

B-cell Lymphoma 6 (BCL6): From Master Regulator of Humoral Immunity to Oncogenic Driver in Pediatric Cancers



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ABSTRACT

B-cell lymphoma 6 (*BCL6*) is a protooncogene in adult and pediatric cancers, first identified in diffuse large B-cell lymphoma (DLBCL) where it acts as a repressor of the tumor suppressor *TP53*, conferring survival, protection, and maintenance of lymphoma cells. *BCL6* expression in normal B cells is fundamental in the regulation of humoral immunity, via initiation and maintenance of the germinal centers (GC). Its role in B cells during the production of high affinity immunoglobins (that recognize and bind specific antigens) is believed to underpin its function as an oncogene. *BCL6* is known to drive the self-renewal capacity of leukemia-initiating cells (LIC), with high *BCL6* expression in acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and glioblastoma (GBM) associated with disease progression and treatment resistance.

The mechanisms underpinning *BCL6*-driven therapy resistance are yet to be uncovered; however, high activity is considered to confer poor prognosis in the clinical setting. *BCL6*'s key binding partner, *BCL6* corepressor (*BCOR*), is frequently mutated in pediatric cancers and appears to act in concert with *BCL6*. Using publicly available data, here we show that *BCL6* is ubiquitously overexpressed in pediatric brain tumors, inversely to *BCOR*, highlighting the potential for targeting *BCL6* in these often lethal and untreatable cancers. In this review, we summarize what is known of *BCL6* (role, effect, mechanisms) in pediatric cancers, highlighting the two sides of *BCL6* function, humoral immunity, and tumorigenesis, as well as to review *BCL6* inhibitors and highlight areas of opportunity to improve the outcomes of patients with pediatric cancer.

Introduction

BCL6 encodes a zinc finger transcription repressor that is frequently translocated in diffuse large B-cell lymphomas (DLBCL; ref. 1). A master regulator of humoral immunity, the *BCL6* protein plays a critical role in the initiation and maintenance of the germinal centers (GC). GCs are microstructures formed in secondary lymphoid tissues (such as lymph nodes, tonsils, and spleen) and are the sites of antibody diversification and affinity maturation. Reiterative cycles of somatic hypermutation (SHM) in immunoglobulin gene (Ig) variable regions produce high-affinity antibodies in response to T-cell dependent antigen presentation, and hence, are vital to humoral immunity (2).

Transcription of tumor suppressors, high-fidelity DNA repair genes, and genes implicated in cell differentiation are repressed by *BCL6*. Simultaneously, *BCL6* promotes the expression of genes linked with proliferation, immune avoidance, antiapoptosis, cell-cycle arrest, and cell differentiation, hallmarks of cancer cell biology. The many

cancer-associated functions linked to *BCL6* activity promote a “perfect oncostorm”, deregulated transcriptional programs with increased tolerance to genome insult and instability. *BCL6* acts similarly in B cells, as an engine of cell turnover, where it promotes SHM and mediates immune cell metamorphosis in response to encountering foreign antigens (3).

The oncogenic functions of *BCL6* have been characterized in lymphoma as early as 1993 (4). Notably, *BCL6* has since been implicated in pediatric cancers, including acute lymphoblastic leukemia (ALL; refs. 5, 6), acute myeloid leukemia (AML; ref. 7), and solid cancers such as high-grade glioma (HGG; refs. 8, 9) and sarcoma (discussed in Section *BCL6* in Pediatric Cancers). With *BCL6* playing an important role in transcriptional repression, *BCL6* rearrangement (10) and/or overexpression (11) is associated with poor overall survival, and resistance to chemotherapies (5, 12).

Despite the important roles outlined above, targeting *BCL6* as an anticancer approach is yet to be effectively executed in the clinic. Drawing on what is known of *BCL6* (role, effect, mechanisms) in pediatric cancers, and humoral immunity, we review the utility of *BCL6*-targeted therapies in the hope of improving future outcomes in the pediatric cancer setting.

BCL6 Structure

The *BCL6* gene is located on chromosome 3q27 and encodes a 95 kDa protein that acts as a master regulator, necessary for the formation of GCs in B-cell follicles of lymphoid tissues in response to antigen encounter (13, 14). The *BCL6* protein harbors a trimodular structure consisting of (i) an N-terminus Broad-complex, Tramtrack and Bric-à-brac/poxvirus and zinc finger (BTB/POZ) domain, a docking site for corepressor proteins; (ii) a central transcriptional repressor (RD2) domain containing a proline (P), glutamic acid (E), serine (S), and threonine (T) – PEST region, important in the control of protein

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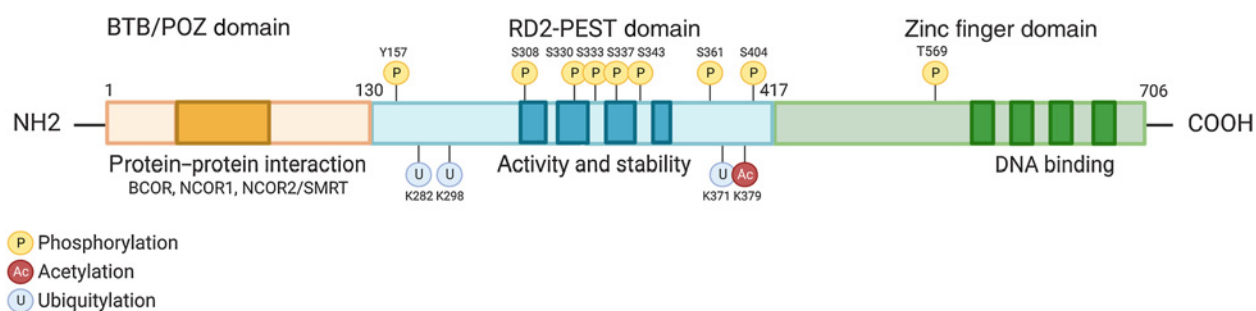


Figure 1.

Structure and function of BCL6. BCL6 harbors a trimodular structure consisting of a N-terminus Broad-complex, Tramtrack and Bris-à-brac/poxvirus and zinc finger (BTB/POZ) domain for protein interactions such as corepressor proteins (darker orange). A central transcriptional repressor domain (RD2) containing a proline (P), glutamic acid (E), serine (S), and threonine (T) - PEST region, which dictates the proteins activity and stability by binding regulatory proteins that determine its half-life (darker blue). A series of C-terminal Krüppel-like C2H2-type zinc fingers which bind DNA to repressed gene expression (darker green).

half-life and interactions with auto regulatory proteins (15); and (iii) a series of C-terminal Krüppel-like C2H2-type zinc fingers that bind DNA (Fig. 1; ref. 16).

The BCL6 homodimer binds to target gene promoters to influence transcriptional programs during the formation of GCs. This is regulated following recruitment of corepressor proteins that bind to an exposed surface at the interface of the two chains of the BCL6 dimer, referred to as the lateral groove BTB domain (Fig. 1; ref. 17). BCL6 corepressor (BCOR), nuclear receptor corepressor 1 and 2 (NCOR1, NCOR2/SMRT) form a multifunctional ternary corepressor complex - BCL6 binds at gene promoters, with BCOR bound to polycomb repressive complex 1 (PRC1) and NCOR2/SMRT bound to histone deacetylase 3 (HDAC3; Fig. 2; refs. 18, 19). Not only does this drive the deacetylation of histone H3 at lysine 27 (H3K27), but also recruits the H3K27 methyltransferase Enhancer of zeste homolog 2 (EZH2) through tethering to noncanonical PRC1-BCOR-CBX8 complexes. EZH2 catalyzes the trimethylation of H3K27 (H3K27me3) and the formation of bivalent chromatin, resulting in epigenetic silencing of the cell-cycle arrest gene, cyclin-dependent kinase inhibitor 1A (*CDKN1A*), and retinoblastoma RB/E2F1 tumor suppressor pathway (Fig. 2; ref. 20).

It is of little wonder that BCL6 regulatory BTB domain has become an attractive target for the development of BCL6 inhibitors. The

unique lateral groove at the surface of the BTB domain differentiates BCL6 from other BTB proteins (Fig. 1; refs. 14, 21) and hence, competitive inhibitors have the potential to be BCL6 specific (Table 1). Peptomimetic inhibitors and competitive peptide aptamers of the BTB have been shown to compete with corepressor binding, and effectively eliminate the repressor functions of BCL6, and increase expression of BCL6 target tumor suppressor genes (discussed in Section BCL6 as a Therapeutic Target; ref. 22).

BCL6 in Humoral Immunity

Regulation of the GCs

The formation of GCs in secondary lymphoid tissues (lymph nodes, tonsils, spleen, Peyer's patches, and mucosa-associated lymphoid tissues) is critical in the production of long-lived antibody-secreting plasma cells and memory B cells in response to infection, and as such, underpin the function of the humoral immune system (13). In lymphoid organs, upon antigen presentation, B cells undergo SHM, followed by Ig class switch recombination (CSR), resulting in mass proliferation of mature B cells with foreign antigen affinity. BCL6 is known to play an essential role in the maturation of B cells and the formation of GCs, as *BCL6*^{-/-} knockout mice fail to form GCs (13).

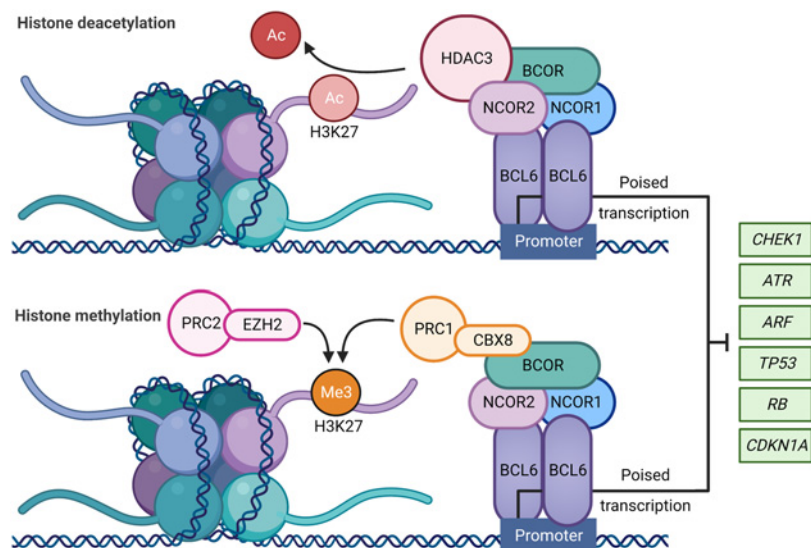


Figure 2.

BCL6 role in transcriptional regulation: BCL6 homodimer binds to target gene promoters to repress the transcriptional programs required for tumor suppression and apoptosis during the formation of the GCs. This is regulated via the recruitment of BCL6 corepressor (BCOR), nuclear receptor corepressor (NCOR1), and nuclear receptor corepressor 2 (NCOR2) to form a multifunctional ternary corepressor complex. BCL6 represses or poises transcription through BCOR and NCOR2 binding to histone deacetylase 3 (HDAC3), resulting in deacetylation of H3K27. When BCOR binds and recruits non-canonical polycomb repressive complex 1 (PRC1)-CBX8, the polycomb repressive complex 2 (PRC2) complex subunit enhancer of zester homolog 2 (EZH2) catalyzes H3K27 trimethylation resulting in epigenetic silencing. Ac = acetylation, Me3 = trimethylation. (Created with BioRender.com)

Table 1. BCL6 targeted inhibitors.

Type	Name	MW g/mol	Malignancy	IC ₅₀	K _D	In vivo model	In vivo dose	In vivo outcome	Challenges for translation	Reference
Peptide inhibitors	BPI	4,550.19	DLBCL	14.2 μmol/L	20 μmol/L	NR	NR	NR	Easily degraded by proteases	(93)
	FI324	1,765.04	NR	1 nmol/L	0.57 nmol/L	NR	NR	NR	No inhibitory effects <i>in vitro</i>	(95)
	Apt48	1,536.74	BL	NR	NR	NR	NR	NR	No cytotoxic effects, requires cytokines	(22)
Reverse peptide inhibitors	RI-BPI	4,510.12	DLBCL	16.5 μmol/L	16 μmol/L	SU-DHL4 Toledo SCID mice	150 & 500 μmol/L/day	↓ TV ↑ survival	SU-DHL4/6	(94)
	79-6	457.28	DLBCL	24-95.6 μmol/L	NR	JM94	50 mg/kg/day	↓ TS ↑ response	with EGFR1	(9)
Small molecules	FX1	368.82	NSCLC	35 μmol/L	4 μmol/L	OCI-Ly7 Toledo SCID mice	0.5-50 mg/kg/day	↓ TV	OCI-Ly7 SU-DHL6 BCL6 lymphoma specific	(101)
	GSK137	374	DLBCL	10 nmol/L	10 nmol/L	NR	NR	NR	Non cytotoxic	(101)
	BI-3802	485	DLBCL	20 nmol/L	<3 nmol/L	NR	NR	NR	Degrades BCL6	(98,99)
	BI-3812	558	DLBCL	3 nmol/L	<3 nmol/L	NR	NR	NR	Bioavailability	(98,99)

Abbreviations: IC₅₀, inhibitory concentration 50%; K_D, dissociation constant; NR, not reported in cancer models; TS, tumor size; TV, tumor volume.

Activated B cells are called “centroblasts” and form the dark zone of the GCs, upregulating *BCL6* following interactions with T cells/antigens (23). Once differentiated, nondividing activated GC B cells derived from centroblasts are called “centrocytes” and migrate to the light zone of the GCs, where the affinity of their antibody receptors is evaluated with the help of T follicular helper cells (T_{FH}) through interactions with follicular dendritic cells (FDCs; ref. 24).

BCL6 in SHM and CSR

SHM and CSR occur in B cells following immunization or infection through T-cell-dependent (Td) activation of follicular B cells. A consequential and continuous elevation in *BCL6* expression is seen during formation of GCs (25). This rise in *BCL6* expression is essential to direct transcriptional programs that promote SHM while repressing expression of tumor surveillance and antiapoptotic gene signatures, allowing CSR to occur. Not only does *BCL6* target the promoters of protein coding genes, but also microRNAs (miRNA), including *miR-155* and *miR-361*, which target activation-induced cytidine deaminase (AID; ref. 26). AID is part of a family of cytidine deaminases that deaminate deoxycytosine to deoxyuracil (dCs to dUs) on both single-stranded DNA (ssDNA) substrates, and ssDNA generated by the formation of RNA–DNA hybrids (27). Repression of *miR-155* and *miR-361* drives AID expression, which introduces point mutations into the DNA at a relatively high rate (up to 10^{-4} to 10^{-3} per base per division) in both Ig and non-Ig genes (26, 28). AID is a key factor driving CSR and therefore an adaptive immune response.

Central to sustained DNA damage, antiapoptosis, and increased proliferation of mature B cells during SHM and CSR, *BCL6* represses DNA repair and tumor suppressor gene expression. Repression of multiple genes including *CHEK1*, *ATR*, *ARF*, *TP53*, *RB*, and *CDKN1A* switch off high-fidelity DNA repair and apoptosis, driving the cell cycle (Fig. 2). These mechanisms have evolved to evade premature apoptosis and to reprogram B cells to allow for the selective specificity necessary to generate antibodies for the infinite number of possibly encountered foreign antigens (29).

Regulation of BCL6 expression in B cells

Inhibition of *BCL6* function is a fundamental process in the normal immune response, occurring once GC B cells have matured with the correct antigen affinity, and is controlled via several mechanisms (30). The Myocyte enhancer-binding factor 2B (*MEF2B*) is thought to be the master transcription factor for *BCL6* in B cells, binding to the promoter approximately 1 kilobase upstream of the *BCL6* gene following T-cell activation (31). Activity of *MEF2B* is regulated by interactions with its corepressor phosphatase *CABIN1* (Calcineurin binding protein 1; Fig. 3; ref. 32). Mutations within the C-terminus of *MEF2B* are associated with escape from phosphorylation-mediated negative regulation (31), with *MEF2B* deletion reducing the formation of GCs (33). *MEF2B* is mutated in approximately 10% to 12% of DLBCLs and follicular lymphomas (FL; ref. 31). *MEF2B* binds predominantly to chromatin at histone marks suggestive of active promoters, enhancers or superenhancers ($H3K4me3^+$ and $H3K27ac^+$) to regulate transcription of genes influencing DNA replication and repair, cell cycle, apoptosis, and GC B-cell confinement (Figs. 2 and 3; ref. 34). Interestingly, like *BCL6*, *MEF2B* plays an important role in regulating epigenetic modifiers, chromatin marks that are also modulated by the formation of *BCL6* corepressor complex (Figs. 2 and 3, discussed in *BCL6* in Humoral Immunity).

Recent studies have shown an indirect mechanism of *BCL6* expression in GCs during B-cell differentiation. Following IL4 stimulation, metabolic reprogramming sees the accumulation of α -ketoglutarate

(α KG), produced by its conversion in the tricarboxylic acid (TCA) cycle from isocitrate. Isocitrate is converted to α KG using the Isocitrate dehydrogenase (IDH) enzymes (IDH2 and IDH3; Fig. 3). α KG acts as a substrate for epigenetic modifier Lysine-specific demethylase 6A (KDM6A), also known as Ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX). Signal transducer and activator of transcription 6 (STAT6) recruits KDM6A to the *BCL6* locus, reducing methylation of repressive histone mark $H3K27me3$, leading to euchromatin and active transcription of *BCL6* and B-cell differentiation in the developing GCs (35).

Following an immune response, transient activation of the B-cell receptor (BCR), cluster of differentiation 40 (CD40), and IL2 receptors drive increased NF- κ B activity and the dissociation of the *BCL6*–*NCOR* complex, alleviating the negative regulation of c-MYC by *BCL6*, driving the expression of cell-cycle genes (34). MYC activates both Cyclin D2 (CCND2) and Cyclin D3 (CCND3) resulting in a CCND2-dependent proliferation (36). Transient activation of surface receptors (such as CD40) lead to the nuclear translocation of NF- κ B and induction of Interferon regulatory factor 4 (IRF4; ref. 34). IRF4 binds to the promoter region of *BCL6* to suppress its expression (37) and is a member of the interferon-regulatory factor family of transcription factors (required for plasma cell differentiation). Hence, the expression level and activity of CD40, their respective T_{FH} , promotes memory B-cell differentiation from GC B cells, ending the requirement for *BCL6*'s repressor activity (38). CD40 stimulation has also been reported to dysregulate *BCL6* transcriptional repression of the DNA repair gene Serine/threonine-protein kinase (*ATR*; Fig. 2), responsible for the detection of DNA damage to be repaired, or in the case of genotoxicity, to trigger apoptosis (30). A subset of DLBCLs harboring *BCL6* translocations or mutations (depending on the position of the chromosomal breakpoints) have been reported to be refractory to CD40-IRF4-induced *BCL6* repression (34). Mutations in the *IRF4*-responsive region of the *BCL6* promoter are identified in 60% of DLBCLs harboring *BCL6* translocations (approximately 30% of all DLBCL diagnoses). With this mutation directly linked to pathogenesis and malignant growth of DLBCL subsets (34), it is therefore important to understand the regulation of *BCL6* if we are to develop strategies to improve the treatment of *BCL6*-driven neoplasms.

Members of the STAT family are promiscuous transcription factors that are phosphorylated and activated by receptor-associated tyrosine kinases (39), causing them to dimerize and cross the nuclear membrane to bind to promoters and enhancers of regulatory genes, including *BCL6*. IL2, IL4, and IL10 induce growth of B cells through activation of JAK/STAT signaling pathways, whereas IL4 drives STAT6 activity to promote CSR (40) and upregulate *BCL6* expression by enhancing RNA polymerase II recruitment and initiating transcription (41). The IL4/IL4R axis is known to activate STAT3, STAT5, and STAT6 (discussed in Regulation of *BCL6* expression in B Cells), and is further enhanced by IL4-dependent metabolic reprogramming, driving KDM6A activity and demethylation of repressive $H3K27me3$ marks, increasing STAT6-dependent *BCL6* expression (Fig. 3; ref. 35). STAT5 is a potent oncogenic transcription factor in acute hematologic malignancies that has been shown to outcompete STAT3 for binding to the enhancers of *BCL6* at one of the two shared regulatory sites (41), highlighting the potential of targeting *BCL6* in malignancies characterized by high STAT5 activity.

BCL6 in Pediatric Cancers

Over the past four decades, mortality rates for children diagnosed with cancer have reduced by more than 50% (42). This is underpinned

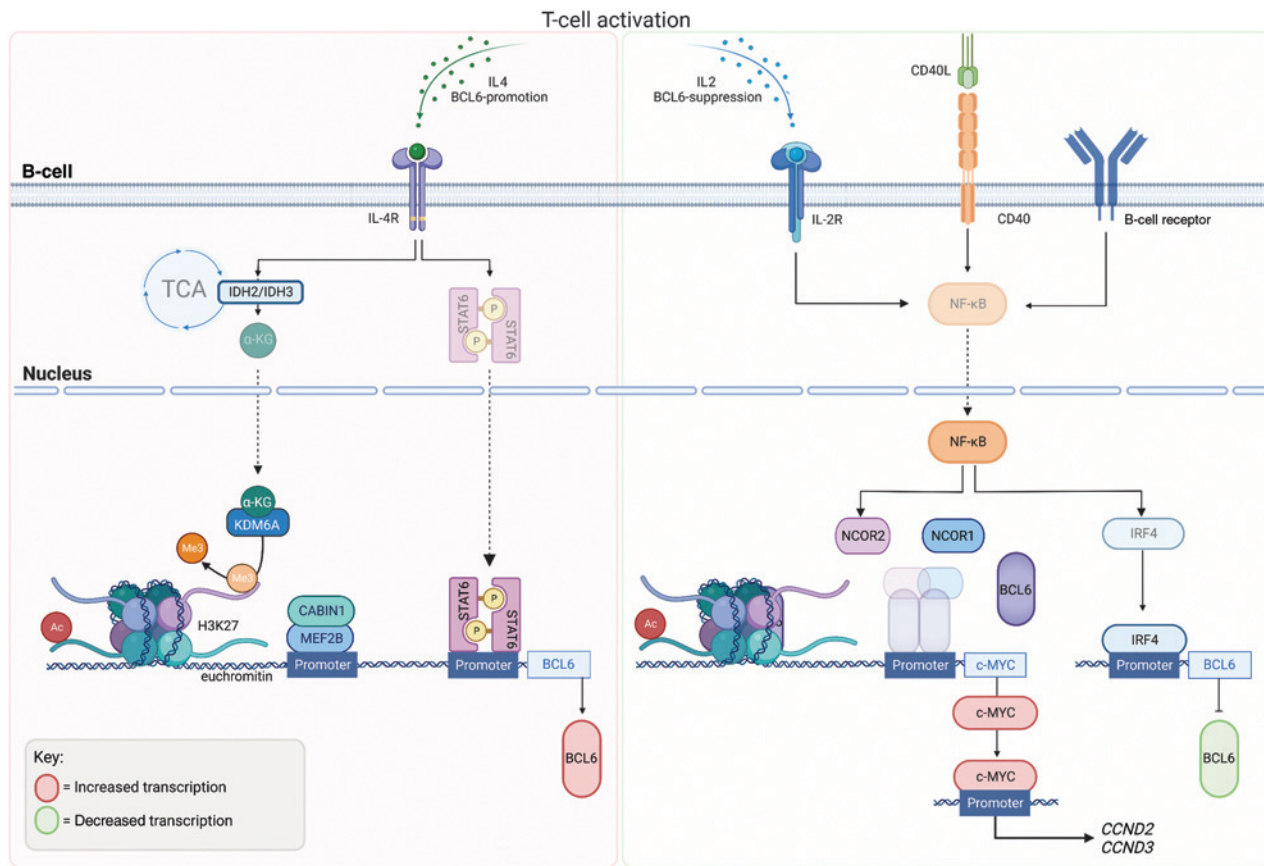


Figure 3.

BCL6 Regulation: BCL6 expression in B cells during GC formation occurs once B cells have matured with correct antigen affinity. T-cell activation and IL4 stimulation via cytokines drives metabolic tricarboxylic acid (TCA) cycle reprogramming through IDH2 and IDH3 and hence the production of alpha ketoglutarate (α KG), which acts as a substrate for Lysine-specific demethylase 6A (KDM6A) leading the loss of repressive H3K27me3 marks. KDM6A is recruited by phosphorylated Signal transducer and activator of transcription (STAT6), to the BCL6 locus causing the demethylation of H3K27me3, leading to euchromatin and active transcription of BCL6. Myocyte enhancer-binding factor 2B (MEF2B), the master regulator of BCL6 expression is regulated via its corepressor phosphatase, CABIN 1 (Calcineurin Binding Protein 1) and binds to BCL6 promoter, following T-cell activation. MEF2B binds predominantly to chromatin with histone marks, H3K4me3 (trimethylation, Me3) and H3K27ac (acetylation, Ac) suggestive of active marks. Following IL2 stimulation, CD40 activation and B-cell receptor activation, NF- κ B is translocated to the nucleus where it suppresses the formation of the BCL6-NCOR complex, alleviating the negative regulation of the c-MYC promoter by BCL6. NF- κ B activation promotes IRF4 to bind to the BCL6 promoter to negative regulate its transcription. (Created with BioRender.com)

by sophisticated improvements to protocol-driven clinical trials that utilize combinations of chemotherapies that attempt to address the somatic and epigenetic heterogeneity of these cancers. However, for some of the most aggressive types of childhood malignancies, there remains no effective pharmacologic intervention and children succumb to their disease within a short timeframe (39, 43–45). As 35% to 40% of children’s malignancies originate in cells of the lymphoid lineage where BCL6 plays a critical role in their maturation, there is a potential for targeting BCL6 in combination with therapies focused on the recurring somatic alterations responsible for disease initiation and progression. Furthermore, the opportunity to increase p53 activity (and hence tumor suppression) through BCL6 inhibition is an attractive paradigm in the pediatric setting, as somatic loss-of-function mutations in TP53 are only seen in 4% of cases, compared with adults where TP53 mutations are the most common mutation (>50% of unselected sporadic tumors; ref. 46). However, this is not to say that p53 shows ubiquitous tumor suppression in pediatric cancers. Rather, p53 dysregulation occurs via the altered activity of cell cycle regulators

(CDKN2A or CDKN2B) and through mutations in genes implicit in modulating the pediatric cancer epigenome (TP53, H3K27M, IDH2/3, SETD2), with these recognized as some of the most recurring mutations in children’s cancers (47). Furthermore, mutations in the direct regulators of BCL6 activity, that is, BCOR, are common in pediatric cancers of the central nervous system (CNS; discussed in Pediatric central nervous system cancers). BCOR interacts selectively with the POZ domain of BCL6, and interestingly, these mutations lead to reduced affinity for BCL6 which drives cell proliferation, Protein kinase B (AKT) phosphorylation, the expression of IL2, and importantly, inhibition of TP53 through gene repression.

Using publicly available pediatric cancer genome and RNA expression data from the St. Jude Cloud (48), we analyzed the expression and mutation of BCL6 and BCOR across the three main types of pediatric cancer categories: hematopoietic malignancies (HM), brain tumors (BT), and solid tumors (ST), in the *de novo* and relapse setting (Fig. 4). Interestingly, there is a significant increase in mRNA expression of BCL6 in BT at diagnosis and relapse compared with HM or ST

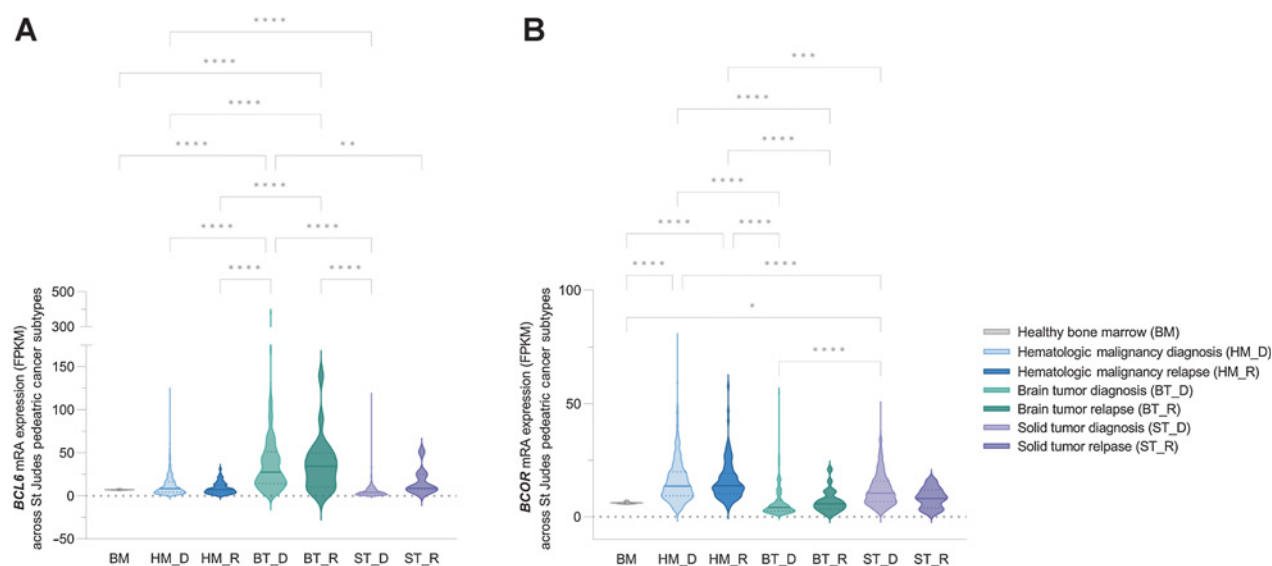


Figure 4.

BCL6 and *BCOR* shows hyperexpression in pediatric brain cancers. **A**, *BCL6* and **B**, *BCOR* mRNA expression data from 1,853 patients was downloaded from the St Jude cloud database (48) and grouped into three pediatric tumor types, HM, BT, and ST. Healthy bone marrow (BM) mRNA expressions from the BEAT AML dataset (102) was used as a control for the analysis. Within each cancer type diagnosis (HM_D, $n = 1173$; BT_D, $n = 232$; ST_D, $n = 453$) and relapse (HM_R, $n = 96$; BT_R, $n = 22$; ST_R, $n = 9$) data are identified. *BCL6* mRNA expression showed increased expression in BTs both at diagnosis and relapse compared with other cancer types. *BCOR* mRNA expression showed an inverse expression correlation, and significantly reduced in BT both at D and R compared with other tumor types. In both BT and ST, *BCL6* mRNA expression showed a nonstatistical trend toward increased expression in the relapse setting. *BCL6* mRNA expression in HM showed no difference to the BM control. No significant difference was seen between HM_D and HM_R in both *BCL6* and *BCOR* expression (one-way ANOVA performed and corrected for multiple comparison using Tukey statistical hypothesis testing; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

(Fig. 4A). The reciprocal trend was seen for *BCOR* mRNA expression with higher expression in HM and ST compared with BT (Fig. 4B). This is not surprising given that high-grade pediatric cancers of the CNS are often caused by epigenetic alterations, driving hypomethylation of repressive chromatin marks resulting in the formation of euchromatin and potentially high expression of *BCL6* (43, 45).

Lymphomas

Non-Hodgkin lymphoma

Accounting for an estimated 7% of childhood cancers in the developed world, pediatric non-Hodgkin lymphoma (NHL) diagnoses are the fifth most common in children aged 15 years and under (49). The majority of B-cell non-Hodgkin lymphomas derive from the dysregulation of GC or post-GC B cells (14). B cells transitioning to GCs manifest many of the hallmark features of cancer: rapid proliferation, clonal expansion, inactivation of tumor suppressors, genome instability, and inhibition of DNA damage repair pathways (discussed in *BCL6* in Humoral Immunity); hence, it is of no surprise that the development of cancer occurs in these lymphoid organs.

DLBCL

DLBCLs makes up 10% to 20% of pediatric non-Hodgkin lymphomas and comprises about 8% of childhood malignancies (50). Although DLBCL shows epigenetic and somatic heterogeneity, most recurrently, 45% of patients harbor 3q27 translocations in the *BCL6* gene (51), which does not appear to alter *BCL6* mRNA expression (52). The first line of treatment for DLBCL is an immunotherapy regimen consisting of a short 9-week course of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP; ref. 53). R-CHOP achieves a complete response (CR) in 65% of cases; however, relapse is seen in 30% to 40% of

patients, particularly those deemed to have “high-risk” or “high-grade” B-cell lymphoma (HGBL) at diagnosis (53). Two other genes frequently mutated in DLBCL (either singularly or concurrently) are the *MYC* (2%–20%) and *BCL2* (20%) oncogenes. Patients harboring *MYC* rearrangements along with concurrent *BCL2* and/or *BCL6* mutations are classified as having HGBL; these combinations are commonly referred to as “double-hit” (DHL) or “triple-hit” lymphomas (THL; ref. 51). Conjecture persists about the prognosis for patients harboring either *BCL2* or *BCL6* translocations. One study showed patients with DLBCL harboring *MYC/BCL6* rearrangements experienced reduced overall survival (OS) compared with *MYC/BCL2* rearrangements; 4.5 months for those with *BCL6* aberrations compared with 34.6 months for patients with *BCL2* mutations (54). Early-stage clinical trials have evaluated the toxicity and benefit of adding a *BCL2* inhibitor (venetoclax) to standard-of-care treatment in the adult population, citing promising preliminary results in patients with HGBL; however, clinical trials introducing *BCL6* inhibitors are yet to be established (55).

Follicular lymphoma

FL accounts for 35% of all NHLs, and 70% of indolent lymphomas, portending a 10-year OS of approximately 80% (56). In adult FL, the vast majority of cases contain a t(14;18) translocation at diagnosis that leads to overexpression of the antiapoptotic protein *BCL2* (56). However, pediatric FL lack abnormal *BCL2* protein expression or the *BCL2* gene rearrangements that characterize the majority of adult FL cases (57). Rather, *BCL2*-independent lymphogenesis is driven by Bcl-2-like protein 1 (*BCL2L1* also known as *BCL-XL*), *BCL2*-associated agonist of cell death (*BAD*), and *BCL6* (58). Patients with FL harboring *BCL6* translocations are reported to experience earlier transformation to DLBCL and face a less favorable prognosis (59). One proposed

mechanism of early progression is BCL6-driven antagonism of NOTCH signaling (60). Notch receptor 2 (NOTCH2) is preferentially expressed in mature B cells and is inversely correlated with BCL6 expression following terminal B-cell differentiation in the GCs. This is also seen in FLs that are dependent on BCL6 expression. Hence, pharmacologic or genetic inhibition of BCL6 drives NOTCH2 expression to suppress growth of human FL xenografts *in vivo*, and primary human FL specimens *ex vivo*.

Burkitt lymphoma

Burkitt lymphomas are rare and aggressive cancers primarily diagnosed in children. Burkitt lymphoma represents 30% of childhood NHL compared with only 1% of adult NHL diagnoses. Although multiple genetic abnormalities are implicated in the pathogenesis of Burkitt lymphoma, chromosomal translocations affecting the *MYC* oncogene family are considered a biological hallmark of the disease (61). A retrospective karyotype study of 34 *MYC*-immunoglobulin rearranged lymphomas (including 24 Burkitt lymphoma cases) concluded that BCL6 expression and noncomplex karyotype were independent predictors of better overall survival (62), contrary to what we have seen in other cancers.

Primary central nervous system lymphoma

This particularly rare form of NHL is highly aggressive with the tumor confined to the CNS (63). Primary central nervous system lymphoma (PCNSL) accounts for approximately 1% of all lymphoma diagnoses (64) and of this, around 1% are pediatric cases (65). PCNSLs harbor similar morphology to DLBCLs; *BCL6* rearrangements occur in 20% to 40% of cases (66). Prognosis for PCNSL patients that harbor *BCL6* rearrangements are suggested to be better than for PCNSL patients with *BCL2*, human GC-associated lymphoma (*HGAL*), or LIM domain only-2 (*LMO2*) mutations (67); however, this study was conducted using adult patients samples and with a small cohort size of 69.

Leukemias

ALL

Five-year survival rates for patients diagnosed with ALL have dramatically increased following the inclusion of multimodal treatment regimens; 89% of patients live >5 years if diagnosed under the age of 20. However, for patients diagnosed >20 years, 5-year survival rate is only 38%. Interestingly, *BCL6* appears to be an oncogene in patients that are either refractory to treatment, or relapse with an aggressive and resistant form of ALL. The standard-of-care (SOC) treatment for ALL patients includes combinations of chemotherapies, including tyrosine kinase inhibitors (TKI). TKIs are an effective treatment, however, they are unable to eradicate the rare population of leukemia-initiating cells (LIC), which often lead to relapse even after an initial period of response. BCL6 expression is increased in ALL cells treated with TKIs and drives transcriptional inactivation of the TP53 pathway. This aids in leukemic cell protection from DNA-damaging chemotherapies (5).

LICs originate in the bone marrow. The bone marrow plays pivotal roles in leukemogenesis, but also may promote the activity of BCL6, in disease initiation and during the development of treatment resistance (6). Clonal evolution drives treatment resistance through the acquisition of additional genetic lesions caused by DNA damage. ALLs harboring molecular deletion of *BCL6* harbor reduced mutational profiles compared to ALL overexpressing BCL6, suggesting that increased sensitivity to DNA damage limits clonal evolution in the absence of *BCL6* (5).

AML

AML is the second most common acute leukemia in children, with a 5-year survival rate of 67% (68). Currently, there is limited research on the role of BCL6 in AML. A recent study using cell lines and primary patient samples demonstrated variable, but often, high-level expression of BCL6 in different subtypes of AML (particularly those with a less differentiated phenotype) compared with either hematopoietic stem cells or granulocyte-monocyte progenitor cells (69). Interestingly, BCL6 expression was induced by treatment with the SOC chemotherapy cytarabine, both at the mRNA and protein level. This resulted in the development of drug resistance, suggested to be due to the increased blast cell tolerance to DNA-damaging chemotherapies (69). Furthermore, inhibition of BCL6 decreased the number of leukemic stem cells (LSC). Knockdown of *BCL6* in primary cells resulted in a significant reduction of leukemia-initiating capacity in mice, while BCL6 inhibition had no effect on normal cells of hematopoietic phenotype (69). As BCL6 plays a role in leukemia-initiating capacity in both ALL and AML (5), it is interesting to consider whether combining SOC and pharmacologic inhibition of BCL6 is a potential therapeutic strategy that may reduce leukemia repopulation and improve outcomes.

Pediatric central nervous system cancers

The role of BCL6 in pediatric brain tumors is also largely unknown, although a correlation between increased BCL6 expression and poor survival has been reported in adult studies (70). BCL6 plays an important role in neurogenesis, a key regulator of differentiation of progenitor cells to neurons (71). In somatic transgenic glioma models, BCL6 expression is also required for glioblastoma cell-cycle progression by silencing *TP53* (9). As discussed above, BCL6 requires homodimerization and the formation of a complex with its cofactors, including the BCOR. Recurring mutations in *BCOR* are found in pediatric CNS neuroepithelial tumors (72), and diffuse midline gliomas (DMG), including those diagnosed in the pontine region, diffuse intrinsic pontine glioma (DIPG; ref. 73). However, uncertainty remains regarding the genomic landscape and significance of *BCOR* alterations in pediatric CNS malignancies and their influence on BCL6 function.

Pediatric HGG

Glioblastoma. The most frequently diagnosed HGGs in children are anaplastic astrocytomas (WHO grade 3) followed by glioblastoma (WHO grade 4). These highly aggressive tumors are often resistant to treatment; median OS survival estimated at just 15 months (74). *BCL6* translocations have been identified in patients diagnosed with glioblastoma (36.6% $n = 11/30$; ref. 8). BCL6 overexpression at both the transcript and protein level is also seen in patients harboring *BCL6* translocations, with expression correlating with decreased apoptotic processes (8). More recent studies showed that molecular inhibition of *BCL6* expression in glioblastoma cell lines decreased expression of BCL2, and Cyclin D1 (CCND1), and proteins influencing the diffuse migratory and invasive growth features of these tumors influenced by matrix metalloproteinases- MMP2 and MMP9. Commensurate, *BCL6* knockdown increased the expression of proapoptotic protein BAX and CDKN1A to potentiate p53 activity in response to radiation and decreased Extracellular signal-regulated kinase (ERK) signaling (9, 75). The Tyrosine-protein kinase receptor (AXL) is a transcriptional target of BCL6 and responsible for the decreased ERK activity following BCL6 or AXL inhibition in glioblastoma. The coupling of BCL6 inhibition with SOC radiation and chemotherapeutic temozolomide enhanced the therapeutic effect of these therapies *in vitro* and

in vivo (12), highlighting the potential for BCL6 inhibition as a strategy to improve treatment outcomes for this patient population facing such poor prognoses.

The BCL6 transcriptional target *miR-361-5p* (76) is also repressed in high-grade glioma cells and in tumor tissue, when compared with normal tissue and low-grade gliomas (77). Overexpression of *miR-361-5p* in glioblastoma cell lines significantly inhibited glioma cell migration, invasion, and epithelial–mesenchymal transition (EMT). The transcription factor Twist-related protein 1 (TWIST1) is a target of *miR-361-5p*, with overexpression rescuing the inhibitory effect of *miR-361-5p* on EMT. TWIST1 is a STAT3 target gene directly required for the development of T_H cells and is involved in the regulation of BCL6 expression, resulting in a negative feedback loop (78).

DMG. DMGs, including DIPG, are pediatric CNS tumors recognized as the most lethal of all children's cancers (44, 45). Palliative radiotherapy is the only approved treatment, with median survival just 9 to 11 months postdiagnosis (79). DMG is an immunologically cold tumor (80) characterized by recurring somatic mutations in H3 genes including, *HIST1H3B/C* (H3.1K27M) or *H3F3A* (H3.3K27M), or through overexpression of EZH inhibitory protein (EZHIP) *EZHIP* in patients harboring wildtype H3 – all of which lead to hypomethylation of H3K27 (45). *TP53* is the second most recurring lesion in H3.3K27M+ DMG (60%–80%; ref. 43); however, it is only seen in 13% of H3.1K27M and 11% of EZHIP DMGs (73). Both H3.1K27M+ and EZHIP+ DMG harbor recurring mutations to *BCOR* and *BCORL1* (45). Although yet to be fully characterized in DMG, these recurring aberrations may act comparably with *TP53* mutations in H3.3K27M+ DMG, leading to increased BCL6 activity, and hence, decreased p53-driven tumor suppression and poor outcomes.

Medulloblastoma

Medulloblastoma is the most common malignant brain tumor in children, accounting for over 20% of all pediatric brain tumors with 70% of medulloblastoma cases occurring in children under the age of 10 (81). Treatment regimens for medulloblastoma are complex and usually include a mixed modality approach: surgical resection, craniospinal irradiation, combination chemotherapies. Interestingly, *TP53* mutations are rarely seen in cases of medulloblastoma (82). However, recurring cytogenetic mutations in isochromosome 17q resulting from the loss of the short arm (p) cause a gain of genetic material from the long arm (q). In addition, recurring deletions in the short arm are also seen, leading to loss of heterozygosity of 17p (83).

Interestingly, medulloblastoma is characterized by sonic hedgehog (SHH) pathway activation where BCL6 acts as a tumor suppressor. Indeed, transient overexpression of *BCL6 in vivo* delays or inhibits medulloblastoma formation and tumor growth (84), through suppression of NOTCH signaling and epigenetic transcriptional silencing of the glioma-associated oncogenes, Zinc finger protein *GLI1* and *GLI2*. BCL6 recruits *BCOR* and *SIRT1* (to achieve transcriptional repression), which in turn promotes neurogenic conversion (84). Although recurring mutations in *BCL6* have not been identified in patients with medulloblastoma, recurrent *BCOR* mutations have been identified and may play a role in medulloblastoma formation (85).

CNS high-grade neuroepithelial tumor

CNS high-grade neuroepithelial tumors (HGNET) with *BCOR* alteration (CNS HGNET-*BCOR*) are rare pediatric tumors that occur

mostly in the supratentorial region of the brain (less frequently in the infratentorial region), suggested to harbor inferior survival outcomes compared with non *BCOR* altered HGNETs (72). HGNET-*BCOR* are considered a certain type of neuroepithelial tumor, like a glioma, due to the HGNET-*BCOR* tumors exhibiting glial cell morphology, ependymoma-like perivascular pseudorosettes and palisading necrosis (86). A recurring somatic internal tandem duplication in the 3' end of *BCOR* gives rise to CNS HGNET-*BCOR* tumors. These mutations are also seen in clear cell sarcomas of the kidney (CCSK) and soft tissue undifferentiated round cell sarcomas/primitive myxoid mesenchymal tumors of infancy (discussed in Pediatric non-CNS solid tumors; Sarcoma) and are reported to share similar pathological features (86). Immunohistopathologic analysis identified CNS HGNET-*BCOR* to harbor positive expression of glial fibrillary acidic protein (GFAP) and oligodendrocyte transcription factor 2 (OLIG2), which are indicative of glial malignancy. Although the functional consequences of expression of BCL6 in CNS HGNET-*BCOR* is yet to be determined, other tumors that harbor *BCOR* internal tandem duplications coexpress BCL6 in most of their nuclei, suggestive of cooperating oncogenic roles.

Pediatric non-CNS solid tumors

Sarcoma

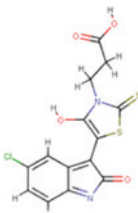
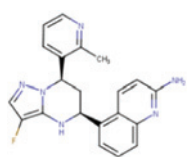
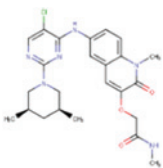
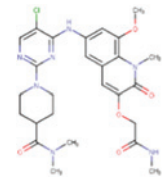
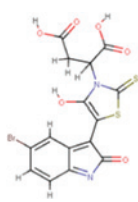
Sarcomas represent over 20% of all pediatric solid malignancies (87), including over 50 different subtypes identified to date. They originate in the mesenchymal tissue and therefore can arise almost anywhere in the body. Almost 80% of all sarcomas originate from soft tissue with the remaining 20% originating in bone (88). Rhabdomyosarcoma, a soft tissue located in the striated muscle, account for 50% of pediatric cases, while the remaining soft tissue malignancies are commonly classified according to the normal tissue types where they are derived.

The broad spectrum of sarcoma subtypes makes diagnosis, treatment, and research complex; it is difficult to gain significance in small population subtypes in this sense. In the soft tissue sarcoma primitive myxoid mesenchymal tumor of infancy (PMMTI), Santiago and colleagues reported *BCOR*-internal tandem duplications (ITD) and high *BCOR* and BCL6 immunoreactivity in 11 of 12 cases studied (89). In addition, in 9 of the 11 pediatric clear cell sarcomas (CCS) cases, *BCOR* immunoreactivity was again strongly detected (90). *BCOR*-ITD mutations appear to be chromosome X-linked, with cases diagnosed in males two times more frequently than in females (89, 91, 92). Recurring, *BCOR*-ITD mutations are also seen in up to 85% of small cell carcinoma of the kidney (SCCK) cases, CNS high-grade neuroepithelial tumors and undifferentiated round cell sarcomas of the bone. These results highlight not only the potential for improved diagnosis of these rare tumors, but also enables future informed targeted treatment that may improve reduce relapse rates and increase overall survival.

BCL6 as a Therapeutic Target

BCL6 plays an oncogenic role across a variety of cancer types, highlighting the potential for targeted inhibition to improve outcomes. BCL6's unique structure, its crucial involvement in the repression of tumor suppressors, and its role in the activation of oncogenes, highlights a unique opportunity to develop novel and selective anticancer therapies. Several peptidomimetic and small molecule inhibitors have been developed and explored in cellular and animal models of cancer, but none have yet progressed into the clinic or been investigated under clinical trial settings.

Table 2. Brain penetration prediction for BCL6 small-molecule inhibitors.

Name	FX1		GSK137		BI-3802		BI-3812		79-6	
Chemical structure										
CNS MPO Parameter	Actual	Score	Actual	Score	Actual	Score	Actual	Score	Actual	Score
LOGP	1.90	1.00	2.40	1.00	3.30	0.85	1.28	1.00	1.75	1.00
LOGD	-3.12	1.00	2.38	0.81	3.30	0.35	1.28	1.00	1.75	1.00
MW	-3.12	1.00	374.42	0.90	484.99	0.11	558.04	0.00	457.27	0.31
TPSA	90.20	0.99	81.65	1.00	99.69	0.68	129.23	0.00	127.50	0.00
HBD	2.00	0.50	3.00	0.25	2.00	0.50	2.00	0.50	3.00	0.25
PKA	-0.63	1.00	6.11	1.00	4.32	1.00	4.32	1.00	0.77	1.00
CNS MPO Score	5.43/6		4.96/6		3.48/6		3.50/6		3.56/6	

Abbreviations: CNS MPO, central nervous system multiparameter optimization, score ranges from 0 to 6, higher than 4 aims to predict CNS penetration ability (97); HBD, hydrogen bond donor; LogD, distribution coefficient; LogP, partition coefficient; MW, molecular weight; PKA, acid dissociation constant; TPSA, topologic polar surface area.

Peptide inhibitors

The first anticancer therapeutics to be developed targeting BCL6 were peptide inhibitors. In 2004, Polo and colleagues (93), published data on the first BCL6 lateral groove inhibitor. Developing a wild-type BCL6-targeted peptide and mutant-targeted peptide that would compete for corepressor binding at the BTB/POZ binding domain (BBD) (Table 1). The wild-type peptide contained the SMRT BTB motif sequence along with a polyhistidine-tag, pTAT protein transduction domain and a hemagglutinin epitope tag. The mutant peptide incorporated point mutations in the SMRT binding motif, which reduced its affinity to BCL6. Although the wild-type peptide demonstrated the ability to penetrate cells and selectively bind BCL6, inhibit corepressor recruitment, and reactive expression of BCL6 target genes such as *CD80* and *CD69* (93), it also resulted in complete loss of biological BCL6 function, which mimicked the *BCL6*-null phenotype *in vivo*. *BCL6* knockout mice develop a lethal inflammatory condition that causes fierce migration of macrophage and T cells into tissues, as well as an inability to form GCs (93). Thus, the need for specific peptide inhibitors, that explicitly target the BTB domain of BCL6, while ensuring BCL6 is still active, would minimize unwarranted immune side effects, while still promoting anticancer activity by inhibiting BCL6 repression of antitumor genes.

In 2006, Chattopadhyay and colleagues (22) developed a peptide aptamer (small peptides with both terminal ends anchored to a scaffold protein), Apt 48, which binds selectively to the POZ domain of BCL6, although outside the known corepressor and dimerization interface (Table 1). In Ramos B lymphocyte cell lines, Apt 48 successfully reinstated gene expression of BLIMP1 (PR domain zinc finger protein 1, *PRDM1*) and CCND2 (known targets of BCL6 repression); however, *in vivo* data on Apt 48 is yet to be published.

Following these studies, research then focused on inverting the original 17 amino acid sequence of the NCOR2-BBD peptide inhibitor, resulting in the development of the “retro-inverso” BCL6 peptide inhibitors (RI-BPIs) (Table 1). This category of BCL6 inhibitor showed success across multiple cancers types, with activity charac-

terized similarly to their predecessor BBD peptides, with the advantage of RI-BPIs lacking immunomodulatory side effects (14, 21). Using BCL6-dependant DLBCL patient-derived xenograft mouse models (SU-DHL6, SU-DHL4), RI-BPI effectively induced cell death and growth arrest and was deemed nontoxic as treatment was continued for over a year in immunocompromised mice with no reported side effects (94). RI-BPI were also tested in multiple glioblastoma models (U87, U251, DA66, DBTRG, JM94, SN172) all of which harbored high BCL6 expression, and showed *in vitro* and *in vivo* efficacy (9). Promisingly, RI-BPI's sensitivity in glioblastoma was similar to DLBCL (OCI-Ly3 and SU-DHL-4) both *in vitro* and *in vivo* (9). RI-BPI alone, and in combination with STAT3 and tyrosine kinase inhibitors, has also been shown to be effective in other cancer models such as FL, ALL, and breast cancer (3, 5, 60). Phage-display technologies identified the peptide inhibitor F1234, showing higher affinity and potency than all other peptide inhibitors (Table 1); however, to our knowledge, the anticancer effects of F1324 have not been tested (95).

Small-molecule inhibitors

This series of BCL6 inhibitors were discovered using computer-assisted, small-molecule drug screen of the BTB domain lateral groove to identify low molecular weight, potent, BCL6 inhibitors (96).

79-6 is a small-molecule compound that was first developed in response to this computer-aided drug discovery approach, and elicits its inhibitory effect by binding to the lateral groove, once again inhibiting recruitment of NCOR and SMRT to the BTB domain of BCL6 (Table 1; ref. 21). 79-6 has had success in inducing apoptosis and reducing EMT in aggressive triple-negative breast cancer cell lines (SK-MDA-MB-468 and BT-549), as well as showing selective killing of BCL6-dependant DLBCL cells (OCI-Ly7), *in vitro* (Table 1; refs. 3, 14, 96). Unfortunately, 79-6 is predicted to show limited utility for the treatment of brain tumors based on CNS multiparametric optimization desirability (CNS-MPO; Table 2; ref. 97).

FX1 was developed in response to 79-6 with the view of identifying a drug with a higher affinity to specific areas of the BTB domain lateral groove than endogenous corepressors (21). FX1 was shown to out-

compete every other BCL6 inhibitor based on affinity for the lateral groove of BTB domain and in pharmacodynamic assays (14, 21). In immunocompromised mice, FX1 inhibited the growth of BCL6 dependent DLBCL cells (OCI-ly7), and inhibited proliferation of non-small cell lung cancer in cells (H1299 and H383) engineered to express high *BCL6* mRNA levels (Table 1; ref. 21). Interestingly, CNS-MPO (97) predicts FX1 to harbor the highest potential to show activity in the brain (Table 2) of all BCL6 inhibitors described (Table 1). This is potentially important information for the choice of BCL6 inhibitor to test in preclinical brain tumor models.

BI-3802 and BI-3812 were developed using structure-based drug design methods and high-throughput screening, using high-affinity binding to the BTB domain as the selection criterion (98). BI-3802 inhibits corepressor binding to BCL6 PTP/POZ domain and promotes rapid BCL6 degradation by the formation of BCL6 filaments, to drive ubiquitination by the SHAH1 E3 ubiquitin ligase (99). BI-3812 is a structural analog of BI-3802, however, inhibits rather than degrades BCL6. Although both compounds BI-3802 and BI-3812 demonstrated BCL6 inhibition and reestablishment of tumor suppressor gene transcription such as BLIMP1 in DLBCL cell lines (SU-DHL-4, OCI-ly7, OCI-Ly-1, and Farage), poor bioavailability has limited their use in preclinical animal models (Table 1; ref. 98). Furthermore, neither of these two therapies are predicted to show activity in the brain when delivered systemically (Table 2).

GSK137, was recently discovered using a high-throughput screening approach, again selected based on affinity for the BCL6 BTB-POZ domain (100). GSK137 binds the lateral groove to antagonize corepressor binding, including NCOR2/SMRT; however, unlike BI-3802, it does not induce BCL6 degradation. Unfortunately, GSK137 caused little change to the viability or proliferation of BCL6-expressing B-cell lymphoma lines (Farage, Karpas422, ULA, VAL) *in vitro* (Table 1). Despite this, *in vivo* studies were still conducted to determine GSK137's pharmacokinetic profile and determined that it suppressed GC-dependent antibody response (male CD1 mice). Promising *in vivo* studies demonstrated that GSK137 suppressed immunoglobulin G responses following immunization of naïve mice with the hapten trinitrophenol, reducing the numbers of GCs and GC B cells, suggesting an on-target effect (100). The oral bioavailability and pharmacokinetic profile of GSK137, is the most promising agent to date, and therefore, although it is ineffective at driving apoptosis in cancer cells as a single agent, it may prove to be a promising drug when used in combination with SOC (as described in Sections Leukemias, AML and Pediatric HGG, *Glioblastoma*, *DMG*). GSK137 is predicted to show some activity in the brain, warranting further investigation in preclinical models of brain tumors (Table 2).

Conclusion

BCL6's role in transcriptional silencing of tumor suppressor and checkpoint regulators during the formation of GCs is also fundamental in the way it acts as an oncogene in cancer. BCL6 suppresses DNA repair genes and antiapoptotic signatures to promote B cell SHM and CSR, leading to the production of high-affinity immunoglobulins necessary to recognize and bind to specific antigens, triggering their destruction. This is largely coordinated by the recruitment of repressor proteins, including NCOR1, NCOR2, BCOR, PRC1, and HDAC3, critical regulators of epigenetic signaling and gene silencing. BCL6 translocations were originally described as the driver of the lymphomagenesis of DLBCL. However, when we take a closer look at BCL6 in pediatric cancers (St Jude Pediatric Cancer database, $n = 1,985$ samples), it is clear that BCL6 translocations/mutations are

not necessarily the main driver of increased BCL6 expression and oncogenesis in the majority of cases. 36.6% of glioblastoma cases harbor a BCL6 translocation, corresponding with increased mRNA and protein expression; however, 45% of DLBCL cases harbor a BCL6 translocation, with no change in mRNA expression. Sarcomas and medulloblastoma are rarely BCL6 mutant: however, BCOR mutations are highly prevalent in both cancer types, which can also drive BCL6 expression. Overall, increased expression of BCL6 mRNA appears a predictor of worse prognosis in most pediatric cancers; however, survival studies using protein expression have not been widely conducted. In two rare cancers including medulloblastoma and Burkitt lymphoma, overexpression of BCL6 conferred better prognosis, albeit in only a small sample size. The mechanisms underpinning the role of BCL6 in the pediatric cancer population remains poorly understood, but as it appears to be a notable player in many cancer subtypes, highlighting the importance of more research in this area. Molecular inhibition of BCL6 *in vivo* and *in vitro* decreased leukemia-initiating cell capacity, increased chemotherapy sensitivity, and increased expression of the master tumor suppressor p53, rarely mutated in pediatric cancers (except DMG; ref. 45), as well as promoted expression of cell-cycle checkpoint genes such as CDKN1A (9). These observations highlight the potential for targeting BCL6, particularly in combination with DNA-damaging therapies including chemotherapies and radiotherapy. It is becoming even clearer that combinations of treatments are necessary to achieve long-term positive outcomes for many aggressive pediatric cancers, and we propose targeting/evaluation of BCL6 warrants consideration. Development of nonimmunosuppressive BCL6 therapies may even open the door to new combinations, including radiotherapy and immunotherapy. Radiotherapy promotes the expression of checkpoint proteins that underpin the efficacy of immune checkpoint inhibitors, the latter having revolutionized the treatment of aggressive adult cancers including melanoma, non-small cell lung cancer and Hodgkin lymphoma (80). In the context of pediatric tumors such as DMG, for which radiotherapy is the only recognized treatment (43, 45), BCL6 inhibition may serve to enhance the therapeutic benefit and increase the survival of this poor outcome patient cohort.

As it stands, BCL6 remains a challenging target; however, the field is moving closer to the development of specific anti-BCL6, nonimmunosuppressive therapies. Over the coming years, we hope that new trials will open to test the efficacy of the next generation of BCL6 inhibitors.

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