Bromodomain Inhibitors: What Does the Future Hold?

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Abstract: Cancer cells are addicted to mutations that cause gain of function in oncogenes and loss of function in tumor suppressors, so that these cells are reliant on aberrant signaling pathways and transcription. Protein-protein and DNA-protein interactions that cause chromatin remodeling are another source of the deregulation of critical signaling and transcriptional regulators, altering epigenetic signatures and creating additional vulnerabilities. Owing to mutations in multiple epigenetic regulators in hematologic malignancies, cancer cells are highly addicted to altered transcription. These vulnerabilities have been targeted by several epigenetic drugs, including hypomethylating agents, but the idea of targeting bromodomain proteins has emerged relatively recently. Because bromodomain proteins recognize acetylated lysine on histones and recruit transcription complexes on the chromatin, targeting these proteins may serve as a strategy to target transcription, irrespective of the presence of epigenetic mutations. Here, we review the existing literature to explain the rationale of using bromodomain inhibitors in hematologic malignancies. We discuss the evolution of bromodomain inhibitors, with an in-depth evaluation of bromodomain and extraterminal domain (BET) proteins, the most prominent bromodomain family, and also highlight the prospect of targeting non-BET proteins. In the later sections, we comment on the combinatorial targeting of BET proteins to overcome the effects of multiple signaling pathways. Finally, we emphasize the newer concepts, such as dual-kinase inhibition and selective bromodomain targeting, and technologies, such as protein degradation, that are expected to influence the future generation of bromodomain inhibitors.

Transcriptional Regulation of Gene Expression

Gene expression is highly regulated in a spatiotemporal manner. DNA in eukaryotic cells is packaged by the core histone proteins (H2A, H2B, H3, and H4) forming the chromatin structures. The less-condensed form of chromatin, euchromatin, is transcriptionally more active than the more-condensed form, heterochromatin. In simple terms, the open chromatin is more accessible for the assembly of multiprotein transcriptional complexes, marking the DNA
The Histone Code Editors: Readers, Writers, and Erasers

The proteins that modify and recognize the histone code (such as the PTMs), and thereby influence transcription, are categorized as readers, writers, and erasers. Writers are histone acetyltransferases (HATs), histone methyltransferases (HMTs), kinases, and E3 ubiquitin (Ub) ligases, which can add acetyl, methyl, phosphate, or Ub moieties to N- and C-terminal tails of histones on lysine (K), K and arginine (R), serine/threonine/tyrosine (S/T/Y), and K residues, respectively. Various PTM patterns have differential effects on transcription and assembly of the transcription complex. For example, although monomethylation at K9 on histone H3 (H3K9me1) marks active transcription, dimethylation and trimethylation marks on the same residue (H3K9me2 and H3K9me3) are repressive. Playing the role of erasers are the histone deacetylases (HDACs), histone demethylases (HDMs), phosphatases, and deubiquitinating enzymes (DUBs). DNA modifications, in conjunction with the histone modifications made by writers and erasers, create the epigenetic code. This is interpreted by the readers to regulate transcription. The readers generally contain unique domains or motifs that identify the specialized modifications on histones and DNA to accomplish functional specificity. Some examples are chromo-like domains (chromo, tudor, and MBT), which recognize methylation; conserved bromodomains (BDs), which recognize acetylation; and the 14-3-3 protein domain, which recognizes phosphorylation events.

Vulnerability of Oncogenes to Transcription Inhibition

Owing to genetic or functional perturbations in several transcription regulators—such as transcription factors (TFs), coactivators, repressors, chromatin regulators and remodelers, signaling proteins, and enhancer elements—transcription is deregulated in cancer. Cancer cells are addicted to this deregulation. These perturbations are context-specific. Thus, targeting regulators of transcription is a strategic approach to cancer drug discovery. Several examples show that cancer cells depend on oncogenic drivers such as MYC and RAS, and on effectors such as the antiapoptotic proteins BCL2, BCL-xL, and MCL1. Oncogenic TFs recruit transcription complexes at enhancer and superenhancer (SE) sites and/or remodel the chromatin to drive the cancer phenotype. The concerted action of writers, erasers, and readers through histone and DNA modifications determines the organization of transcription complexes on the chromatin and the repertoire of recruited TFs. Therefore, interest is increasing in therapeutically targeting chromatin modifiers (readers, writers, and erasers) and other epigenetic regulators.

Transcriptional Targets in Hematologic Malignancies

Genetic alterations procreate an aberrant epigenetic landscape in cancer. Together with altered functions of oncogenes and tumor suppressors such as MYC, RAS, p53, BCL2, BCL-xL, and MCL1, these have immense implications in the biology of the disease. Aberrant protein expression results from gene amplifications, translocations, changes in upstream signaling, or transcription. Resulting gene and protein expression patterns differ considerably in cancer and normal cells, providing a strategic opportunity to target. In this section, we discuss the most frequent transcriptional alterations in the context of hematologic malignancies.

Oncogenic Drivers

Mutations and translocations of several of these factors are seen in different leukemias and lymphomas.

MYC. MYC overexpression results from gene amplifications, translocations (IGH-MYC fusion) in B-cell malignancies, and mutations in upstream signaling (NOTCH1) in T-cell acute lymphoblastic leukemia (T-ALL). MYC coordinates cellular proliferation and metabolic adaptations acquired by cancer cells.

NRAS/KRAS. RAS frequently shows dominant somatic mutations in cancers. Mutations in NRAS and KRAS, and associated factors c-KIT, PTPN11, CBL, and BCR-ABL, cause aberrant RAS/RAF/MEK/ERK signaling that acts as an initiation trigger. Mutations in NRAS, KRAS, and NF1 are strongly associated with myeloid malignancies, specifically juvenile myelomonocytic leukemia (JMML), chronic myelomonocytic leukemia (CMML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPNs), and multiple myeloma (MM). They are also associated to a lesser extent with lymphoid malignancies, namely T-ALL. These mutations cooperate with driver kinases such as FLT3 and c-KIT in leukemogenesis.
MLL. Rearrangements and mutations in the mixed lineage leukemia (MLL) gene are drivers in hematologic malignancies. Approximately 70 fusion partners of MLL exist in leukemias, most of which retain the N-terminal part of the protein (required for binding to the chromatin). The most common translocations are t(4;11), t(9;11), and t(10;11), which give rise to fusion oncogenes MLL-AF4, MLL-AF9, and MLL-AF10, respectively, whereas translocation t(11;19) produces 2 fusions, MLL-ENL and MLL-ELL.15 MLL1 is more relevant in AML and ALL, whereas MLL2 is more important in follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL).10,16 The fusion proteins upregulate HOX genes, leading to epigenetic reprogramming–induced differentiation block. This promotes self-renewal and leukemic stem cell (LSC) maintenance.17,18 Most of the MLL fusion partners, AF4, AF5, LAF4, and ENL, bind either directly to RNA polymerase II (RNAPII) or indirectly as a member of the superelongation complex (SEC) or polymerase-associated factor complex (PAFc), which bind to RNAPII. Thus, MLL fusions play a major role in regulating transcriptional elongation.19 Because of the major role of MLL in transcription-dependent transformation, these complexes are important targets in hematologic malignancies.15

Other histone acetyltransferases. Mutations in CREB-binding protein (CBP) and p300 have been found in lymphomas (FL and DLBCL) and acute leukemias (ALL and AML).10

EZH2 and other PRC2 members. The most frequently mutated chromatin regulator in hematologic malignancies is EZH2, with somatic mutations found in lymphomas (FL and DLBCL), myeloid (MDS, MPN, myelofibrosis, and AML), and lymphoid (T-ALL) malignancies. The other PRC2 components, EED and SUZ12, are also found to be mutated in T-ALL.10

ASXL1, DNMT3A, and TET2. ASXL1 mutations are present in MDS and CMML,20 MPN, secondary AML, and de novo AML.19 Almost 20% of patients with AML have mutated DNMT3A, most frequently at the R882 locus.21 TET2 mutations (deletions, truncations, and nonsense and nonsense mutations) are associated with gene inactivation in AML, MDS, MPN, and CMML. TET1 is also an MLL fusion partner.22

IDH. IDH1 and IDH2 mutations are mutually exclusive to TET2 mutations. Recurrent mutations in IDH (IDH1-R132, IDH2-R140, and IDH2-R172) have been identified in AML and MDS. Mutated genes express enzymes to produce a unique “oncometabolite,” 2-hydroxyglutarate, that inactivates TET.22,23

**Superenhancers**

TFs bind to upstream promoter regions and engage distal cis-acting elements (enhancers) by virtue of protein-protein and DNA-protein complexes, driving transcription.24 Large clusters of enhancers form SEs, which control the transcription of several oncogenes.25 Selectively targeting these SEs is a feasible strategy in cancer cells, because here SEs are enriched by regulators of oncogenic drivers.8 SEs are several-fold larger than regular enhancers and are enriched many-fold by the occupancy of readers such as bromodomain-containing protein 4 (BRD4). Some of the SE-regulated genes in the context of hematologic cancers are MYC, IRF4, PRDM1/BLIMP1, XBP1, PIM1, MCL1, and BCL-xL.25

Thus, the epigenetic and transcriptional addiction of hematologic malignancies, in addition to oncogenic addiction, generates a strong rationale to target epigenetic and chromatin regulators for drug discovery. As a proof of concept, the use of DNMT and HDAC inhibitors to target epigenetic addictions in hematologic malignancies has been successful.25 Some of the US Food and Drug Administration (FDA)-approved epigenetic drugs are: (1) DNMT inhibitors azacitidine and decitabine for MDS and AML; (2) HDAC inhibitor vorinostat (Zolinza, Merck) for cutaneous T-cell lymphoma; and (3) enasidenib (Idhifa, Celgene), a first-in-class inhibitor of mutant IDH2, for advanced IDH2-mutant hematologic malignancies (NCT01915498).26

Because the BD-containing proteins can anchor to acetylated lysine (Ac-K) on histone molecules and act as scaffolds to recruit active transcription complexes, targeting this family may serve as a strategy to target transcription, irrespective of the presence of epigenetic mutations. Strengthening this concept, in a pioneering RNA interference–based screen, BRD4, a member of the BET family, was identified as a critical factor in AML disease maintenance and could be efficiently targeted by the small-molecule inhibitor JQ1.18 In the following sections, we discuss BD proteins and methods to target them in hematologic malignancies.

**Bromodomain Family of Proteins**

**Function in Transcription**

The BD proteins are “readers” of Ac-K at N-terminal histone tails. As epigenetic regulators, these proteins fulfill 2 very important functions. First, identification of Ac-Ks on histones, enabling attachment to chromatin. Second, recruitment of transcriptional coactivators forming multiprotein transcription complexes (SECs).27 This facilitates modulation of chromatin dynamics, to ultimately diversify gene expression.28 Members of this family comprise nuclear proteins such as HATs, HMTs, chromatin remodelers,
helices, transcription coactivators and mediators, and scaffold proteins. These are divided into 8 subfamilies (I-VIII) according to structure and sequence similarities. All of these proteins contain 1 or more BDs, accounting for a total of 61 BDs, which comprise approximately 110 amino acids and remain highly conserved evolutionarily.28 The BD Ac-K binding sites are deep hydrophobic pockets with a conserved asparagine residue in 79% cases. The other 21% of cases have either tyrosine, threonine, or aspartate. This makes them highly druggable.28,29 The most common approach in drug development for this family has been to develop small-molecule mimics that disrupt the interactions between BDs and Ac-Ks on chromatin.37 Because of easy druggability, this family is an attractive target in cancer, inflammation, fibrosis, and heart disease.12

**The BET Family of Bromodomain Proteins**
The best-studied subfamily is number II, BET proteins, which has 4 members—BRD2, BRD3, BRD4, and BRDT. In addition to 2 bromodomains, BD1 and BD2, these proteins carry an extraterminal (ET) domain that is responsible for protein-protein interactions, enabling the BET proteins to act as scaffolds for the recruitment of TFs and regulators.12 This facilitates diversification of gene expression by BETs and also makes them attractive targets for drug design.

**Targeting BET and Other Bromodomain Proteins**
The first BD inhibitors were NP1, which targets transcriptional coactivator PCAF,30 and MS7972, an inhibitor of CBP-BD that hinders its binding to acetylated p53.31 These were followed by other small-molecule BD inhibitors targeting BET proteins: JQ1,32 I-BET151,33 I-BET762,34 PFI-1,35 OTX015,35 TEN-010 or CPI-203,36 BI-894999,37 CPI-0610,38 FT-1101,39 GS-5829,40 INCB-54329,41 ABBV-075,42 and ABBV-0744.3 (See Table 1 for details.) In phase 1/2 dose-escalation studies of several of these drugs, the major dose-limiting toxicities included hematologic events such as thrombocytopenia, neutropenia, and anemia. The associated nonhematologic events included diarrhea, nausea, fatigue, vomiting, headache, altered taste, and rashes (see Table 2 for detailed trial-based descriptions). Observed toxicity profiles have been adequately discussed elsewhere.44

Efforts at identifying drug targets for BDs beyond the BET family are highlighted in some recent studies. These identified drugs targeting other BD subfamilies: SGC-CBP30 and I-CBP112 (targeting p300/CBP of subfamily III); LP99 and I-BRD9 (targeting BRD7 and 9 of subfamily IV); OF-I and GSK compound 3 (targeting BRPF1, 2, and 3 of subfamily IV); BAZ2-ICR and GSK2801 (targeting BAZ2A and B of subfamily IV); PFI-3 (targeting SMARCA2, SMARCA4 and PB1 of subfamily VIII); and the pan-BD inhibitor bromosporine. Thus, BD-containing proteins have emerged as exciting novel targets over the last few years (Table 1).27,29,45

**BET Targeting in Hematologic Malignancies**
With the discovery of JQ1 and I-BET, drug discovery for BET inhibitors boomed with advanced knowledge of how BD proteins function and mechanistic insights into their inhibitor activities. Here, we discuss some of the biological ramifications of BET targeting in the context of hematologic malignancies.

**Targeting MYC and Antiapoptotic Proteins**
Despite the central role of MYC in multiple hematologic malignancies, direct targeting of MYC has not been successful yet, owing to lack of enzymatic activity and globular domains. Because MYC heterodimerizes with MAX for transcriptional activation, several attempts have been made to interfere with MYC/MAX to disrupt its DNA binding activity (described elsewhere).46 Because MYC recruits several chromatin modifiers (HATs) and cofactors (cyclin-dependent kinase CDK9 and BETs) to enhance RNAPII activity at promoters and enhancers, indirectly targeting MYC activity instead becomes logical. BET inhibition was found to downregulate MYC transcriptional activity in varied oncogenic contexts. Interestingly, studies with the BET inhibitor JQ1 showed that expression of MYC was profoundly affected, given that the SE driving MYC expression is rich in BRD4. Antiapoptotic proteins BCL2 and MCL1 were also downregulated, either by direct transcription repression or as a downstream consequence.12 Given that MYC is a central transcription regulator and is downstream to several upstream factors such as KRAS, MAPK, phosphoinositide 3-kinase (PI3K), Wnt/β-catenin, Janus kinase/signal transducer and activator of transcription (JAK/STAT), and NOTCH, targeting MYC with BET inhibitors has been successful in preclinical models of several cancers. Because all these signaling pathways show aberrations in hematologic malignancies, inhibition of BET proteins is promising.46

**Targeting NOTCH1**
T-ALL, harboring NOTCH1 mutations, often become resistant to gamma-secretase inhibitors (GSIs). Because BRD4 was found to be a vulnerability in this context, JQ1 was used and it successfully downregulated MYC and BCL2. There were beneficial effects of JQ1 and GSI combination in primary human leukemia in vivo.47 In another approach to tackling MYC, vincristine was successfully combined with JQ1 in T-ALL, with beneficial outcomes.13
Table 1. Bromodomain-Targeting Drugs in Hematologic Malignancies

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target(s)</th>
<th>Strategy</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>JQ1</td>
<td>BET members (BRD2, BRD3, BRD4)</td>
<td>Diazepine-based small-molecule Ac-K mimics</td>
<td>Triazolobenzodiazepine-based; first BD inhibitor with profound anticancer activity affecting MYC and its targets.</td>
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<tr>
<td>I-BET762 (GSK525762)</td>
<td></td>
<td></td>
<td>Triazolobenzodiazepine-based inhibitor by GSK. In clinical trial for relapsed/refractory hematologic malignancies (NCT01943851).</td>
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<tr>
<td>OTX015 (MK-8628)</td>
<td></td>
<td></td>
<td>Thienotriazolodiazepine-based inhibitor developed by OncoEthix. In clinical trials: NCT02698189 (AML), NCT02303782 (AML, in combination with azacitidine), and NCT01713582 (DLBCL).</td>
</tr>
<tr>
<td>TEN-010 (RO6870810) or CPI-2036</td>
<td>3,5-Dimethylisoxazole-based small-molecule Ac-K mimic</td>
<td></td>
<td>Primary amide analogue of JQ1. In clinical trials: NCT02308761 (AML and MDS), NCT03068351 (relapsed/refractory MM). Also shows effect in T-cell leukemias.</td>
</tr>
<tr>
<td>I-BET151 (GSK1210151A)</td>
<td>3,5-Dimethylisoxazole-based small-molecule Ac-K mimic</td>
<td></td>
<td>Inhibitor with high level of efficacy in MLL-rearranged context and with broad anti-inflammatory effects.</td>
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<tr>
<td>PFI-127,29</td>
<td>Dihydroquinoxalinone-based small-molecule Ac-K mimic</td>
<td></td>
<td>In clinical trials: NCT01949883 (lymphoma), NCT02157636 (MM), NCT02158858 (AML).</td>
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<tr>
<td>CPI-0610</td>
<td>Benzoisoxazolopipperazine-based small-molecule Ac-K mimic</td>
<td></td>
<td>In clinical trial for solid malignancies, including DLBCL (NCT02516553).</td>
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<tr>
<td>BI-894999</td>
<td>Small-molecule Ac-K mimics</td>
<td></td>
<td>In clinical trial for AML (NCT02543879).</td>
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<tr>
<td>FT-1101</td>
<td>PROTAC-based degrader (heterobifunctional molecule)</td>
<td>PROTAC-based degrader (heterobifunctional molecule)</td>
<td>Targets signaling pathways and their effectors: JAK/STAT pathway; MYC; BCL2 family of proteins; NFkB and its targets cIAP2, XIAP, cFLIP, TNFAIP3, BCL-xL, IL-10, TNF-α, and BTK; and microenvironment (leukemia-stroma interactions).</td>
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<tr>
<td>dBET1</td>
<td>BRD4</td>
<td>Phthalimide conjugation–based degrader (bifunctional molecule)</td>
<td>BRD4 degradation seen within 1 to 2 hours, downregulation of BRD4 targets MYC and PIM1.</td>
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<tr>
<td>dBET6</td>
<td>BET</td>
<td>Phthalimide conjugation–based degrader (bifunctional molecule)</td>
<td>Phenocopies the effect of CDK9 inhibitor.</td>
</tr>
<tr>
<td>Dinaciclib</td>
<td>BRD4, CDKs</td>
<td>Dual-kinase inhibitors</td>
<td>CDK inhibitor with very low level of affinity for BETs.</td>
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<tr>
<td>LY2940023</td>
<td>BRD4, PI3K</td>
<td>PI3K inhibitor with very low level of affinity for BETs.</td>
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<tr>
<td>ABBV-075</td>
<td>BET</td>
<td>Small-molecule</td>
<td>In clinical trial for advanced hematologic malignancies and solid tumors (NCT02391480).</td>
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<tr>
<td>ABBV-744</td>
<td>BDII</td>
<td>Second generation BDII specific small-molecule</td>
<td>In clinical trials for metastatic castration resistant prostate cancer and relapsed/refractory AML (NCT03360006). Improved oral bioavailability and enhanced tolerability.</td>
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BROMODomain inhibitors: What does the future hold?

### Non-BET inhibitors

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<tr>
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<tr>
<td>NP1&lt;sup&gt;30&lt;/sup&gt;</td>
<td>PCAF</td>
<td>Small-molecule Ac-K mimics</td>
<td>Inactivation of transcription coactivation function.</td>
</tr>
<tr>
<td>MS7972&lt;sup&gt;21&lt;/sup&gt;</td>
<td>CBP</td>
<td></td>
<td></td>
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<tr>
<td>LP99 and I-BRD9&lt;sup&gt;27,29&lt;/sup&gt;</td>
<td>BRD7, BRD9</td>
<td></td>
<td>Lead molecules showing druggability of other BD-containing protein families (targets subfamily IV).</td>
</tr>
<tr>
<td>OF-I&lt;sup&gt;29&lt;/sup&gt; and GSK compound 3&lt;sup&gt;27,29&lt;/sup&gt;</td>
<td>BRPF1, BRPF2, BRPF3</td>
<td></td>
<td></td>
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<tr>
<td>BAZ2-ICR and GSK2801&lt;sup&gt;27,29,45&lt;/sup&gt;</td>
<td>BAZ2A, BAZ2B</td>
<td></td>
<td>High level of affinity for BAZ2 (targets subfamily V).</td>
</tr>
<tr>
<td>PFI-3&lt;sup&gt;27,29,45&lt;/sup&gt;</td>
<td>SMARCA2, SMARCA4, PB1</td>
<td></td>
<td>Examples of effective inhibitors of subfamily VIII.</td>
</tr>
<tr>
<td>SGC-CBP30&lt;sup&gt;27,29,45&lt;/sup&gt;</td>
<td>CBP</td>
<td>3,5-Dimethylisoxazole–based small-molecule Ac-K mimic</td>
<td>High level of CBP affinity.</td>
</tr>
<tr>
<td>I-CBP11&lt;sup&gt;27,29,45&lt;/sup&gt;</td>
<td>CBP, p300</td>
<td>Benzoazepine-based small-molecule Ac-K mimic</td>
<td></td>
</tr>
<tr>
<td>dBRD9&lt;sup&gt;97&lt;/sup&gt;</td>
<td>BRD9</td>
<td>Phthalimide conjugation–based degrader (bifunctional molecule)</td>
<td>Ensures total abrogation of protein function.</td>
</tr>
<tr>
<td>dTRIM24&lt;sup&gt;98&lt;/sup&gt;</td>
<td>TRIM24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IACS-9571&lt;sup&gt;96&lt;/sup&gt;</td>
<td>TRIM24, BRPF1</td>
<td>Dual-affinity chemical probe</td>
<td>Uses structure-guided approach to drug development.</td>
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</table>

### BET and Non-BET inhibitors

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<tr>
<th>Inhibitor</th>
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<tbody>
<tr>
<td>BI2536&lt;sup&gt;27,45,76&lt;/sup&gt;</td>
<td>BRD4, PLK1, TAF1, TAF1L, CBP, p300</td>
<td>Dual-kinase inhibitor</td>
<td>PLK1 inhibitor with potent activity toward BRD4, TAF1, and TAF1L, and moderate activity toward CBP and p300.</td>
</tr>
<tr>
<td>TG101209 and TG101348&lt;sup&gt;27,45,76&lt;/sup&gt;</td>
<td>BRD4, JAK2, CBP, p300</td>
<td></td>
<td>JAK2 inhibitors with highly potent activity toward BRD4 and moderate activity toward CBP and p300.</td>
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</table>

*The table shows different drugs targeting BD proteins. The drugs are classified on the basis of whether they have BET or non-BET targets. Information regarding targets, chemical structure, design strategies, in vitro and in vivo applications, and available clinical trial details has been provided for each drug.*

Ac-K, acetylated lysine; AML, acute myeloid leukemia; BD, bromodomain; BET, bromodomain and extra-terminal domain; BRD, bromodomain-containing protein; BTK, Bruton tyrosine kinase; CBP, cAMP response element-binding protein (CREB)-binding protein; CDK, cyclin-dependent kinase; DLBCL, diffuse large B-cell lymphoma; GSK, GlaxoSmithKline; MDS, myelodysplastic syndrome; IL, interleukin; MLL, mixed lineage leukemia gene; MM, multiple myeloma; NFκB, nuclear factor kappa B; PCAF, p300/CBP-associated factor; PI3K, phosphoinositide 3-kinase; PROTAC, proteolysis targeting chimera; TNF-α, tumor necrosis factor α.
Targeting BET in the MLL-Rearranged Context

As a proof of concept, either RNAi–mediated knockdown or JQ1 shows the benefit of targeting BET in the context of MLL-translocated AML (MLL-AF9 model). This strategy also inhibits MYC activity.18 MLL translocation–driven cancer cells are addicted to aberrant SEC recruitment and transcription. Because BET proteins are associated with chromatin complexes, BET inhibitors such as I-BET151 efficiently displace BRD3 and BRD4 from the chromatin complexes. This transcriptionally downregulates key oncogenes—BCL2, MYC, and CDK6—and eliminates leukemic cells and LSCs, providing survival benefits in MLL-rearranged leukemias.33

Other Relevant Targets

In the case of MM, the t(4;14) translocation, which involves rearrangement of the MMSET gene, leads to overexpression of MMSET. This in turn globally increases H3K36me2 and decreases H3K27me3, resulting in a more open chromatin structure, enhancing the recruitment of transcription complexes, to drive the disease.48 Given that BET has been reported to interact with MMSET, targeting BET proteins in MM may be of benefit in this context.33

The activating JAK2 V617F mutation is a characteristic feature of MPN and is also important in MDS and AML, especially in cases that have progressed from...
In a model of post-MPN secondary AML, simultaneous JAK2 and BET inhibition converges partially on the transcriptional profiles. JAK2 inhibitor resistance was overcome by I-BET151 treatment by suppressing the JAK2 target LMO2. In B-cell ALL (B-ALL), in which JAK/STAT signaling plays a significant role, JQ1 exhibits cytotoxicity via the interleukin 7 receptor (IL7R).

The cytokine receptor-like factor 2 (CRLF2) gene is often overexpressed in ALL and is associated with activating mutations in IKZF1, JAK1, JAK2, and IL7RA, leading to activation of the JAK/STAT pathway. Patients with these mutations have higher rates of relapse and poor overall survival. In this context, BRD4 inhibition leads to MYC suppression and compromises BRD4 occupancy on the promoter of IL7RA. Also, the entire pathway is inactivated owing to a decrease in STAT5 and JAK2 phosphorylation.

In addition to JAK2 activation and CRLF2 rearrangements, ETV6-PDGFRB, IGH-BCL1, TCF3-PBX1, and BCR-ABL fusions and IKZF1 mutations are additional drivers of B-ALL. These are also sensitive to JQ1, and show downregulation of the proteins BIRC3, FAIM3, SENP1, ALKBH8 and CARD6, and the cytokines IL-2, IL-7, IL-10 and IL-17. They also inhibit DNA replication. Moreover, in vitro and in vivo models of B-ALL show JQ1-mediated sensitization of leukemic cells to standard therapy (dexamethasone).

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is another aggressive hematologic malignancy arising from plasmacytoid dendritic cells (pDCs), which are innate immune cells. Transcription factor 4 (TCF4) was found to be an essential driver for BPDCN, as it is necessary for lineage commitment of the DC progenitors. TCF4 suppression induces apoptosis in these cells. BET inhibition also induces apoptosis in these cells. Gene expression studies showed downregulation of TCF4 and its targets MYC, BCL2, and TLR9 by BET inhibition. Analysis of SEs showed high co-occupancy of BRD4 and TCF4 on SEs in BPDCN, regulating TCF4 targets and TCF4 itself. TCF4 SEs were also enriched in BPDCN primary samples. This proves that TCF4-dependent BRD4 activity is necessary for the BPDCN oncogenic program. As a result, targeting BET proteins can have benefits by regulating drivers such as TCF4 in BPDCN.

**Targeting Leukemia Stem Cells**

JQ1, with its antileukemic effects in vitro and in vivo, can eliminate LSCs and induce differentiation by critically regulating MYC expression. In leukemic cells, self-renewal capacity is maintained by sustained activity of hematopoietic TFs—PU.1, FLI1, ERG, C/EBPα, C/EBPβ and MYB—which act as oncogenic drivers. These TFs recruit p300/CREB, which maintains Ac-K and facilitates BRD4 recruitment at the promoter. Thus, BET inhibition can block the TF-dependent self-renewal capacity of LSCs. This finding highlights the possibility of targeting p300/CREB along with BRD4 to achieve total effect and to overcome BRD4 rebound activity.

BRD4 inhibition in IDH2-mutant AML in vivo has shown efficacy by inducing differentiation and cell death.

**Epigenetic drugs.** JQ1 with the HDAC inhibitor panobinostat (Farydak, Novartis), OTX015 with the demethylating agent azacitidine, and I-BET151 with the HMT (DOT1L) inhibitor SGC0946 have shown synergistic effects in leukemia cell lines, primary samples, and mouse models, which has led to the evaluation of such combinations in clinical trials.

**Tyrosine kinase inhibitors.** Ibrutinib (Imbruvica, Pharmacyclics/Janssen), a potent tyrosine kinase inhibitor (TKI) targeting B-cell receptor (BCR) signaling, is effective in regulating IKK protein complex to inhibit nuclear factor κB (NFκB) nuclear translocation and activation (NFκB signaling). BET inhibition was also found to target IKK, and combining JQ1 and ibrutinib was highly synergistic in killing the activated B-cell subset of DLBCL (ABC-DLBCL) cells. This combination is also synergistic in mantle cell lymphoma (MCL) models. A CPI-203 and ibrutinib combination showed efficacy in ABC-DLBCL. GS-5829 showed synergy in combination with the Bruton tyrosine kinase (BTK) inhibitor GS-4059, inducing antitumor activity by downregulating MYC, IL-10, and IL-6 in ABC-DLBCL cell lines. Apart from showing single-agent activity in chronic lymphocytic leukemia, GS-5829 was synergistic with a BCR inhibitor, as shown in lymphoma studies.

**FLT3 mutations.** FLT3 mutations occur in about one-third of AML patients. Despite initial response to FLT3 inhibitors, the disease eventually recurs, showing elevated levels of activated STAT5, Akt, and ERK1/2. FLT3 mutations and FLT3-TKI resistance in AML models were successfully targeted by combining JQ1 and FLT3-ITD inhibitor quizartinib.

**Cell cycle–modulating kinase inhibitors.** CDKs regulate the cell cycle in association with cyclins. Whereas
CDKs 1, 2, 3, 4, and 6 predominantly regulate the cell cycle, some of the noncanonical functions (transcription and DNA damage repair) involve CDKs 7 and 9. Targeting both functions of CDKs has proved to have positive effects in conjunction with BET inhibitors. The CDK4/6 inhibitor palbociclib (Ibrance, Pfizer) is synergistic with JQ1 in MCL cell lines and in vivo. At active transcription sites, BRD4 recruits positive transcription elongation factor b (P-TEFb) complex. CDK9 is a core component of this assembly. BET inhibitor BI-894999 shows profound synergy with CDK9 inhibitors alvocidib and LDC000067 in multiple models of hematologic malignancies. Recently, BI-894999 was shown to act synergistically with the polo-like kinase (PLK) inhibitor volasertib in preclinical AML models. The synergy of cell cycle and transcription regulators with BET inhibitors is an interesting avenue for future research because the chromatin structure changes dynamically throughout the cell cycle, enabling a variety of epigenetic modulations. Concomitant targeting of BET proteins in this context may increase the vulnerability of cancer cells.

**BCL2 inhibitor.** The combination of JQ1 and venetoclax (ABT-199, Venclexta, AbbVie/Genentech), is synergistic in MCL models. GS-5829 is also synergistic with ABT-199 in MCL and DLBCL models. Similarly, CPI-203 is synergistic with ABT-199 in aggressive double-hit lymphoma models, which have overexpression of both MYC and BCL2. ABT-199 resistance could be overcome by CPI-203–mediated downregulation of BCL2A1. ABBV-075, a first-in-class BET inhibitor, showed antiproliferative effects on a panel of cancer cell lines, and hematologic cell lines showed higher sensitivity. Further, a subclass of DLBCL showing venetoclax resistance and higher BCL2 expression undergoes apoptosis with a combination of venetoclax and ABBV-075. These promising results are currently being evaluated in clinical trials.

**Proteasome inhibitor.** The proteasome inhibitor bortezomib (Velcade, Millennium/Takeda Oncology) is an effective treatment strategy in MM, but resistance emerges. A study has shown that combining bortezomib with CPI-203 is a promising strategy for clinical trials in MM. A similar context of bortezomib resistance also arises in MCL, and is associated with upregulation of IRF4, BLIMP-1, MYC, etc, and the plasmacytoid differentiation phenotype. In this context, treatment with lenalidomide (Revlimid, Celgene) showed significant activity by inhibiting IRF4, whereas CPI-203 inhibited MYC. Thus, the combination was found to be highly synergistic in inducing apoptosis in bortezomib-resistant myeloma cells. A similar concept may also be applicable in bortezomib-resistant DLBCL.

**Immunomodulatory drugs.** As previously discussed, the immunomodulatory drug lenalidomide has been successfully combined with CPI-203 to overcome bortezomib resistance. Studies have shown that lenalidomide in combination with dexamethasone, an established regimen for MM patients, can potentiate the apoptotic activity of CPI-203 in MM models.

**Microenvironment-modulating agents.** The role of CXCR4 and its receptor CXCL12 in leukemia microenvironment signaling is well established. Although our studies with the BET degrader ARV-825 showed a decrease in CXCR4 surface expression, another study shows that the CXCR4 inhibitor IQS-01.01 prevents CXCR4 nuclear import and is associated with reduced levels of activated AKT and ERK1/2, and MYC. The combination of this agent with CPI-203 showed significant synergy in DLBCL. Thus, the combination provides a logical strategy for successfully targeting cell intrinsic and extrinsic factors.

**The Future of Bromodomain Inhibitors: Newer Drugs**

Treatment with small-molecule BET inhibitors can cause a rebound increase in protein levels, which is a mode of resistance. Another mechanism of resistance is downregulation of the suppressive PRC2 complex by BET inhibitors, resulting in upregulation of the Wnt/β-catenin pathway. This in turn provides alternate drivers for MYC transcription. However, newer approaches, such as the use of dual-kinase inhibitors and BET degraders, are now changing the landscape of drug development in this field. Dual-kinase inhibitors can target multiple kinases and BRD4. Degraders completely abrogate protein activity over a prolonged time. These approaches are likely to dominate the future of research in BD inhibitors.

**Phosphorylation Inhibitors**

Recently, the BET proteins have been associated with atypical kinase activity. Moreover, it has been shown that several kinase inhibitors can bind to the Ac-K binding pockets of BET proteins to inhibit their activity. This gave rise to a new concept of dual-kinase inhibitors, which has led to the identification of kinase inhibitors against BET and other BD proteins, to target their Ac-K binding function. The CDK inhibitor dinaciclib and the PI3K inhibitor LY294002 have shown modest effects, whereas the PLK1 inhibitor BI2536 and the JAK2 inhibitor TG101209 are highly potent BRD4 inhibitors. To further broaden the applicability of the dual-kinase inhibitor concept, the inhibitors identified...
during initial screens were further profiled against 32 BD proteins. BI2536 and the JAK inhibitor TG101348 showed moderate activity with CREBBP and p300. BI2536 also has high activity toward TAF1 and TAF1L76 (Table 1).

New Technologies

The development of BD inhibitory drugs started in the early 2010s with the discovery of small molecular mimics that inhibited Ac-K and BD interactions. Research in this field has led to newer concepts and technologies. In addition to inhibitors, now there are protein degraders. The advantages of targeted protein degradation are on-target effect and complete abrogation of function. Owing to the catalytic nature, the process is highly specific and sensitive. The activity is fast with sustained effects and rapid turnover of the catalyst. The problem of target protein overexpression as a feedback mechanism causing resistance during prolonged treatment with inhibitors can be overcome. Also, larger proteins and proteins with scaffolding functions that are “undruggable” can be targeted. The concept of degraders is rapidly developing in the context of BD inhibitors, with several favorable reports of effectiveness in hematologic malignancies.

The first approach to protein degradation, described by researchers at Yale University and later patented by Arvinas, is the proteolysis targeting chimera (PROTAC) platform. PROTAC is a heterobifunctional molecule wherein a linker connects an E3 ligase–recruiting moiety to a ligand that recruits the target protein. This leads to effective recruitment of the target protein, degradation, and reuse of the PROTAC for the next molecule of the target. Because the PROTAC is available for later use, activity is sustained. The BET PROTACs that have been currently developed are ARV-771 and ARV-825. Each PROTAC has a BET-recruiting ligand linked to an E3 ligase–binding moiety specific for VHL (in case of ARV-771) or cereblon (in case of ARV-825). We and others have demonstrated profound antiproliferative and apoptotic activity of these PROTACs. These exhibit efficacy and sustained effects in both in vitro and in vivo models of leukemia and lymphoma and are more effective than the existing BET inhibitors.72,81,82 We have also shown that ARV-825 can target both cell intrinsic factors, such as MYC and BCL2, in leukemic cells and LSC populations, and extrinsic factors, such as the microenvironment, to modulate leukemia-stroma interactions in AML72 and T-ALL82 (Table 1).

In post-MPN secondary AML, BET PROTACs significantly downregulate MYC, the JAK/STAT pathway, and BCL2 family proteins, so this strategy may be beneficial in patients with secondary AML who have JAK2 mutations or those that become resistant to ruxolitinib (Jakafi, Incyte) treatment. Preclinical studies have shown possible benefits of combining the BET PROTACs with ruxolitinib.83 Other potential combinations, with the HSP90 inhibitor AUY922 or the BCL2/BCL-xL antagonist ABT-263, may also produce benefit in post-MPN secondary AML.84 Like the other BET inhibitors mentioned earlier, BET PROTACs can also inhibit NFκB signaling. So, it has been successfully combined with the BTK inhibitor ibrutinib in MCL cell lines that show enhanced NFκB activity as a resistance mechanism.81

Another approach to protein degradation, developed at Dana Farber Cancer Institute and Harvard Medical School, is phthalimide conjugation of small molecules to generate a bifunctional molecule, dBET1. Here, a BRD4 (ligand)–binding moiety is linked to thalidomide for binding cereblon (the E3 ligase). dBET1 induced rapid BRD4 degradation, downregulated BRD4 targets MYC and PIM1, and induced apoptosis in AML and lymphoma cell lines. dBET1 was also active in primary AML cells and in mouse models of AML.85 Based on this technology, several other molecules, such as dBET6,86 dBRD9,87 and dTRIM24,88 have been developed. These display profound effects in various leukemia models. The development of dBRD9 and dTRIM24 is very encouraging, as these show possibilities of targeting other BD families. dBET6 also proves the possibility to target multiple-domain proteins (Table 1).88

Other protein degradation technologies include Shield,89 SNIPER,90 and hydrophobic tagging,91 reviewed extensively elsewhere.92-95 A dual-affinity chemical probe targeting both TRIM24 and BRPF1 presents another structure-guided approach to drug development.96

Along with newly acquired insights into the alterations of BD proteins, such as BRD1, BRD3, BRD4, BRWD3, CREBBP, MLL, PCAF, SP140, and others that occur in the biology of hematologic malignancies,97 the application of different strategies would make it possible to target additional BD proteins. The functional roles of these molecules could be elaborated in greater detail, eventually leading to further preclinical and clinical studies.79

New Concepts

Recent efforts have led to second-generation small molecules, such as ABBV-744, that show selectivity in targeting the BD2 domain of BET proteins. Preclinical studies show that the drug is comparable to pan-BET inhibitors in efficacy, but has significantly improved oral bioavailability and tolerability. An ongoing phase 1 clinical trial is evaluating reduced drug toxicities in this context.99

This field is expanding with the emergence of new concepts and technologies for the discovery and development of targeted molecular drugs.
References


