ELSEVIER

KEYNOTE (GREEN)

Inhibition of bromodomain and extra-terminal motif (BET) proteins in pediatric sarcoma: A *systematic review of in vitro* and *in vivo* studies

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BET inhibitors (BETi), especially those targeting BRD4, show promising preclinical activity against pediatric sarcomas by disrupting oncogenic transcription. This systematic review of 26 studies highlights the anti-proliferative and pro-apoptotic effects of BETi across five pediatric sarcoma types. *In vivo* data show a decrease in tumor growth, with better results when combined with other therapies. This systematic review revealed that, whereas early investigations mostly rely on pan-BETi, recent studies focus on newer and more specific agents. Accordingly, we reported that ABBV-744 and RVX-208, which selectively target the BD2 domain, and GNE-987, a specific BRD4 degrader, are the most promising inhibitors. However, ABBV-075, a pan-BETi, also exhibits high efficacy, being effective at low doses. Nevertheless, translating these experimental findings into clinical practice remains difficult because of resistance, toxicity, and inconsistent responses. Future approaches include using biomarkers for patient selection, developing isoform-specific BETi, and designing rational combination therapies to enhance treatment for these aggressive pediatric cancers.

Keywords: BET inhibitors; pediatric sarcoma; epigenetics; transcriptional regulation; targeted therapy; fusion oncogenes

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Introduction

Sarcomas are rare mesenchymal tumors that can originate from bone or soft tissues. They can occur across all age groups, but have a relatively higher prevalence in children and adolescents aged 0–14 years, accounting for approximately 12–15% of all pediatric cancers.[p1] Currently, sarcoma treatment typically involves a multimodal approach, including chemotherapy, surgery, radiation therapy, immunotherapy, and/or targeted therapy,[p2] leading to 5-year event-free survival rates of approximately 60–70%. However, despite these comprehensive therapeutic strategies, survival rates for metastatic sarcomas remain below 20–30% and decrease further to 10–20% in cases of relapse.[p1],[p2]

Unlike many adult cancers that display a high mutational burden, pediatric sarcoma onset mainly depends on epigenetic and transcriptional alterations. Despite having a low mutation rate, pediatric sarcoma tumorigenesis is often driven by specific chromosomal translocations or fusion oncogenes,[p3] such as Ewing sarcoma (EWS)–friend leukemia integration 1 (FLI1) in Ewing sarcoma (ES)[p4] or paired box gene (PAX)3/7–forkhead box O1 (FOXO1) in alveolar rhabdomyosarcoma (RMS).[p5] These fusion proteins often function as aberrant transcription factors or transcriptional co-regulators, causing widespread disruption of gene expression programs.[p6] Aberrant transcriptional activity is linked to epigenetic changes and abnormal chromatin remodeling, including histone modification patterns and DNA methylation, which lead to alterations in cell identity, malignant transformation, and therapy resistance.[p7],[p8],[p9]

In this context, epigenetic regulators have emerged as attractive therapeutic targets.[p2] One of the most extensively studied families is the highly conserved bromodomain and extra-terminal motif (BET) domain protein family, which includes bromodomain containing (BRD)2, BRD3, BRD4, and BRDT.[p10],[p11] These proteins recognize acetylated lysine residues on histone tails and act as epigenetic readers, promoting transcriptional activation. BET proteins recruit transcriptional machinery to chromatin, modulating chromatin structure and enhancer–promoter looping, thereby influencing three-dimensional genome organization.[p11] Structurally, BET proteins contain two N-terminal bromodomains (BD1 and BD2) that bind acetyl-lysine and an extra-terminal (ET) domain that recruits transcription factors (Figure 1). Additionally, BRD4 and BRDT feature a C-terminal domain (CTD) that facilitates interaction with transcriptional regulators, such as positive transcription elongation factor b (P-TEFb) (Figure 1).[p12] Functionally, BRD2 and BRD3 are involved in transcription activation, cell-cycle progression, and differentiation.[p12],[p13] BRDT is expressed in testicular tissue and participates in the removal and replacement of acetylated histones during spermatogenesis.[p13] BRD4, the most well-characterized member of the BET family, regulates transcription initiation and elongation by binding to chromatin regions enriched with acetylated lysine 27 on histone 3 (H3K27ac), interacting with the mediator complex, and recruiting P-TEFb.[p14] It plays a vital role in chromatin looping, facilitating long-range enhancer–promoter interactions and promoting super-enhancer (SE) activity under physiological conditions.[p15],[p16] Notably, abnormal BRD4 association with SEs is critical in oncogenesis. Indeed, BRD4 works in conjunction with core transcription factors that establish autoregulatory loops and cooperatively increase the expression of oncogenic drivers, such as c-MYC and B-cell lymphoma 2 (BCL2), thereby influencing cell-cycle progression and apoptosis pathways.[p15],[p17],[p18] Consequently, BET proteins have attracted significant clinical interest for their role in key pro-tumorigenic mechanisms across various cancers, including oral, breast, prostate, lung, and colorectal cancers, as well as acute myeloid leukemia (AML).[p12],[p19],[p20],[p21],[p22],[p23] Fusion-positive (FP) sarcoma has been recently discovered, where BRD4 binds to H3K27ac-enriched SEs associated with EWS–FLI1 (ES) and PAX3–FOXO1 (FP-RMS; P3F), recruiting P-TEFb to promote and amplify oncogene expression.[p24],[p25],[p26] Therefore the emerging central role of BRD4 in driving pro-tumorigenic

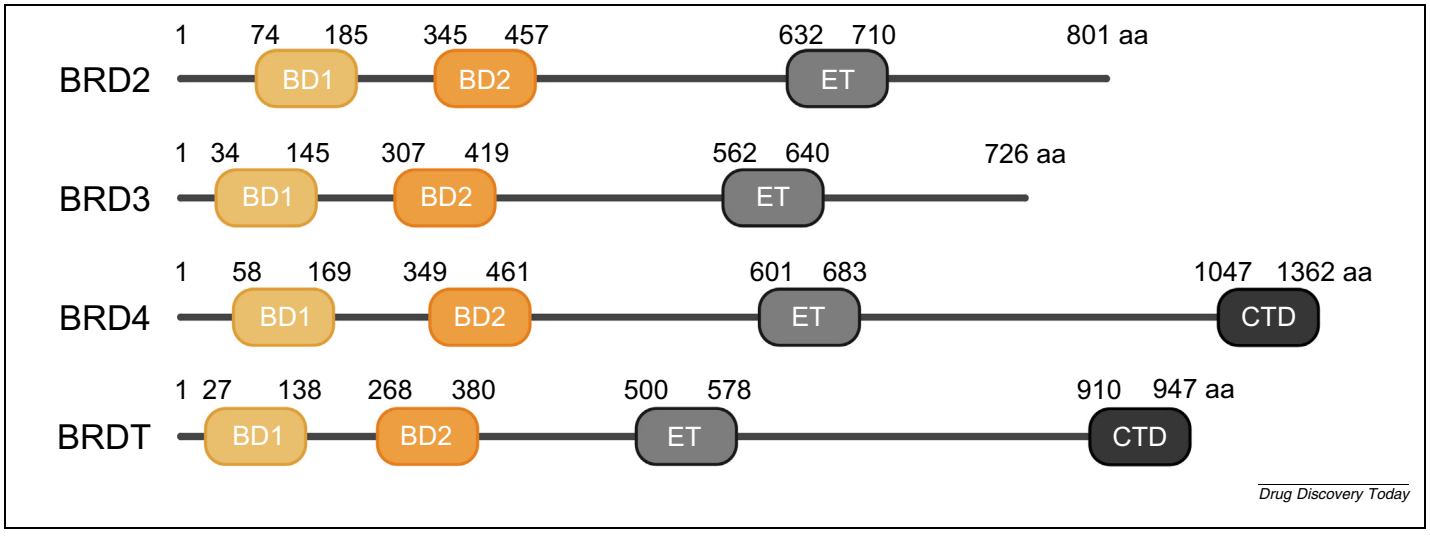


FIGURE 1

Schematic representation of BET proteins. BD1, bromodomain 1; BD2, bromodomain 2; ET, extra-terminal domain; CTD, C-terminal domain.

transcriptional programs has provided the rationale for the development of BET inhibitors (BETi) as potential anticancer agents, both as single treatments and in combination strategies. The development of selective inhibitors began in 2010 with the design of the first two BETi. I-BET was described as a benzodiazepine derivative with anti-inflammatory effects.[p27] At the same time, another BETi, JQ1, emerged as the prototypical BETi, demonstrating potent anticancer effects by displacing BRD4 from chromatin, inducing G1 cell-cycle arrest, and promoting apoptosis.[p11],[p28] Additionally, JQ1 treatment exhibits antitumor activity *in vivo* by halting tumor growth and promoting apoptosis in patient-derived xenograft models (PDXs). Despite its widespread use in preclinical research, the short half-life and limited pharmacokinetic profile of JQ1 have restricted its clinical applicability, prompting the development of more potent, selective, and clinically deliverable BETi.[p29]

BETi are categorized based on their chemical structure (Table 1), selectivity for bromodomains BD1 and BD2 (pan-BETi, BD1- or BD2-selective inhibitors), and mechanism of action.[p30] Most of them function as acetyl-histone mimetics, competitively binding to the acetyllysine pocket of BET bromodomains. Among the pan-BETi, JQ1 remains one of the most studied, despite its pharmacological limitations. Structural analogs with improved pharmacokinetics, such as OTX015, i-BET762, i-BET151, and ZEN-3694, have been developed, although clinical responses remain modest, with remission rates below 30%, primarily because of dose-limiting toxicities requiring intermittent treatment schedules.[p30]

To overcome these challenges, new agents have been developed with greater bromodomain-specific selectivity. RVX-208 (apabetalone), a BD2-selective inhibitor, exhibits narrower gene expression profile modulation and reduced toxicity.[p31] Additionally, ABBV-744, another BD2-selective inhibitor, has shown significant antiproliferative effects in AML and prostate cancer.[p32] Recently, more advanced classes of compounds have been developed. Proteolysis-targeting chimeras (PROTACs) are bifunctional molecules that, by linking an E3 ligase recognition ligand to a BET-targeting small molecule, promote proteasome-dependent degradation of BET proteins.[p33]

Given the evidence mentioned above and the central role of transcriptional dysregulation and epigenetic reprogramming in pediatric sarcomas, BET inhibition presents a promising avenue for therapeutic intervention.

This systematic review aims to provide a comprehensive overview of the current landscape of BETi applications in pediatric sarcomas. We summarize their mechanisms of action, *in vivo* and *in vitro* preclinical evidence, and rationale for ongoing and future clinical investigations. We seek to clarify the therapeutic potential of BET inhibition, whether as monotherapy or in combination, to improve outcomes for children and adolescents affected by these aggressive malignancies.

BET inhibition in pediatric sarcoma

The systematic review process collected data from 26 selected studies that involve the use of 14 distinct BETi, with JQ1 being the most widely used (19 studies) (Tables 1 and 2; and see Table S1 in the supplementary material online). Following JQ1, I-BET-151 was used in three studies, OTX015 in three, and I-BET-762 in two. All of the remaining inhibitors covered in this work (I-BET-762, bromosporine, TW09, SF1126, SF2523, SF2523P, RVX-208, ABBV-744, ABBV075, GNE987) were only tested in one work each (Tables 1, 2, and S1).

Considering the heterogeneity and rarity of each sarcoma subtype, in 2015, Teicher *et al.* screened a panel of 63 sarcoma cell lines, belonging to 17 sarcoma subtypes, against 100 FDA-approved compounds and 345 agents under investigation.[p34] This screening demonstrated that some drugs, including JQ1 and iBET151 BETi, were viable therapeutic options for treating these sarcomas, opening the possibility for further studies. Indeed, synovial sarcoma (SS), which was the most sensitive subgroup, along with osteosarcoma (OS) and RMS, showed low IC_{50} values in the nanomolar range.

Ewing Sarcoma (ES)

ES is a rare and aggressive cancer primarily affecting children and adolescents, with an incidence of 1.5 per million annually.[p35] It mainly targets bones, but can also involve soft tissues.[p36],[p37],[p38] In 85% of ES cases, the disease is linked to nonrandom chromosomal translocations, most commonly t(11;22) (q24;q12), which results in the fusion of the Ewing sarcoma breakpoint region 1 (EWSR1) gene on chromosome 22 with the FLI1 gene on chromosome 11.[p39] EWS–FLI1 is an oncogenic transcription factor that interferes with normal gene regulation and cell growth,[p40],[p41],[p42] affecting several pathways including cell differentiation, survival, and proliferation.[p41] Primary treatments for ES include surgery, chemotherapy, and radiation therapy. However, new therapies are emerging, such as tyrosine kinase inhibitors, CAR-T-mediated immunotherapy, and treatments that specifically target the fusion protein or its downstream effectors.[p35],[p43] In recent years, BET proteins have become promising targets because of their role in regulating EWS–FLI1 activity.[p44]

The first study on the effectiveness of BETi in ES was carried out by Hensel *et al.* in 2015.[p45] Using ES cells, they found that JQ1 treatment downregulates EWS–FLI1 and ES-specific gene expression, but does not reduce c-MYC levels in any of the ES lines tested. Furthermore, they showed that JQ1 can influence the growth of ES lines by causing cell-cycle arrest in the G1 phase and triggering apoptosis. Additionally, apoptosis induced by JQ1 increased when ES cells were treated with a combination of JQ1 and the phosphoinositide 3-kinase inhibitor (PI3Ki) BEZ235. Indeed, the combination of PI3K and BET inhibition synergistically suppresses MYC-driven oncogenic signaling at both transcriptional and post-translational levels. In particular, BET inhibition reduces *MYC* transcription, whereas PI3K pathway inhibition interferes with MYC protein stability and translation.[p46] Moreover, in several cancer types, the combination of these agents also prevents compensatory feedback activation of the PI3K pathway, resulting in a more durable inhibition and enhanced antitumor activity.[p47] Interestingly, they also observed that knocking down both BRD3 and BRD4 produced effects similar to those seen with JQ1 treatment, indicating that the action of JQ1 is likely exerted through displacing these proteins. The therapeutic potential of JQ1 was confirmed in a subcutaneous xenograft mouse model, which showed a significant

TABLE 1

Summary of BET inhibitors discussed in the present systematic review.

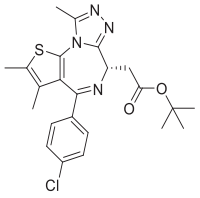
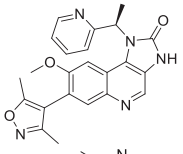
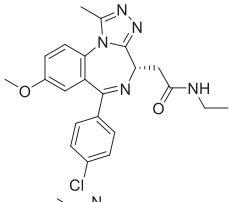
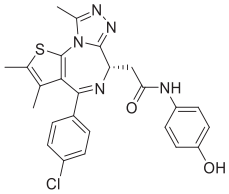
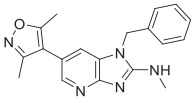
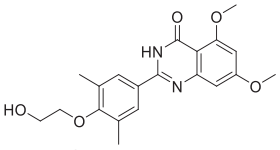
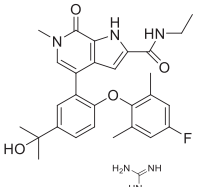
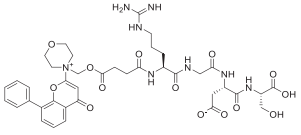
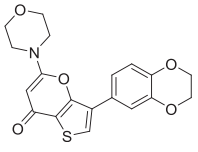
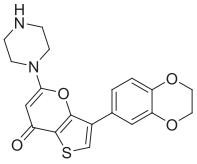
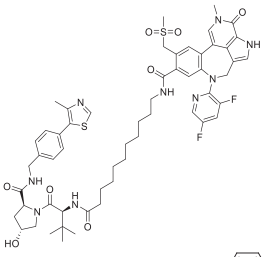
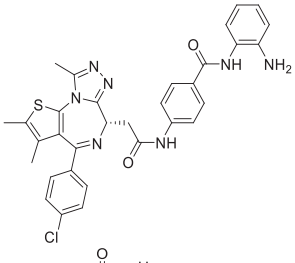
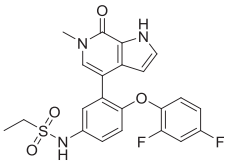
Name	Chemical structure	Simplified chemical descriptor	Type/class	Mechanism of action	Therapeutic relevance	Cancer type	Refs
JQ1		Benzotriazolodiazepine	Pan-BETi	Competitive BD1/BD2 bromodomain inhibition	Induces apoptosis, downregulates c-MYC, EWS–FLI1, angiogenesis, enhances combination therapy	ES, OS, RMS, GIST	(p11),(p25), (p28),(p45), (p48),(p49), (p52),(p53), (p54),(p59), (p64),(p65), (p66),(p67), (p70),(p73), (p76),(p77), (p79),(p80), (p82)
I-BET151 (GSK1210151A)		Imidazoquinoline	Pan-BETi	BD1/BD2 inhibition	Suppresses AURKA, synergistic with alisertib and vincristine	ES, GIST	(p30),(p52), (p59)
iBET762 (GSK525762A, molibresib)		Benzotriazolodiazepine	Pan-BETi	BD1/BD2 inhibition	Synergistic antiproliferative effect with other agents	ES, GIST	(p30),(p49), (p59)
OTX015 (birabresib)		Thienotriazolodiazepine	Pan-BETi (clinical)	BD1/BD2 inhibition	Reduces proliferation, enhances radiation response, affects DNA repair	OS, RMS	(p25),(p30), (p59),(p68), (p76)
ZEN-3694		Imidazopyridine	Pan-BETi	BD1/BD2 inhibition	Improved PK over JQ1, tested in clinical trials	Mentioned, no specific tumor	(p30)
RVX-208 (apabetalone)		Quinazolinone	BD2-selective	Selective BD2 bromodomain inhibition	Reduced toxicity, narrower gene modulation, combination with mTOR inhibitors	RMS, GIST	(p31),(p59), (p79)
ABBV-744		Pyrrolopyridinone	BD2-selective	Selective BD2 bromodomain inhibition	Strong antiproliferative synergy in RMS with mTOR inhibitors	RMS	(p32),(p79)
SF1126		Chromone PI3K inhibitor conjugated to Arg–Gly–Asp peptide	Dual PI3K/BRD4	Dual inhibition	Reduces AKT phosphorylation, EWS downregulation, blocks bone destruction	ES	(p54)
SF2523		Thienopyranone	Dual PI3K/BRD4	Dual inhibition	Most potent among tested, reduces p-AKT and EWS	ES	(p54)

TABLE 1 (*CONTINUED*)

Name	Chemical structure	Simplified chemical descriptor	Type/class	Mechanism of action	Therapeutic relevance	Cancer type	Refs
SF2523P		Thienopyranone	Dual PI3K/BRD4	Modified dual inhibition	Decreases EWS expression, not p-AKT	ES	(p54)
GNE-987		Triazolobenzodiazepine linked to VHL ligand	PROTAC	BRD4 degradation via VHL-mediated ubiquitination	Depletes BRD4, lowers H3K27ac at SEs, targets oncogenes	OS	(p69)
TW09		CI994 fused with thienotriazolodiazepine	Dual BET/HDACi	Dual inhibition (BET and HDAC)	Induces mitochondrial apoptosis, affects BCL2 family	RMS	(p77)
ABBV-075 (mivebresib)		Pyrrolopyridinone	Pan-BETi	BD1/BD2 inhibition	More effective than JQ1, modulates apoptotic proteins	SS	(p86)

Arg, arginine; Asp, aspartic acid; BETi, bromodomain and extra-terminal domain inhibitors; BD1, bromodomain 1; BD2, bromodomain 2; ES, Ewing sarcoma; GIST, gastrointestinal stromal tumor; Gly, glycine; OS, osteosarcoma; RMS, rhabdomyosarcoma; SS, synovial sarcoma.

reduction in ES tumor growth and size in treated mice as compared with controls.

Jacques *et al.*, in 2016, tested the effect of JQ1 on cell viability in a panel of 11 ES cell lines, revealing highly heterogeneous results among the different lines.[p48] Indeed, TC32, SKES-1, EW7, and RDES turned out to be the most sensitive, whereas A673 was the least sensitive. The functional effects of JQ1 treatment on ES cell lines include a reduction in clonogenic potential of more than 45% compared with untreated cells and a decrease in the migratory capabilities of both TC71 (moderately sensitive to JQ1) and A673 (less sensitive to JQ1) ES cells. Consistent with previous work,[p45] reports also indicated G1-phase cell-cycle arrest and induction of apoptosis. Jacques *et al.* also confirmed the downregulation of EWS–FL11 expression at both the mRNA and protein levels in ES cells treated with JQ1 in a dose- and time-dependent manner. Furthermore, BRD4 chromatin immunoprecipitation (ChIP) revealed specific enrichment of BRD4 within the EWS–FL1 promoter region and showed that JQ1 treatment can induce the release of BRD4 from its binding sites. As a result, EWS–FLI1 and its target genes, including GLI family zinc finger 1 (GLI1), nuclear receptor subfamily 0 group B member 1 (NR0B1), FOXM1, and vascular endothelial growth factor A (VEGA), were downregulated. *In vivo* treatments with JQ1 confirmed its antitumor effect in a pretibial ES mouse model (TC-71 cells), where the treatment increased overall survival and decreased tumor growth. Interestingly, they also observed a reduction in tumor vascularization and VEGA expression, an angiogenic factor, in the JQ1-treated group of mice.

Accordingly, in the same year, Bid *et al.* investigated the effect of JQ1 as an anti-angiogenic drug in both ES and RMS models.[p49] The anti-angiogenic effect of JQ1 was initially evaluated in subcutaneous xenograft ES mouse models (EW-5 and EW-8 cell lines), where the treatment significantly reduced tumor growth. Furthermore, protein expression analysis of 55 angiogenesis-related factors revealed a significant reduction in key pro-angiogenic molecules, including VEGF, angiopoietin, and fibroblast growth factor 1 (FGF-1), in tumors from JQ1-treated mice compared with those from untreated controls.

In 2016, Loganathan *et al.* further explored the therapeutic potential of the BETi JQ1 and i-BET762 (GSK525762, which target BD1 and BD2 domains) in *in vitro* ES models, and only JQ1 in *in vivo* models.[p50] They initially assessed the molecular effects of JQ1 treatment using RNA sequencing analysis, which showed a rapid and direct suppression of EWS–FLI1-dependent transcription. Among EWS–FLI1 target genes, they observed downregulation of cyclin D1 (CCND1), protein phosphatase 1 regulatory

TABLE 2

Characteristics of the selected articles. Information on tumor types, cell lines, and dosages of specific inhibitors utilized *in vitro* and *in vivo* is reported.

Refs	Tumor	Cell lines	Inhibitor	*In vitro* range	*In vivo* range
(p34)	Several		JQ1, iBET151	0.065–10 μM	NA
(p45)	ES	A673, SK-N-MC, TC-71	JQ1	2 μM	50 mg/kg, IP, twice a day
(p48)	ES	A673, TC-71	JQ1	0.4–10 μM	50 mg/kg, IP, twice a day
(p49)	ES	ES-1, ES-2, ES-4, ES-6, ES-7, ES-8, EW-8, TC-71	JQ1	0.5 μM	50 mg/kg, OG, once a day
	RMS	RH4, RH5, RH10.RH18, RH28, RH30, RH36, RH41			
(p50)	ES	A673, TC32. TC71	JQ1	0.1–2.5 μM	50 mg/kg, IP, twice a day
			iBET762	1 μM	
(p52)	ES	SK-ES, ES2, TC71	iBET151	0.2–8 μM	20 mg/kg, IP, once a day
(p53)	ES	A673, A673r, EW7, SKNMC, SKNMCr	JQ1	0.5–1 μM	50 mg/kg, IP, once a day
(p54)	ES	A673, EWS502, SK-N-MC, SK-PN-DW, RDES	SF1126	5–25 μM	50 mg/kg, IF, once a day
			SF2523	0.1–5 μM	NA
			SF2523P	0.1–5 μM	NA
(p59)	GIST	GIST-T1, GIST430, GIST48, GIST48B, GIST226, GIST882	JQ1	50–500 nM	50 mg/kg, IP, 5 days per week
(p64)	OS	MNNG/HOS, KHOS, U2OS, MG63	JQ1	0.1–10 μM	50 mg/kg, IP, twice a day
(p65)	OS	K7M2	HAp/BP/JQ1	5 mg/mL of particles (0.624 nM)	NA
		K7M2	JQ1	0.624 nM–1 μM	NA
(p66)	OS	MNNG, 143B	JQ1	250 nM	NA
(p67)	OS	U2OS	JQ1	0.5 μM	NA
(p68)	OS	HT96	OTX015	NA	25 mg/kg, PO, 5 days a week, twice a day
(p69)	OS	HOS,143B, MG-63, U2OS	GNE-987	2.5–40 nM	0.2 mg/kg, subcutaneous, once every 2 days
(p25)	RMS	RH4, RH3, RH5, RH41, SCMC, RD, CTR	JQ1	0.5–1 μM	50 mg/kg, IP, once a day
			OTX015	NA	NA
			iBET726	NA	NA
			iBET151	NA	NA
			iBET762	NA	NA
			Bromosporine	NA	NA
(p73)	RMS	RD, RH30	JQ1	0.5–2 μM	NA
(p74)	RMS	RH30, RH36, RH41, RD	JQ1	0.5–2 μM	NA
(p76)	RMS	RH4, RH30	OTX015	1–3 μM	NA
(p77)	RMS	RD, RH30	TW09	2.5–5 μM	NA
			JQ1	1–5 μM	NA
(p78)	RMS	RH30, RH36, RH41, RD	JQ1	0.25–2 μM	NA
(p10)	RMS	RD, SJCRH30, H69, RH4, A204	JQ1	0.02–5 μM	NA
(p79)	RMS	RD, RH30	RVX-208	10 μM	60 mg/kg, OG, once a day
			ABBV-744	NA	NA
(p80)	RMS	RD, RH30, RH41	JQ1	100–500 nM	NA
(p82)	RMS	RD	JQ1	50 nM	NA
(p86)	SS	ASKA-SS, Yamato-SS, HS-SY-II, SYO-1	ABBV-075	0.01–1 μM	1 mg/kg, OG, once a day

ES, Ewing sarcoma; GIST, gastrointestinal stromal tumor; IF, intrafemoral; IP, intraperitoneal; NA, no data available; OG, oral gavage; OS, osteosarcoma; PO, per os; RMS, rhabdomyosarcoma; SS, synovial sarcoma.

subunit 1A (PPP11R1A), protein kinase C beta (PRICE,) and GLI1, all previously reported as direct targets of BRD4 and key drivers of tumor development in ES.[p51] Unlike previous reports, no changes were seen in the gene or protein expression of either c-MYC or EWS–FLI1 after BETi treatment in any of the ES cell lines tested. Interestingly, transcriptomic analysis revealed that JQ1 treatment downregulated the expression of insulin-like growth factor 1 (IGF1), an EWS–FLI1 target gene that, through an autocrine mechanism, activates the insulin-like growth factor 1 receptor (IGF1R) signaling pathway, promoting the growth and proliferation of metastatic ES. Loganathan *et al*. also confirmed the effects of BETi on cell proliferation and survival in ES cell lines, except for the A673 ES cell line, which exhibits higher resistance to JQ1 treatment. This resistance seems to be correlated with the low expression of IGF1R in the A673 cell line. These findings suggest that the effectiveness of BETi in ES critically depends on disrupting the IGF1 autocrine signaling loop, and that reduced IGF1R levels in A673 cells contribute to their decreased sensitivity to JQ1. Additionally, the study conducted by Loganathan *et al*. noted a significant reduction in colony formation and activation of caspase-3 following treatment in the colony formation assay. The effects on caspase-3 activation, along with tumor growth reduction, were also evaluated in subcutaneous ES xenograft mice treated with JQ1.[p50]

Tomino *et al*., in 2019, investigated the effects of the BETi I-BET151, alone and in combination with the aurora kinase A (AURA) inhibitor alisertib, on ES cell lines.[p52] AURA is crucial for controlling the cell cycle and division, and, in ES, it is a direct

target of EWS–FLI1 transcriptional control and might contribute to c-MYC stabilization. Using three different ES cell lines, they demonstrated that the two drugs synergistically inhibit both cell proliferation and viability across various concentrations. They observed that I-BET151 treatment decreases the expression of genes induced by alisertib, such as AURA, c-MYC, cyclin-dependent kinase (CDC)4, CDK6, BCL2, and myeloid cell leukemia 1 (MCL1), showing that the combination treatment counteracts alisertib-mediated upregulation. At the protein level, I-BET151 results in the downregulation of AURA, CDK4/6, and c-MYC, along with increased caspase-3 cleavage; these effects are even more pronounced when ES cells are treated with both alisertib and I-BET151. To assess the therapeutic efficacy of the combination, mice with subcutaneous ES xenografts (TC71SK-ES and ES2 cells) were treated with alisertib and iBET151, alone or in combination. The combined treatment extended the survival of the mice compared with single treatments, although no tumor regression was observed. Survival improved further when mice received combined alisertib, I-BET151, and the cytotoxic agent vincristine.

The study by Richter *et al.* in 2020 examined the combined effects of JQ1 and CDKI-73, a CDK9i, in ES cell lines as a strategy to overcome the JQ1 resistance that cells developed after several weeks of treatment.[p53] Combined inhibition of BRD4 and CDK9 might synergistically disrupt tumor cell survival by blocking transcriptional regulation, because BRD4 directly interacts with CDK9 to repress apoptosis and promote survival pathways. JQ1 and CDKI-73 both affected cell-cycle progression and apoptosis when used as single agents, but at low doses, they were generally ineffective. In this study, Richter *et al.* demonstrated that CDK9i are still effective in blocking ES cell proliferation even in those cell lines that developed BETi resistance after prolonged JQ1 treatment (A673r, SKNMCr). These findings suggest that CDK9 inhibition can overcome BETi resistance by targeting partially distinct transcriptional pathways. Interestingly, Richter *et al.* also revealed that the combination of BETi and CDK9i at low doses showed strong synergism in inhibiting cellular proliferation. This synergy was also evident in the increased expression of apoptosis markers, such as cleaved caspase-7 and poly (ADP-ribose) polymerase (PARP), after 24 or 48 h of treatment. The study explored the *in vivo* therapeutic potential of combining low-dose JQ1 and CDKI-73 in a subcutaneous ES xenograft mouse model (A673). The results showed that this treatment was more effective at slowing tumor growth compared with control groups and the single-agent treatments. Notably, the effects were comparable with high-dose JQ1 treatment. Although both monotherapies delayed tumor growth, they ultimately resulted in tumor sizes similar to those in the control group. However, in a separate experiment with SKNMC cells, no synergistic effect was observed, likely because of biological differences between the cell lines. Immunohistochemical analysis of tumors revealed increased cleaved caspase-3 expression in treated tumors, indicating enhanced apoptosis, but no significant differences were highlighted among the different treatment groups.

In 2021, Goldin *et al.* examined the effects of dual PI3K/BRD4 inhibitors (SF1126, SF2523, SF2523P) on ES cells *in vitro*.[p54] The inhibitors were tested on A673, EWS502, SK-N-MC, SK-PN-DW, and RDES cell lines, and all the tested drugs effectively suppressed cell viability in a dose-dependent manner, except for RDES cells. The IC_{50} values for SF1126, SF2523, and SF2523P varied across the cell lines, with SF2523 being the most potent. Phosphorylation of the key signaling molecule protein kinase B (AKT), mediated by PI3K, also decreases in a dose-dependent manner following SF2523 and SF1126 treatment. EWS protein expression is affected only after high doses of SF2523. Conversely, SF2523P is not able to reduce phosphorylated (p)-AKT levels, but decreases EWS protein expression in A673 cells. *In vivo*, in intrafemorally injected ES xenograft models (A673), treatment with SF1126 significantly reduced tumor growth and tumor volume. SF1126 was shown to decrease p-AKT and EWS expression in mouse tumors. Additionally, imaging analysis using micro-computed tomography (micro-CT) revealed reduced bone destruction in the SF1126-treated mice compared with controls, suggesting that the drug could mitigate tumor-induced bone damage. Overall, the study demonstrates the efficacy of SF1126 in reducing tumor growth, signaling pathways involved in ES progression, and bone destruction, indicating its potential as a therapeutic agent for ES.

Gastrointestinal stromal tumor (GIST)

Gastrointestinal stromal tumor (GIST) is one of the most frequent mesenchymal tumors.[p55] It originates from interstitial cells of Cajal in the gastrointestinal tract. The incidence of GIST is estimated at 10–20 cases per million people annually.[p56] The pathogenesis of the tumor is mainly linked to activating oncogenic mutations in the KIT [cluster of differentiation (CD)117] gene (3/4 of cases) or the platelet-derived growth factor alpha (PDGFRA) gene, leading to ligand-independent receptor tyrosine kinase (RTK) signaling.[p2],[p56],[p57] Complete surgical resection remains the best treatment option for localized disease, with an overall survival rate of 95%, but options are limited for patients with advanced GIST.[p58] In cases of recurrent or metastatic disease, imatinib, an RTKi, has shown good results, with a survival rate of 75–85% after 2 years of treatment.[p56]

In 2019, Hemming *et al.* first aimed to analyze the epigenetic landscape of KIT and observed that KIT expression is regulated by an SE upstream of the KIT transcription start site.[p59] Using a dead Cas9–Krüppel-associated box dCas9–KRAB system, they showed that GIST cell lines depend on the KIT-associated SE. To mimic sgRNA-mediated repression of SEs, JQ1 was employed to disrupt the oncogene-associated SEs. Indeed, JQ1 treatment reduced KIT protein levels and significantly increased apoptosis in four different KIT-dependent cell lines compared with KIT-independent ones. Further studies examined the effect of JQ1 on the KIT SE, and as expected, after JQ1 treatment, a reduction in H3K27ac at the SE and depletion of BRD4 at the KIT locus were observed. Accordingly, the combination of JQ1 and imatinib was evaluated. Interestingly, this combination showed a synergistic effect, leading to a higher apoptotic response in KIT-dependent cell lines. These findings were also confirmed in a subcutaneous *in vivo* xenograft mouse model (GIST-T1) treated daily with imatinib and/or JQ1, resulting in reduced tumor mass. Moreover, Hemming *et al.* assessed the effects of other BETi (such as iBET151, OTX015, RG6146, iBET762, and RVX-208) on GIST cell lines and found that some cell lines exhibited resistance to RG6146 and MT1. Further investigation into the resistance

mechanism revealed overexpression of the ATP-binding cassette subfamily B member 1 (*ABCB1*) gene, which encodes the efflux pump P-glycoprotein (Pgp). Confirming the role of multidrug resistance and efflux pump upregulation in the mechanism, sensitivity was restored after treatment with tariquidar, a Pgp inhibitor.

Osteosarcoma (OS)

OS, the most common bone malignancy in children, originates from osteoblasts, with an incidence of 2–5 cases per million per year.[p60] Nearly three-quarters of cases occur in individuals between the ages of 15 and 25 years. The standard of care, which involves adjuvant and neoadjuvant chemotherapy combined with surgery, offers a 5-year survival rate of 70% for patients with localized disease, but this drops to 20% when pulmonary metastases are present at diagnosis.[p61],[p62]

Genetic and epigenetic dysregulations play a crucial role in oncogenesis, tumor progression, and metastasis formation in OS. In particular, altered expression of oncogenic drivers, such as c-MYC and runt-related transcription factor 2 (RUNX2), has been shown in several studies to be associated with oncogenesis.[p63] Therefore a therapeutic strategy targeting the altered expression of these genes is one of the most extensively studied treatment approaches. For this reason, researchers assessed the efficacy of BETi to inhibit both tumor development and metastatic processes in OS.

In 2014, Lamoureux *et al.* demonstrated that treatment with a BRD4i could be a promising therapeutic strategy in OS.[p64] They observed for the first time a positive correlation between *c-MYC* and *BRD4* expression in human OS patient samples. However, the limited size of the cohort did not allow any correlations between gene expression and clinical outcome. *c-MYC* and *BRD4* correlation was further evaluated in several patient-derived and murine OS cell lines, highlighting a higher variability. Moreover, Lamoureux *et al.* evaluated the apoptotic effect of JQ1 on several OS cell lines, demonstrating that cell lines with low expression of both *BRD4* and *c-MYC* were the most resistant, whereas cells with high expression of *BRD4* and *c-MYC* were the most sensitive. To confirm the correlation between BRD4 inhibition and oncogenic driver regulation, BRD4 release from SE was assessed after JQ1 treatment, leading to *c-MYC* downregulation. Later, the treatment effectiveness was assessed *in vivo* using two different models: a human xenograft derived from the MNNG/HOS cell line inoculated by an intramuscular injection in athymic mice, and a syngeneic model generated with the POS-1 cell line, subcutaneously implanted into C3H mice. In these models, mice treated with JQ1 exhibited reduced tumor size, increased survival compared with control mice, and alleviation of bone deficiencies caused by the tumor. Simultaneously, *in vivo* models were used to validate the potential of JQ1 to block osteoclastogenesis and osteoblastogenesis without adverse effects on healthy tissues.

In 2017, Wu *et al.* evaluated the potential of generating a carrier to deliver JQ1, aiming to extend its half-life and lower its associated toxicity. Unexpectedly, ionic hydroxyapatite demonstrated a limited loading efficiency for hydrophobic JQ1 (0.624 nM).[p65] However, bisphosphonate-functionalized hydroxyapatite nanoparticles carrying 0.624 nM of JQ1 showed an effect comparable with 1 μM of JQ1 alone at 72 h because of enhanced uptake and sustained drug release. Additionally, this system decreases the viability of differentiated OS cells, can induce necrosis in OS cells, and reduces the migration and invasion of OS spheroids.

In 2018, Morrow *et al.* studied the differences between primary and metastatic tumors.[p66] They identified chromatin regions with increased or decreased histone H3 lysine 4 monomethylation (H3K4me1) and concomitant alteration of histone H3 lysine 27 acetylation (H3K27ac), called metastatic variant enhancer loci (Met-VELs). Therefore considering that gained Met-VLs exhibit H3K27ac enrichment, BETi were evaluated as a therapeutic option. Treatment with JQ1 demonstrated a tumor cell-specific antiproliferative effect associated with downregulation of genes regulated by Met-VEL(s), highlighting the potential to use JQ1 to block the expression of Met-VEL(s) and thereby inhibit the metastatic process in OS.

In the same year, Chen *et al.* studied the role of c-MYC in OS and identified a correlation between *c-MYC* expression, poor prognosis, and metastasis formation.[p67] Furthermore, they found that c-MYC binds to SEs that regulate genes involved in epithelial–mesenchymal transition and the tumor metastasis process. In this context, they treated OS cell lines with JQ1 and observed an apoptotic effect, along with a significant reduction in the migration and invasion capacity of the treated cells.

Considering the need for a new model to understand tumor mechanisms better and identify new therapeutic targets, in 2022, Pandaya *et al.* established PDXs from patients with OS and RMS at diagnosis and after treatment. These PDXs were used to validate the efficiency of a novel approach that integrates DNA, RNA, protein, and pathway enrichment analysis. Results from comparing OS PDXs at diagnosis and after several passages revealed a copy number gain/amplification of *c-MYC*, also confirmed by increased protein levels. Based on this, the mouse model generated with OS PDX HT96 and treated with OTX015 showed a reduction in tumor size compared with the control, without significant weight loss associated with the treatment. Meanwhile, analysis of the other RMS PDXs they examined showed no changes in *c-MYC* expression over time that would support treatment with a specific compound.[p68]

In 2024, Wu *et al.* also studied BRD4 expression on tissue microarrays generated from OS patient samples, demonstrating that BRD4 protein levels in the nucleus were higher in OS samples compared with paracancerous normal tissues.[p69] Considering this, they compared the effects of three BETi: JQ1, a pan-BETi, and MZ1 and GNE-987, two PROTACs based on von Hippel–Lindau (VHL) tumor suppressor, on HOS and 143B cell lines. The results highlighted that cells treated with GNE-987 had a lower IC_{50} value than those treated with other inhibitors. Moreover, they showed that GNE-987 exhibits higher cytotoxicity and better proliferation inhibition. Wu *et al.* investigated the molecular mechanism behind the antitumor effects of GNE-987. They found that the VHL tumor suppressor is critical for GNE-987 to induce BRD4 degradation and decrease H3K27ac levels on SEs that regulate tumorigenesis-related genes in OS, such as KRT80, which is downregulated following GNE-987 treatment. These results were also validated in an *in vivo* model created by subcutaneously inoculating HOS cells and treating

them with GNE-987 every 2 days. The results also indicated that treatment with GNE-987 showed no toxicity.

Rhabdomyosarcoma (RMS)

RMS is the most common pediatric soft tissue sarcoma (STS), accounting for 5% of malignant solid tumors in children. RMS originates from mesenchymal cells and is linked to skeletal muscle lineage. Recently, this tumor has been classified into two molecular subtypes: FP-RMS, characterized by *PAX3/7:FOXO1* translocation and associated with poor prognosis, and fusion-negative (FN-RMS), which lacks any fusion gene but features alterations in the RAS pathway.[p70],[p71] Although conventional therapies offer a good survival rate in localized disease (70%), patients with metastasis are often resistant to treatment or relapse within the first 5 years of treatment.[p9],[p72]

The sensitivity of several RMS cell lines to JQ1 treatment was first assessed in 2016 by Bid *et al.*, demonstrating that RMS cell lines can respond heterogeneously to JQ1.[p49] They also confirmed that JQ1 treatment induces cell-cycle arrest in the G0/G1 phase, with a corresponding decrease in cells in the S phase. Interestingly, unlike the results seen in ES, it was noted that JQ1 reduces MYCN expression in FP-RMS cell lines. Additionally, as previously shown in ES, the impairment of angiogenesis caused by JQ1 treatment was also confirmed in RMS xenograft models generated with RH10 and RH28 cells subjected to JQ1.

Later, in 2017, Gryder *et al*. studied the role of the fusion protein P3F in the epigenetic dysregulation of FP-RMS.[p25] They demonstrated that P3F is a pioneering transcription factor, as it not only recognizes specific SEs but also recruits BRD4 to specific chromatin sites. This results in conformational changes, chromatin opening, and the recruitment of coactivators necessary for activating transcription. Additionally, working with other master transcription factors such as myogenic differentiation 1 (MYOD), myogenin (MYOG), and MYCN, which are upregulated in FP-RMS, they create autocrine loops that regulate the expression of specific oncogenes. These findings suggest that BETi could be a promising therapeutic approach to block P3F and BRD4 activity in FP-RMS. JQ1 and the clinical analog OTX015 were the most effective BETi tested on several FP-RMS and FN-RMS cell lines, showing lower IC_{50} values in FP-RMS regardless of MYCN amplification status. The *in vitro* results of JQ1 treatment were confirmed in an orthotopic xenograft *in vivo* model (RH4 cells).

Given the involvement of histone deacetylases (HDACs) in the oncogenic process in several tumors, in 2018, HDAC inhibitors were tested as potential compounds to use in combination with JQ1 on RMS cell lines. Enßle *et al*. used JQ1 in combination with various HDAC inhibitors, such as quisinostat (JNJ-26481585), vorinostat (SAHA), and panobinostat (LBH589), all pan-HDAC inhibitors, and entinostat (MS-275), a class-I HDAC inhibitor, on both FP-RMS and FN-RMS cell lines.[p73] Based on the IC50s obtained from the analyzed combinations, they selected JNJ-26481585 (10 nM) and JQ1 (1 μM) as the best combination capable of synergistically inducing apoptosis. This combination increases the expression of the pro-apoptotic proteins BCL2-associated X protein (BAX) and BCL2-antagonist killer (BAK), decreases the anti-apoptotic B-cell lymphoma-extra large (BCL-xL), and affects external mitochondrial membrane permeabilization. Additionally, it was observed that the overexpression of BCL-2 and MCL-1 could induce resistance in RMS cell lines, confirming the role of the pro- and anti-apoptotic BCL2 family proteins in the response to the combination therapy.

Similarly, in 2020, Boedicker *et al*. evaluated JQ1 in combination with the p110α-isoform-specific PI3K inhibitor BYL719 (alpelisib). They observed that BETi downregulates the hedgehog pathway,[p74] as previously demonstrated for PI3Ki,[p75] thereby suggesting a potential synergistic effect on apoptosis in both FP- and FN-RMS cell lines. The study of the molecular mechanism underlying the antitumor effect revealed that the combination treatment caused MYC to decrease and FOXO3a to increase. Specifically, the reduction of FOXO3a phosphorylation promoted the transcription of pro-apoptotic BCL2 family proteins, leading to cell death.

In 2020, Camero *et al*. investigated the molecular mechanisms associated with OTX015 treatment in FP-RMS, demonstrating its ability to reduce tumor cell proliferation and migration while downregulating pro-stemness genes [e.g. guanine nucleotide binding protein-like 3 (*GNL-3*)] and *c-MYC* in FP-RMS.[p76] OTX015 induces apoptosis by suppressing BCL2 and upregulating phosphatase and tensin homology (PTEN), thereby inhibiting AKT activation. Additionally, OTX015 impairs the DNA damage repair mechanism, as evidenced by increased gamma-H2A histone family member X (γH2AX) and decreased RAD51 expression levels. The study also highlighted the role of miR-124-3p in enhancing OTX015-induced DNA damage and apoptosis in FP-RMS cells. Furthermore, its combination with irradiation (IR) (4 Gy) led to increased γH2AX accumulation, further decreased RAD51, reduced colony formation, and proliferation, and caused cell-cycle arrest in the G2 phase, demonstrating its potential to sensitize cells to IR-induced double-strand break accumulation and cell death.

To gain insight into off-target effects and resistance related to JQ1 monotherapy, in 2020, Laszig *et al*. tested the dual BET/HDAC inhibitor TW09, a dual inhibitor based on JQ1 and the class-I HDAC inhibitor CI994.[p77] They observed that TW09 reduces cell viability, impairs clonogenic survival, and induces dose-dependent cell death in both FP- and FN-RMS cells. Similar to co-inhibition with JQ1 and MS-275, TW09 downregulates BET and HDAC target proteins, including c-MYC, and decreases H3 acetylation, while inducing comparable levels of cell death at equal molar concentrations. Mechanistically, TW09 promotes a pro-apoptotic shift in BCL2 family proteins, leading to BAK and BAX activation and caspase-dependent apoptosis. Notably, genetic silencing of apoptotic regulators, BCL2 overexpression, or caspase inhibition can rescue TW09-induced cell death. These findings suggest that TW09 effectively triggers the intrinsic pathway of apoptosis and could be a promising therapeutic approach for RMS treatment.

In 2022, Erdogdu *et al*. validated another promising approach involving JQ1 and BCL2 homology domains 3 (BH3) mimetics.[p78] Among all the BH3 inhibitors, the BCL-XL inhibitor A-1331852 and the MCL-1 inhibitor S63845 were selected for this study. The results showed that the combination of BH3 mimetics and JQ1 increased caspase-dependent apoptosis compared with single treatments by rebalancing BCL2 protein expression and activating BAX and BAK.

Marchesi *et al.*, in the same year, demonstrated that JQ1 treatment induces G0/G1 cell-cycle arrest and triggers apoptosis in RMS cell lines.[p10] To better predict potential *in vivo* efficacy, spheroids generated with A204 or RH4 cells were also used as models. Notably, their findings also suggest that BETi efficacy correlates with c-MYC expression levels, but no correlation was found between c-MYC levels and P3F fusion status or RMS subtypes.

In 2022, Srivastava *et al.* evaluated a combinatorial approach using the BETi RVX-207 or ABBV-744 with the dual mechanistic target of rapamycin complex (mTORC)1/2 inhibitors OSI-027 or PP242.[p79] The combination synergistically reduced colony formation and spheroid size at selected concentrations. Interestingly, the combinatorial approach with RVX-2017 and OSI-027 induced a stronger reduction of proteins involved in the AKT/mTOR signaling pathway. Supporting this result, ChIP-qPCR results showed a decrease in H3K27ac and H4K8ac in the promoter regions of genes related to mTOR. Additionally, the combination promoted necroptosis-mediated cell death. These findings were validated *in vivo* using xenograft mice treated with RVX-207, PP242, or OSI-027, which also showed a reduction in tumor size following the combination approach.

In 2023, Shackleford *et al.* tested BETi as a treatment to overcome IGF1R-antibody resistance, whether intrinsic or acquired, in RMS cell lines.[p80] For the first time, they identified overactivation of the RTK pathway as the molecular mechanism underlying the acquired and intrinsic resistance to IGF1R. Considering that the involvement of bromodomain readers in this mechanism has been previously shown,[p81] the combination of JQ1 and/or LY2874455 [pan-fibroblast growth factor receptor (FGFR) inhibitor] and TZ1 (an antagonist of IGF-1) was tested on RMS cell lines. The combined treatment results showed downregulation of the RTK target, except for FGFR2, and an increase in apoptosis reaching up to 71% when using all three compounds together.

Das *et al.* investigated BRD4 isoforms and their roles in RMS, showing that BRD4 long isoform (BRD4-L) and BRD4 short isoform (BRD4-S) are upregulated in BRD4-positive RMS samples.[p82] They found that BRD4-L promotes apoptosis and inhibits myogenic differentiation, a process regulated by BRD4-S. Notably, BRD4-S can counteract BRD4-L activity in FN-RMS, but not in FP-RMS, indicating an isoform-specific regulatory mechanism different from other tumor models. They also tested the pan-BRD4 inhibitor JQ1 on RD cells, noting decreased proliferation and increased myogenic differentiation, shown by higher MYOG and MyHC levels after treatment. However, JQ1 unexpectedly enhanced cell migration and invasion, suggesting possible adverse effects of pan-BRD4 inhibition. These effects might be caused by the simultaneous inhibition of BRD2 and BRD3, which could have opposing roles, or by disruption of the antagonistic functions of BRD4 isoforms. Overall, their results highlight the importance of understanding the precise role of the BRD4 isoform to improve BRD4-targeted therapies in RMS.

Synovial Sarcoma (SS)

SS is a high-grade STS accounting for 5–10% of all STS cases.[p83] Originating from mesenchymal cells, SS can develop in various parts of the body.[p84] It is commonly associated with the t (X:18) translocation (present in 90–95% of cases), which produces the synovial sarcoma translocation (SS18)–synovial sarcoma (SSX) fusion protein.[p85] This protein interacts with the chromatin remodeling switch/sucrose non-fermentable (SWI/SNF) BAF complex, influencing gene expression. Complete surgical resection is the most effective treatment for localized SS, whereas neoadjuvant chemotherapy and adjuvant radiotherapy can be considered based on tumor location, size, and subtype. For advanced disease, treatment typically includes doxorubicin combined with ifosfamide; however, overall survival rates remain poor.[p83]

In 2024, Kotani *et al.* demonstrated that ABBV-075 has a more potent antitumor effect on SS cell lines compared with JQ1.[p86] Further analysis revealed G0/G1 cell-cycle accumulation and a decrease in the anti-apoptotic BCL-xL, along with an increase in the pro-apoptotic BIM, following ABBV-075 treatment. The antitumor effect of ABBV-075 was also confirmed *in vivo* using subcutaneous xenografts generated with SYO-1 cell lines, showing a reduction in tumor volume in the ABBV-075-treated group compared with the vehicle group. Additionally, Kotani *et al.* showed that the fusion protein SS18–SSX influences sensitivity to ABBV-075 through the intrinsic apoptotic pathway.

Discussion

This systematic review highlights the growing interest and preclinical success of BET inhibition in pediatric sarcomas. A key finding is the consistent downregulation of oncogenic transcription factors such as EWS–FLI1, c-MYC, P3F, and tumor-promoting pathways across various sarcoma types.[p4],[p5] Unfortunately, the therapeutic effectiveness is inconsistent. JQ1, the most studied BETi, shows the broadest activity spectrum, but suffers from suboptimal pharmacokinetics.[p29] More selective BD2 inhibitors, like ABBV-744 or PROTACs such as GNE-987, demonstrate greater potency in some models and offer a promising therapeutic option for overcoming resistance mechanisms.[p69]

Resistance to BETi can be intrinsic or acquired through adaptive mechanisms. Although the resistance mechanisms of BETi are not yet fully understood, some known mechanisms include dysregulation of PI3K/AKT signaling, increased Pgp-mediated drug efflux, kinome reprogramming, and overactivation of RSK3, a member of the p90 ribosomal S6 kinase family, caused by miR-548d-3p loss.[p53],[p59],[p87] Preclinical data demonstrate that targeting these pathways via combination therapies can overcome BETi resistance. Indeed, JQ1 and imatinib co-treatment reverses BETi resistance in GIST,[p59] whereas combining CDK9i and JQ1 restores sensitivity and enhances apoptosis in ES.[p53]

Additional promising combination strategies have also been investigated to work synergistically with BETi and boost their ability to induce apoptosis. These include pan-HDACi such as quisinostat (JNJ-26481585), vorinostat (SAHA), and panobinostat (LBH589), as well as the class-I selective HDACi entinostat (MS-275) and the dual BET/HDAC inhibitor TW09. Other approaches involve mTORC1/2i like OSI-027 and PP242, PI3Ki, and triple-agent regimens that combine JQ1 with FGFR inhibitors (e.g. LY2874455) and IGF1R antagonists (e.g. TZ1).[p77],[p79],[p80],[p81] These findings highlight the potential of BETi-based combination therapies to enhance antitumor efficacy while

mitigating side effects by enabling dose reduction, supporting their continued clinical development. The response to BETi varies significantly among and within sarcoma types. For instance, high BRD4 and c-MYC expression levels are associated with sensitivity in OS, whereas P3F-driven FP-RMS shows greater sensitivity compared with FN-RMS. Despite encouraging laboratory results, translation of this approach into clinical practice remains limited.[p10],[p25] Indeed, all the extracted data rely solely on cell lines cultured in *in vitro* and *in vivo* xenografted models, which lack the complexity of human pediatric sarcoma. Thus knowledge about BETi efficacy should be deepened through the use of a more reliable model that includes multicellular complexity, such as patient-derived organoids or humanized mouse models. Major challenges include BETi-related toxicity, a lack of pediatric-specific pharmacokinetic data, and the absence of validated biomarkers to predict treatment response. Additionally, most clinical trials exploring BETi are still primarily focused on adult cancers, with minimal inclusion of pediatric cohorts, partly because of ethical concerns.

To address these gaps, future research should focus on developing isoform-selective and next-generation BETi agents, which could potentially provide improved efficacy and safety. To this end, improving BETi efficacy through the development of new potent BET PROTACs could pave the way for more efficacious and well-tolerated therapies. These molecules, by linking an E3 ligase recognition ligand to a BET-targeting small molecule, promote specific proteasome-dependent degradation of BET proteins, exponentially increasing BETi potency and efficacy, leading to the possibility of reducing therapeutic doses and limiting toxic adverse effects. Furthermore, selective targeting of the BD1 or BD2 domain is an emerging avenue that can reduce off-target effects and related toxicity compared with pan-BETi. Accordingly, the development of innovative peptide inhibitors targeting understudied domains, like the ET domain, is a promising avenue for overcoming current limitations.[p88] Analyzing patients based on their molecular profiles, such as fusion status and MYC amplification, is essential for a targeted and personalized medicine approach, as is considering combining BETi with immunotherapy or agents that target DNA-damage response pathways. Ultimately, pediatric-specific early-phase clinical trials with comprehensive pharmacodynamic and pharmacokinetic monitoring are crucial for advancing BETi in the treatment of pediatric sarcomas.

Clinical perspective

Although preclinical data are promising, translation of BETi at the pediatric patient's bedside has been limited. A few early-phase trials are investigating BETi in adults and young adults with solid tumors or hematologic malignancies. Pediatric participation in BETi research remains limited. To date, eight clinical trials including pediatric patients have been initiated: two are currently recruiting, three have been completed, two were withdrawn, and one is no longer available (Table 3). These studies often involve pediatric patients from the earliest phases, typically within basket or umbrella trial designs that encompass multiple age groups, allowing earlier access but potentially limiting the depth of pediatric-specific data. Thus improving pediatric enrollment through basket trials with age-stratified cohorts could address the current lack of age-adjusted formulations and the related ethical concerns over toxicity testing in children, by integrating pediatric and adult data while ensuring dose safety.

TABLE 3

Summary of BET inhibitors in pediatric clinical trials.

NCT Number	Study status	Age	Phases	Interventions	Conditions	Response rate
NCT05301972	No longer available	Child, adult, older adult	–	–	–	NA
NCT03936465	Completed	Child, adult	I	BMS-986158 BMS-986378	Solid tumor, lymphoma Brain tumor	NA NA
NCT05372640	Recruiting	Child, adult, older adult	I	ZEN-3694 + abemaciclib	Breast carcinoma, malignant solid neoplasm	NA
NCT03925428	Withdrawn	Child, adult, older adult	I	GSK525762C + entinostat	Advanced lymphomas, advanced malignant solid neoplasm, refractory malignant solid neoplasm, refractory lymphomas	NA
NCT02419417	Completed	Child, adult, older adult	I/II	BMS-986158 ± nivolumab	Advanced tumors	CR 0% PR 2.4% SD 28.9% PD 51.8%
NCT01587703	Completed	Child, adult, older adult	I	GSK525762	Carcinoma, midline	CR 0% PR 2% SD 34% PD 41%
NCT05019716	Recruiting	Child, adult, older adult	I/II	ZEN003694 + etoposide + cisplatin	Advanced NUT carcinoma, metastatic NUT carcinoma, unresectable NUT carcinoma	NA
NCT04116359	Withdrawn	Child, adult, older adult	I/II	Molibresib ± etoposide + cisplatin	Metastatic NUT carcinoma, unresectable NUT carcinoma	NA

CR, complete response; NA, no data available; NUT, nuclear protein in testis; PD, progressive disease; PR, partial response; SD, stable disease.

Furthermore, the challenges in preclinical research aimed at discovering and developing new treatments for pediatric sarcoma are complex. Key issues include a lack of robust data from patient-derived *ex vivo* models that accurately reflect the disease. This aspect, caused by the rarity of these pediatric sarcomas, strongly limits studies on drug effectiveness, absorption, distribution, metabolism, and action in relevant pediatric models.[p89] Furthermore, there is a notable deficiency in research exploring how new compounds work alongside existing standard therapies, which complicates their integration into current treatment protocols. Thus preclinical findings frequently struggle to translate effectively into clinical outcomes, particularly in pediatric patients. As a result, pediatric patients might experience unexpected toxicities or side effects not predicted by adult data or preclinical models. The challenge of tailoring dose and timing specifically for a child's developing body often leads to safety concerns and limits the effectiveness of therapies that initially showed promise in preclinical studies.[p89] Focused efforts on increasing pediatric enrollment, optimizing dosing strategies, and developing predictive biomarkers are crucial for advancing the use of BETi in pediatric sarcoma treatment.

Concluding remarks

BET proteins, especially BRD4, are crucial for maintaining an oncogenic transcriptional profile in pediatric sarcomas. BETi demonstrate remarkable antitumor effects in both laboratory and animal studies, particularly in fusion-driven sarcomas such as ES and FP-RMS. However, variability in response and the development of resistance emphasize the importance of biomarker-guided strategies. Our systematic review provides, for the first time, a comprehensive integration of data specifically obtained in pediatric sarcoma on the use of BETi as a promising strategy to improve outcomes for pediatric patients. The literature supports the hypothesis that BET proteins are valuable pharmacological targets in these pediatric rare tumors, but the efficacy and safety of these inhibitors need further exploration and improvement. To this end, the most promising avenue could be the further development of new next-generation agents such as PROTACs. Future research should focus on translating isoform-selective BETi into clinical practice, exploring rational combination therapies, and designing pediatric-specific trials.

Declarations of interest

No interests are declared.

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CRediT authorship contribution statement

Erika Ferraro: Writing – original draft, Visualization. **Elisa Macrì:** Writing – original draft, Visualization. **Clemens Zwergel:** Resources, Formal analysis, Data curation, Funding acquisition. **Chiara Lambona:** Resources, Formal analysis, Data curation. **Giovanni Barillari:** Resources, Formal analysis, Data curation. **Cinzia Marchese:** Resources, Formal analysis, Data curation. **Franco Locatelli:** Resources, Formal analysis, Data curation. **Rossella Rota:** Resources, Formal analysis, Data curation. **Matteo Cassandri**: Writing – review & editing, Funding acquisition, Conceptualization. **Silvia Pomella:** Writing – review & editing, Funding acquisition, Conceptualization.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.drudis.2025.104516.

Data availability

Data will be made available on request.

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