

## Original Article

# BETting on next-generation bromodomain inhibitors

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**Abstract:** Within the last decade, bromodomain and extraterminal (BET) domain inhibitors were introduced as the first in a wave of new agents known as bromodomain inhibitors. These original examples exhibited anti-inflammatory and anticancer properties, and some have progressed to human clinical trials. BET proteins and their conserved N-terminal bromodomains, BD1 and BD2, have been implicated in the regulation of transcription. The early-generation BET inhibitors showed equal affinity for BD1 and BD2, and therefore the differential roles of BD1 and BD2 remain poorly understood. A recent study published in *Science* by Gilan *et al.* outlines the transcriptional and phenotypic effects of inhibiting BD1 and BD2 individually, specifically in the context of cancer and immunoinflammatory pathologies. These findings suggest that BD1 and BD2 have separate and distinct roles in transcriptional regulation, and that BD1- and BD2-selective agents may exhibit higher clinical efficacies in solid tumors, such as prostate cancer, with fewer off-target side effects seen with early generation compounds.

**Keywords:** Bromodomain inhibitor, BET inhibitor, immunoinflammation, BD1, BD2, epigenetics

The bromodomain and extraterminal (BET) domain family of proteins is comprised of the epigenetic reader proteins BRDT, BRD2, BRD3, and BRD4, each of which contains two N-terminal bromodomains (BD1 and BD2) [2]. Early studies utilizing first-generation BET inhibitors showed this family of proteins is involved in the coordination and regulation of transcriptional programs essential to normal development, injury and infection response, and oncogenic gene expression [3, 4]. While early pre-clinical studies showed the efficacy of BET inhibition in both nonmalignant [5-7] and malignant [8-10] pathologies, limited duration of responses and off-target side effects have prevented their approval and subsequent therapeutic use [11, 12].

The first-generation BET inhibitors demonstrate equal affinity for the two N-terminal bromodomains BD1 and BD2. As such, little is currently understood about the individual and differential roles of BD1 and BD2 in transcriptional regulation. A recent study published in *Science* by Gilan *et al.* outlines the development and characterization of novel next generation BET inhibitors, iBET-BD1 and iBET-BD2, that exhibit

high specificity for BD1 and BD2, respectively [1]. Using a pan-BET inhibitor (I-BET151) as a comparator, the researchers identified differential requirements of BD1 and BD2 in induction and maintenance of gene expression in *in vitro* and *in vivo* models of cancer and immunoinflammatory pathologies.

In this study, surface plasmon resonance (SPR) binding showed domain selectivity for iBET-BD1 and iBET-BD2, while time-resolved fluorescence resonance energy transfer (TR-FRET) assays demonstrated the specificity of these inhibitors to the bromodomains of the BET family of proteins. These inhibitors maintained their selectivity and specificity in various cellular orthogonal assays. In comparison, most BET inhibitors lack the specificity and/or potency to discriminate between BD1 and BD2 inhibition.

The authors then sought to determine the effects of each inhibitor and the role of their respective target in cancer. In cancer cell proliferation studies, iBET-BD1 demonstrated high potency in inhibiting cell proliferation and inducing cell cycle arrest and apoptosis, mimicking the effects of the pan-BET inhibitor I-BET151.

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iBET-BD1-treated cells also displayed a global transcriptome profile similar to cells treated with I-BET151, while transcription levels were largely unaffected by treatment with iBET-BD2. iBET-BD1 was able to displace chromatin-bound BRD2, BRD3, and BRD4, while iBET-BD2 was mostly ineffective. In an *in vivo* acute myeloid leukemia (AML) mouse model, treatment with iBET-BD1 led to increased survival when compared to treatment with iBET-BD2. Taken together, these results suggest that BD1 plays a larger role than BD2 in affecting transcriptional changes that lead to cancer cell survival and proliferation, which provide evidence for BD1 as a therapeutic target for certain malignancies.

In contrast to the evidence supporting BD1 having a greater impact than BD2 on cancer cells, a study published in *Nature* by Faivre et al. illustrates the effects of BD2 inhibition on preclinical models of prostate cancer [13]. The authors demonstrate the antiproliferative activity of ABBV-744, a preferential BD2 inhibitor, predominantly in prostate cancer cell lines that express the full length androgen receptor (AR). Xenograft studies show the consistency of the anticancer properties of ABBV-744 *in vivo*, reducing tumor volume while mitigating toxicities seen with pan-BET inhibitors. Further studies are needed to determine the cause of the inconsistency of anticancer properties between ABBV-744 and iBET-BD2.

In the nonmalignant setting, Gilan et al. illustrate the effects of these inhibitors on the immunoinflammatory response. Using a model that measures transcriptional responses to stimulation with interferon- $\gamma$  (IFN- $\gamma$ ), the researchers show that I-BET151, iBET-BD1, and iBET-BD2 all inhibited the cell-surface expression of major histocompatibility complex I (MHC-I) and the underlying IFN- $\gamma$ -responsive transcripts, which include those in the MHC-I-presenting pathway. Only iBET-BD2 left the baseline transcriptional level unaltered. Chromatin immunoprecipitation sequencing (ChIP-seq) analyses additionally showed reduced IFN- $\gamma$ -induced recruitment of BET proteins to target genes in the presence of any of the three inhibitors individually. Using functional *in vitro* assays, the authors then demonstrated that treatment with iBET-BD2 inhibits the production of several key proinflammatory mediators while leaving cell

viability and proliferation largely unaltered. *In vivo*, iBET-BD2 reduced production of T-cell dependent primary antibodies (IgM), displaying similar immunomodulatory properties to iBET-BD1 and I-BET151. These results combined suggest that BD2 inhibition prohibits the induction of gene expression, while leaving baseline transcription programs unaltered, and provide evidence for BD2 as a potential therapeutic target in various immunoinflammatory pathologies.

To investigate the efficacy of BD2 inhibition in preclinical models of inflammatory diseases, the authors developed another BD2-selective inhibitor, GSK620, with improved oral bioavailability compared to iBET-BD2. In an inflammatory arthritis model in rats, a mouse model of imiquimod-induced psoriasis, and a mouse model of nonalcoholic fatty liver disease, GSK620 displayed improved efficacies in reducing the respective disease-relevant proinflammatory genes and their associated symptomatic manifestations compared to experimental comparators and/or clinical standards of care. This demonstrates the preclinical value of BD2 inhibition and the subsequent prevention of inducible gene expression in immunoinflammatory diseases.

BET inhibitors represent a novel class of potential therapeutics in the treatment of various cancers and immunoinflammatory diseases. Due to their robust preclinical activities, a multitude of newly-developed BET inhibitors are currently under investigation in open clinical trials for solid and hematological malignancies. Most of these BET inhibitors under clinical investigation are pan-BET inhibitors, and the BD1- or BD2-selective agents remain predominantly in the preclinical stages of development [14]. ABBV-744 represents the lone BET inhibitor selective for BD2 under clinical investigation, currently in Phase I for Acute Myeloid Leukemia (AML) (ClinicalTrials.gov identifier NCT03360006). As newer-generation BET inhibitors progress into clinical investigation, efficacy and tolerability profiles of BD1- and BD2-selective agents will begin to take shape. Further mechanistic studies are required, however, to fully elucidate the roles of BD1- and BD2-dependent transcriptional programs in both malignant and nonmalignant pathologies to reveal additional therapeutic opportunities.

Previous studies support the clinical investigation of BET inhibitors in patients with castration-resistant prostate cancer refractory to second-generation antiandrogens (abiraterone or enzalutamide) as they were shown to inhibit AR splicing and persistent AR signaling [15]. The development of selective next generation BET inhibitors is highly anticipated and should be a promising therapeutic approach to overcome AR aberrant signaling and resistance to existing approved therapies.

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### Disclosure of conflict of interest

None.

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