Therapeutic Targeting of BET Proteins in Sarcoma

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ABSTRACT

Bromodomain and extraterminal (BET) domain protein family are epigenetic readers that regulate gene transcription, cell-cycle progression, and DNA damage response (DDR), making them attractive therapeutic targets for sarcomas, which are epigenetically dysregulated and genomically unstable. Sarcomas are molecularly heterogeneous with a high propensity for metastasis, resulting in poor clinical outcomes. BET inhibitors (BETi) hold promise for the treatment of sarcomas, for they block interaction of BETs with acetylated lysines, modify gene expression, and create an imbalance in transcription and replication kinetics. BETis also disrupt transcriptional programs driven by oncogenic fusion proteins found in some sarcomas. Preclinical studies demonstrate efficacy of BETis in inducing apoptosis, disrupting DDR, and reducing tumor growth, either as monotherapy or in combination with chemotherapy or other targeted agents, such as PI3K, histone deacetylases, and CHK1 inhibitors. Favorable results have been observed in clinical trials, but more studies are required to fully assess safety and efficacy as well as identify biomarkers of response and resistance. Ongoing research is focused on optimizing BETi safety and selectivity and exploring combination therapies, such as BETis with DDR inhibitors. This review summarizes the preclinical studies on BET inhibition and discusses clinical trial activity, providing insights into the potential of BETis in sarcoma therapy.

Introduction

Sarcomas originate from mesenchymal tissues and are characterized by high heterogeneity and poor clinical outcomes (1). Advances in cancer biology have identified epigenetic dysregulation as a critical factor in the progression of various cancers, including sarcomas (1, 2). Among the epigenetic regulators, the bromodomain and extraterminal (BET) domain family of proteins have emerged as a promising therapeutic target (3). BETs BRD2, BRD3, BRD4, and BRDT are involved in the regulation of gene expression by recognizing acetylated lysine residues on histones and interacting with transcription factors, thereby modulating chromatin structure and transcriptional activity (4). BRD4 is the best characterized and most studied member of this family. It has also been shown to be involved in various other cellular functions besides transcription regulation, such as DNA damage response (DDR) and immune responses (5). Dysregulation of BETs has been implicated in oncogenesis, making BET inhibition a potential strategy for targeting cancer-related transcriptional programs (4). Although the use of BET inhibitors (BETi) has been reported in clinical trials for many malignancies, especially in hematologic cancers and selected solid tumors (6), here, we will explore the role of BETis in sarcomas. This review will focus on preclinical and clinical studies that highlight their therapeutic potential across different sarcoma subtypes.

Structure and Function of BET Proteins

BETs are epigenetic readers recognizing acetylated lysine residues on histone and nonhistone chromatin factors (5, 7). BETs include BRD2, BRD3, and BRD4, which are ubiquitously expressed, and BRDT, which is specific to male germ cells (8). All BETs are comprised of two conserved amino-terminal bromodomains (BD1 and BD2), which are responsible for recognizing the acetylated lysine, and an extraterminal domain that recruits transcriptional regulatory proteins (**Fig. 1A**; ref. 8). BD1 recognizes and binds to acetylated histone lysine and BD2 to acetylated nonhistone proteins (9). Although the sequence conservation of BD1 or BD2 is very high among the BET paralogs, they differ functionally because of differential acetyl-lysine binding preferences (9, 10).

BETs play a key role in regulating gene expression during cellular proliferation and differentiation. They recognize and bind to acetylated histone residues, positioning them at active enhancers and promoters. By doing so, they help recruit transcriptional coactivators and the transcription machinery (**Fig. 1B**; ref. 4). In addition to binding acetylated histones, BETs can also be tethered to chromatin through direct interaction with key transcription factors and proteins, most notably c-Myc (7). This BET–c-Myc partnership helps stabilize enhancer–promoter loops and drives transcription of c-Myc target genes (7). Overall, BET-mediated recruitment of coactivators and transcription machinery, along with direct interaction with proteins, ensures robust enhancer activity and regulates transcription of critical genes involved in cell growth and proliferation.

Other cellular processes regulated by BETs include DNA repair, splicing, DDR, immune response, and replication stress. Hence, dysregulation of BET-mediated gene regulation can play a critical role in diseases, including cancer (4). BD1 is primarily responsible for steady-state gene expression, maintaining the baseline transcriptional programs essential for cellular identity and function (10,

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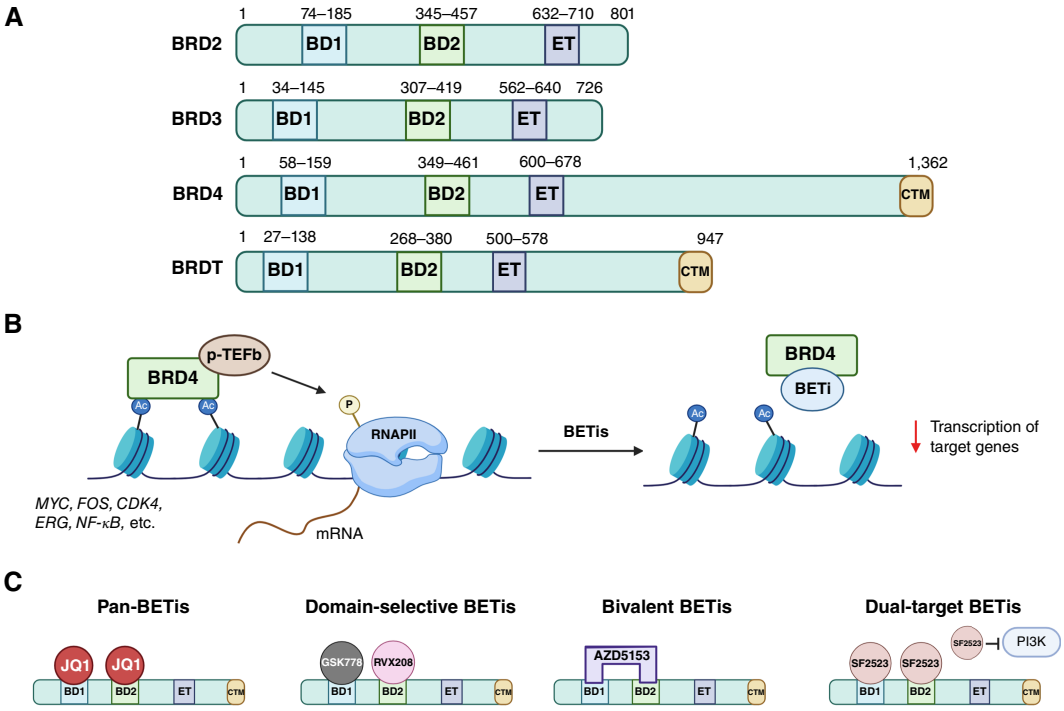

Figure 1.

Structure and function of BET proteins, BETi classes, and BETi interference with transcription. **A,** Arrangement of domains in BETs. BETs contain two highly homologous N-terminal bromodomains (BD1 and BD2) and are crucial for their direct recruitment to nucleosomes by binding to acetylated lysine. They also contain an extraterminal (ET) domain. The C-terminal motif (CTM) is specific to BRD4 and BRDT. **B,** BRD4 binds to acetylated histones and facilitates the recruitment of the positive transcription elongation factor b (P-TEFb) to chromatin, promoting transcriptional initiation and elongation. BETis bind to BETs and block interactions with acetylated histones, thus repressing BET activity. **C,** Different types of BETis.

11). BD1 binds more effectively to acetylated histones, particularly histone H4 (11). Inhibition of BD1 interactions closely mimics the effects of pan-BETis in various cancer models, demonstrating its critical role in oncogenic gene expression (10, 11). Conversely, broader binding specificity of BD2 allows it to engage with a wider range of acetylated nonhistone proteins, facilitating quick transcriptional responses to environmental changes (11). For example, BD2 plays a specialized role in the rapid induction of gene expression in response to external stimuli, such as inflammatory signals (11).

BRD4 and BRDT have an additional C-terminal domain that recruits positive transcription elongation factor b and activates transcription by cyclin-dependent kinase 9 (CDK9) by phosphorylation of serine 2 on RNA polymerase II (RNAPII; **Fig. 1B**; refs. 8, 12–15). BRD4 has also been shown to possess intrinsic kinase activity, enabling it to phosphorylate the RNAPII directly on the C-terminal domain and leading to the activation of RNAPII (16). Moreover, the extraterminal domain of BRD4 independently recruits transcription-modifying factors, such as glioma tumor suppressor candidate region gene 1; NSD3, an SET domain–containing histone methyltransferase; JMJD6, a histone arginine demethylase; and CHD4, a catalytic component of the NuRD nucleosome remodeling complex (17). BRD4 marks transcriptional start sites of multiple M–G_1 genes during mitosis and facilitates cell-cycle transition to the S-phase (18). Dey and colleagues (19) reported that BRD4 is required for the G_2–M cell-cycle transition based on microinjection of BRD4-specific antibodies and subsequent cell-cycle arrest induction. BRD2 and BRD3 modulate cellular processes in a positive transcription elongation factor b–independent manner through recruiting E2F proteins, histone deacetylases (HDAC), histone H4-specific acetyltransferase, and other chromatin remodeling proteins (17).

Types of BETis

As previously mentioned, due to BETs' role in regulating multiple oncogenic cellular processes, BETis have emerged as promising therapeutic agents for cancer treatment (3). These inhibitors target BETs' bromodomains by disrupting their interactions with chromatin and transcription factors, which leads to the disruption of BET-mediated regulatory mechanisms involved in transcription, replication stress, and DDR. This, ultimately, can lead to downregulating oncogenic gene expression and inhibiting cancer cell growth. Different classes of BETis exist based on their selectivity toward different bromodomains (**Fig. 1C**; ref. 20).

(i) Pan-BETis

These inhibitors were designed to bind to either the BD1 or BD2 domain of a given BET protein (21). JQ1 was one of the first pan-BETi developed based on thienotriazolodiazepine compounds (22) and targets either BD1 or BD2 of all BETs [BRD2, BRD3, BRD4, and BRDT (20)], with 40 to 50 times higher affinity for BD1 versus BD2 (21). Although JQ1 has been a valuable preclinical

tool for investigating BET inhibition, it has not progressed into clinical trials because of its short half-life (23). JQ2 (TEN-010), an optimized derivative of JQ1, was then designed and evaluated in two phase I clinical trials for advanced hematologic malignancies and certain solid tumors (NCT01987362 and NCT02308761), with early data suggesting that the treatment is well tolerated and beneficial for specific patient subsets (24). In addition, other pan-BETis, such as ABBV-075, ODM-207, I-BET762, GS-626510, OTX-015, PLX51107, CPI-0610, and BMS-986158, that are structurally unrelated to benzodiazepine inhibitors have been developed with some advancing to clinical trials in sarcoma (**Table 1**; refs. 25–28).

(ii) Domain-selective BETis

Early pan-BETis induced dose-limiting toxicities in clinical trials; therefore, drug development has focused on inhibitors that selectively block BD1 or BD2 of BETs (20, 29). BD1-selective inhibitors GSK789 and GSK778 have shown broad anticancer activities in different cancer cell lines (30). In contrast, BD2-selective inhibitors such as RVX-208 and ABBV-744 are most effective in specific cellular contexts that rely on BD2-driven transcriptional programs, especially beneficial in tumors in which immune or inflammatory signals play a prominent role in tumor progression (30). ABBV-744 is being tested in relapsed or refractory acute myeloid leukemia and myelodysplastic syndromes (NCT03360006), as well as certain advanced solid tumors including castration-resistant prostate cancer (NCT04454658).

(iii) Bivalent BETis

This class of BETis was designed to simultaneously block interactions of both BD1 and BD2 on the BETs (30). Bivalent BETis displace the whole BET protein, specifically BRD4, with greater potency than monovalent BETis at relatively lower concentrations (21). Pharmacokinetic optimization of bivalent BETis MT1 and MS654 led to the development of AZD5153 (31, 32), which has shown excellent *in vivo* potency and is currently under investigation in clinical trials (**Table 1**; refs. 6, 30, 33, 34).

(iv) Dual-target BETis

To tackle aggressive cancers such as sarcomas, molecularly guided combination therapeutic approaches will be necessary. Therefore, there is growing interest in developing dual inhibitors that block both BETs and other cancer targets (20) such as HDACs (35), EZH2 (36), and PARP (37) and kinases such as JAK (38), CK2 (39), ALK (40), and PI3K (41). For example, Wang and colleagues (37) synthesized a dual-target inhibitor targeting both PARP and BRD4 called III-16 and showed promising antitumor efficacy in pancreatic cancer cells and xenografts by arresting cell-cycle progression, inhibiting DNA damage repair, and promoting autophagy-associated cell death. However, these inhibitors have not yet advanced into clinical settings.

BET Inhibition in Sarcoma

Rhabdomyosarcoma

Rhabdomyosarcoma (RMS) is a prevalent soft-tissue sarcoma (STS) predominantly affecting children and young adults and is characterized by the malignant proliferation of skeletal muscle precursor cells (42). Two primary RMS types are embryonal (eRMS) and alveolar (aRMS), each displaying distinct molecular characteristics and clinical behaviors (43). For instance, eRMS typically affects younger children and often arises in the head and neck region, whereas aRMS tends to occur in older children and adolescents and commonly involves the trunk and extremities (43). Moreover, aRMS is associated with significantly worse outcomes compared with eRMS, primarily owing to its aggressive nature and higher rate of metastasis (42).

Despite advancements in treatment, metastatic RMS remains a significant cause of morbidity and mortality in pediatric oncology (44). BETis have emerged as promising therapeutic candidates for RMS. c-Myc oncoprotein, frequently overexpressed in RMS tumors and regulated by BETs, is associated with poor prognosis and is effectively targeted by BETis such as JQ1 (45, 46). Higher expression of c-Myc correlates with increased sensitivity to JQ1 in RMS (46). Marchesi and colleagues (46) demonstrated that JQ1 leads to cell-cycle arrest on RMS cells with high c-Myc expression, whereas apoptosis is associated with c-Myc downregulation.

Furthermore, aRMS subtypes with the PAX3–FOXO1 transcription factor fusion are found in ~60% of patients (47) and exhibit a unique dependency on BRD4 function (45, 48, 49). Gryder and colleagues (45) found that the PAX3–FOXO1 fusion protein recruits BRD4 to super-enhancers (SE), making BRD4 an essential factor for the transcriptional activity of PAX3–FOXO1. Thus, this dependency creates a therapeutic vulnerability as targeting BRD4 with BETis can disrupt the oncogenic function of PAX3–FOXO1, leading to reduced RMS tumor growth (45). Chin and colleagues (48) extended these findings by showing that BRD4 inhibition or degradation results in a global reduction in transcription and selective downregulation of core regulatory transcription factors associated with PAX3–FOXO1 fusion. This effect is achieved without disrupting the three-dimensional structure of RNAPII clusters, indicating that BETis can effectively suppress the oncogenic activity of PAX3–FOXO1 without altering the overall chromatin architecture (48).

Moreover, growing evidence supports the synergistic effects of BETis in combination with other agents in RMS treatment (50–52). Combinations of BETis with HDAC inhibitors (HDACi) and PI3Kα-specific inhibitors have shown enhanced efficacy in triggering mitochondrial apoptosis and suppressing tumor growth in RMS cells (50–52). These combinations modulate the balance of proapoptotic and antiapoptotic proteins, leading to BAK- and BAX-dependent apoptosis, and hold promise for overcoming drug resistance and improving therapeutic outcomes in aggressive forms of RMS (50–53).

Both HDAC and PI3K pathways are implicated in RMS pathogenesis, promoting tumor cell growth and survival (54, 55). Completed and ongoing clinical studies of PI3K inhibitors (NCT03458728 and NCT03155620) and HDAC inhibitors (NCT04299113, NCT04308330, and NCT00112463) in RMS demonstrate their promising therapeutic potential in RMS (55–57). It has been demonstrated that co-targeting of BETs and HDACs or PI3Kα using BETi JQ1 in combination with HDACi quisinostat or vorinostat, or PI3Kα inhibitor BYL719, results in a synergistic induction of apoptosis in RMS cells (50, 51). These combinations effectively disrupt the function of key survival pathways, thereby enhancing cell death (50, 51). The simultaneous inhibition of BET and HDAC/PI3Kα triggers a more robust apoptotic response than either agent alone by modulating the transcriptional landscape, leading to the downregulation of antiapoptotic proteins like BCL-2 and BCL-xL and the upregulation of proapoptotic proteins such as BIM and

Table 1. Clinical trials of BETis in sarcoma.

Drug	Study	Phase	Tumor type	Status	Result
ODM-207	NCT03035591	I	Solid tumors and sarcoma	Completed	Reported (25)
PLX51107	NCT02683395	II	Hematologic cancers, solid tumors, and sarcoma	Terminated	Reported (26)
BMS-986158 BMS-986378	NCT03936465	I	Pediatric cancer including sarcoma	Ongoing	Not reported
AZD5153	NCT05253131	I/II	MPNST, NF1, and sarcoma	Ongoing/not yet recruiting	Not reported
CPI-0610	NCT02986919	II	MPNST	Withdrawn	Not reported

Pan-BETis: ODM-207, PLX51107, BMS-986158, BMS-986378, and CPI-0610; bivalent BETi: AZD5153.

NOXA (50, 51). This change in the BCL-2 family protein expression balance promotes mitochondrial outer membrane permeabilization and caspase-dependent cell death (50, 51).

In addition to targeting c-Myc, BETis have been explored in the context of epigenetic regulation and signaling pathways implicated in RMS pathogenesis (52, 58). Dual inhibition of BETs and mTORC1/2 signaling, which regulate critical oncogenic pathways in RMS, has shown significant antitumor activity in preclinical models (58). Furthermore, Srivastava and colleagues (58) reported that combination treatment with BETis and mTORC1/2 inhibitors effectively inhibited aggressive RMS growth by abrogating PI3K/mTOR signaling and suppressing proliferation in both *in vitro* and in a human xenograft murine model. Although more *in vivo* testing for safety and efficacy is required, these findings underscored the potential of BETis as part of novel therapeutic interventions for RMS, either as monotherapy or in combination with other targeted agents.

More recently, the distinct roles of BRD4 isoforms, BRD4-L (long) and BRD4-S (short), were investigated with respect to tumor progression and metastasis in eRMS (59). Studies revealed that BRD4-L promotes tumor growth and inhibits myogenic differentiation by regulating the expression of myostatin, a negative regulator of muscle differentiation (59). Depletion of BRD4-L in eRMS cells led to reduced tumor growth and increased differentiation, highlighting its oncogenic role (59). Conversely, BRD4-S, which does not possess the C-terminal motif, seemed to act as a gatekeeper of metastasis; its depletion resulted in enhanced metastasis, likely owing to increased enrichment of BRD4-L and RNAPII at integrin gene promoters, which are associated with cell migration and invasion (59). This study demonstrated that BRD4-S loss upregulates several integrin genes and downregulates metastasis suppressors, facilitating a metastatic phenotype (59). These findings underscored the isoform-specific functions of BRD4 in eRMS, with BRD4-L driving tumor growth and BRD4-S preventing metastasis, thereby providing information into potential therapeutic strategies targeting these distinct roles to treat eRMS effectively (59).

Insights into the molecular mechanisms underlying BETi action in RMS are paving the way for developing more targeted and effective therapeutic strategies (48, 60). By elucidating the role of BETs in regulating transcriptional programs and chromatin remodeling in RMS, researchers are uncovering novel vulnerabilities that can be exploited for therapeutic benefit (48, 60). Continued research efforts focused on BETis hold promise for advancing RMS treatment and overcoming resistance.

Ewing sarcoma family of tumors

The Ewing sarcoma family of tumors (ESFT) is characterized by highly malignant bone or soft-tissue tumors that primarily afflict children and young adults (61). The genetic landscape of the ESFT is defined by balanced chromosomal translocations, most commonly the t(11;22; q24;q12) translocation, which results in the fusion of the *EWSR1* gene with genes encoding ETS family transcription factors, notably FLI1 (61–63). The EWS–FLI1 fusion protein functions as a transcription factor, is found in ~85% of patients with Ewing sarcoma, and drives the oncogenic transcriptional program (62, 63).

Despite therapeutic advances in the ESFT treatments (64), directly targeting EWS–ETS fusion proteins has been challenging, and proteins in this class are often regarded as undruggable owing to the absence of small molecule–binding pockets (63, 65). Consequently, researchers have turned their attention to alternative strategies, such as targeting epigenetic regulators implicated in modulating the aberrant transcriptional program orchestrated by EWS–ETS fusion proteins (62, 63). One such promising avenue involves the inhibition of BETs. Recent studies using JQ1 have demonstrated encouraging results in preclinical models of Ewing sarcoma (61–63). JQ1 disrupts the interaction between BET bromodomains and acetylated histones, thereby downregulating the expression of genes associated with the EWS–ETS transcriptional program (62, 63). BET inhibition represses EWS–FLI1 expression and induces anticancer effects in *in vitro* and *in vivo* models of the ESFT (61–63). In addition, Loganathan and colleagues (63) demonstrated that JQ1 impaired the insulin-like growth factor 1 (IGF1) autocrine mechanism leading to significant inhibition of the IGF1R/AKT pathway critical to ESFT cell proliferation and survival. Moreover, JQ1 significantly downregulated the expression of *GLI1*, a direct EWS–FLI1 downstream target gene and a key regulator of the EWS–FLI1–dependent transcriptional network that drives tumorigenesis in the ESFT (63). This downregulation significantly reduced cell viability and impaired the clonogenic survival of the ESFT cells (63). Additionally, JQ1 induced cell-cycle arrest and increased apoptosis, demonstrated by activated caspase 3/7 and PARP cleavage, further supporting the potential of BETis as a viable therapeutic strategy for the ESFT (63).

Furthermore, combination strategies involving BETis and inhibitors of key signaling pathways implicated in the ESFT pathogenesis, such as the PI3K/AKT/mTOR pathway or the IGF1R pathway, have shown synergistic effects in preclinical models, suggesting potential combinatorial therapeutic strategies for Ewing sarcoma (66, 67). For instance, Loganathan and colleagues (66) demonstrated *in vitro* and *in vivo* that targeting the IGF1R/PI3K/AKT pathway sensitizes the ESFT cells to BETis. IGF1R inhibitors significantly enhanced BETi efficacy by inducing potent apoptosis (66). This combination disrupted the IGF1 autocrine loop and attenuated the downstream PI3K/AKT/mTOR signaling, which is crucial for the survival and proliferation of the ESFT cells (66). Additionally, the combination of BETis and mTOR inhibitors

exhibited a synergistic effect, especially in cells with constitutively activated AKT signaling, further underscoring the therapeutic potential of targeting multiple pathways simultaneously (66).

Recent studies have also investigated the molecular mechanisms underlying resistance to BETis in ESFTs and identified potential strategies to overcome this resistance (66, 67). For instance, Richter and colleagues (67) explored the interaction between BETs and CDK9. They found that combining BETis with CDK9 inhibitors resensitized the ESFT cells to BET inhibition (67). This combination was more effective than individual drugs in preclinical models (67). Moreover, Loganathan and colleagues (66) showed that the ESFT cells with constitutively activated AKT signaling exhibit intrinsic resistance to BETis, emphasizing the need to understand the interplay between BET inhibition and key signaling pathways in tumor cells. These insights suggest that combining BETis with agents targeting the PI3K/AKT/mTOR pathway or other epigenetic regulators could provide more effective treatment strategies for ESFTs (66, 67).

Overall, the therapeutic potential of BET inhibition in ESFTs holds promise, offering a novel approach to targeting the underlying oncogenic transcriptional program driven by EWS–ETS fusion proteins (61–63, 66, 67). Further research aimed at elucidating the intricate molecular mechanisms involved in BET inhibition and exploring rational combination strategies holds the potential to advance the field and ultimately improve outcomes for patients with the ESFT.

Osteosarcoma

Osteosarcoma, the most common primary malignant bone tumor, predominantly affects children and adolescents, with peak incidence during periods of rapid bone growth (68, 69). Arising from primitive bone-forming mesenchymal cells, osteosarcoma exhibits considerable histologic heterogeneity, the most common of which include osteoblastic, chondroblastic, and fibroblastic subtypes (68, 69). Genomic studies have revealed complex karyotypes with numerous genomic instabilities. Frequent alterations in tumor suppressor genes such as *TP53*, *RB1*, and *CDKN2A/B* along with recurrent amplifications and deletions involving various chromosomal regions are observed in osteosarcoma (70–72). Despite advancements in multimodal therapy comprising surgery, chemotherapy, and occasionally radiotherapy, prognosis remains suboptimal, particularly in cases of metastatic or recurrent disease (72, 73).

Epigenetic dysregulation is one hallmark of osteosarcoma pathogenesis, with aberrant histone modifications and chromatin remodeling contributing to oncogenic transcriptional programs (74–76). Notably, BETs have garnered significant attention as potential therapeutic targets in osteosarcoma (77–79). By modulating key oncogenic pathways and disrupting the expression of critical genes implicated in tumor progression, BETis hold promise as a novel therapeutic approach for combating osteosarcoma.

BETis, including JQ1, OTX-015, and I-BET762, have demonstrated significant antitumor effects against osteosarcoma cell lines by targeting the BRD4 protein (77, 78, 80, 81). The efficacy of BETis has been demonstrated in cell culture models and animal studies (77–79, 81). *In vivo* experiments have shown that BET inhibition as monotherapy can effectively reduce the size of osteosarcoma xenografts and prolong the probability of survival in mouse models of osteosarcoma (78, 79, 81).

Pandya and colleagues (77) developed an integrated multi-omics pipeline to identify cancer-associated pathways in a panel of pediatric as well as adolescent and young adult (AYA) patient-derived xenografts (PDX) of osteosarcoma. CDK4/6 and BETs were identified as biomarkers of therapeutic response in osteosarcoma PDXs with pertinent molecular signatures (77). This study demonstrated that the BETi, OTX-015, effectively suppressed osteosarcoma PDX growth, confirming the role of BETs in osteosarcoma pathogenesis (77). In addition, combination targeted therapies demonstrated that BET inhibition potentiated the antitumor activity of rapamycin, an mTOR inhibitor, doxorubicin, CHK1, and CDK inhibitors and synergistically inhibited osteosarcoma cell growth and survival both *in vitro* and *in vivo* (78, 80, 81). Pandya and colleagues (81) demonstrated that dual inhibition of BET and CHK1 using OTX-015 and SRA737, respectively, resulted in significant tumor growth suppression and improved survival in osteosarcoma PDX models. This study identified the MYC-RAD21^{+} genetic signature as a critical driver of poor prognosis and replication stress and showed that targeting both BETs and CHK1 effectively blocked tumor progression by increasing DNA damage and activating apoptosis pathways (81). This combination therapy was well tolerated in PDX models and offered a promising new approach for treating patients with osteosarcoma with high-risk signatures, who typically have limited therapeutic options and poor outcomes (81).

Mechanistically, Lamoureux and colleagues (79) showed that BET inhibition using JQ1 suppresses the expression of c-Myc. However, we and others have shown that anticancer properties of BET inhibition have been associated with induction of apoptosis and G_1 cell-cycle arrest, independent of c-Myc downregulation (78, 80, 81). In addition, JQ1 suppresses RUNX2 and FOSL1, while upregulating the tumor suppressor p21, contributing to its antiproliferative effects in osteosarcoma cells (78–80). The key role of oncogenic replication stress in BETi efficacy was emphasized in Zhang and colleagues (5) study, in which they demonstrated that BET inhibition (JQ1 or AZD5153) disrupts DNA replication checkpoint signaling and leads to decreased CHK1 phosphorylation, impaired DNA replication checkpoint response, and increased replication stress–induced DNA damage (5). The study also showed that combining BET inhibition with ATR inhibitors significantly enhanced DNA damage and cell death across multiple cancer types including osteosarcoma, providing a mechanistic rationale for using BETis in tumors with high replication stress (5).

Furthermore, BETs have been targeted for degradation using proteolysis-targeting chimeras (PROTAC) in osteosarcoma (82, 83). The BET PROTAC molecule BETd-260 effectively depleted BETs and suppressed cell viability in multiple osteosarcoma cell lines, including MNNG/HOS, Saos-2, MG-63, and SJSA-1 (82). BETd-260 induced apoptosis by inhibiting antiapoptotic proteins Mcl-1 and Bcl-xl and upregulating the proapoptotic protein Noxa, leading to substantial cell death (82). Shi and colleagues (82) demonstrated that treatment with BETd-260 results in complete degradation of BRD2, BRD3, and BRD4 in an osteosarcoma xenograft model, significantly inhibiting tumor growth and increasing apoptosis markers such as cleaved PARP. In addition, Lang and colleagues (83) explored the efficacy of combining PROTACs with cytotoxic drugs to enhance therapeutic responses in osteosarcoma. The results demonstrated that BET degraders showed synergistic activity when combined with most chemotherapeutics, except for the combinations involving topotecan/gemcitabine, with the highest synergy shown in Saos-2 cells (83). Moreover, BET depletion reduced expression of AXL, BCL-X, e-cadherin, CAIX, EpCAM, ErbB2, and vimentin in HOS and Saos-2 cell lines (83). Although BET

PROTACs hold promise, clinical development has encountered obstacles primarily related to toxicity (84). Efforts are ongoing to optimize the pharmacokinetics and selectivity of PROTACs to minimize adverse effects while retaining their therapeutic potency (84).

Additionally, recent studies have highlighted the role of enhancer elements in driving the metastatic phenotype of osteosarcoma, indicating their potential as therapeutic targets (85). Morrow and colleagues (85) conducted extensive epigenomic profiling and identified substantial differences in enhancer activity between primary and metastatic osteosarcoma tumors. These regions, termed metastatic variant enhancer loci, were found to drive coordinated waves of gene expression during the metastatic colonization of the lung (85). In their study, Morrow and colleagues (85) showed that BET inhibition disrupted the expression of genes associated with metastasis, including coagulation factor III/tissue factor (F3). This disruption was achieved using BETi JQ1, which effectively blocked the binding of BETs to acetylated histones at metastatic variant enhancer loci, thereby suppressing the transcriptional activation of metastasis-related genes (85). The inhibition of these enhancer-driven genes significantly impeded osteosarcoma lung metastasis in the MG63.3 lung colonization model (85).

In conclusion, BET inhibition represents a promising therapeutic strategy for the treatment of osteosarcoma. Targeting BETs using small-molecule inhibitors or PROTACs has demonstrated significant antitumor activity in preclinical models, as monotherapy and in combination with other agents. Further research into the molecular mechanisms underlying BET inhibition in osteosarcoma and the development of BET-targeted therapies are warranted and will lead to the development of clinical trials that may improve clinical outcomes in patients with osteosarcoma.

Other sarcomas

Uterine leiomyosarcoma

Uterine leiomyosarcoma is an aggressive sarcoma that originates from the smooth muscle layer of the uterus, with limited treatment options (86). A BETi, GS-626510, has shown efficacy in inhibiting the growth of uterine leiomyosarcoma PDXs harboring genetic abnormalities involving the homologous recombination repair pathway, alternative lengthening of telomere, and the PI3K/AKT/mTOR signaling (87). In a study conducted by Choi and colleagues (87), 83 uterine leiomyosarcoma samples were analyzed by next-generation sequencing, which led to the identification of recurrent somatic mutations in *TP53*, *MED12*, and *PTEN* genes, with significant somatic copy number variations, including amplifications in *TERT*, *C-MYC*, and *MYOCD*, among others. Notably, the study found that monotherapy with BETi GS-626510, PARP inhibitor olaparib, and PI3K inhibitor copanlisib significantly inhibited the growth of uterine leiomyosarcoma PDXs with specific genetic derangements (87). These findings outline the genetic landscape of uterine leiomyosarcoma and suggest that at least a subset of these tumors could benefit from BETis.

Undifferentiated pleomorphic sarcoma

Undifferentiated pleomorphic sarcoma (UPS), a subtype of STS, is characterized by a lack of lineage differentiation markers and aggressive behavior. Rivera-Reyes and colleagues reported that a combination of HDACis (vorinostat) and BETis (JQ1) can partially restore differentiation in skeletal muscle UPS cells (88). This combination treatment reduced tumorigenesis by modulating the Hippo pathway transcriptional effector YAP1 and NF-κB. Additionally, it influences circadian clock function and metabolic processes, supporting its therapeutic potential (88). This combination strategy activated the unfolded protein response and autophagy, processes crucial for cellular homeostasis (88). In UPS cells, YAP1 suppresses these pathways, contributing to the cancerous phenotype. Inhibition of YAP1/NF-κB upregulates unfolded protein response and autophagy-related genes, shifting cellular metabolism away from glycolysis toward more efficient processes like fatty acid oxidation (88). This shift promotes muscle differentiation and function, highlighting the potential of HDACis and BETis as an effective combinatorial treatment strategy for UPS (88).

Malignant peripheral nerve sheath tumors

Malignant peripheral nerve sheath tumors (MPNST) are highly aggressive sarcomas with a poor prognosis, particularly affecting patients with neurofibromatosis type 1 (NF1; ref. 89). These tumors are characterized by rapid growth, high recurrence rates, and resistance to conventional therapies, making them a significant therapeutic challenge (90). MPNSTs frequently arise in the context of NF1, a genetic disorder that predisposes patients to a variety of tumors owing to mutations in the *NF1* tumor suppressor gene (91).

Preclinical studies have highlighted the potential of BETis, particularly those targeting the BRD4 protein, in treating MPNSTs (92, 93). BRD4 has been identified as a crucial target in these tumors, with its inhibition leading to significant antitumor effects (92). Patel and colleagues (92) showed that BET inhibition induces apoptosis in MPNST cells by regulating the proapoptotic protein Bim. This mechanism shifts the balance of anti- and proapoptotic molecules in favor of cancer cell death, offering a promising therapeutic avenue (92). However, the effectiveness of BETis alone is limited by the development of resistance (93). Some studies suggest that BET/BRD4 inhibition as a monotherapy may not be sufficient to achieve sustained therapeutic effects (93, 94). This has led to exploring combination therapies and alternative strategies to overcome resistance. For instance, Cooper and colleagues (94) demonstrated that genetic depletion of BRD4 via CRISPR/Cas9-based genome editing combined with pharmacologic BET inhibition resulted in synergistic effects in blocking growth of MPNST cells. They identified that elevated BRD4 protein levels are associated with resistance to BETis and can be targeted through combinatory therapies or with PROTACs, which simultaneously degrade BRD4 and inhibit bromodomain activity (94). These findings suggest that BRD4 addiction can be a pivotal resistance mechanism and that directly reducing the BRD4 level can help overcome BETi resistance.

Gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GIST) are primarily driven by activating mutations in the receptor tyrosine kinases KIT and PDGFRA, which play a crucial role in tumor growth and survival (95). Although kinase inhibition is an effective treatment for many patients with GISTs, disease progression due to kinase-resistant mutations remains a major clinical challenge (96). Hemming and colleagues (96) demonstrated that BETi JQ1 targets enhancer domains critical for *KIT* gene expression. By disrupting these enhancer domains, BET inhibition induces cell-cycle arrest, apoptosis, and cell death of GIST cells (96). This therapeutic strategy not only decreases *KIT* mRNA and protein levels but also affects mediators of KIT signal transduction, leading to a significant reduction in tumor cell viability (96).

Furthermore, combining BETis with tyrosine kinase inhibitors (TKI) has shown promising results in TKI-resistant GISTs (96). The

synergistic effects of BETis and TKIs result in more pronounced cytotoxic effects, both *in vitro* and *in vivo*, compared with using either agent alone (96). This combination therapy effectively reduces tumor growth in TKI-resistant GIST xenografts, underscoring the potential of BET inhibition as a valuable addition to current GIST treatment regimens (96).

Chondrosarcoma

Chondrosarcoma (CHS), the second most frequent primary bone malignancy among sarcomas (97), exhibits a notable resistance to conventional therapies such as chemotherapy and radiotherapy (98). BETi JQ1 has shown considerable efficacy in preclinical studies by significantly inhibiting CHS cell growth (99). This is achieved through inducing G_1-phase cell-cycle arrest and promoting apoptosis via the regulation of the YAP/p21/c-Myc/Bcl-xL signaling axis (99). Specifically, JQ1 leads to the upregulation of cell-cycle inhibitors p21 and p27 and downregulation of cell-cycle promoters cyclin E2 and c-Myc (99). The suppression of c-Myc alongside the inhibition of antiapoptotic proteins like Bcl-xL underscores the potential of BETis in the treatment of CHS (99).

In addition, Zhang and colleagues (100) revealed that resistance to BETis, like JQ1, in CHS is linked to the YAP1/p21 axis. They found that although BETis induce senescence in cancer cells through the upregulation of p21, the survival of these senescent cells is a potential resistance mechanism (100). YAP1 depletion enhances this senescence, but persistent p21 expression allows these senescent cells to avoid apoptosis, contributing to resistance (100). To overcome this resistance, they suggest a "one-two punch" approach: first inducing senescence with BETis or YAP1 targeting, followed by targeting p21 or using senolytic agents like ABT263 to eliminate the senescent cells, thereby enhancing the therapeutic efficacy of BETis in CHS (100).

Liposarcoma

Liposarcoma is characterized by adipocytic differentiation, with specific subtypes exhibiting distinct transcriptional dependencies (101, 102). In myxoid liposarcoma, BETs cooperate with the FUS–DDIT3 fusion protein, which is seen in approximately 90% of patients (103), playing a critical role in maintaining the malignancy of these cells (104). This collaboration involves the regulation of SE regions that drive the expression of oncogenic transcription factors and other essential genes (104). Genetic depletion or pharmacologic inhibition of BETs in myxoid liposarcoma has been shown to disrupt these SE-driven transcriptional programs, leading to reduced cell proliferation and tumor growth (104).

In dedifferentiated liposarcoma, BETs are part of a core transcriptional regulatory circuitry that includes FOSL2, MYC, and RUNX1 (104). This circuitry is essential for the survival and proliferation of dedifferentiated liposarcoma cells (104). BETis, such as the BET PROTAC ARV-825, have demonstrated significant efficacy in targeting these transcriptional dependencies (105). Treatment with ARV-825 leads to the degradation of BETs, resulting in the downregulation of vital oncogenic transcription factors and the induction of apoptosis (104). This approach not only mitigates the malignancy of liposarcoma but also shows promise in overcoming resistance to traditional chemotherapy agents like trabectedin, providing a potential new therapeutic strategy for patients with liposarcoma (104).

Synovial sarcoma

Synovial sarcoma (SS) is a rare and aggressive type of STS, with ~95% of patients with SS possessing the oncogenic SS18–SSX fusion gene (106). Treatment options include surgical resection and chemotherapy but only provide limited efficacy (107, 108, 109). Kotani and colleagues (109) demonstrated that pan-BETi ABBV-075 induces G_1-phase cell-cycle arrest and apoptosis in SS cells by modulating the expression of key cell-cycle regulators such as upregulation of p21 and downregulation of c-Myc, CDK4, and CDK6. Additionally, RNA sequencing, Western blot, and qPCR analysis indicated that ABBV-075 downregulates the antiapoptotic genes such as *BCL-xl* and upregulates proapoptotic genes such as *BIM* and *PUMA* (109). Knockdown of SS18–SSX reduced BCL-2 expression and consequently decreased sensitivity to BETis, suggesting that BETis effectively target the intrinsic apoptotic pathway modulated by SS18–SSX (109). The findings suggest that BETis could represent a promising therapeutic approach for SS, particularly by targeting pathways involving BCL-2 and c-Myc (109). This study highlights the potential for BETis to fill the gap in effective treatments for SS, providing a basis for further clinical research and development.

Clinical Trials of BETis in Sarcoma

The first-in-human phase I open-label study of the BETi ODM-207 (pan-BETi) was designed to evaluate its safety, pharmacokinetics, and antitumor activity in patients with advanced solid tumors, including sarcoma (NCT03035591). The study involved a dose-escalation phase and an evaluation of the effect of food on pharmacokinetics (25). ODM-207 was administered orally, with dose escalation proceeding up to 2 mg/kg, which was determined to be the maximal tolerated dose (MTD; ref. 25). Common adverse events included thrombocytopenia, nausea, anorexia, fatigue, and vomiting (25). Although no partial or complete responses were observed, six patients achieved stable disease, and platelet count reduction correlated with drug exposure (25). The study concluded that ODM-207 has a manageable safety profile at doses up to 2 mg/kg but exhibits a narrow therapeutic window (25).

Another notable trial was the phase Ib/IIa study of PLX51107 (pan-BETi), which was designed to evaluate the safety, tolerability, pharmacokinetics, and efficacy of BET inhibition in patients with advanced hematologic malignancies, solid tumors, and sarcoma (NCT02683395). This study aimed to establish the MTD and the recommended phase II dose through a dose-escalation method (26). The trial included both a phase Ib component, which focused on identifying dose-limiting toxicities and understanding the pharmacokinetic profile, and a phase IIa component, which involved expansion cohorts to assess further the safety and efficacy at the established recommended phase II dose in specific malignancies (26). Early results have shown that PLX51107 has a manageable safety profile with adverse events consistent with BETis. Preliminary efficacy data indicated promising antitumor activity in certain hematologic malignancies, solid tumors, and sarcoma (26).

An ongoing phase I/II trial will continue to build on these findings by evaluating the combination of the BETi AZD5153 with selumetinib (an MEK inhibitor) and durvalumab (a PD-L1 inhibitor) in patients with several types of sarcomas (NCT05253131). The trial aims to determine the safety, tolerability, and efficacy of these combinations. The study includes patients with desmoplastic small round blue cell tumors, malignant fibrous histiocytoma, osteosarcoma, neurofibroma, MPNSTs, ESFTs, SS, and RMS. This study is expected to be completed by 2030.

Final stages of a phase I trial on BETi BMS-986158 and BMS-986378 (pan-BETi) in pediatric patients with relapsed or refractory central nervous system tumors, other solid tumors, and sarcomas are

being evaluated (NCT03936465). This trial has focused on determining dose-limiting toxicities, pharmacokinetics, and objective response rates over 2 years, providing foundational data on the safety and potential efficacy of BETis in treating these challenging cancers (110). This study was expected to be completed in March 2024.

In addition, a phase 2 clinical trial of BETi CPI-0610 was planned for treatment of patients with MPNST, but the study was withdrawn owing to lack of participation (NCT02986919; ref. 111).

Notably, there are newly developed BETis, such as ZEN-3694 and PLX2853, that are showing promising results in clinical trials in various cancers but have not yet been explored in sarcoma (112, 113).

These clinical trials, both completed and ongoing, demonstrate significant advancements in the exploration of BETis as a therapeutic option for sarcoma. The results of these studies will be crucial in shaping future treatment protocols and improving patient outcomes.

Summary and Future Perspective

The development of BETis remains an active area of research, particularly in treating cancers such as sarcomas, in which BETs play critical roles in oncogenesis. BETis exert broad anticancer effects by modulating the expression of key oncogenes and survival pathways. BETs, particularly BRD4, play a crucial role in regulating genes involved in cell-cycle progression, apoptosis, and proliferation in sarcomas. By disrupting these transcriptional programs, BETis can suppress tumor growth and induce cell death (77–79, 92). In addition, BETis can block essential oncogenic transcriptional programs, including the function of fusion proteins in sarcomas such as the ESFT (EWS–FLI1) and RMS (PAX3–FOXO1; refs. 45, 48, 62, 63). This approach is particularly appealing owing to the lack of effective therapies directly targeting these oncogenic fusion proteins, which are often difficult to inhibit with traditional small molecules. By disrupting the interaction between BETs and acetylated histones, BETis prevent the expression of oncogenic fusion-driven genes, offering a novel therapeutic strategy. These wide-ranging effects make BETis a versatile tool in the fight against sarcoma, not only by targeting oncogenic drivers but also by interfering with essential cellular processes that sustain tumor growth.

Beyond their role in transcriptional regulation, BETs, particularly BRD4, play a critical role in the DDR. BRD4 participates in repairing DNA damage, regulating replication stress, and maintaining genomic stability (5, 81, 114). Given the elevated levels of genomic instability and replication stress commonly observed in sarcomas, BET inhibition offers a promising therapeutic strategy. By disrupting BRD4's involvement in DDR, BETis can exploit these vulnerabilities, particularly in cancers with heightened replication stress. However, the role of BETs in DDR within sarcoma remains somewhat understudied and represents a crucial future direction that needs further exploration. Understanding how BET inhibition interacts with DDR pathways in sarcoma could open new avenues for combining BETis with other agents that induce or enhance DNA damage and replication stress, such as PARP inhibitors or ATR inhibitors, to further enhance therapeutic effects in sarcoma.

Despite these encouraging results, the clinical translation of BETis faces challenges, primarily concerning the balance between efficacy and safety. Although preclinical models have shown strong antitumor effects, including the ability to induce apoptosis and inhibit tumor growth, concerns about toxicity and tolerability remain. For example, studies in mice have reported adverse events such as thrombocytopenia and gastrointestinal issues, and similar dose-limiting toxicities have been observed in early human trials (6, 25, 28). These findings underscore the need for further refinement of dosing regimens to maintain efficacy while minimizing harmful side effects.

One potential solution to improve the therapeutic window of BET inhibition involves using BETis in combinatorial strategies to enhance efficacy while potentially managing toxicity. ZEN-3694 is a promising monovalent BETi that is being actively investigated in combination therapies across multiple clinical trials owing to its encouraging safety and efficacy profile (112). This drug is being evaluated in a variety of cancers, with the aim of improving therapeutic outcomes when used in combination with other agents. One notable example is the phase I/II study (NCT05372640), in which ZEN-3694 is being evaluated in combination with abemaciclib, a CDK4/6 inhibitor, in patients with advanced solid tumors, including breast cancer, and the results will shed more light on the future of BETis in combination therapies.

Another promising avenue is bivalent BETis, such as AZD5153, which are designed to engage both bromodomains of BETs simultaneously. These inhibitors have shown increased potency, allowing for effective inhibition at lower drug doses, potentially reducing the risk of adverse effects. By focusing on combination therapies and bivalent inhibitors, the therapeutic potential of BET targeting could be further optimized if regimens that are efficacious and well tolerated can be designed.

Looking ahead, BET inhibition in sarcoma treatment will likely focus on optimizing drug design to enhance efficacy, selectivity, and reduce toxicity. Also, investigations on the utility and safety of BETis in combating metastatic disease in sarcomas from which most patients succumb are a priority. To strengthen the rationale for advancing this class of drugs to expanded testing in clinical trials, investigations in preclinical sarcoma models of metastatic disease will be critical. Additionally, integrating BETis with other targeted therapies, such as kinase inhibitors or immunotherapies, could provide a more comprehensive approach to combating sarcoma. Moreover, continued exploration of PROTACs and their ability to degrade BETs offer a promising avenue for overcoming current limitations and achieving more durable patient responses. Further clinical trials will be essential to determine the long-term efficacy and safety of these therapies, ultimately improving outcomes for patients with aggressive sarcomas.

Authors' Disclosures

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