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TARGETING BCL-6 IN DIFFUSE LARGE B-CELL LYMPHOMA: WHAT DOES THIS MEAN FOR THE FUTURE TREATMENT?

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The BCL6 (B-cell lymphoma 6) transcriptional repressor has emerged as a critical therapeutic target in diffuse large B-cell lymphomas (DLBCL). Although a sequence-specific transcription factor, which were traditionally considered to be “undruggable”, in depth mechanistic studies have facilitated the development of BCL6 targeted therapies, which are the first rationally designed transcription factor inhibitors.

The properties of BCL6 as a therapeutic target stem from its normal function in the humoral immune system, where it plays important roles enabling the survival of germinal center (GC) Bcells, which are the cell of origin of DLBCLs [1]. After T-cell dependent antigen stimulation, Bcells migrate within lymphoid follicles, and form GCs within which they undergo rapid proliferation while at the same time enduring somatic hypermutation of their immunoglobulin loci [1]. BCL6 is required for GC B-cells to proliferate and tolerate the DNA damage that occurs as a byproduct of this process of immunoglobulin affinity maturation [1]. BCL6 mediates these effects by directly binding and repressing the replication checkpoint and DNA damage sensor encoding gene *ATR* as well as key checkpoint genes *CHEK1*, *TP53*, *CDKN1A*, *CDKN2A* and *p14ARF* [2–5]. GC B-cells that have generated high affinity immunoglobulins are subsequently selected for terminal differentiation into antibody producing plasma cells or memory B-cells through the actions of follicular T-helper and follicular dendritic cells. BCL6 also represses genes involved in terminal differentiation such as IRF4 and PRDM1 [1,4], and so must be downregulated for exit from the GC reaction to occur. Hence BCL6 not only enables but also maintains the GC B-cell phenotype.

The checkpoint suppression properties of BCL6 are inherently pro-oncogenic and accordingly BCL6 is almost universally expressed in DLBCLs. DLBCLs can be subclassified according to gene expression profiles into various disease subtypes. Among these the ABC (activated B-cell)-DLBCLs are generally considered to be derived from late GC B-cells in which BCL6 downregulation would normally occur [6]. Accordingly ABC-DLBCLs feature more frequent translocation of the BCL6 locus to heterologous promoters allowing for its constitutive expression. Although ABC-DLBCLs are often thought of as being BCL6-negative, this is likely a due to the relatively low sensitivity of standard immunohistochemistry methods and indeed BCL6 protein can be detected in ABC-DLBCL cells when evaluated by more sensitive methods such as immunoblotting. The more

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prevalent GCB-type DLBCLs tend to express higher BCL6 protein levels even in the absence of translocations, reflecting their origin from GC B-cells [6]. Altogether, a majority of ABC and GCB type DLBCLs require and are hence “addicted” to BCL6 to maintain their proliferation and survival, reflecting its function in normal GC B-cells and supporting the notion that BCL6 is a broadly relevant therapeutic target for DLBCLs [7–9].

Biochemical and functional studies have provided the basis and rational for development of highly specific and non-toxic BCL6 inhibitors. From the biochemical standpoint BCL6 contains a BTB-POZ domain at its N-terminus, which has autonomous transcriptional repressor activity [10]. The BTB/POZ domain represses transcription by recruiting three corepressor proteins: SMRT, NCoR and BCoR to a specific groove motif that is formed by BCL6 BTB domain homodimers [10]. Notably the BCL6 BTB domain surface residues that interface with these corepressors are unique to BCL6. Point mutations of key surface residues abrogate corepressor binding and hence repressor activity of the BTB domain. The reciprocal 18 aminoacid regions of SMRT, NCoR and BCoR that binds to BCL6 are also unique and do not associate with other BTB domains [10]. Thus the physical contacts between the BCL6 BTB domain and its cofactors appear to be exclusive to BCL6, providing a pharmacological basis for rational design of specific BCL6 BTB domain inhibitors unlikely to affect other BTB proteins.

BCL6 knockout mice are unable to form germinal centers, but of more concern from the therapeutic standpoint display a lethal phenotype manifesting as a rapidly fatal inflammatory syndrome due to hyperactivity of T-cells and macrophages [1]. BCL6 deficient macrophages are also linked to the development of accelerated atherosclerosis in mice [11]. These serious consequences of BCL6 deficiency could temper enthusiasm for development of BCL6 inhibitors due to concerns for potential toxicity. However more recent studies suggest that these concerns may be unwarranted, since in fact BCL6 may function through distinct biochemical mechanisms in different cell types. Remarkably, mice engineered to express BCL6 with point mutations that disrupt the BTB domain-corepressor complex live normal healthy lives without inflammation, but still exhibit failure to form GC B-cells [12]. Closer examination of lymphoid follicles in BCL6-BTB mutant animals revealed failure of activated B-cells to proliferate and survive after antigen stimulation. In contrast, functions of BCL6 in macrophages and T-cells, which are responsible for the inflammatory phenotype, were perfectly intact [12]. Instead the inflammatory macrophage function appeared to be more dependent on BCL6 competition with STAT proteins for binding to DNA, since both factors share similar DNA binding sequences [12]. Targeting the BCL6 BTB domain corepressor interface could thus specifically suppress B-cell survival without inducing acute or chronic inflammatory effects that instead would be expected to occur with the complete loss of BCL6.

Along these lines proof of principle studies using recombinant peptide technology demonstrated that BCL6 repression complexes could be disrupted *in vitro* and *in vivo* by SMRT BCL6 binding domains fused to TAT protein transduction domains [7]. In an effort to transform this peptide into a more drug-like molecule a series of stepwise design improvements were undertaken that ultimately yielded a stable and potent synthetic retro-inverso BCL6 peptide inhibitor (RI-BPI) constructed with D-amino acids [9]. The D-amino

acid composition endows RI-BPI with greater stability and inherent resistance to cellular and serum proteases. Just five doses of RI-BPI were sufficient to eradicate establish human DLBCL xenografts in mice [13]. RI-BPI had favorable pharmacokinetics and no toxic effects in even after one year of continuous administration in mice [9]. There was also no evidence of an immune response elicited against RI-BPI in animals. The success of RI-BPI inspired deeper computational modeling efforts leading to the first BCL6 small molecule inhibitors including 79-6, with powerful activity against BCL6-dependent DLBCLs and again with no evident toxicity [14]. Collectively these molecules, their derivatives and other BCL6 inhibitors in development will soon be ready for testing in the clinic.

The principal effect of BCL6 inhibitors on DLBCL cells is rapid induction of cell death. Apoptosis is typically observed within 24 hours *in vitro* and *in vivo* [9], and occurs in large part because BCL6 inhibitors release from BCL6-mediated transcriptional repression a variety of cell death checkpoint effectors and modulator genes such *ATR, p53 and EP300* [2,15,16]. It is likely the combinatorial effect of multiple simultaneous checkpoint gene reactivations that delivers such a powerful killing effect to lymphoma cells. This mechanism of action raises several possible scenarios for how BCL6 targeted therapy could be deployed to the clinic. Indeed, since chemotherapy drugs such as doxorubicin kill at least in part through checkpoint activation, it would be anticipated that exposure to BCL6 inhibitors could enhance response to cytotoxic drugs. Indeed powerful synergy between BCL6 and drugs such as doxorubicin has been observed in pre-clinical studies supporting a rationale for sequential RI-BPI / RCHOP combination therapy [17].

Other aspects of BCL6 mechanism could also be harnessed to design combined targeted therapy regimens. For example, BCL6 directly represses the *BCL2* gene and hence BCL6 inhibitors like RI-BPI could have the unintended effect of inducing this pro-survival factor thus enabling at least a subset of lymphoma cells to survive. Targeting BCL2 might prevent these pro-survival effects and indeed combinations of BCL6 and BCL2 inhibitors were reported to by highly synergistic in DLBCL cells [18]. BCL6 protein stability and transcriptional repression function is partially dependent on a stress-activated isoform of Hsp90. Combinatorial therapy of BCL6 inhibitors with the stress-activated isoform Hsp90 specific inhibitor PUH71 were highly synergistic in vitro and in vivo [16]. BCL6 directly represses the p300 gene which encodes a lysine acetyltransferase that activates p53 function and suppresses Hsp90 activity [16]. BCL6 inhibitors accordingly synergistically killed DLBCL cells when combined with various histone deacetylase inhibitors, and this was linked to enhancement of p53 acetylation and Hsp90 activity [16]. Finally BCL6 may help to maintain B-cell receptor signaling through repression of the SYK inactivating phosphatase PTRPROt [19]. It is possible that BCL6 targeted therapy may enhance the anti-tumor effects of drugs targeting aberrant B-cell receptor activity such as SYK or BTK inhibitors. The apparently minimal toxicity of BCL6 inhibitors suggests that these drugs could be safely administered concurrently with chemotherapy and/or additional targeted therapy drugs. Taken together with the fact that BCL6 is broadly required by both GCB and ABC-type DLBCLs and its many intersections with other key lymphomagenic pathways it seems that BCL6 inhibitors could serve as a crucial building block for novel rationally

designed combinatorial therapies geared towards more effectively eradicating lymphomas with less toxicity than current regimens.

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