

# Pediatric sarcomas: translating molecular pathogenesis of disease to novel therapeutic possibilities

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Pediatric sarcomas represent a diverse group of rare bone and soft tissue malignancies. Although the molecular mechanisms that propel the development of these cancers are not well understood, identification of tumor-specific translocations in many sarcomas has provided significant insight into their tumorigenesis. Each fusion protein resulting from these chromosomal translocations is thought to act as a driving force in the tumor, either as an aberrant transcription factor (TF), constitutively active growth factor, or ligand-independent receptor tyrosine kinase. Identification of transcriptional targets or signaling pathways modulated by these oncogenic fusions has led to the discovery of potential therapeutic targets. Some of these targets have shown considerable promise in preclinical models and are currently being tested in clinical trials. This review summarizes the molecular pathology of a subset of pediatric sarcomas with tumor-associated translocations and how increased understanding at the molecular level is being translated to novel therapeutic advances.

**S**arcomas are a rare, heterogeneous group of neoplasms presumed to be of mesenchymal origin. Tumors can arise in bone or soft tissues such as muscle and fat and can develop anywhere in the body. They account for only 1% of all malignancies, and although the incidence of malignancy is higher in adults, sarcomas occur with higher frequency in children. Each year, between 1,500 and 1,600 children and young adults in the United States develop these malignant bone and soft tissue tumors comprising ~13% of cancers afflicting patients below the age of 20 (1,2). The overall 5-year survival rate for pediatric sarcomas is ~60% and falls closer to 20–30% for recurrent and metastatic cases despite aggressive surgery, multiagent chemotherapy, and/or radiation.

Sarcomas can be divided into two groups based on the underlying molecular events that initiate tumorigenesis. The first group is characterized by the presence of specific chromosomal translocations (Table 1) or activating mutations whereas the second is more cytogenetically complex. Sarcomas with complex karyotypes predominantly afflict older patients and translocations tend to be observed with higher frequency in pediatric cases. Pediatric sarcomas with tumor-associated

translocations include Ewing sarcoma (ES), rhabdomyosarcoma (RMS), synovial sarcoma (SS), and dermatofibrosarcoma protuberans (DFSP). The genetic aberrations in these neoplasms produce defined fusions that are critical for sarcomagenesis. Depending on the genes involved in the fusion, the resulting protein can promote tumor progression through transcriptional modulation, epigenetic modifications, or activation of oncogenic signaling pathways.

Due to the rarity of sarcomas, especially when considering the prevalence of individual subtypes, they are understudied cancers. As the fusion proteins present within translocation-associated sarcomas are inherent to tumor development, they provide an avenue of research for development of improved and targeted treatments. This review focuses on four pediatric sarcomas with tumor-associated translocations and will discuss the molecular genetics of these malignancies, potential therapeutic targets, and the status of agents directed against these targets in clinical trials.

## ES

ES is part of a group of bone and soft tissue small-round blue cell malignancies that predominantly occur within the second decade of life. ES is the second most common bone malignancy in children and adolescents, with an annual incidence of 2.93 per million in the United States (3); it accounts for 3% of pediatric cancers. The 5-year survival rate is nearing 70%, although this number shrinks to ~20–30% in patients with metastatic, refractory, and/or recurrent disease (4). The current treatment of ES is multimodal consisting of intensive multiagent chemotherapy, surgery, and/or high-dose radiation therapy. The standard systemic chemotherapeutic regimen includes cycles of vincristine, cyclophosphamide, and doxorubicin with ifosfamide and etoposide.

ES occurs primarily as tumors of the bone, with less than 15% of cases arising in extraosseous locations (5). ES primary osseous sites are split between the extremities and the central axis, with an increased tendency for incidence in the shaft of long tubular bones, pelvis, and rib. The histogenesis of ES has long been disputed, as evidence has been provided for both a neural crest and mesenchymal stem cell origin.

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**Table 1.** Sarcomas with defined chromosomal translocations

Tumor type	Translocation	Fusion
ES	t(11;22)(q24;q12)	<i>EWS-FLI1</i>
	t(21;22)(q22;q12)	<i>EWS-ERG</i>
	t(7;22)(p22;q12)	<i>EWS-ETV1</i>
	t(17;22)(q21;q12)	<i>EWS-ETV4</i>
	t(2;22)(q33;q12)	<i>EWS-FEV</i>
	t(16;21)(p11;q22)	<i>FUS-ERG</i>
	t(2;16)(q35;p11)	<i>FUS-FEV</i>
	t(1;22)(p36.1;q12)	<i>EWS-ZSG</i>
	t(20;22)(q13;q12)	<i>EWS-NFATc2</i>
	ES-like tumors (CD-99 negative)	t(6;22)(p21;q12)
t(1;22)(q36.1;q12)		<i>EWS-PATZ1</i>
t(2;22)(q31;q12)		<i>EWS-SP3</i>
t(4;19)(q35;q13)		<i>CIC-DUX4</i>
Clear-cell sarcoma	t(12;22)(p13;q12)	<i>EWS-ATF1</i>
	t(2;22)(q33;q12)	<i>EWS-BREB1</i>
Desmoplastic small-round-cell tumor	t(11;22)(p13;q12)	<i>EWS-WT1</i>
	t(21;22)(q22;q12)	<i>EWS-ERG</i>
Myxoid liposarcoma	t(12;16)(q13;q11)	<i>FUS-DDIT3</i>
	t(12;22)(q13;q12)	<i>EWS-DDIT3</i>
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22-31;q11-12)	<i>EWS-NR4A3</i>
	t(9;17)(q22;q11)	<i>TAF15-NR4A3</i>
	t(9;15)(q22;q21)	<i>TCF12-NR4A3</i>
	t(9;22)(q22;q15)	<i>TFG-NR4A3</i>
Low-grade fibromyxoid sarcoma	t(7;16)(q33;p11)	<i>FUS-CREB3L2</i>
	t(11;16)(p11;p11)	<i>FUS-CREB3L1</i>
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11)	<i>FUS-ATF1</i>
	t(12;22)(q13;q12)	<i>EWS-ATF1</i>
	t(2;22)(q33;q12)	<i>EWS-CREB1</i>
Alveolar rhabdomyosarcoma	t(2;13)(q35;q14)	<i>PAX3-FOXO1</i>
	t(1;13)(q36;q14)	<i>PAX7-FOXO1</i>
	t(2;2)(p23;q35)	<i>PAX3-NCOA1</i>
	t(2;8)(q35;q13)	<i>PAX3-NCOA2</i>
	t(8;13;9)(p11.2;q14;9q32)	<i>FGFR1-FOXO1</i>
Alveolar soft part sarcoma	t(X;17)(p11;q25)	<i>ASPSL-TFE3</i>
Congenital fibrosarcoma	t(12;15)(p13;q25)	<i>ETV6-NTRK3</i>
Congenital mesoblastic nephroma	t(12;15)(p13;q25)	<i>ETV6-NTRK3</i>
Inflammatory myofibroblastic tumor	t(1;2)(q25;q23)	<i>TPM3-ALK</i>
	t(2;19)(q23;q13)	<i>TPM4-ALK</i>
	t(2;17)(q23;q23)	<i>CLTC-ALK</i>
	t(2;2)(p23;q13)	<i>RANBP2-ALK</i>
Synovial sarcoma	t(X;18)(p11;q11)	<i>SS18-SSX1</i>
	t(X;18)(p11;q11)	<i>SS18-SSX2</i>
	t(X;18)(p11;q13)	<i>SS18-SSX4</i>
	t(X;20)(p11;q13)	<i>SS18L1-SSX1</i>
Endometrial sarcoma	t(7;17)(p15;q21)	<i>JAZF1-SUZ12</i>
	t(6;7)(p21;p15)	<i>JAZF1-PHF1</i>
	t(6;10)(p21;p11)	<i>EPC1-PHF1</i>
Dermatofibrosarcoma protuberans	t(17;22)(q22;q13)	<i>COL1A1-PDGFB</i>
Giant cell fibroblastoma	t(17;22)(q22;q13)	<i>COL1A1-PDGFB</i>

ES, Ewing sarcoma.

### Molecular genetics

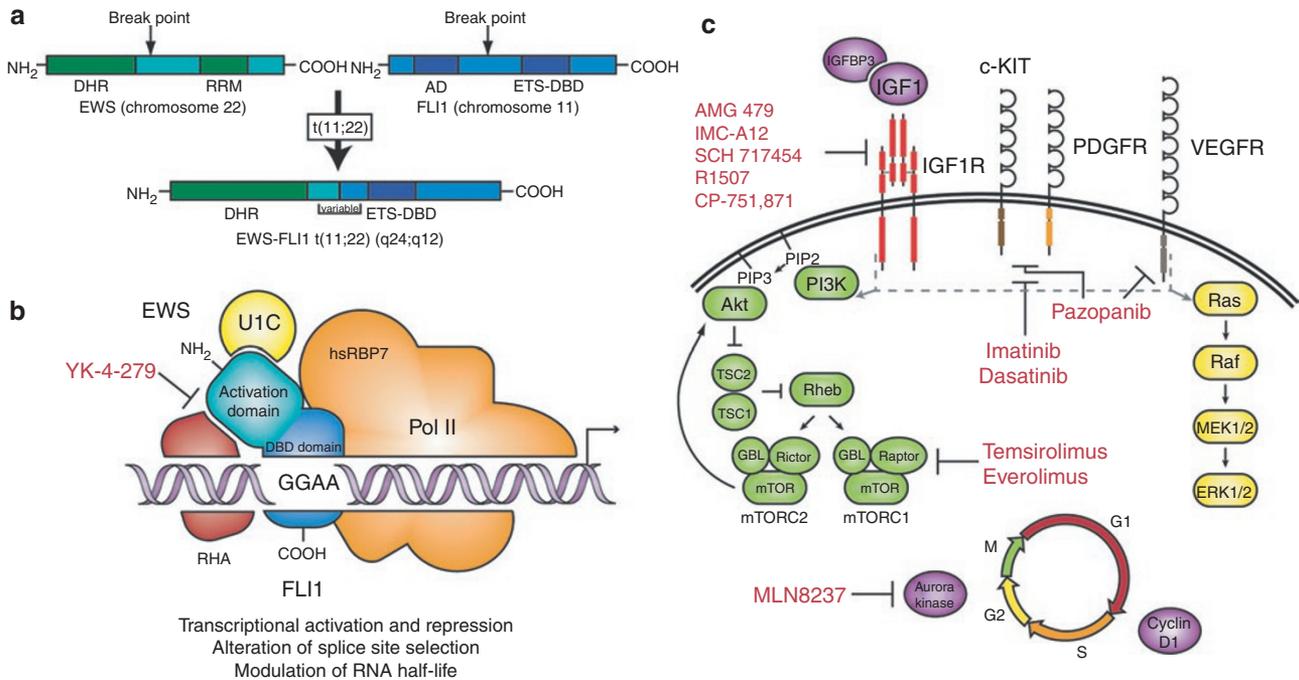
The pathognomonic genetic aberration in ES fuses the *EWS* gene (also known as *EWSR1*, ES break point region 1) to one of five ETS (erythroblast transformation-specific) transcription factors (TFs) (**Table 1**). *FLI1* (Friend leukemia virus integration 1) is the fusion partner in ~85% of cases and *ERG* (v-ets erythroblastosis virus E26 oncogene homolog) in about 10%, whereas others each account for less than 1% (6). In very rare cases, *FUS* combines with *ERG* or *FEV* (ETS oncogene family), and *EWS* is juxtaposed to non-ETS genes (7) (**Table 1**). Adding to the complexity of multiple translocations, variation in the location of the chromosomal break point results in numerous types of each fusion. Initial reports suggested *EWS-FLI1* type 1 fusions confer a prognostic advantage to patients with localized disease, but more recent studies have demonstrated there is no difference in clinical outcome based on fusion type (8).

The most common *EWS-ETS* fusion, *EWS-FLI1*, is generated from the t(11;22)(q24;q12) reciprocal translocation that combines the N-terminal *EWS* activation domain and the C-terminal ETS DNA-binding domain of *FLI1* (9) (**Figure 1a**). *EWS* is a member of the FET (*FUS*, *EWS*, and *TAF15*) family of proteins, which may be involved in transcription and mRNA splicing as they contain both an activation and RNA-binding domain (10). The ETS family constitutes a group of 30 TFs characterized by the presence of a highly conserved ETS domain that mediates site-specific DNA binding. They also possess either an activation or a repression domain and are involved in various cellular processes such as cell proliferation and differentiation (11). Because the more potent *EWS* activation domain replaces that of *FLI1* while the *FLI1* DNA-binding domain remains intact, *EWS-FLI1* is thought to primarily act as an aberrant TF. As most ETS TFs bind to a core consensus (GGAA/T), *EWS/FLI1* has also been shown to interact with DNA in a site-specific manner through association with GGAA microsatellites (12). Interactions with splicing factors and the ability to alter splice-site selection suggest *EWS-FLI1* also plays a role in RNA splicing (13,14). The capacity of *EWS-FLI1* to function post-transcriptionally is further demonstrated by modulation of target gene RNA half-life (15) (**Figure 1b**).

### Target genes and targeted therapies

Ectopic expression of *EWS-FLI1* in heterologous cell types or small interfering RNA-mediated knockdown of the fusion in ES cell lines have both been used to discover potential target genes. These studies have identified both up- and downregulated genes, demonstrating the function of *EWS-FLI1* as both a transcriptional activator and a repressor. Multiple direct targets have been confirmed through demonstration of *EWS-FLI1* binding to their promoters including *insulin-like growth factor (IGF) binding protein 3* (16) and the *Aurora A* and *B kinases* (17).

Repression of IGF binding protein 3 by *EWS-FLI1* is one of the multiple connections between the IGF pathway and ES pathogenesis. In addition to targeting IGF binding protein 3, *EWS-FLI1* has been shown to upregulate IGF1 in mesenchymal progenitor cells (18). In addition, ES cell lines ubiquitously



**Figure 1.** Molecular genetics and targeted therapies in ES. (a) Schematic of EWS-FLI1 t(11;22)(q24;q12) translocation. The EWS-FLI1 fusion includes the N-terminal activation domain of EWS, which contains multiple degenerate hexapeptide repeats (consensus SYGQQS), and the C-terminal ETS DNA-binding domain (ETS-DBD) of FLI1. The RNA recognition motif (RRM) of EWS and the activation domain (AD) of FLI1 are not retained in the fusion. Variation in the sites of chromosomal break points leads to multiple fusion types (bracketed region). (b) Putative molecular function of the EWS-FLI1 protein and selected protein-protein interactions. As an aberrant transcription factor, EWS-FLI1 regulates genes in part by binding to GGAA microsatellites upstream of target genes. EWS-FLI1 has been shown to interact with the splicing factor U1C (also known as SNRPC, small nuclear ribonucleoprotein polypeptide c), RNA helicase A (RHA), and the hRBP7 subunit of RNA polymerase II (Pol II), which links the protein to splicing and transcription. The small molecule that blocks the EWS-FLI1-RHA interaction is indicated in red. (c) Signaling pathways and targeted therapies in ES. EWS-FLI1 modulates IGF1R, PDGFR, c-KIT, and VEGFR leads to downstream signaling through the PI3K and MAPK pathways (indicated by gray dashed line and arrows). EWS-FLI1 upregulates Aurora kinase A and cyclin D1, promoting progression through the cell cycle. Targeted therapeutic agents used in recent clinical trials for ES are indicated in red. Genes modulated by EWS-FLI1 are indicated in purple. Receptors overexpressed in ES are indicated in red. ES, Ewing sarcoma; ETS, erythroblast transformation-specific; FLI1, Friend leukemia virus integration 1; IGFBP, insulin-like growth factor binding protein; IGF1, insulin-like growth factor 1; IGF1R, IGF1 receptor; MAPK, mitogen-activated protein kinase; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PI3K, phosphoinositide-3-kinase; VEGFR, vascular endothelial growth factor receptor.

express the IGF1 receptor (IGF1R) and display autocrine production of IGF1. Moreover, IGF1R is necessary for EWS/FLI1-mediated cellular transformation and inhibition of IGF1R suppresses tumor growth *in vitro* and *in vivo* (19). In the clinic, phase I trials of monoclonal antibodies targeting IGF1R (Figure 1c) have shown partial and complete responses in patients with ES (20). Although these early results were promising, recent phase II studies showed limited response rates of ~10% for patients with recurrent or refractory ES (21–23). To improve the efficacy of anti-IGF1R therapies, future work is being directed toward identification of predictive biomarkers associated with patients with ES who benefit from treatment or combination therapy with other targeted agents (Table 2).

Although the IGF pathway has received the most attention, other EWS-FLI1 target genes or interacting proteins provide potential therapeutic targets. EWS-FLI1 upregulates both Aurora A and Aurora B, cell cycle-regulated serine/threonine kinases that are overexpressed in multiple cancers (17). Preclinical testing revealed a maintained complete clinical response for an Aurora kinase A inhibitor (Figure 1c) in an ES

xenograft model (24). A phase II trial evaluating the effects of this drug in pediatric leukemias and solid tumors, including ES, is currently under way (NCT01154816, Table 2). It has also been shown that RNA helicase A, a protein involved in the regulation of transcription and splicing, binds to EWS-FLI1 and enhances its transcriptional activity (25). Utilization of a small molecule inhibitor to block the RNA helicase A-EWS/FLI1 interaction (Figure 1b) induces apoptosis in ES cells and reduces tumor growth in xenografts (26). This promising preclinical evidence suggests these agents may be effective in clinical trials.

### ALVEOLAR RMS (ARMS)

RMS is the most common pediatric soft tissue sarcoma, accounting for 5–7% of all malignancies in children and adolescents less than 20 years of age. The overall 5-year survival rate is ~60%, although it is only 30% for those with metastatic disease (27). Current therapy for RMS is similar to that for ES, employing a combination of adjuvant intensive chemotherapy with surgery and/or radiation to the primary and metastatic sites of disease.

**Table 2.** Targeted agents undergoing clinical testing for pediatric sarcomas

Target	Drug	Phase	NCT number	Eligible sarcoma types	Status
ALK, c-MET	Crizotinib (PF-02341066)	I/II	NCT01182896	Sarcoma, OS	Recruiting
Aurora kinase A	Alisertib (MLN8237)	II	NCT01154816	RMS, OS, ES, NRSTS	Recruiting
BCL-2	Oblimersen (G3139)	I	NCT00039481	ES, OS, SS, DSRCT	Completed
c-MET	Tivantinib (ARQ 197)	II	NCT00557609	CCS, ASPS	Completed
Death receptor-5	Conatumumab (AMG 655)	I/II	NCT00626704	Locally advanced, unresectable or metastatic STS	Active
EGFR	Cetuximab (IMC-C225)	II	NCT00148109	EGFR-positive bone and STS	Active
HDACs	PCI-24781	I/II	NCT01027910	Metastatic or unresectable sarcoma	Recruiting
HDACs	SB939	II	NCT01112384	Translocation-associated, metastatic sarcomas	Recruiting
IGF1R	Cixutumumab (IMC-A12)	II	NCT00668148	ES, RMS, LMS, LS, SS	Active
IGF1R	Cixutumumab (IMC-A12)	II	NCT00831844	OS, ES, RMS, SS	Recruiting
IGF1R	Figitumumab (CP-751,871)	I/II	NCT00560235	ES	Active
IGF1R	Ganitumab (AMG 479)	II	NCT00563680	ES, DSRCT	Active
IGF1R	Teprotumumab (R1507)	II	NCT00642941	ES, OS, SS, RMS, ASPS, DSRCT, EMC, CCS, MLS	Active
IGF1R, mTOR	Cixutumumab; temsirolimus	I	NCT00880282	Childhood solid tumor	Recruiting
IGF1R, mTOR	Cixutumumab; temsirolimus	II	NCT01016015	Metastatic, locally advanced, or locally recurring bone and STS	Recruiting
IGF1R, mTOR	Figitumumab; everolimus	I	NCT00927966	Advanced sarcoma	Active
mTOR	Everolimus (RAD001)	II	NCT01048723	Soft tissue extremity and/or retroperitoneal sarcomas	Recruiting
mTOR	Everolimus (RAD001)	II	NCT01216839	RMS and other STS (children and adolescents)	Recruiting
PDGFR- $\alpha$	Olaratumab (IMC-3G3)	I/II	NCT01185964	STS	Recruiting
PDGFR, c-Kit	Imatinib (Gleevec)	II	NCT00031915	ES, OS, SS, RMS, LS, MPNST, FS, AS	Completed
PDGFR, c-Kit	Imatinib (Gleevec)	II	NCT00085475	DFSP, GCF	Recruiting
RAF, VEGFR, PDGFR, c-Kit	Sorafenib	II	NCT00837148	SS, LMS, MPNST	Recruiting
RAF, VEGFR, PDGFR, c-Kit	Sorafenib	II	NCT00330421	Bone and STS	Completed
PDGFR, c-Kit, Src kinases, Eph kinases	Dasatinib	II	NCT00464620	RMS, MPNST, CS, ES, ASPS, C, EPS, GCTB, HPC, GIST	Active
VEGF	Bevacizumab	I/II	NCT01106872	Locally advanced, unresectable, or metastatic STS	Recruiting
VEGF	Bevacizumab	II	NCT00643565	RMS and NRSTS (children and adolescents)	Recruiting
VEGF; HDACs	Bevacizumab, valproic acid	I/II	NCT01106872	Locally advanced, unresectable, or metastatic STS	Recruiting
VEGFR	Cediranib	II	NCT00942877	ASPS	Recruiting
VEGFR, PDGFR, c-Kit	Sunitinib	II	NCT00400569	LS, LMS, FS	Active
VEGFR, PDGFR, c-Kit; VEGFR	Sunitinib; cediranib	II	NCT01391962	ASPS	Recruiting
VEGFR, PDGFR, c-Kit	Axitinib	II	NCT01140737	AS, LMS, SS, RMS, MPNST, fibroblastic, fibrohistiocytic	Recruiting
VEGFR, PDGFR, c-Kit	Pazopanib	I	NCT00929903	STS, DSRCT, extraosseous ES	Recruiting
VEGFR, PDGFR, c-Kit	Pazopanib	II	NCT00297258	STS (LMS, SS, adipocytic tumors)	Active
VEGFR, PDGFR, c-Kit	Pazopanib	II	NCT01059656	DFSP	Not yet recruiting
VEGFR, PDGFR, c-Kit	Pazopanib	III	NCT00753688	Metastatic STS	Active

AS, angiosarcoma; ALK, anaplastic lymphoma receptor tyrosine kinase; ASPS, alveolar soft part sarcoma; BCL-2, B-cell CLL/lymphoma 2; C, chordoma; CCS, clear-cell sarcoma; CS, chondrosarcoma; c-MET: met proto-oncogene (hepatocyte growth factor receptor); c-Kit, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; DFSP, dermatofibrosarcoma protuberans; DSRCT, desmoplastic small-round-cell tumor; EGFR, epidermal growth factor receptor; EMC, extraskeletal myxoid chondrosarcoma; EPS, epithelioid sarcoma; ES, Ewing sarcoma; FS, fibrosarcoma; GCF, giant cell fibrosarcoma; GCTB, giant cell tumor of bone; GIST, gastrointestinal stromal tumor; HDACs, histone deacetylases; HPC, hemangiopericytoma; IGF1R, insulin-like growth factor 1 receptor; LMS, leiomyosarcoma; LS, liposarcoma; MLS, myxoid liposarcoma; MPNST, malignant peripheral nerve sheath tumor; mTOR, mammalian target of rapamycin; NRSTS, nonrhabdomyosarcoma soft tissue sarcoma; OS, osteosarcoma; PDGF, platelet-derived growth factor; RAF, v-raf-1 murine leukemia viral oncogene homolog 1; RMS, rhabdomyosarcoma; SS, synovial sarcoma; Src, v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian); STS, soft tissue sarcoma; VEGFR, vascular endothelial growth factor receptor.

RMS is divided into histologic subtypes. Embryonal RMS (ERMS) and ARMS are the two major subtypes, accounting for ~60% and ~20% of cases, respectively (2). RMS tumors can arise anywhere in the body, although frequency of primary sites varies with histological subtype and age of diagnosis. Tumors in unfavorable sites (e.g., extremities) are more common in ARMS, which predominantly afflicts adolescents and young adults and confers a worse prognosis than ERMS (28). For patients who have refractory, recurrent, and/or metastatic ARMS, long-term survival is truly the exception rather than the rule. Although the expression of skeletal muscle markers and location of tumors in skeletal muscle suggest a myogenic origin, the exact cell of origin for ARMS remains uncertain. Mouse models and *in vitro* cell culture systems have provided evidence for both skeletal muscle and mesenchymal stem cell origins.

### Molecular genetics

In addition to histologic differences, ARMS is distinguished from ERMS by the presence of specific chromosomal translocations present in the majority of ARMS tumors. The predominant translocation, t(2;13)(q35;q14), fuses *PAX3* (paired box 3) to forkhead in human rhabdomyosarcoma (*FOXO1*) (forkhead box O1, also known as *FKHR*) (29). Less commonly, the t(1;13)(p36;q14) translocation fuses another *PAX* gene, *PAX7* (paired box 7), to *FOXO1* (30). A recent study also identified rare, noncanonical t(2;2)(p23;q35), and t(2;8)(q35;q13) translocations that unite *PAX3* with the nuclear receptor transcriptional coactivators *NCOA1* or *NCOA2* (31) (Table 1).

*PAX3* and *PAX7* are part of the paired box family of TFs, which is involved in embryonic development and myogenesis (32). *FOXO1* is a member of a subfamily of forkhead TFs regulated by the PI3K (phosphoinositide-3-kinase) pathway and is believed to play a role in myogenic growth and differentiation (33). Both translocation break points consistently occur within the seventh intron of the *PAX* gene and the first intron of *FOXO1*, resulting in a chimeric TF that contains the *PAX* DNA-binding domain and transcriptional activation domain of *FOXO1*. Fusion type has been found to correlate with clinical outcome, as patients with *PAX7-FOXO1*-positive tumors had better overall survival rates than those with tumors containing the *PAX3-FOXO1* fusion (34). More recently, clinical characteristics and prognosis of fusion-negative ARMS were found to be more similar to ERMS than fusion-positive ARMS, implying that the presence of the *PAX-FOXO1* fusion is more crucial than histology to the underlying biology of the tumor (35).

### Target genes and targeted therapies

Initial *PAX3-FOXO1* target genes were identified by evaluating the expression of known *PAX3* target genes in ERMS cells transduced with *PAX3-FOXO1*. Upregulation of *MET* (met proto-oncogene/hepatocyte growth factor receptor) upon *PAX3-FOXO1* expression and an observed correlation between *MET* and *PAX3-FOXO1* in tumor samples suggested *MET* is a downstream target (36). The role of *MET* in ARMS tumorigenesis was further characterized by experiments that

demonstrated *MET* is required for *PAX3-FOXO1*-mediated transformation of mouse embryonal fibroblasts, and short hairpin RNA knockdown of *MET* results in decreased tumor growth *in vitro* and *in vivo* (37). More recently, studies have shown that *MET* is posttranscriptionally regulated, as low levels of the microRNAs miR-1 and miR-206 result in derepression and upregulation of *MET* in RMS cells. Furthermore, overexpression of these miRNAs promoted myogenic differentiation and inhibited tumor growth *in vivo* (38). Additionally, a preclinical study demonstrated the ability of a c-*MET* small molecule inhibitor to hinder growth of ARMS cell lines (39).

Components of the IGF system are also potential therapeutic targets in ARMS. Heterologous expression of the *PAX3-FOXO1* fusion results in an increase in IGF1R levels and overexpression of IGF2 and IGF1R has been observed in ARMS and ERMS tumors and cell lines (40,41). Elevated IGF2 levels in ERMS result from loss of heterozygosity at the 11p15.5 locus whereas in ARMS it may be transcriptionally upregulated. Introduction of *PAX3-FOXO1* into NIH3T3 cells identified a myogenic transcriptional signature distinct from *PAX3* alone that included genes such as those encoding *MyoD*, myogenin, and IGF2 (42). Multiple studies have demonstrated suppression of tumor growth *in vivo* and *in vitro* with small molecular inhibitors and monoclonal antibodies targeting IGF1R. This has led to both phase I and II clinical trials to evaluate anti-IGF1R therapy in RMS. In phase I trials involving multiple tumor types, responses were observed only in patients with ES. However, preliminary phase II data have shown three objective radiological responses for patients with RMS (41).

The platelet-derived growth factor  $\alpha$  receptor (PDGFR $\alpha$ ) is also transcriptionally upregulated by *PAX3-FOXO1* (43). PDGFR $\alpha$  is overexpressed in human ARMS and ERMS tumors as well as in mouse models of ARMS. Small interfering RNA downregulation of PDGFR $\alpha$  resulted in decreased cell growth *in vitro* and PDGFR $\alpha$  inhibition in mouse models using imatinib mesylate (Gleevec) or a PDGFR $\alpha$  neutralizing antibody led to disease stabilization and in some cases resistance to therapy (44). Resistance has also been observed with anti-IGF1R therapy, which may be due to activation of other growth factor receptors such as human epidermal growth factor receptor 2 (also known as ERBB2) and PDGFR $\alpha$  (45,46). Because both IGF1R and PDGFR $\alpha$  are potential therapeutic targets that have shown resistance as single agents, combination therapy may enhance patient response. Combination of anti-IGF1R therapy with mTOR (mammalian target of rapamycin) inhibitors is currently being evaluated in a phase I trial for pediatric solid tumors (NCT00880282) and in combination with cytotoxic chemotherapy in a Children's Oncology Group study (ARST08P1) for patients with high-risk RMS (Table 2).

### SS

SS is the most common non-RMS soft tissue sarcoma in adolescents and young adults, accounting for ~8% of all soft tissue sarcomas (28). The overall 5-year survival rate is between 70 and 80%, but as with most sarcomas, this number drops sharply for those patients with metastatic and recurrent disease. Other

than complete surgical resection of localized SSs, there is no standard of care for treatment and approach to these tumors may vary from center to center. Radiation therapy may be used as an adjuvant therapy to improve local control, and chemotherapy is generally reserved for larger tumors or patients with metastatic disease.

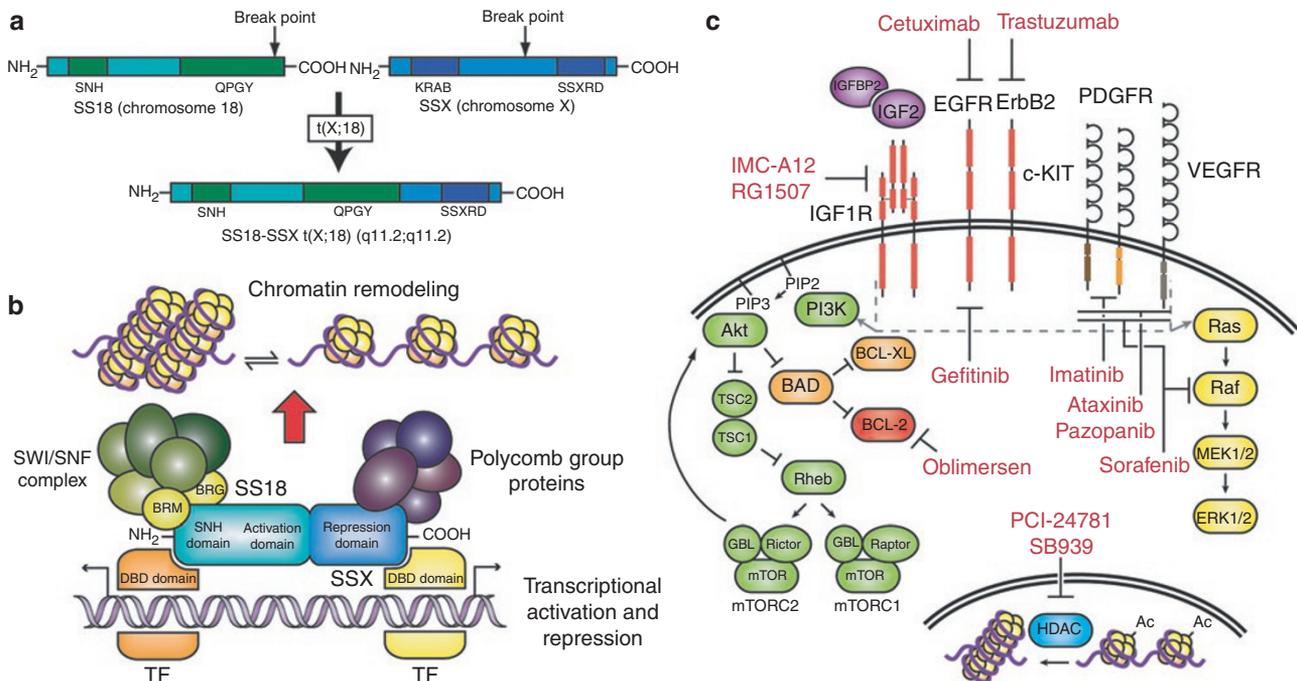
SS is histologically unique, displaying both biphasic and monophasic tumors. Monophasic tumors are mesenchymal in origin, exhibiting a spindle cell morphology that is difficult to distinguish from fibrosarcoma. Biphasic tumors contain cells of epithelial differentiation that form a glandular component within the mesenchymal spindle cells (28). The designation of SS originated from tumor proximity to large joints, as most tumors arise in the extremities, and microscopic resemblance to synovial tissue. However, this is a misnomer as the histogenesis is not of synovial origin (46). Mouse models utilizing myogenic regulatory factor-driven expression of fusion genes suggest SS arises shortly after differentiation from a muscle stem cell (47).

### Molecular genetics

The underlying genetic aberration in SS results from a specific t(X;18)(p11.2;q11.2) translocation that fuses *SS18* (SS translocation, chromosome 18, also known as *SYT*) to *SSX1*, *SSX2*, or *SSX4* (SS X break point 1, 2, or 4 (48–51)) (Figure 2a). SS18 is

a ubiquitously expressed, nuclear protein that contains a novel SYT N-terminal homology domain that allows for interaction with chromatin remodeling factors and a C-terminal QPGY domain that resembles the transactivation domain within the FET family of proteins. Despite the absence of a DNA-binding domain, SS18 is thought to function as a transcriptional activator and may play a role in signal transduction via its SH2- (Src homology 2) and SH3 (Src homology 3)-binding motifs. The *SSX* genes encode a family of highly homologous proteins located on the X chromosome. They are believed to act as transcription repressors due to the presence of a Kruppel-associated box domain and an SSX repression domain. The *SSX* proteins also lack a DNA-binding domain so must rely on protein-protein interactions to mediate transcriptional repression (52).

The t(X;18) translocation fuses the C-terminus of SS18 to all but the last eight amino acids of SS18, generating a chimeric protein that contains both transcriptional activation (QPGY) and repression (SSX repression domain) domains as well as the SYT N-terminal homology domain (Figure 2a). The SSX repression domain allows SS18-SSX proteins to colocalize with components of the polycomb group chromatin remodeling repressor complex whereas the SYT N-terminal homology domain facilitates interaction with members of the switch/sucrose nonfermentable (SWI/SNF) complex (53,54) (Figure 2b). This



**Figure 2.** Molecular genetics and targeted therapies in SS. (a) Schematic of the SS18-SSX t(X;18)(q11.2;q11.2) translocation. The SS18-SSX fusion contains both the SNH and QPGY activation domains of SS18 as well as the SSXRD. The SSX KRAB domain is not retained in the fusion. (b) Putative molecular function of SS18-SSX and selected protein-protein interactions. The SNH domain facilitates binding to components of the SWI/SNF complex while the SSXRD interacts with polycomb group proteins, which results in chromatin remodeling. Interactions with transcription factors (TFs) may also lead to transcriptional activation and repression. (c) Signaling pathways and targeted therapies in SS. Activation of growth factor receptors leads to downstream signaling through the PI3K and MAPK pathways (indicated by gray dashed line and arrows). Histone deacetylases (HDACs) remove acetyl groups (Ac) from histones, resulting in condensed chromatin. Targeted therapeutic agents used in recent clinical trials for SS are indicated in red. Genes modulated by SS18-SSX are indicated in purple. Receptors overexpressed in SS are indicated in red. EGFR, epidermal growth factor receptor; IGF1, insulin-like growth factor 1; KRAB, Kruppel-associated box; MAPK, mitogen-activated protein kinase; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide-3-kinase; SNH, SYT N-terminal homology; SS, synovial sarcoma; SSXRD, SSX repression domain; SWI/SNF, switch/sucrose nonfermentable; SYT, synovial sarcoma translocation; VEGFR, vascular endothelial growth factor receptor.

suggests the fusion drives tumor progression through epigenetic chromatin remodeling. The SS18-SSX1 fusion protein is able to transform rat fibroblasts, and association with the hBRM/hSNF2 $\alpha$  (also known as SMARCA2, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2) chromatin remodeling factor is required for transformation (55). Its presence is required for sarcomagenesis as small interfering RNA downregulation of SS18-SSX expression inhibits tumor growth *in vitro* and *in vivo* (56).

SS18 is fused to either SSX1 or SSX2 in >90% of SS cases and is only rarely observed bound to SSX4. Although initial studies showed patients with SS18-SSX2 fusions had improved survival rates, an expanded study performed more recently concluded there was no correlation between fusion variant and survival (57). Although fusion type may not determine survival, it is strongly associated with histology as biphasic and monophasic tumors contain the SS18-SSX1 and SS18-SSX2 transcripts, respectively (58).

#### Target genes and targeted therapies

As with RMS, IGF2 plays a role in SS biology. cDNA microarray analysis of SS and closely related spindle cell tumors revealed that genes such as those encoding IGF binding protein 2, human epidermal growth factor receptor 2 (ERBB2), and IGF2 are upregulated in SS (59). Evidence for the role of IGF2 in SS pathogenesis is further supported by studies in which SS18-SSX fusions were exogenously expressed in heterologous cell systems. Gene expression profiling determined IGF2 to be the most highly upregulated gene upon SS18-SSX2 expression in 293T cells (60) and SS18-SSX1 expression in human primary lung fibroblasts (61).

SS18-SSX2 has been shown to interact with Brahma-related gene 1 (BRG1) (also known as SMARCA4, SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4), a component of the SWI/SNF complex, and BRG1 was found to bind to the *IGF2* promoter. These data combined with active chromatin marks observed upon induction of SS18-SSX2 suggest the fusion interacts with BRG1 to epigenetically modulate IGF2 expression (60). In addition, SS18-SSX2 is necessary for maintenance of IGF2 expression, and IGF2 is required for SS18-SSX1-mediated tumor formation (61). IGF2 expression results in activation of IGF1R and phosphorylation of the downstream proteins Akt (v-akt murine thymoma viral oncogene homolog) and mitogen-activated protein kinase. Providing evidence that the IGF pathway could be a therapeutic target, treatment of SS cell lines with the IGF1R inhibitor NVP-AEW541 resulted in impaired cell growth and increased apoptosis (62).

Immunohistochemical and molecular studies have demonstrated high expression levels of the antiapoptotic protein BCL-2 (B-cell CLL/lymphoma 2) in the majority of SS tumors (63). Due to the absence of genomic amplifications or rearrangements, overexpression of BCL-2 may result from transcriptional activation (64). Although *BCL-2* has not been shown to be a direct target of SS18-SSX, lower mRNA and protein levels in SS tumors and cell lines lacking the t(X,18) translocation suggest an association does exist (65). Furthermore,

BCL-2 antisense oligonucleotide treatment of a translocation-positive SS cell line resulted in increased sensitivity to doxorubicin treatment, implying BCL-2 may be a promising therapeutic target (66). In a phase I trial assessing the effectiveness of BCL-2 antisense therapy (Figure 2c) in combination with chemotherapy in childhood solid tumors, two patients with SS displayed prolonged stable disease (67).

Epidermal growth factor receptor (EGFR) is also overexpressed in SS. Microarray analysis of a set of 41 soft tissue tumors and subsequent clustering analysis identified EGFR as part of cluster that showed SS-specific expression (68). Immunohistochemical studies and molecular characterization have confirmed the presence of EGFR in SS (69,70). These data led to a phase II trial to establish the efficacy of the EGFR inhibitor gefitinib (Figure 2c) in EGFR-positive, chemoresistant SS. In this trial, stable disease was the best observed response, suggesting EGFR is not required for tumorigenesis (71). Gene expression profiling and immunohistochemical studies have identified another member of the EGFR family, human epidermal growth factor receptor 2 (ERBB2), that is upregulated in SS and that may provide an alternative target for the treatment of this disease (59,70) (Figure 2c).

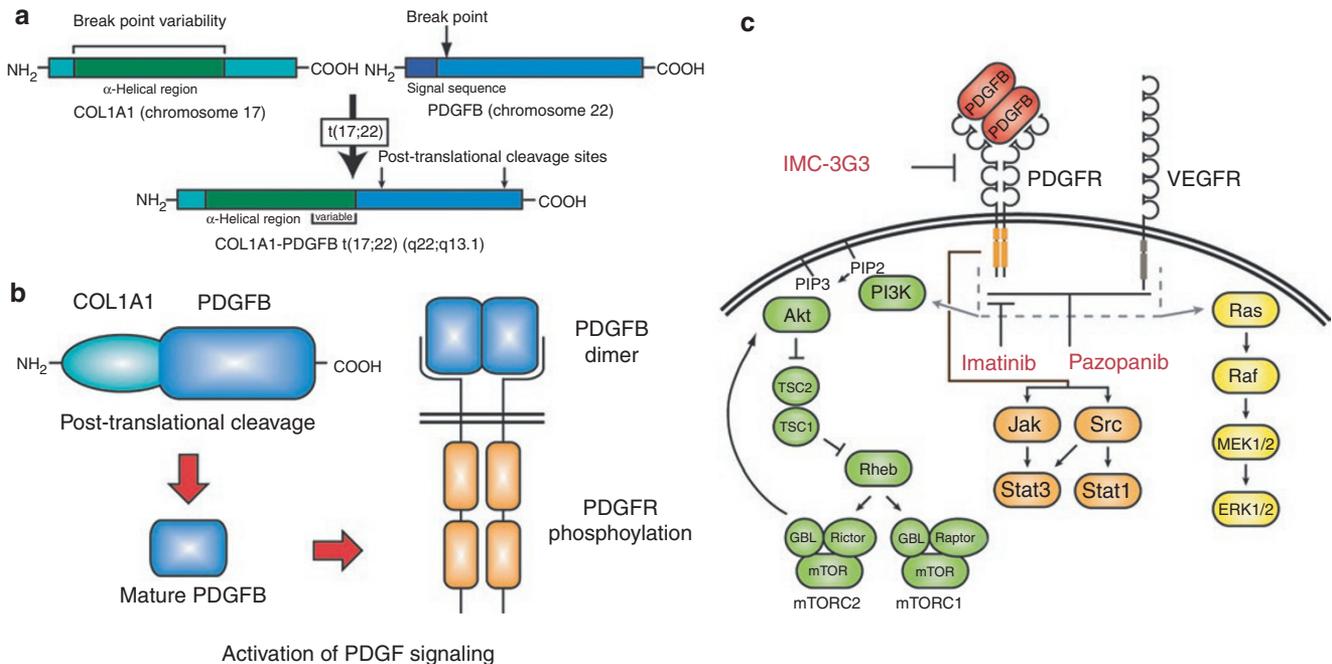
#### DFSP

DFSP is a relatively rare cutaneous malignancy, accounting for ~0.1% of all cancers and ~1% of soft tissue sarcomas. DFSP has an annual incidence rate of 4.2 per million and primarily afflicts adults between the ages of 30 and 50 years (72). Pediatric cases, both congenital and in young children, do occur, but with much less frequency than adult cases. As suggested by its name, DFSP tumors arise in the dermis, infiltrating the dermal stroma and often breaching the subcutaneous fat. Although studies have provided evidence for a fibroblastic, neural, and histiocytic origin, the histogenesis of DFSP remains uncertain (73). Primary-site locations can occur throughout the body, although the trunk, proximal extremities, and head and neck are the most common (72). The 5-year survival rate of patients with DFSP exceeds 95% and metastases occur in less than 5% of patients (72,74).

Treatment of DFSP, like other soft tissue sarcomas, centers on achieving a complete surgical resection. Radiation is used when surgical margins are positive and a re-resection is not feasible. Chemotherapy is used only in metastatic cases, but there is increasing evidence that imatinib therapy can be used in an adjuvant setting in patients with recurrent, refractory, and/or metastatic disease.

#### Molecular genetics

DFSP tumors contain either the t(17;22)(q22;q13.1) reciprocal chromosomal translocation or a supernumerary ring chromosome derived from t(17;22). Both of these karyotypic aberrations result in a fusion of the genes *COL1A1* (encoding the pro- $\alpha$ 1 chain of type I collagen) on 17q21-22 and *PDGFB* (encoding the PDGF B chain) on 22q13.1 (75) (Figure 3a). Rings are predominantly found in adult cases, although occasionally translocations are identified. In contrast, all pediatric



**Figure 3.** Molecular genetics and targeted therapies in DFSP. **(a)** Schematic of the COL1A1-PDGFB t(17;22)(q22;q13.1) translocation. The COL1A1-PDGFB fusion joins the  $\alpha$ -helical region of COL1A1 to PDGFB lacking its signal sequence. The COL1A1 N-terminal signal sequence replaces that of PDGFB. Break points occur throughout the  $\alpha$ -helical region in COL1A1 but occur only within the first intron of PDGFB. PDGFB post-translational cleavage sites are retained in the fusion. **(b)** Putative molecular function of the COL1A1-PDGFB fusion. The COL1A1 signal sequence allows for protein export and posttranslational cleavage results in the generation of mature PDGFB. Ligand binding of the PDGFB homodimer dimer results in receptor dimerization, autophosphorylation, and activation. **(c)** Signaling pathways and targeted therapies in DFSP. Activation of PDGFR by the PDGFB dimer results in downstream signaling through the PI3K, MAPK, and Jak/Stat (Janus kinase/signal transducer and activator of transcription) pathways. VEGFR is also activated in DFSP and signals through the PI3K and MAPK pathways. Targeted therapeutic agents in current clinical trials for DFSP (imatinib and dasatinib) and soft tissue sarcomas expressing PDGFR- $\alpha$  (IMC-3G3) are indicated in red. Growth factors overexpressed in DFSP are indicated in red. DFSP, dermatofibrosarcoma protuberans; MAPK, mitogen-activated protein kinase; PDGFB, platelet-derived growth factor B chain; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide-3-kinase; VEGFR, vascular endothelial growth factor receptor.

tumors contain translocations. DFSP variants and related malignancies such as giant cell fibroblastoma have also been found to contain the *COL1A1-PDGFB* fusion.

The t(17;22) break point occurs upstream of the second exon of the *PDGFB* gene and within the  $\alpha$ -helical region of *COL1A1*. This results in the removal of the both the PDGFB inhibitory regulatory elements and signal peptide in the *COL1A1-PDGFB* fusion and placement of remainder of *PDGFB* locus, beginning with exon 2, under the control of the *COL1A1* promoter. Although the *PDGFB* break point is invariably located in the first intron, the one in the *COL1A1* locus can occur within multiple exons, probably due to the repetitive nature of the  $\alpha$ -helical region. Most of the *COL1A1* coding sequence is postulated to be functionally irrelevant as PDGFB is posttranslationally cleaved at sites retained in the fusion to generate the mature growth factor. In contrast, the replacement of the *PDGFB* repressor elements with the *COL1A1* promoter allows for aberrant expression of the protein. The COL1A1 N-terminal signal sequence, also retained in the fusion, permits PDGFB secretion, resulting in constitutive activation of the PDGFR $\beta$  pathway (76) (Figure 3b). The finding that there is no correlation between *COL1A1-PDGFB* fusion type and clinical response or histology supports the theory that the *COL1A1* portion of the fusion only provides a mechanism for PDGFB overexpression (77).

### Signaling pathways and targeted therapies

The COL1A1-PDGFB fusion has been shown to transform NIH3T3 cells (78). Additionally, stable transfection of the fusion in a Chinese hamster lung fibroblastic line led to growth factor-independent growth and tumor formation in nude mice (76). Both of these studies demonstrated activation of the PDGFR $\beta$  receptor, indicating the involvement of PDGF signaling in DFSP tumorigenesis. To block this pathway, NIH3T3 cells transformed with COL1A1-PDGFB and DFSP tumor-derived primary cultures were treated with imatinib mesylate (Gleevec or STI-571), which inhibits the PDGF receptor (PDGFR) (79) (Figure 3c). Growth inhibitory effects were observed *in vitro* and *in vivo*, suggesting imatinib could be an effective therapy in the treatment of DFSP.

Initial case reports demonstrating patient response to imatinib led to a study of its activity in 10 patients with locally advanced or metastatic DFSP. Despite low levels of PDGFR phosphorylation in patient tumors, imatinib was shown to be an effective therapy as four patients with locally advanced disease displayed a complete clinical response (80). On a larger scale, the efficacy of imatinib treatment is currently being evaluated in a phase II trial for DFSP and giant cell fibrosarcoma (NCT00085475, Table 2). In addition to imatinib, another tyrosine kinase inhibitor, pazopanib, which targets vascular endothelial growth

factor receptor in addition to PDGFR (Figure 3c), has just begun phase II testing for DFSP (NCT01059656, Table 2).

## CONCLUSIONS

Despite aggressive therapeutic approaches to patients with pediatric sarcomas, we have reached a plateau in the survival rate. Although the molecular genetics of these tumors reveal they are distinct entities, they are often treated as a homogeneous group when it comes to standard therapy. Fusion gene transcriptional targets, downstream signaling pathways, and overexpressed growth factor receptors provide novel therapeutic targets that are currently being investigated in clinical trials. Certain targets have shown promise, but tumor resistance is a common problem, suggesting combination therapy may be required for effective treatment. Although initial trials and *in vitro* studies have paved the way for advances in targeted therapy in sarcoma, further work is needed to better characterize tumors at the molecular genetic level to tailor therapies to individual tumors.

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