shown in stick form. The lateral groove surface that contacts the BBD is colored in dark blue and dark red according to the contributing chain. **Panel D:** Top view of the BCL6 BTB dimer, indicating the charged pocket at the dimer interface. The portions of the charged pocket contributed by each monomer are colored in dark blue and dark red according to the contributing chain.

Figure 4. Potential strategies for therapeutic targeting of BCL6

Panel A: BCL6 expression can be downregulated by siRNA or antisense oligonucleotides.

Panel B: Some of the potential ways to inhibit BCL6 functions include 1) blockade of the BCL6 lateral groove by BPI; 2) blockade of the charged pocket, as seems to be the case with Apt48; 3) blocking the interaction of BCL6 with MTA3; 4) inhibiting HDACs so that BCL6 becomes constitutively acetylated, which could reduce its association with MTA3. Since HDACs are also associated with the NuRD and SMRT/N-CoR repression complexes this could further impair BCL6 repressor activity; 5) blockade of the ETO-BCL6 interaction; and 6) blockade of the BCL6-DNA interaction. **Panel C:** Another way to target BCL6 is to induce reactivation or functional activity of BCL6 target genes. For example, p53 activating peptides

or small molecules can induce p53 activity and cause cell death in BCL6 positive lymphoma cells, and chemotherapy drugs can kill lymphoma cells through induction of a p53 response. Combination of the approaches shown in A or B with that shown in C might have enhanced anti-tumor effects.