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UNDERSTANDING THE PATHWAY

What Is the BET on Solid Tumors?

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Transcription is a tightly regulated process that involves the spatial and temporal assembly of the RNA-polymerase-II (RNApol-II) complex. This process is mediated by transcription factors (TFs) that direct the polymerase to specific DNA sequences and scaffolding cofactors that facilitate the assembly of the transcriptional apparatus. Recruitment often is mediated by effector protein interaction modules, which specifically recognize post-translational modifications, in chromatin-associated proteins, including histones and DNA. This complexity offers opportunities for therapeutic intervention, and one of the most promising in recent years has been through pharmacologic targeting of epigenetic readers of the bromo and extraterminal (BET) family (bromodomain-containing protein 2 [BRD2], BRD3, BRD4, and the testis-specific BRDT).[1-6]

BET proteins are characterized by two BRD protein interaction modules that mediate specific molecular recognition of acetyl lysine (Kac). Additional protein recruitment domains offer a scaffolding template for docking of transcriptional regulators. For example, association with chromatin-modifying enzymes, such as the methyltransferase NSD3, the CHD8 remodeler, or the hydroxylase JMJD6, results in changes of local chromatin accessibility, which promotes additional recruitment of regulators and elongation factors.[7,8] Transcriptional elongation is stimulated by the recruitment of the positive transcription elongation factor b, which facilitates phosphorylation of RNApol-II, a required event for pause-release and transcriptional initiation.[9-14]

BETs control the expression of many genes that are central to the pathogenesis of human cancer, including cell-cycle and proliferation-related genes such as cyclins,[15] E2F1 targets,[16] and the *MYC* oncogene[17-19] (Fig 1). BETs associate with Kac-rich chromatin during mitosis, which leads to proper segregation and cytokinesis of chromosomes through direct regulation of AURKB, the kinase component of the chromosomal passenger complex.[20,21] BETs remain tethered to chromatin during mitosis by acting as bookmarks that accelerate postmitotic gene activation.[22,23] They also drive the expression of genes involved in self-renewal and pluripotency, including *NANOG*, *OCT4*, *PRDM14*, *SOX2*, and *WNT5A*, which contribute to stemness, development, and hematopoiesis.[24-30] Loss of either *BRD2* or *BRD4* is embryonic lethal in animals,[31,32] whereas knockdown of *BRDT* prevents germ differentiation, which leads to sterility in mice.[33]

BETs act as coactivators of diverse transcription factors, including nuclear factor-κB, GATA1, TWIST, and ERG, by directly binding and stabilizing their chromatin recruitment.[30,34-36] Genome-wide studies found BRD4 enriched at superenhancers,[37,38] nucleosome-depleted regions with a high density of enhancer elements bound by multiple TFs[39,40] that drive the expression of key oncogenes (*MYC*, *RUNX1*, *FOSL2*, and *CCND1*) and co-operate with lineage-specific TFs at these sites.[41] Finally, BETs also control the expression of lineage-specific genes in nonhematopoietic cells, for example, neuroendocrine progenitor cells.[42]

Alterations that affect BET levels or function have been found in human cancers.[43] High mRNA levels of BRD4 were shown to modulate the expression of extracellular matrix genes, which drives metastasis in mouse xenograft models and correlates with disease progression.[44] In non–small-cell lung cancer (NSCLC), BRD4 regulates growth through control of eIF4E expression,[45] and BRD4 protein levels have been shown to correlate with disease progression.[46] In NUT midline carcinomas (NMCs), *BRD4* and *BRD3* are found fused to *NUT*, a testis-specific gene.[47] The BET-NUT fusion product recruits factors that promote local chromatin hyperacetylation, which further aggregates BRD4 and leads to transcriptional activation of prosurvival and antidifferentiation genes,[48,49] a phenotype that is rescued by genetic ablation of the fusion product.[50]

Pharmacologic targeting of BET BRDs has shown promise in preclinical cancer models,[4,6] and > 20 clinical trials have been initiated since 2010 that target BETs in hematopoietic and solid tumors. BET bromodomain inhibitors (BETi) compete for the same binding site that recognizes Kac, which effectively displaces BET proteins from their interaction sites on chromatin and other interacting partners (eg, TFs) and results in disruption of their controlled programs. In the article that accompanies this Understanding the Pathway, Lewin et al[51] report the results of a phase Ib dose escalation study of birabresib (OTX015/MK-8628) in patients with castration-resistant prostate cancer (CRPC), NMC, and NSCLC tumors. The major dose-dependent toxicity observed was reversible thrombocytopenia, which agrees with results from other phase I reports after treatment with birabresib in leukemias,[52] multiple myelomas,[53] lymphomas,[53] glioblastomas,[54] CRPCs,[55] NSCLCs,[55] and NMCs[55,56] as well as in other BETi (TEN-010, CPI-0610, BAY1238097, GSK525762) in lymphomas[57] and other advanced solid tumors.[58] Patients with NMC showed short-lived antitumor response, which suggests the existence of secondary resistance mechanisms that support the need for synergistic combination studies.

Opportunities for rational combination therapies are beginning to emerge from the study of BETi resistance. In leukemias, activation of a WNT/β-catenin axis or loss of RPC2 function restores the transcription of BET target genes,[59,60] including *MYC*, a hallmark of BET inhibition.[18,19] In triple-negative breast cancers, BETi resistance is associated with bromodomain-independent

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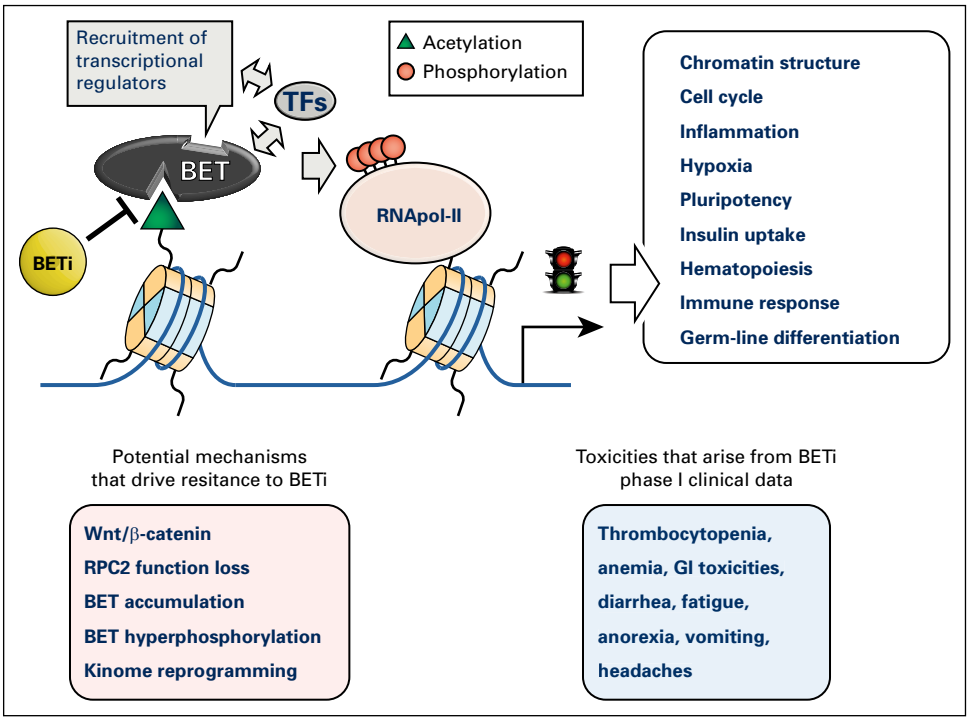

Fig 1. Bromo and extraterminal (BET) proteins contain a modular architecture that facilitates the recruitment of diverse transcriptional regulators (including methyltransferases, such as NSD3; remodelers, such as CHD8; and kinases, such as CDK9) together with a broad range of transcription factors (TFs; such as nuclear factor-κB, GATA1, TWIST, and ERG) at acetylated promoters and enhancers of genes, which activate RNA polymerase II (RNApol-II) that leads to the expression of key lineage-specific genes involved in diverse processes, including cell cycle, hypoxia, pluripotency, hematopoiesis, and germ-line differentiation as well as oncogenes, such as *MYC*, *RUNX1*, *FOSL2*, and *CCND1*. Pharmacologic targeting with BET inhibitors (BETi), which block the specific recognition of lysine acetylation by BET bromodomains, dissociates BET proteins from chromatin, which perturbs expression of their controlled genes and associated transcriptional programs. Resistance to BETi has been evaluated in model systems, whereas the use of BETi in the clinic has uncovered dose-limiting, albeit manageable, toxicities.

transcription and tumor cell proliferation.[61] In prostate cancer, speckle-type POZ protein (SPOP), a component of the BCR E3 ligase complex responsible for protein degradation, interacts with and targets BETs for degradation, which effectively reduces BET protein levels. SPOP mutations prevent BET degradation that leads to accumulation and resistance to BETi.[62] In ovarian cancers, resistance to BET inhibition was shown to develop by elevation of PI3K/ERK activity, which resulted in subsequent stabilization of MYC and FOSL1 expression.[63] The downregulation of survival pathways after BET inhibition may provide a strategy to overcome BETi resistance. In CRPC, BETi-resistant cells were sensitive to enzalutamide, CDK9, and poly (ADP-ribose) polymerase inhibitors.[64] Similarly, in NSCLCs, combination with proapoptotic chemotherapeutic agents improved antitumor efficacy in mice.[65]

In summary, the broad study of BETi has identified opportunities for additional exploration of these agents, and early clinical experience suggests that these drugs can be given safely. Although clinically used BETi are highly selective over other BRD-containing proteins, toxicities must be considered carefully given the prominent role of these proteins in many cellular functions and potential off-target effects. Recent proteomic data, for example, have suggested that FYTTD1, an adaptor of the TREX complex implicated in mRNA export to the cytoplasm, and NUDT1, a key enzyme responsible for nucleotide sanitization, also are targeted by specific BETi.[66] The use of several distinct chemotypes that target the same BET sites may be able to address and alleviate some of these concerns as data continue to emerge from ongoing trials.

AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the author are available with this article at jco.org.

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No relationship to disclose

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