

A review of soft-tissue sarcomas: translation of biological advances into treatment measures

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Abstract: Soft-tissue sarcomas are rare malignant tumors arising from connective tissues and have an overall incidence of about five per 100,000 per year. While this diverse family of malignancies comprises over 100 histological subtypes and many molecular aberrations are prevalent within specific sarcomas, very few are therapeutically targeted. Instead of utilizing molecular signatures, first-line sarcoma treatment options are still limited to traditional surgery and chemotherapy, and many of the latter remain largely ineffective and are plagued by disease resistance. Currently, the mechanism of sarcoma oncogenesis remains largely unknown, thus necessitating a better understanding of pathogenesis. Although substantial progress has not occurred with molecularly targeted therapies over the past 30 years, increased knowledge about sarcoma biology could lead to new and more effective treatment strategies to move the field forward. Here, we discuss biological advances in the core molecular determinants in some of the most common soft-tissue sarcomas – liposarcoma, angiosarcoma, leiomyosarcoma, rhabdomyosarcoma, Ewing's sarcoma, and synovial sarcoma – with an emphasis on emerging genomic and molecular pathway targets and immunotherapeutic treatment strategies to combat this confounding disease.

Keywords: sarcoma, molecular pathways, immunotherapy, genomics

Introduction

Soft-tissue sarcoma (STS) is a diverse group of rare cancers that arise from pathological transformations in the mesenchyme, which is the mesodermal portion of the embryo that develops into connective and skeletal tissues. These rare cancers account for <1% of all adult malignancies, and an estimated 12,000 new cases of STS are diagnosed in the US each year, with approximately 5,000 deaths.^{1,2} While the exact cause of carcinogenesis has remained elusive and these cancers can arise from any body part, most STSs are diagnosed in the extremities (59.5%), followed by the trunk (17.9%).³ At the time of initial diagnosis, distant metastases are rarely present, but blood is the most common route for the disease to spread, most frequently to the lungs.⁴

Current treatments for STS often involve multiple modalities, including surgery, radiation, and chemotherapy. There are a few US Food and Drug Administration (FDA)-approved chemotherapeutic drugs for treating STS, such as eribulin, trabectedin, ifosfamide, anthracyclines, and taxanes, but toxicity and partial responses remain significant limitations. Little progress has been made with respect to targeted therapeutics. The 5-year overall survival rate for STS is 90% for stage I, 81% for stage II, and 56% for stage III.⁵ Beyond TNM stage and histologic grade,^{5,6} additional prognostic factors include surgical margins, age, anatomic site, and histologic subtype.⁷

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According to the World Health Organization classification, over 100 distinct histological subtypes have been categorized.⁸ Given this diversity, the appropriate course of treatment could be guided by a better understanding of the disease pathobiology at the molecular level. In this review, we focus on several of the most common histologic subtypes

in adults: liposarcoma (LPS), angiosarcoma, leiomyosarcoma (LMS), rhabdomyosarcoma (RMS), Ewing's sarcoma (ES), and synovial sarcoma (SS). We summarize current knowledge and advances in STS biology in terms of molecular pathways and genomics (Figure 1 and Table 1). We also consider translational implications of new targeted and immunotherapeutic

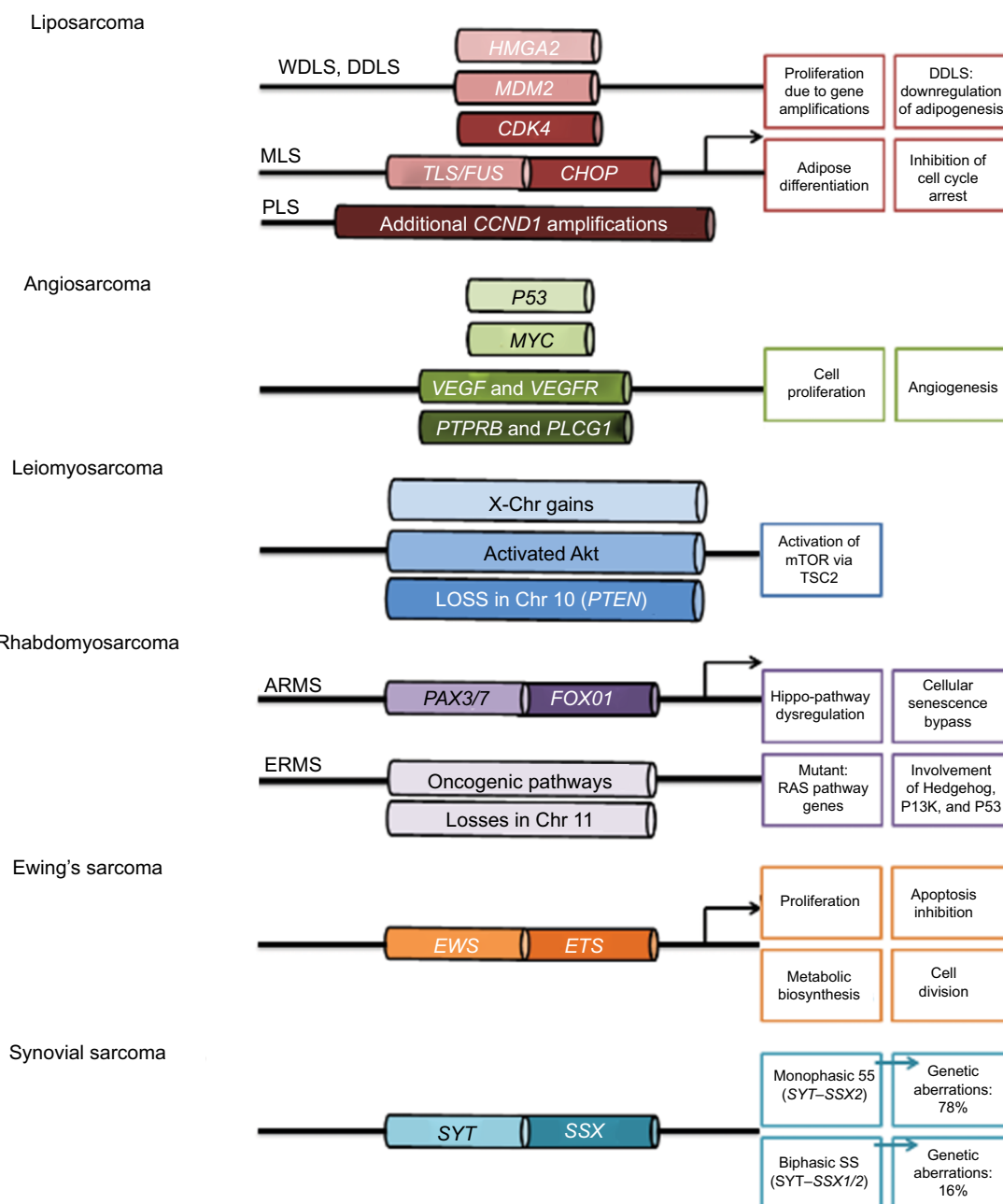


Figure 1 Genomic changes in soft-tissue sarcoma.

Notes: Liposarcomas consist of four subtypes: well-differentiated liposarcoma (WDLS), dedifferentiated liposarcoma, myxoid liposarcoma (MLS), and pleomorphic liposarcoma (PLS). A common characteristic of WDLS, DDLS, and PLS is amplifications in *HMGA2*, *MDM2*, and *CDK4*. PLS bears additional *CCND1* amplifications. MLS, on the other hand, harbors a fusion of *TLS/FUS-CHOP*, which is responsible for pathogenesis. Angiosarcomas are diverse malignancies and bear aberrations in *MYC*, *VEGF/VEGFR*, *PTPRB*, and *PLCG1*. Leiomyosarcomas have frequent X-chromosome (Chr) gains, constitutively activated Akt and losses in Chr 10, which bears the *PTEN* gene. The two latter aberrations lead to mTOR activation via TSC2 and are instrumental in disease pathology. Rhabdomyosarcoma can be subtyped into alveolar rhabdomyosarcoma (ARMS) and embryonic rhabdomyosarcoma (ERMS). The former is associated with *PAX3/7-FOXO1* fusions and cause Hippo-pathway dysregulation accompanied by bypass of cellular senescence, and the latter is distinguished by losses in Chr 11, along with gene mutations in the Ras pathway. Other pathways involved include Hedgehog, PI3K, and p53. Ewing's sarcoma is characterized by *EWS-ETS* gene fusion, and this potent transcription factor induces genes associated with proliferation, apoptosis inhibition, and metabolic changes to favor biosynthesis and cell division. Synovial sarcoma (SS) is associated with *SYT-SSX* fusions: *SYT-SSX2* for monophasic SS and *SYT-SSX1/2* for biphasic SS. Arrows indicate gene transcription.

Table 1 Soft-tissue sarcoma genomic landscape

Sarcoma type/sub-type	Gene/chromosome alteration	Frequency
LPS (WDLS, DDLS)	<i>HMG2A</i> amplification	76%
	<i>MDM2</i> amplification	87%
	<i>CDK4</i> amplification	95%
LPS (MLS)	13q21–13q32 amplifications	24%
	Telomerase reactivation	69%
	Telomerase reactivation	39%
	C228T <i>TERT</i> mutation	74%
AS	<i>TP53</i> mutation	4%
	8q24.21 amplification	50%
	10p12.33 amplification	33%
	5q35.3 amplification	11%
	VEGF overexpression	21%–25%
	Inactivating <i>PTPRB</i> mutations	26%
	Likely activating <i>PLCG1</i> mutations	9%
	<i>PIK3CA</i> mutations	3%
	<i>FLT4</i> mutations	3%
	<i>H1K/NRAS</i> mutations	13%
	Genomic imbalances	88%
LMS	Aberrant chromosome numbers and structures	60%
	Promoter hypermethylation of <i>RASSF1A</i>	39%
	X-chromosome gains	48%
LMS (ULMS)	10q chromosome region loss	62%
	13q chromosome region loss	Most
	Ras pathway mutations	35%–45%
RMS	<i>TP53</i> mutations	5%–22%
	<i>MDM2</i> amplification	10%–17%
	<i>CDKN2A/B</i> focal deletion	23%
RMS (ERMS)	<i>FGFR4</i> activating mutations	20%
	<i>NFI</i> locus deletions	15%
	Ras family activating mutations	12%–42%
	High <i>GLI1</i> expression	21%
	<i>FGFR4</i> mutations	9%
	<i>PIK3CA</i> mutations	5%
	<i>PAX3–FOXO1</i> gene fusions	55%
RMS (ARMS)	<i>PAX7–FOXO1</i> gene fusions	22%
	<i>EWS–FLI1</i> translocation	Characteristic
ES	<i>SYT–SSX1</i> translocation fusion	Characteristic
SS	Genetic aberrations	78%
Monophasic SS	Genetic aberrations	5%
Poorly differentiated SS	Genetic aberrations	16%
Biphasic SS	Overexpression: <i>KRT5</i> , <i>KRT7</i> , <i>KRT8</i> , <i>KRT14</i>	Preferentially expressed in biphasic samples
	Overexpression: <i>EST</i> , <i>ELF3</i>	Preferentially expressed in biphasic samples

Abbreviations: LPS, liposarcoma; WDLS, well-differentiated liposarcoma; DDLS, dedifferentiated liposarcoma; MLS, myxoid liposarcoma; AS, angiosarcoma; LMS, leiomyosarcoma; ULMS, uterine leiomyosarcoma; RMS, rhabdomyosarcoma; ERMS, embryonic rhabdomyosarcoma; ARMS, alveolar rhabdomyosarcoma; ES, Ewing's sarcoma; SS, synovial sarcoma.

strategies under investigative development that could potentially permit longer survival and a better quality of life for those with STS (Table 2).

Liposarcoma

LPSs are mesenchymal-derived cancers that originate from adipose precursors, named so because of the resemblance they bear to fat cells when examined under microscopy.⁹

These tumors are typically large and bulky, with extensions that branch off from the confines of the main tumor mass. LPS is the most common STS subtype, comprising 20% of all adult STS.¹⁰ They most frequently occur in adults over age 40 years, and the 5-year survival rates range from 56% to 100% depending upon tumor histology.¹¹ Surgery is the standard of care for LPS, but recurrence is common and resistance to chemotherapeutics underscores a critical need

Table 2 Soft-tissue sarcoma targets, therapeutics, and clinical status

	Target(s)	Therapeutic	Status	Trial ID
LPS	CDK4/6	Palbociclib (PD0332991)	Phase II trial completed (2017)	NCT01209598
LPS	VEGFR and PDGFR	Pazopanib	Phase II trial completed (2017)	NCT01506596
LPS (MLS)	NY-ESO1	CAR T cells	Phase II trial ongoing	NCT02992743
LPS (MLS), SS	Class I MHC expression	Recombinant IFN γ	Pilot study ongoing	NCT01957709
LPS (DDLs), LMS	mTOR and CDK4/6	Everolimus + ribociclib	Phase II trial ongoing	NCT03114527
LPS, LMS	PDL1 and DNA repair	Avelumab (PDL1 mAb) + trabectedin	Phase II trial ongoing	NCT030743
LPS, ES, AS (UPS)	PDI and mTOR	Nivolumab (PDI mAb) + ABI009 (mTOR inhibitor)	Phase II trial ongoing	NCT0319017
AS	VEGF	Bevacizumab (VEGF mAb)	Phase II trial ongoing	NCT00288015
LMS, SS	EGFR	Anlotinib (AL3818)	Phase III trial ongoing	NCT03016819
RMS	NY-ESO1, MAGEA4, PRAME, survivin, and SSX	TAA-specific CTLs	Phase I trial ongoing	NCT02239861
RMS, ES	Immunomodulated lysis	Recombinant vaccinia GM-CSF (JX594)	Phase I trial completed	NCT01169584
RMS, SS	CD56 and tubulin	Lorvotuzumab mertansine	Phase II trial ongoing	NCT02452554
ES	EWS-FLI1	TK216	Phase I trial ongoing	NCT02657005
SS	G6PD	DHEA	Phase II trial ongoing	NCT02683148
SS	mTOR, c-Kit, and PDGFR	Everolimus + imatinib mesylate	Phase II trial completed	NCT01281865
SS	NY-ESO1-expressing tumor cells	Autologous dendritic cells loaded with allogeneic tumor lysate expressing NY-ESO1	Phase I/II ongoing	NCT01883518
Advanced STS postchemotherapy	VEGFR1 and VEGFR2, VEGFR3, PDGFR, and c-Kit	Pazopanib	FDA-approved (2012)	—
Several	Immunomodulated lysis	HSV1716	Phase I trial ongoing	NCT00931931
Several	Tubulin and mitotic spindle	Eribulin	FDA-approved (2015)	—
Several	DNA repair	Trabectedin	FDA-approved (2016)	—
Several	PDI and CTLA4	Nivolumab $\pm$ ipilimumab (CTLA4 mAb)	Phase II trial ongoing	NCT02500797
Several	mTOR	Ridaforolimus	Phase II trial completed (2015)	NCT00112372
Several	VEGFR, PDGFR, and DNA replication	Pazopanib + topotecan	Phase II trial ongoing	NCT02357810
Several	Topoisomerase 2 and PDGFR	Dexrazoxane + doxorubicin + olaratumab	Phase II trial ongoing	NCT025843
Several	Raf, VEGFR1, VEGFR2, VEGFR3, PDGFR B, and c-Kit	Sorafenib	Phase II trial completed	NCT00217620
Several	PDL1 and NY-ESO1	Atezolizumab (PDL1 mAb) + CMB305	Phase II trial ongoing	NCT026099
Several	Histone-lysine methyltransferase EZH2	Tazemetostat	Phase II trial ongoing	NCT02601950

Abbreviations: LPS, liposarcoma; MLS, myxoid liposarcoma; SS, synovial sarcoma; DDLs, dedifferentiated liposarcoma; LMS, leiomyosarcoma; mAb, monoclonal antibody; ES, Ewing's sarcoma; AS, angiosarcoma; UPS, undifferentiated pleomorphic sarcoma; RMS, rhabdomyosarcoma.

for the identification of novel therapeutic targets.¹² The most frequent chromosome gains at the genomic level are in chromosome regions 1q, 12q, and 13q.¹³

Liposarcoma: subtypes

The four recognized histological subtypes of LPS are categorized based on clinicopathological and molecular genetic characteristics: well-differentiated LPS (WDLS), dedifferentiated LPS (DDLs), myxoid LPS (MLS), and pleomorphic LPS (PLS).^{10,12} The most common are WDLS, DDLs,¹⁴ and MLS.⁹ Those with greatest metastatic potential include DDLs and PLS.¹² In the following sections, we present recent molecular discoveries in these biological subtypes of LPS.

Well-differentiated and dedifferentiated liposarcoma

WDLS and DDLs occur either in the retroperitoneal region or in the extremities.¹² Patients with retroperitoneal WDLS or DDLs have higher rates of local recurrence and disease-related deaths than those with extremity tumors.¹² WDLS and DDLs share common underlying genetic alterations, but WDLS in particular consists predominantly of mature adipocytes, along with mixtures of primitive lipoblasts and atypical stromal cells.^{10,12} In contrast, DDLs is believed to be more aggressive and more likely a metastatic progression of WDLS with poorer outcomes. DDLs develops due to the deregulation of normal adipocyte differentiation programs, and is thus characterized by a lack of mature adipocytes.¹⁰ The exact genetic events

that prompt this evolution are unclear.¹² WDLS and DDLS are best treated with surgical resection;¹² metastatic DDLS is commonly resistant to chemotherapy and radiation.¹⁰

Myxoid liposarcoma

MLS and round-cell LPS constitute 30% of all LPS.¹⁴ These subtypes frequently occur in the lower extremities,¹⁴ with metastases observed commonly in the lungs, soft tissue, and bones.¹⁵ Histologically, these cancers consist of round to oval mesenchymal cells, and MLS is characterized by the presence of lipoblasts, which are adipose precursors at different stages of differentiation, with distinct plexiform capillary patterns and a myxoid or mucous matrix.⁹

Pleomorphic liposarcoma

PLS comprises 5% of all LPS, making it the rarest subtype.¹⁴ Along with DDLS, PLS is also aggressive with metastatic potential and associated with increased disease-related deaths.⁹ These high-grade tumors arise frequently in the retroperitoneum⁹ and the lower extremities, and have a high risk of recurrence.¹⁴ PLS has unusual histological features⁹ similar to malignant fibrous histiocytoma, but with the presence of adipose differentiation.^{14,16}

Liposarcoma: genomic landscape

Genomic characteristics of WDLS and progression into DDLS

A common characteristic of both WDLS and DDLS is the presence of a supernumerary ring (where the two arms of the chromosome are fused together) or a giant marker chromosome (where no structural parts of the chromosome can be identified) with amplifications in the chromosome region 12q13–12q15.^{13–15,17–19} This causes amplification of such genes as *HMG2*, *MDM2*, and *CDK4*.^{13–15,17–19} The occurrence of both *CDK4* and *MDM2* amplification is associated with higher local recurrence rates (47% versus 12.5% in *MDM2*-exclusive amplifications).²⁰

Although WDLS is locally aggressive, it has relatively little metastatic potential¹² and exhibits fewer copy number aberrations (CNAs) relative to DDLS: 5.7% in WDLS versus 21% in DDLS.^{10,21} Of the 11 chromosomes amplified in WDLS, the most frequent is 12q13–12q15, found in 95% of cases with *CDK4* amplifications, 87% with *MDM2* amplifications, and 76% with *HMG2* amplifications.¹⁰ Progression from WDLS to DDLS involves additional genomic alterations¹⁰ and importantly the downregulation of adipocyte differentiation programs.¹⁰ Nine CNAs, termed

progression-associated CNAs, which are differentially expressed between the two subtypes, could potentially have roles in the progression of WDLS to DDLS.¹⁰

A major element of dedifferentiation from WDLS to DDLS is the loss or downregulation of adipogenesis.^{10,21–24} Adipocyte-metabolic genes such as *LIPE*,^{10,21,23} *PLIN*,²³ and *PLIN2*,²¹ among others, are also uniquely absent in DDLS,²³ thus displaying a distinctive genomic landscape with global suppression of adipogenesis. Expression of genes related to apoptosis (*BAX*, *BIRC5*, *SULF1*), cytoskeleton arrangement and maintenance (*CTNNA1*, *MARKS*, *TMP4*, *PLEC*), Ras-related genes (*RAB23*, *HRASLS3*, *RAB20*), transcription factors (*TLE4*, *FOXF2*, *SOX11*), and cell-cycle control (*MAPK1*, *CDC2*, *CCNB2*) are differentially expressed between DDLS and WDLS.²³

Genomic characteristics of MLS

Interestingly, MLS displays very few genomic imbalances and in particular lacks high amplifications commonly observed in the other subtypes.^{14,25} MLS is characterized by the presence of a unique reciprocal translocation of bands 13q, which encodes for *CHOP*, and p11, which encodes for *TLS/FUS* on chromosomes 12 and 16, respectively.^{9,24,25} The resulting translocation, t(12;16)(q13;p11), forms the fusion protein TLS/FUS–CHOP, which may play a role in adipose differentiation and inhibition of G₁/S cell-cycle arrest induced by native CHOP proteins.⁹ Amplifications of 13q, specifically 13q21–13q31 and 13q32, are also observed frequently in MLS and are associated with poor overall survival.²⁵ Telomerase reactivation is moderate in MLS (39%),¹⁹ but the *TERT* promoter mutation C228T occurs commonly in MLS cases (74%).²⁶

Genomic characteristics of PLS

PLS is distinguished in having the most chromosome imbalances,^{14,16,25} with more gains and deletions of chromosome regions than any other LPS subtype, occurring on all chromosomes.²⁵ Unlike MLS, PLS has not been associated with any translocations;^{9,27} instead, frequent CNA amplifications occur in a number of chromosome regions.²⁷ Specifically, amplification of 13q31–13q32 (frequent in PLS but not other subtypes) is associated with poor patient survival and increased tumor-related death, with a median survival of 35 months versus 78 months in those with no 13q gain.²⁵ PLS displays differentially high amplifications of *CCND1* and similarly high amplification of *CCND2*, *MYB*, *MDM2*, *GLI1*, and *CDK4* to DDLS.²⁸

Chemotherapeutics for LPS: eribulin and trabectedin

In 2015 and 2016, the FDA approved two chemotherapeutic agents specifically for LPS treatment: eribulin and trabectedin. Eribulin acts by inhibiting the polymerization of tubulin, preventing the formation of microtubules, and interfering with the mitotic spindle required for cell division. A Phase II clinical trial showed measurable tumor shrinkage and RECIST (response evaluation criteria in solid tumors) scores in LPS patients treated with eribulin.²⁹ About 47% of patients with DDLS treated with eribulin showed complete or partial response or stable disease.²⁹ Approximately 45% of patients with other LPS subtypes (eg, PLS and MLS) showed stable disease.²⁹ In a large Phase III multicenter clinical trial, eribulin treatment significantly extended overall survival in patients by 2 months compared to dacarbazine, a DNA cross-linking agent.³⁰ Overall survival was improved in LPS patients treated with eribulin compared to dacarbazine.³⁰

Trabectedin exerts its antitumor effect by interfering with DNA repair machinery and by causing DNA breakage and cell-cycle arrest. In 2007, a clinical trial with exclusively MLS patients showed efficacy (51% objective response with progression-free survival at 6 months in 88% of patients) of the drug in MLS, and specifically for those who carried the type I and II variants of the TLS/FUS-CHOP fusion products.³¹ The efficacy of trabectedin specifically against type I and II variants was confirmed in xenograft models, showing that trabectedin prevented and prolonged the binding of variant types I and II to target genes *PTX3* and *NFI*.³²

Liposarcoma: targeted therapeutics

Despite the relatively large amount of genomic information garnered for LPS subtypes, there is currently no approved targeted therapy for LPS.

CDK4/6 for WDLS/DDLS

WDLS and DDLS both harbor *CDK4* amplifications,^{13,14,15,17–19} making CDK4 a promising therapeutic target. A Phase II WDLS/DDLS-specific clinical trial showed PD0332991, a CDK4 oral inhibitor, to be effective in the treatment of these subtypes, with 60% of patients showing no disease progression at 12 weeks and median progression-free survival of 17.9 weeks.³³ These initial findings of CDK4 inhibition as a potential targeted therapy for LPS are promising, and several clinical trials are ongoing in patients with WDLS/DDLS.^{34,35} Furthermore, PD0332991, also known as palbociclib, has been approved for the treatment of ER-positive and human EGFR2-negative breast cancer. Given that it is the first CDK inhibitor to be approved as a cancer therapy

by the FDA, its use in WDLS/DDLS could become a reality in the near future.

VEGFR/PDGFR inhibition

Treatment with pazopanib, a small-molecule inhibitor of VEGFR and PDGFR, showed promising results for patients with high-intermediate-grade disease that was surgically unresectable or metastatic.³⁶ Overall, 2.4% of patients had a partial response to the drug, 41.5% had stable disease, and 43.9% experienced disease progression; overall and progression-free survival were, respectively, 12.62 and 4.44 months.³⁶ A similar trial with pazopanib is ongoing that includes low-grade subtypes, such as WDLS.³⁷ Another clinical trial for all subtypes of LPS is investigating the efficacy of pazopanib in combination with topotecan, a compound that prevents the religation of topoisomerase-dependent DNA-strand breaks during DNA replication.³⁸

Akt-mTOR pathway inhibition

Ridaforolimus, an mTOR inhibitor, influences effector proteins S6K and 4E-BP1, and has shown promising results for several sarcomas, including LPS.³⁹ In a Phase II clinical trial, ridaforolimus was shown to elicit responses in 30% of LPS patients; 27% had progression-free survival at 6 months and median progression-free survival of 14.3 weeks, which is comparable to outcomes from other novel agents, such as trabectedin and pazopanib.³⁹ These promising findings led to a Phase III clinical trial.³⁹ An ongoing clinical trial is investigating the potential use of another mTOR inhibitor, everolimus, along with a CDK4/6 inhibitor, ribociclib, in patients with advanced DDLS.⁴⁰

Liposarcoma: immunotherapy

DDLS and PLS

Currently, two immunotherapeutic drug candidates are in clinical trials for these two high-grade and aggressive subtypes of LPS.^{41,42} Both trials involve monoclonal antibodies against the immunotargets PDL1 and CTLA4, namely nivolumab and ipilimumab.^{41,42} One trial is investigating the potential neoadjuvant effect of these two drugs in patients with surgically resectable tumors.⁴¹ The other trial will examine the efficacy of nivolumab with or without ipilimumab in patients with unresectable or metastatic disease.⁴²

MLS

A pilot study of CAR T-cell therapy is ongoing for MLS patients with recurrent or unresectable disease. Treatment consists of administering the patients' own dendritic cells

genetically engineered to express antigen NY-ESO1.⁴³ Similarly, a Phase II clinical trial is under way looking at the effectiveness of atezolizumab, an anti-PDL1 antibody, combined with CMB305, a dendritic-cell-targeted lentiviral vector containing the NY-ESO1 sequence.⁴⁴ Another ongoing pilot study specific for MLS is investigating whether systemic administration of IFN γ can modulate immune-cell infiltration and expression of class II MHC proteins that are expressed on dendritic cells, phagocytes, and antibody-producing B cells.⁴⁵

All liposarcoma subtypes

Ongoing clinical trials are inclusively testing all LPS subtypes as well, such as an efficacy trial of the combination of trabectedin and avelumab, an anti-PDL1 antibody, in patients with unresectable and/or metastatic disease.⁴⁶ Another study is a Phase I trial of ABI009, an albumin-bound rapamycin compound, along with nivolumab.⁴⁷ A third trial is a noninferiority study looking into the effectiveness of combining the standard of care, doxorubicin, with dexrazoxane, a topoisomerase II inhibitor, and olaratumab, an anti-PDGFR α antibody.⁴⁸

Angiosarcoma

Making up 2% of all STS, angiosarcomas are malignant tumors that develop in the inner lining of blood vessels and lymphatic tissue. They can be found in almost any part of the body, but occur most frequently in the skin, breast, liver, and spleen.⁴⁹ There are several syndromes (neurofibromatosis, Maffucci syndrome, and Klippel–Trénaunay syndrome) and various exogenous chemicals (vinyl chloride, thorium dioxide, arsenic, radium, and anabolic steroids) that are known risk factors for developing angiosarcoma.⁴⁹ Prognosis is generally poor, since most patients are not diagnosed prior to widespread metastasis. The overall 5-year survival rate is 35%,⁴⁹ but varies depending on the primary tumor site. Liver and heart angiosarcomas have been found to have as low as no 5-year survival, whereas breast, skin, and soft-tissue angiosarcomas tend to have 5-year survival of 51%, 43%, and 74%, respectively.⁵⁰ The current standard of care is surgery and chemotherapy. A better understanding of angiosarcoma genomics could lead to targeted therapies.

Angiosarcoma: subtypes

Angiosarcomas include a mild form known as epithelioid hemangioendothelioma and more aggressive forms, simply termed angiosarcomas. Epithelioid angiosarcoma is rare and arises in endothelial cells. Angiosarcoma can be divided into five subgroups related to etiology or anatomic location: soft-tissue angiosarcoma (25%), lymphedema-associated

angiosarcoma, radiation-induced angiosarcoma, primary breast angiosarcoma (8%), and cutaneous angiosarcoma (60%).⁴⁹ As these names imply, angiosarcoma can arise either as de novo tumors (primary angiosarcoma) or as secondary angiosarcomas due to chronic lymphedema or radiotherapy.⁴⁹ Because of this, women with breast cancer who undergo radiation treatment are at 1,000-fold higher risk of developing secondary angiosarcoma,⁵¹ making the breast the most common place where radiation and lymphedema-associated sarcomas form.^{51,52} Furthermore, breast angiosarcoma is more aggressive than many other breast cancers and has the tendency to develop rapidly, making it difficult to treat. Although breast angiosarcoma affects deep soft tissue, it does not typically spread to the muscles of the chest wall. Other common primary angiosarcoma tumor sites include the skin and soft tissue,⁵¹ with the skin of the scalp in elderly patients being the most common site for primary angiosarcoma.^{49,53} Skin angiosarcoma remains refractory to treatment measures and progresses quite rapidly. Soft-tissue angiosarcoma afflicts women and men of all ages equally, and typically presents either as a mass in the affected area or as compression of structures inside the abdomen. Although organ-specific angiosarcomas, such as those of the lungs and heart, share the same fundamental characteristics as other STS, the therapeutic strategy employed is individualized based on the subtype.

Angiosarcoma: genomic landscape

Angiosarcomas are a heterogeneous group of malignancies harboring a wide range of genetic alterations. Although critical in the pathogenesis of many cancers and even in other sarcoma subtypes like LMS and undifferentiated PLS, the role of *TP53* gene alterations in angiosarcoma may be more limited. *TP53* mutation and deletion rates have been shown to be as low as 4% and 0%, respectively, in angiosarcoma,⁵⁴ although other studies indicate higher rates.⁵² Additionally, while mutations reported in the MAPK pathway could serve as potential targets of therapeutic interest,⁵² this review focuses on *MYC*, *VEGF/VEGFR*, *PTPRB*, and *PLCG1*.

MYC

MYC is a proto-oncogene known to play a key role in cell-cycle progression and cell proliferation, differentiation, and apoptosis. Mutated or constitutively activated Myc has been implicated in many human cancers. For angiosarcoma in particular, *MYC* also plays a key oncogenic role, and the most common alterations are amplifications on chromosome 8q24.21 (50%), followed by 10p12.33 (33%) and 5q35.3

(11%).⁵⁵ Furthermore, *MYC* gene amplification and protein overexpression in angiosarcoma is well documented^{52,55–61} and is a useful tool in differentiating between primary and secondary angiosarcomas and atypical vascular lesions, which are potential precursors to angiosarcoma. Of the three most common alterations, *MYC* amplification has been widely shown to occur almost exclusively in secondary angiosarcoma, underscoring the fact that genetic distinction can exist even in morphologically indistinguishable tumors.^{55,57–60} *MYC* amplification in secondary radiation-induced angiosarcoma can be observed in up to 100% of samples, as it is an early but often necessary event,^{58–61} whereas atypical vascular lesions have rarer *MYC* amplifications.^{58–61} Furthermore, *MYC* gene amplification is typically,⁵⁹ but not always, related to Myc protein overexpression, suggesting an alternative potential regulatory pathway of *MYC* expression, such as epigenetic control.⁵⁶ Regardless, the amplification of *MYC* in many cases of angiosarcoma suggests the importance of its role in the pathogenesis of angiosarcoma and its utility as a diagnostic tool, as well as a potential treatment target utilizing recently described BET inhibitors, among other agents.⁶²

VEGFR

VEGF and its receptor VEGFR play important roles in the angiogenesis of tumor tissue. VEGF overexpression has been shown in 21%–25% of STS patients of various subtypes.^{63,64} Although high VEGF expression correlates significantly with increased tumor grade in various sarcomas,^{65,66} increased VEGF serum levels are related to worse prognoses, particularly in LMS patients,^{63,64} although the use of VEGF as an independent predictor of clinical outcome as a whole remains controversial.^{66,67} Itakura et al examined immunohistochemical staining of VEGF-related proteins in 34 angiosarcoma samples and found positive expression of VEGFA (94%), VEGFC (12%), VEGFR1 (94%), VEGFR2 (65%), and VEGFR3 (79%).⁵³ Similarly, Antonescu et al showed that in 42 angiosarcoma tumor samples, 60% expressed VEGFR2 in over 75% of cells, though no CNAs were detected despite this strong protein expression and clear upregulation at the transcriptional level.⁶⁸ Moreover, Amo et al showed that treating an angiosarcoma cell line (ISO-HAS) with forced expression of VEGF, VEGFR1, and VEGFR2 with recombinant VEGF caused cell growth, further suggesting the importance of the VEGF family in the pathogenesis of angiosarcoma.⁶⁹ Targeting these VEGF-related proteins in angiosarcoma could thus prove to be an effective treatment.

PTPRB and PLCG1

Given that aberrant angiogenesis is thought to drive angiosarcoma carcinogenesis, the underlying mutational profile does in fact identify angiogenesis genes *PTPRB* and *PLCG1* in angiosarcoma samples (n=39) examined via unbiased next-generation sequencing.^{70,71} Enrichment of both mutations was highly significant, and Behjati et al showed that 15 of 39 (38%) tumors had at least one driver mutation in signaling genes involved in angiogenesis.^{70,71} More specifically, 10 of 39 (26%) samples had inactivating *PTPRB* mutations, whereas 3 of 34 (9%) samples likely had activating *PLCG1* mutations.⁷⁰ *PTPRB* is a tyrosine phosphatase that inhibits angiogenesis by negatively regulating VEGF tyrosine kinases, including VEGFR2, and can often be truncated in angiosarcoma, which may contribute to disease pathogenesis.⁷⁰ In vitro models have shown that inhibition of *PTPRB* increases angiogenesis,⁷¹ so these inactivating *PTPRB* mutations would be expected to lead to angiogenesis in angiosarcoma, as would the activating *PLCG1* mutations. In contrast, *PLCG1* encodes for PLCγ1, which is a signal transducer of tyrosine kinases. A missense alteration (R707Q) can lead to activation of this enzyme, which has been found in the auto-inhibitory cSH2 domain of the protein.⁷⁰ This is consistent with the idea that overactive PLCγ1 drives angiosarcoma by constitutive signal transduction downstream of receptor tyrosine kinases, reinforcing *PLCG1* as an attractive therapeutic target in angiosarcoma.⁷⁰ In addition to these two genes, other rare mutated genes, such as *PIK3CA* (of 39 cases), *FLT4* (1 of 39 cases) and *H/K/NRAS* (5 of 39 cases) have also been reported in angiosarcoma.⁷⁰

Angiosarcoma: targeted therapeutics

Tyrosine kinase inhibitors

Cytotoxic chemotherapy drugs like ifosfamide, anthracyclines, and taxanes are currently used in treating angiosarcoma.^{68,72} However, recently developed anticancer drugs that target angiogenesis-related proteins are exciting, because of the role of the VEGF family in the pathogenesis of this family of diseases. Overexpressed angiogenesis-related proteins have recently been targeted with tyrosine-kinase inhibitors that inhibit proteins in the VEGF family with the hope that curtailing the blood supply might cause tumor shrinkage. These inhibitors include sunitinib, sorafenib, and pazopanib, which target VEGFR1, VEGFR2, and VEGFR3.⁶⁸

In COS7 cells transfected with two different activating mutant forms of VEGFR2, the tyrosine kinase inhibitors sunitinib and sorafenib were effective in decreasing autophosphorylation of both mutants, suggesting their potential

for use in angiosarcoma patients bearing mutant VEGFR2. This mutation has been reported in 10% of samples.⁶⁸ In a recent Phase II study, von Mehren et al showed that sorafenib treatment led to a progression-free rate of 38% in the vascular sarcoma cohort (63% of which were angiosarcoma patients).⁷³ Additionally, the effectiveness of sorafenib in angiosarcoma appears to be related to baseline circulating VEGFA levels, with lower levels being significantly correlated with better outcomes.⁷⁴

The first Phase I dose escalation study of pazopanib in various advanced cancers showed that it was generally well tolerated and had an antitumor activity in a variety of cancers.⁷⁵ Since then, other clinical trials have confirmed both the safety and the efficacy of pazopanib in STS,⁷⁶ establishing it as a viable new treatment option for select STS patients.⁷⁷ Pazopanib proved particularly effective in patients with elevated levels of VEGFR2 in their tumors, as their median overall survival was 7.2 months compared to 2.3 months in those patients with low expression,⁷⁸ further highlighting the value of targeting VEGFR2 overexpression in angiosarcoma as a treatment option.

Anti-VEGF antibody

Use of the anti-VEGF antibody bevacizumab in angiosarcoma has shown promising results alone and in combination with traditional treatments like surgery, chemotherapy, and radiotherapy. Rosen et al showed that bevacizumab monotherapy in one patient with facial cutaneous angiosarcoma who was unable to undergo traditional treatment showed a well-tolerated and encouraging partial response.⁷⁹ Similarly, a Phase II study of bevacizumab use in angiosarcoma (23 of 30) and epithelioid hemangioendothelioma (7 of 30) patients reported that 9% of angiosarcoma patients showed a partial response, and 48% of angiosarcoma patients had stable disease with an average time to progression of 26 weeks. Bevacizumab was also well tolerated by these patients.⁸⁰ Furthermore, use of bevacizumab in combination with pre-operative radiotherapy followed by resection of the tumor bed in two cases of angiosarcoma of the nose was very effective: both patients had a complete response, no residual disease, and no recurrence after follow-up of 8.5 months and 2.1 years.⁸¹ Fuller et al showed that a combination of bevacizumab with chemotherapy was also effective in even inoperable angiosarcoma, with one patient showing dramatic improvements in appearance and symptoms, which remained stable 11 months after treatment had ended.⁸² Finally, in a Phase II trial of a combination of gemcitabine, docetaxel, and bevacizumab in various STS types, 60% of angiosarcoma

patients showed a partial response to this very exciting group of anticancer drugs.⁸³

Angiosarcoma: immunotherapy

Recently, the advent of immunotherapy has provided promising alternatives to cytotoxic chemotherapeutic agents and targeted therapies in cancer treatment. The most popular immune system targets have been the checkpoint proteins CTLA4 and PD1/PDL1, which can modulate the immune system in the control of cancer progression. Unfortunately, ipilimumab, an anti-CTLA4 antibody, showed no response in six patients with SS.⁸⁴ Immunotargeting of the PD1 receptor and its ligand PDL1 has become increasingly popular in the treatment of multiple cancers, including non-small-cell lung cancer, melanoma, and renal and bladder cancers, and reports of responses to these agents in patients with angiosarcoma have begun to emerge.⁸⁵

Leiomyosarcoma

LMS tumors that originate from smooth muscle connective tissue account for 10% of all soft-tissue sarcoma.⁸⁶ LMS frequently occurs in the extremities, small intestine, or retroperitoneal spaces, or most commonly in the uterus,⁸⁷ hence the categorization into either uterine LMS (ULMS) or nonuterine LMS (NULMS).^{86,88} ULMS, which accounts for 1% of all uterine malignancies⁸⁹ and 40% of all uterine sarcomas,⁸⁶ is highly aggressive, with greater metastatic potential than NULMS;⁸⁶ it is also resistant to chemotherapy and radiotherapy.⁹⁰ Median survival in NULMS and ULMS is about 8 and 4.2 years, respectively.⁸⁶ ULMS can progress de novo⁸⁹ or as a result of transformation from uterine leiomyoma, smooth muscle hyperplasia that occurs in as many as 80% of women.⁹¹ The documented incidence of transformation from uterine leiomyoma to ULMS, however, is rare (<0.1%).⁸⁹ The 5-year survival rate for LMS is 40%, but decreases to 10%–15% for high-grade LMS⁹² and 15%–25% for ULMS.^{90,91} The standard of care for LMS is surgical resection when possible.⁸⁶ LMS can remain dormant for extended periods, and the best outcomes occur after early surgical excision with wide margins.^{92,93} The 5-year rate of relapse is 40%, which is associated with very high mortality.^{92,93}

Leiomyosarcoma: subtypes

Guo et al confirmed the presence of three molecular subtypes of LMS.⁸⁷ Types I and II are linked with extrauterine sites, and type III is closely associated with ULMS. Type I can be identified through immunostaining of overexpressed markers, such

as ACTG2, SLMAP, LMOD1, CFL2, and MYLK. Type II is characterized by overexpression of *ARL4C*, associated with translation, translational elongation, and protein localization, and overexpression of *CDK4*, *CTNNB1*, *AURKA*, *RHEB*, *EGFR*, *CCND1*, *MTOR*, *MAPK1*, *NOTCH2*, and *ROR2* has been reported. Type III is associated with upregulation of pathways involved in metabolic processes, ion transport, and regulation of transcription; overexpressed genes include *MDM4*, *ERBB3*, *EPHA3*, *ESR1*, and *EGFR*. The identification of these molecular subtypes has been fairly recent, and although more investigations are warranted, these differences could have clinical significance related to the use of existing or novel targeted therapies.

Leiomyosarcoma: genomic landscape

Genomic imbalances are observed in 88% of LMS cases,⁹⁴ and 60% of them have aberrant chromosome numbers and structures. More aberrations are reported in higher grade tumors than in lower grade ones.⁹⁵ LMS tumors have pleomorphic histology,⁸⁸ absence of CD44 variant 3,⁹⁶ CD34, c-Kit, and S100 expression,⁹⁴ and complex karyotypes.⁸⁸ Almost half of ULMS cases (48%) have X-chromosome gains, and the associated amplicons are located near regions containing the androgen receptor, which might potentially contribute to ULMS resistance to hormone therapy.⁹⁰ Other associations include the putative oncogenes *ELK1* and *ARAF1*.⁹⁵ Sixty-two percent of ULMS cases display loss of the chromosome region 10q, which harbors the tumor suppressors *PTEN* and *MXI1*, and loss of 10q is associated with recurrent⁹⁵ and higher grade tumors.^{94,95} Most ULMS cases show loss of 13q, the region that houses the tumor suppressor *RB*.⁹⁵ Loss of 13q, however, is associated with better prognosis than loss of 10q and contributes to the early development of LMS.⁹⁵ Promoter hypermethylation of the tumor suppressor *RASSF1A* occurs in 39% of LMS, which is higher than in other sarcomas, such as LPS and malignant fibrous histiocytoma, and is associated with poor prognosis in LMS patients with stage II and III cancers.⁹³

PTEN–Akt–mTOR pathway in leiomyosarcoma

Most LMS cases are reported to have activated Akt,⁸⁸ and as mentioned earlier, loss of 10q, which contains the *PTEN* tumor suppressor gene, is a frequent genomic abnormality found in ULMS and associated with recurrence⁹⁵ and high-grade tumors.^{94,95} Hyperplastic smooth muscle cells that lose *PTEN* expression then show constitutive activation of Akt, which results in malignant progression into LMS through the release and activation of mTOR via TSC2.⁸⁸ Mice deficient in

PTEN in smooth muscle lineage cells have shown decreased life spans, with widespread smooth muscle hyperplasia, mainly in the blood vessels, and urinary and intestinal tracts as early as 1 month.⁸⁸ This was accompanied by rapid onset and an 80% increase in incidence of LMS as early as 2 months after birth.⁸⁸ Interestingly, there was no appearance of ULMS, suggesting an altered molecular pathogenesis.⁸⁸ When these 1-month-old mice were treated with an mTOR inhibitor, rapamycin, there was a significant increase in life span and a decrease in tumor growth accompanied by decreases in pAkt and mTOR target pS6, emphasizing the crucial role of this pathway in LMS tumorigenesis.⁸⁸

Leiomyosarcoma: targeted therapeutics

In addition to its use in LPS, the chemotherapeutic agent trabectedin has been approved by the FDA specifically for the treatment of LMS. However, since more than half of early-stage patients experience relapse after therapy,⁹⁷ and the ULMS subtype is resistant to chemotherapy,⁹⁰ more targeted therapies are required for the treatment of LMS.

Aurora kinase A inhibition

Proteins that regulate the formation of the mitotic spindle during cell division are frequently overexpressed in a number of cancers.⁹⁸ One of the key players in mitotic spindle organization and stability is aurora kinase A (AurKA), and its expression is highly regulated in ULMS compared with benign LMM or normal myometrial tissue.⁹¹ Shan et al illustrated the efficacy of an AurKA inhibitor, MK5108, in a mouse model of LMS in which treatment decreased tumor growth and induced G₂/M cell-cycle arrest and apoptosis.⁹¹ A Phase I clinical trial for the use of MK5108 in solid cancers indicated that it was well tolerated⁹¹ and provides the impetus for testing this inhibitor in LMS and other sarcomas.

Combination of aurora A kinase and mTOR inhibition

As mentioned previously, activation of the Akt–mTOR pathway through the loss of tumor suppressor *PTEN* is crucial for the development of LMS.^{88,94,95} One group is investigating the potential therapeutic benefits of simultaneous AurKA and mTOR inhibition⁹⁷ by using the AurKA inhibitor MLN8237, along with the mTOR inhibitor rapamycin. They found that with a specific schedule of 24 hours of pretreatment with MLN8237 followed by cotreatment for 72 hours with both MLN8237 and rapamycin, tumor volume decreased significantly when compared to MLN8237 or rapamycin alone; this was accompanied by a pronounced decrease in

cell proliferation and an increase in apoptosis.⁹⁷ Interestingly, LMS subtype II shows overexpression of both *MTOR* and *AURKA*, making the dual AurKA and mTOR inhibition regimen a possible personalized therapy for patients with this particular subtype.

ROR2 as potential therapeutic target in leiomyosarcoma

ROR2 is activated by Wnt5A via the noncanonical Wnt pathway and is highly expressed in several sarcoma subtypes, including LMS.⁹⁹ LMS patients with strong ROR2 staining have worse 5-year disease-specific survival than those with weak or undetectable ROR2 staining.⁹⁹ It is noteworthy that ROR2 expression is consistent between primary and metastatic LMS tumors, which might enable a common treatment for both.⁹⁹ Edris et al showed that *ROR2* knockdown led to a 50% decrease in invasiveness of LMS cell lines and a threefold reduction in average xenograft tumor mass in mice.⁹⁹ ROR2 expression is specifically enriched in subtype II, making ROR2 another potential therapeutic target for this subtype. Therefore, the use of Wnt pathway inhibitors, such as OTSA101, an anti-Fzd10 antibody in a Phase I trial for advanced SS treatment, might prove to be effective in LMS.¹⁰⁰

EGFR inhibition

Using testicular LMS as a model, Sette et al uncovered a putative LMS cancer stem-cell population that was resistant to chemotherapy.¹⁰¹ Specifically, this population and differentiated tumor populations have shown high activation of EGFR.¹⁰¹ High EGFR expression is also observed in LMS specimens compared to normal tissue. EGFR inhibition combined with chemotherapy results in a decrease in tumor size accompanied by an increase in apoptosis.¹⁰¹ As such, EGFR inhibitors could be used to target both cancer stem cells and differentiated tumor populations in LMS. This therapeutic modality can potentially be effective for those with subtype III, who have enriched EGFR expression.

LMP2 as a potential target for ULMS

PSMB9/LMP2 encodes a subunit of the proteasome that is involved in antigen processing and frequently genetically altered in ULMS.⁹⁰ *LMP2* loss is observed in 85% of ULMS cases,⁹⁰ and over 30% of samples have essential mutations in *LMP2*.⁹⁰ Female mice with mutated copies of *LMP2* spontaneously develop ULMS by 14 months, with 40% prevalence.¹⁰² One group showed that inoculation of ULMS cells expressing exogenous *LMP2* led to a reduction in tumor growth with no toxicity.⁹⁰ Unfortunately, the proteasome

inhibitor bortezomib alone showed the minimal activity in a Phase II study of STS, but one of the patients studied did have a partial response, indicating that combination therapy with other agents may have better effects.¹⁰³ While surgery is a treatment option for resectable ULMS, targeting and reactivation of *LMP2* could potentially be efficacious for this category of LMS.

Hepatocyte growth factor/scatter factor

When hepatocyte growth factor/scatter factor (HGF/SF) is bound to its receptor, c-Met, it promotes angiogenesis, proliferation, and invasion of cancer cells.¹⁰⁴ Since the c-Met protein is overexpressed in LMS,¹⁰⁴ it may be a prime target amenable to therapeutic intervention. Burgess et al developed a fully human anti-HGF/SF antibody termed AMG102/rilotumumab,¹⁰⁵ which significantly decreased tumor growth when used to treat LMS in tumor-bearing mice.¹⁰⁴ There have been several completed clinical trials for AMG102 in other nonsarcoma cancers,^{106–110} one, in particular, showed promising results when combined with epirubicin, cisplatin, and capecitabine for gastric and esophagogastric cancers.¹⁰⁶ Another clinical trial is ongoing for squamous-cell lung carcinoma,¹¹¹ but no study has yet investigated the efficacy of AMG102 in either LMS or other sarcomas. Based upon the encouraging in vivo mouse work and the promising human clinical trials with other types of malignancies, there is strong rationale for testing AMG102 in LMS and other sarcomas.

Leiomyosarcoma: immunotherapy

Checkpoint inhibition

Pembrolizumab, an anti-PDL1 antibody, was approved by the FDA in 2017 for solid tumors.¹¹² A number of LMS tumors are positive for PD1 expression, and two clinical trials are investigating the effect of pembrolizumab alone¹¹³ or in combination with the immunosuppressor cyclophosphamide¹¹⁴ in LMS. A different anti-PDL1 antibody, nivolumab, was shown to be an effective treatment for one patient with refractory LMS, who had already undergone surgery and multiple rounds of radiation and chemotherapy.¹¹⁵ Furthermore, Paoluzzi et al reported a retrospective study of 24 STS patients treated with nivolumab: seven were diagnosed with LMS, three of whom had stable disease after eight cycles of treatment.¹¹² However, the response to pembrolizumab was dramatically different in different individual tumors in a single ULMS patient.¹¹⁶ Molecular analysis suggested that loss of *PTEN* in LMS may correspond to resistance to PD1 inhibition.¹¹⁶ Since *PTEN* loss or its equivalence is a frequent genetic alteration in LMS,^{88,94,95} other immunotherapeutic

strategies may be considered for patients with these tumors. Overall, immuncheckpoint inhibitors have shown encouraging activity in LMS patients, and studies with expanded cohort enrollment are ongoing to confirm the efficacy of these inhibitors.

CD47 inhibition

CD47 is a cell surface marker that is overexpressed by cancer cells (87% of cases) relative to normal muscle tissues; CD47 prevents cells from being phagocytized by macrophages of the immune system.^{92,117} LMS has also shown high infiltration of tumor-associated macrophages, which can promote cancer-cell aggressiveness, and patients have shown significantly poorer prognoses.^{92,117} Edris et al hypothesized that blocking the antiphagocytosis function of CD47 would allow infiltrated macrophages already present in the LMS tumor to switch from a protumor to antitumor function and eliminate the tumor cells within the tumor.^{92,117} In vitro cell-based studies demonstrated phagocytosis of LMS cells by macrophages when treated with an anti-CD47 antibody.⁹² Anti-CD47 treatment drastically reduced tumor mass in vivo in the range of 5- to 30-fold, with few to no distal metastases compared to the controls; a nearly 70-fold decrease in distal metastases was also reported.⁹² Currently, four different anti-CD47 antibodies are undergoing clinical trials for use in hematological and solid cancers.^{118–123} The aforementioned studies collectively represent an underexploited therapeutic opportunity for treatment of LMS patients with anti-CD47 antibodies.

Rhabdomyosarcoma

RMSs are highly aggressive tumors that typically develop from skeletal muscle cells.¹²⁴ They represent 3%–4% of all childhood cancers and are the most common childhood and adolescent STS,^{124,125} accounting for 40% of pediatric STS.¹²⁶ Although RMS can occur anywhere in the body, it most commonly occurs in the head and neck (10%), orbit (9%), genitourinary tract (24%), extremities (19%), and nasal passage and sinuses (16%).^{124,127} Not only are RMS symptoms tumor site-specific, but prognoses are also linked to primary tumor location. Standard of care depends on primary tumor site and the age of the patient, but can include surgery, radiation, and chemotherapy. RMS frequently metastasizes to the lungs, bone marrow, and bones, and heterogeneity in these tumors makes them confounding and difficult to diagnose, given the lack of strong genetic markers. However, up to 70% of newly diagnosed cases that do not involve metastases can be cured with multimodal therapy. Since survival rates can

vary between 35% and 90% depending on the RMS subtype, a clear diagnosis is essential for disease management.^{128,129} Various environmental risk factors have been associated with increased risk of developing RMS, such as paternal smoking, maternal recreational drug use, advanced maternal age, and X-ray exposure in utero.¹²⁶ Additional genetic risk factors include neurofibromatosis type 1, Li–Fraumeni syndrome, Beckwith–Wiedemann syndrome, hereditary retinoblastoma, nevroid basal-cell carcinoma syndrome, Rubinstein–Taybi syndrome, and Costello syndrome.^{124,126} Though advancements in multimodal chemotherapy have shown large increases in patient survival,^{130,131} toxicity remains an issue, and the 5-year survival rate for metastatic disease remains at 30%,¹³² underscoring the need for additional therapeutic strategies.

Rhabdomyosarcoma: subtypes

RMS has traditionally been categorized into two main types according to histopathological differences. The most common subtype is embryonic RMS (ERMS), which accounts for about 60% of RMS,¹³³ whereas alveolar RMS (ARMS) accounts for ~20% of cases.¹³³ ERMS usually manifests in the head and neck, genitourinary tract, and retroperitoneum of children <10 years of age, whereas ARMS usually occurs in the trunk, arms, and legs of adolescents and young adults.¹²⁴ Clinical outcomes differentiate the two subtypes as well, because outcomes for ERMS are typically considered favorable if the tumor is localized, whereas ARMS has a higher propensity to metastasize and generally has a poorer prognosis.^{124,132} Five-year survival rates for RMS vary depending on the risk group and subtype,¹³⁰ but the overall ERMS 5-year survival rate is 73.4%, whereas the ARMS 5-year survival rate is 47.8%.¹²⁶

Rhabdomyosarcoma: genomic landscape

In RMS, the overall somatic mutation burden is relatively low,¹³² but various chromosomal alterations are key and could serve either as prognostic indicators or as targets for therapy.

Alveolar rhabdomyosarcoma

Eighty percent of ARMS have distinguishing translocations between chromosomes 2 and 13 (t[2;13][q35;q14]) or between chromosomes 1 and 13 (t[1;13][p36;q14]), which correspond, respectively, to *PAX3–FOXO1* and *PAX7–FOXO1* gene fusions.^{124,132–134} *PAX3–FOXO1* gene fusions have been detected in 55% of ARMS patients, whereas *PAX7–FOXO1* gene fusions were found in 22% of ARMS patients.¹³² Although *PAX3*, *PAX7*, and *FOXO1* are typically

transcription factor-encoding genes, the *PAX3–FOXO1* fusion gene produces an even more potent transcription activator than *PAX3*, suggesting a role in the pathogenesis of ARMS through aberrant upregulation of *PAX3* target genes,^{135,136} though *PAX3–FOXO1*-specific targets, such as *PDGFR*, have also been shown to be upregulated.¹³⁷ In vitro and in vivo experiments have confirmed that *PAX3* and *PAX3–FOXO1* compete for the same targets, and higher *PAX3–FOXO1* embryonic expression leads to impaired neural crest migration and development.¹³⁶ Although *PAX3–FOXO1* expression seemingly plays a critical role in pathogenesis, it alone does not seem to be enough to cause ARMS. Mouse models made to express the gene fusion developed physical abnormalities but no tumors,^{135,138} suggesting the need for coexisting alterations. Potential cooperating events seem to include dysregulation of the Hippo pathway¹³⁹ and *PAX3–FOXO1* bypassing cellular senescence by cooperating with loss of *INK4a*.¹⁴⁰ Whole-genome sequencing suggests that the most common cooperating events in *PAX3/7–FOXO1* fusions present in RMS are due to genetic amplifications of *MYCN*, *CDK4*, and *MIR17-92*; deletion of *CDKN2A*; or loss of heterozygosity of chromosome 11p15.5.^{124,132}

Whereas the *PAX3/7* fusion type was not associated with patient outcome among ARMS patients with localized disease, patients with metastatic ARMS with *PAX7–FOXO1* fusion had a 4-year survival rate of 75% compared to an 8% 4-year survival rate for those with the *PAX3–FOXO1* gene fusion.¹³⁴ The presence or absence of these *PAX3/7–FOXO1* fusions has been used to subcategorize RMS more accurately as a whole,^{132,141} since the prognosis and molecular profiling for fusion-absent ARMS patients are nearly indistinguishable from those of ERMS patients, despite their histological differences.¹⁴¹ ARMS samples that were fusion absent also had significantly more somatic mutations than those that were fusion present,¹³² suggesting the need and possibility for alternative treatment strategies between the two. Mutations found in fusion-absent but not fusion-present ARMS include the genes *NRAS* and *PIK3CA*, whereas fusion-positive tumors nearly exclusively showed amplification of the chromosome region 12q13–12q14,¹³² which is associated with worse overall survival.¹⁴²

Embryonic rhabdomyosarcoma

ERMS most characteristically shows allelic loss at chromosome 11p15.5,¹²⁴ a region that appears to include tumor-suppressor genes, and wild-type chromosome 11 transfer into an ERMS cell line causes a decrease in proliferation,¹⁴³ suggesting restoration of tumor suppressor activity.

Studies suggest that in most ERMS, both 11p15.5 alleles are inactivated, with an inactivated paternal allele being conserved and the maternal allele being lost altogether.¹²⁴ Further chromosomal alterations shown in ERMS include gains of chromosomes 2, 7, 8, 11, 12, 13, 17, 18, and 20; losses of chromosomes 10, 14, 15, and 16;^{144,145} and translocations in the 1p11–1q11 region.¹⁴⁶ Moreover, studies have shown that 35% (5 of 14) of ERMS samples contain mutant *NRAS* or *KRAS* genes,¹⁴⁷ and at least 45% of fusion-absent RMS as a whole have mutational activations in the Ras pathway, including *FGFR4*, *RAS*, *NF1*, and *PIK3CA*.¹³² Additional mutations or gene amplifications have also been shown in *TP53*, *MDM2*, *CDKN2A*, *GLI1*, *CTNNB1*, and *PTPN11*.^{124,132,148–150} Though p53 expression has been shown in ample RMS,¹⁵¹ *P53* genetic alteration frequencies in RMS seem to vary. Takahashi et al reported *P53* gene alterations in 22.2% (10 of 45) of samples,¹⁵¹ but Taylor et al reported that only 5% (1 of 20) of tumor samples showed *P53* mutations.¹⁵² Despite these differences, neither source reported a correlation between *P53* mutation status and prognosis.^{127,152} Both sources also reported similar *MDM2* gene amplification frequency in RMS samples, with Takahashi et al reporting amplification in 16.7% (3 of 18) of samples¹²⁷ and Taylor et al reporting 10% (2 of 20).¹⁵² Additionally, Paulson et al showed *CDKN2A/B* focal deletion in 23% (6 of 26) of ERMS, activating *FGFR4* mutations in 20% (5 of 26) of ERMS, frequent low-level gains of a chromosome region containing *GLI1*, deletions in the *NF1* locus in 15% (4 of 26) of ERMS, and *RAS*-family activating mutations in 42% (11 of 26) of ERMS.¹⁴⁹ Similarly, Pressey et al showed that high expression of *GLI1* was present in 21% (15 of 70) of ERMS tumors,¹⁴⁸ and Shukla et al showed *RAS* family mutations in 11.7% (7 of 60) of ERMS samples, *FGFR4* mutations in 9.3% of ERMS samples, and *PIK3CA* mutations in 4.9% of ERMS samples.¹⁵⁰ Collectively, these studies suggest that p53, RAS, Hedgehog, and PI3K pathways are potentially necessary in the pathogenesis of ERMS and could thus be sensitive to targeted inhibition.

Rhabdomyosarcoma: targeted therapeutics

Standard chemotherapy treatment from the Soft Tissue Sarcoma Committee of the Children's Oncology Group consists of stratification of patients based on risk groups and undergoing regimens with combinations of vincristine, dactinomycin, cyclophosphamide, and sometimes irinotecan.¹³¹ Although success has been reported with low-risk and intermediate-risk groups, the 5-year failure-free survival for high-risk

groups has changed little over the past 25 years.^{130,133} While a greater understanding of the molecular basis of RMS has led to new strategies of stratifying patients into more accurate risk groups to improve chemotherapeutic outcomes,^{133,153} alternative targeted therapies also show promise. One study showed RMS cell lines were sensitive to an IGF1 receptor small-molecule inhibitor.¹⁵⁴ Similarly, taking advantage of inhibitor pathway activity in RMS, investigators showed in two studies that combination treatment of Ras–MEK–ERK and PI3K–Akt–mTOR pathway inhibitors led to synergistic RMS inhibition *in vitro* and *in vivo*.^{155,156} Guenther et al used the dual PI3K–mTOR inhibitor PI103 in combination with the MEK inhibitor U0126 on RMS cell lines and found highly synergistic triggering of apoptotic activity in both histological variants, whereas use of only one drug failed to cause cell death.¹⁵⁶ Renshaw et al inhibited the same pathways, but used a combination of the TORC1/2 inhibitor AZD8055 and the MEK inhibitor AZD6244, and were able to show synergistic cell growth inhibition in RMS xenografts.¹⁵⁵ Interestingly, their study also showed a lack of efficacy when just one drug was used, because compensatory cross-talk pathways seemed to render monotherapy ineffective. Finally, Chen et al showed that RMS is susceptible to reactive oxygen species and suggested that therapeutics that increase oxidative stress may synergize with current chemotherapy treatments against RMS.^{157,158}

Rhabdomyosarcoma: immunotherapy

The oncogenic protein Pax3–FoxO1 plays a role in the development of RMS and promotes an immunosuppressive tumor microenvironment¹⁵³ that renders antitumor function by the immune system ineffective. In the following sections are some highlights of recent breakthroughs and current immunorelated clinical trials for RMS.

Selective autologous lymphocyte and immunostimulator regimen

Although localized RMS is quite treatable, recurrent or metastatic disease is associated with disappointing outcomes.¹⁵⁹ In an early clinical trial looking into the efficacy of an adjuvant immunotherapy for recurrent or metastatic RMS, patients received infusions of their own lymphocytes with or without dendritic cells pulsed with the fusion peptide plus IL2, an immunostimulator.^{159,160} The initial results were promising, with 43% 5-year overall survival for those treated with the combination of lymphocytes, peptide-pulsed dendritic cells, and IL2 compared to 31% among those treated with lymphocytes alone.¹⁵⁹ A second-generation clinical trial, which

enrolled similar participants, modified the regimen to induce a greater immunoresponse and antitumor effect.¹⁶¹ The new protocol further enriched and purified the lymphocytes to be depleted of CD25⁺ regulatory T cells that caused immunosuppression, as well as potential residual tumor cells. The mature dendritic cells were pulsed with the patient's own tumor lysate in place of the fusion peptide, and the immunostimulant added was IL7, as opposed to IL2.^{159,161} The regimen was tolerated well, with no treatment-related high-grade adverse effects.¹⁵⁹ The 5-year overall survival rate with this second-generation immunotherapy was 51%, which was significantly higher than the previous regimen, and the progression-free survival was 32%.¹⁵⁹ Interestingly, survival on this regimen was higher for RMS patients than for those with other sarcomas, suggesting the specificity of this regimen to those with metastatic or recurrent RMS.¹⁵⁹

Oncolytic viruses in the treatment of RMS

Two trials are investigating the effectiveness of two different oncolytic viruses in the treatment of RMS.^{162,163} Oncolytic viruses can specifically target dividing, cancerous cells while sparing the differentiated, normally functioning cells, and both trials are in the early phase of safety and dose escalation testing. One trial, though completed with no results posted, investigated the vaccinia virus armed with an immunostimulatory GM-CSF.¹⁶² The other trial, ongoing, is examining the antitumor effect of a herpes virus.¹⁶³

Other current clinical trials

A clinical study of the effect of a tumor lysate vaccine plus the cytokine IL7 showed very promising initial findings, with >50% of participants showing positive immunoresponses.¹⁶⁴ Another clinical trial that specifically enrolled only RMS patients is testing the feasibility of cytotoxic T cells armed with tumor-associated antigens and investigating whether an antitumor response can be specifically launched against cancer cells.¹⁶⁵ The five antigens being tested are SSX, survivin, NY-ESO1, MAGEA4, and PRAME.¹⁶⁵ Lastly, one clinical trial is investigating the effectiveness of a drug-conjugated antibody, lorvotuzumab mertansine, in which an anti-CD56 antibody is conjugated to the drug mertansine, a tubulin inhibitor, in RMS and other sarcomas.¹⁶⁶

Ewing's sarcoma

ES is a neuroectodermal-related malignancy of the bone and soft tissue.^{167,168} ES occurs in children and young adults,¹⁶⁹ with higher frequency in males.^{167,168} Frequent primary ES sites include the paravertebral region, the chest wall, and the

lower extremities.¹⁶⁷ For patients with localized disease, the 5-year relapse-free survival rate is 50% for axial primary sites and 67% for all other sites, but decreases drastically to 21% for those with detectable metastasis at diagnosis.¹⁶⁸ However, 30%–50% of those with localized disease will experience relapse within 3 years.¹⁶⁷ In one study, surgery decreased the relapse rate significantly, from 31% to 15% for axial tumors and from 20% to 4% for other sites, but patients whose disease relapsed within 2 years typically had worse survival rates.¹⁶⁸ The hallmark of ES is the translocation fusion between the chromosome regions of the *EWS–ETS* family of transcription factors.^{169,170} This cancer is grouped together with primitive neuroectodermal tumors and termed the “Ewing family” of tumors due to the presence of similar *EWS–ETS* translocations. The difference between the two is a continuum of neural differentiation, with one end of the spectrum being primitive neuroectodermal tumor with its differentiated neural phenotype predominantly found in soft tissue, and the other being ES with its undifferentiated neural components.¹⁶⁷ The extent of differentiation is not a significant prognostic factor for patients with Ewing family tumors.^{129,171}

ES: subtypes

ES tumors are tightly compacted; comprise small, rounded malignant cells separated by strands of fibrous tissue; and contain little to no intercellular stroma.¹⁶⁹ ES tumors arise from embryonic osteochondrogenic progenitors that positively express *ERG*, *GDF5*, and *PTHLH*.¹⁷² ES and the Ewing family of tumors are characterized by the translocation of *EWS* and the *ETS* family of transcription factors, mainly in *FLII* (≥85% of cases) and *ERG* (10% of cases),^{167,170,173} and even some rarer fusions with *ETV1*, *E1AF*, and *FEV*.¹⁶⁹ (1%–5%).¹⁷³ There are four structural variants of *EWS–ERG* fusion transcripts and up to 18 possible variations of *EWS–FLII* transcripts, with the most common being types I and II.¹⁶⁷ Previously, type I showed significantly higher median overall survival compared to other transcript types (9 versus 2 years),¹⁶⁷ but with recent treatment advancements for ES this disparity is now equalized.¹⁷⁴ There are very few mutated genes observed in ES related to signaling pathways and chromatin-modifying genes.¹⁷⁵ Infrequently but consistently, aberrations are detected in three genes: *STAG2* (15%–17%), *CDKN2A* (12%–22%), and *TP53* (6%–7%).^{175,176} *STAG2* and *CDKN2A* mutations are mutually exclusive and observed in primary tumors, as well as cell lines.¹⁷⁶ Patients carrying *STAG2* or *TP53* mutations or both have much lower survival rates.¹⁷⁶ Interestingly, while only 6% of ES patients show

aberrations in *TP53*, this increases to 25% after treatment, suggesting the role of *TP53* deregulation in treatment resistance and recurrence.¹⁷⁵

ES: genomic landscape

The translocation fusion between *EWS* and *ETS* family members produces a potent oncogenic transcription factor¹⁷⁷ capable of inducing tumorigenesis through increased cell viability and proliferation,^{178–181} apoptosis inhibition,¹⁸⁰ metabolic changes to favor biosynthesis, and subsequent cell division.¹⁸² *EWS–ETS* regulates cell proliferation and anchorage-independent growth in ES cells, but not in a non-ES cell line.¹⁷⁸ It also regulates cell viability through *LRWD1*, which plays a role in stabilizing the origin recognition complex required for precise DNA replication.¹⁸¹ Additionally, *EWS–ETS* induces autophagy in ES through overexpression of an autophagy-related gene, *ATG4B*, which leads to a higher rate of proliferation and lower rate of apoptosis.¹⁸⁰ Metabolism in ES is also altered due to *EWS–ETS* oncogenic regulation, which increases serine biosynthesis via PGHDH upregulation, for the production of proteins, lipids, and nucleic acids to meet the demands of cell proliferation.¹⁸² Interestingly, elevated PGHDH expression is highest in ES compared to other cancer cell lines, as well as normal tissue, and patients who are deemed at high risk show upregulation in PGHDH.¹⁸² Inhibition of PGHDH decreases cell proliferation in ES, but not in other non-ES cell lines, suggesting this PGHDH-dependent metabolic phenotype is found exclusively in ES.¹⁸²

Metastasis is a crucial factor leading to mortality in ES.¹⁶⁸ Recently, Choo et al demonstrated the importance of *TWIST1*, a transcription factor involved in early development, in ES metastasis.¹⁸³ *TWIST1* silencing in an in vivo xenograft model showed decreased metastatic burden, and regardless of metastasis status in patients, positive expression of *TWIST1* showed a trend toward lower survival.¹⁸³ Another recent discovery was the oncogenic potential of *KDM3A*, a histone demethylase, in ES.¹⁸⁴ *KDM3A* silencing in vitro showed a 50% decrease in migration and invasion and a tenfold decrease in metastatic burden compared to controls (in mice).¹⁸⁴ *MCAM*, which is also involved in metastasis in other cancers, is a direct downstream target of *KDM3A*, and when silenced, recapitulated impaired proliferation and metastasis are observed with *KDM3A* silencing. *MCAM* was also significantly associated with poor survival in patients.¹⁸⁴ ES cells with elevated expression of APLP2, a prosurvival mediator, are resistant to irradiation as well as immune-cell killing via lymphokine-activated killer cells,¹⁸⁵ allowing cells to continue to grow after treatment and metastasize.

ES: targeted therapies

Without systemic chemotherapy, most ES patients develop rapid tumor recurrence.¹⁸⁶ Standard chemotherapeutic treatment includes combinations of vincristine, actinomycin D, cyclophosphamide, doxorubicin, etoposide, and ifosfamide.^{186,187} Although the 5-year survival rate of patients with localized disease is relatively high (70%), thanks to recent advancements in diagnosis, surgery, chemotherapy, and radiation, survival rates for patients with metastatic or recurrent disease remain <25%.¹⁸⁶ Long-term toxic effects of treatment continue to be a major issue,^{188,189} further emphasizing the need for new forms of therapy.

Because of the characteristic presence of the fusion gene *EWS-FLII* in ES, it has been the target of various therapeutic attempts that have yielded promising in vitro and in vivo results. Use of a small molecule, YK4-279, to bind to *EWS-FLII*, thereby inhibiting its usual binding and transcriptional modulation, caused apoptosis induction in ES cells and reduced the growth of ES orthotopic xenografts.¹⁹⁰ A recent study found synergism in the inhibition of *EWS-FLII* activity between YK4-279 use in combination with vincristine both in vitro and in vivo.¹⁹¹ The YK4-279 analog TK216 is currently being used in a Phase I clinical trial in patients with relapsed or refractory ES.¹⁹²

Moreover, although trabectedin is a chemotherapeutic, trabectedin sensitivity has been shown to be specifically associated with changes in *EWS-FLII* transcription factor activity, because drug treatment decreased the expression of several downstream targets of the fusion gene.¹⁹³ Despite this, a Phase II clinical trial using trabectedin in patients with recurrent RMS, ES, and non-RMS soft-tissue sarcomas showed the insufficient activity of the drug as monotherapy.¹⁹⁴ By taking advantage of downstream targets, Grohar et al were able to show that trabectedin led to the inhibition of the *EWS-FLII*-downstream Werner syndrome protein, which in turn made ES cell lines hypersensitive to the chemotherapeutic SN38, the active metabolite of irinotecan.¹⁹⁵ Utilizing this combination of trabectedin and subsequent SN38 treatment, Grohar et al were able to cause regression of two ES xenografts with low drug concentrations and minimal toxicity.¹⁹⁵ A clinical trial with this combination has indeed begun, but results have not yet been posted.¹⁹⁶

IGFRI and combination treatments

Insulin-dependent signal transduction plays an important role in the malignancy of ES. Prieur et al demonstrated that the fusion protein *EWS-FLII* can directly bind to the promoter and represses the expression of a regulator of the

IGF-signaling pathway, IGFBP3, which is a tumor suppressor that disrupts interaction between the receptor IGFR1 and its ligand IGF, is crucial to prosurvival pathways.¹⁹⁷ Several anti-IGF1R-inhibiting antibodies have been investigated in clinical trials, and in addition to showing a 10% response rate in ES patients, they were generally well tolerated.^{198,199} Resistance to IGF1R inhibition, however, can occur,²⁰⁰ and according to Lamhamedi-Cherradi et al, cells resistant to dalotuzumab, an IGFR1-inhibiting antibody, upregulated some mTOR pathway components. The combination of IGF1R and mTOR inhibition can synergistically bypass the resistance developed from single treatment of either agent and induce an antitumor response.²⁰⁰ A clinical trial investigating this combination of IGFR1 and mTOR inhibition showed that 29% of ES patients had tumor reduction and two patients' tumors regressed.²⁰¹ One patient, who had been previously treated with another IGF1R antibody but developed resistance, had a complete response with this combination treatment.²⁰¹

PARP inhibition

Another potential therapeutic target for treating ES is PARP, a chromatin-associated enzyme involved in DNA repair. Due to upregulation of PARP, ES has proved to be very sensitive to the PARP inhibitor olaparib.²⁰² Heske et al recently showed that combined inhibition of PARP and NAMPT, an enzyme crucial for the production of NAD⁺, a PARP substrate, resulted in delayed tumor growth and increased survival.²⁰³ A Phase II clinical trial for PARP inhibition in refractory ES showed the treatment was safe and well tolerated, though there was no significant response, potentially because the small cohort consisted of patients who had previously been treated with chemotherapy.²⁰⁴ Several ongoing clinical trials are examining the efficacy of PARP inhibition specifically in patients with defective DNA damage repair pathways²⁰⁵ or in combination with other chemotherapeutic drugs.²⁰⁶

ES: immunotherapy

Breakthroughs in immunobased treatments, notably with regard to PDL1/PD1-inhibiting antibodies, have led to successful treatments in several cancers. Recently, the FDA approved a fourth PDL1-specific antibody – durvalumab. Following are some of the major advances in immunotherapy for the treatment of ES.

Immunoblockage: PDL1 status and efficacy

Recently, Spurny et al discovered a lack of PDL1 expression in ES primary samples as well as established cell lines, though

surprisingly over half the samples showed positive staining for the receptor – PD1.¹⁹² Raj et al showed that 30% of tumors from ES patients expressed PDL1, and PDL1 expression was associated with treatment response.²⁰⁷ Le et al recently showed that responsiveness to PDL1 inhibition was due to the lack of a mismatch repair mechanism regardless of tumor type,²⁰⁸ raising the possibility of the mismatch repair pathway being implicated in the efficacy of this treatment in ES. A Phase II clinical trial has been set to investigate the efficacy of PDL1 inhibition in a variety of cancers, including ES with mismatch repair status as the selection criteria.²⁰⁹

CHM1 and EZH2

CHM1, an endochondral bone protein, was reported by von Heyking et al to be highly expressed in ES^{210,211} and is implicated in stemness, enhanced proliferation, invasiveness, and metastasis.²¹⁰ CHM1 recognition by T cells can launch an immunoresponse against CHM1-expressing ES cells and significantly inhibit lung and liver metastatic burdens in vivo, suggesting the clinical potential of CHM1 as a target in ES treatment.²¹² Another potential target is EZH2, a histone methyltransferase, shown by Thiel et al to induce an immunoresponse and ES-specific cytotoxicity when primed with allorestricted T cells.²¹¹

Vaccine-based immunotherapy

A pilot study investigating the effect of the Vigil/FANG vaccine showed promising results.²¹³ Vigil/FANG consists of autologous tumor cells transfected with recombinant GM-CSF and short-hairpin RNA against furin.²¹³ When administered to the tumor site, GM-CSF can induce immunoresponses, whereas furin silencing can block activation of immune tolerance activity of TGFβ₁/TGFβ₂, ultimately resulting in tumor destruction.²¹³ Most of the participants had a complete knockdown of TGFβ₁/TGFβ₂, all had systemic tumor-specific immunoresponses, and it was estimated that 75% would survive past 1 year.²¹³ The vaccine was well tolerated, and this led to a Phase II clinical trial of the vaccine combined with two chemotherapeutic drugs: temozolomide and irinotecan.²¹⁴

Another ongoing clinical trial is looking into the efficacy of a vaccine containing CD25-depleted lymphocytes with tumor lysates and primed dendritic cells with or without IL7 in patients with high-risk ES.²¹⁵ Initial findings showed 57% of those vaccinated with immunostimulatory IL7 had a positive immunoresponse, and 40% of the patients were reported to be stable or without progressive disease.²¹⁵

Synovial sarcoma

SS gets its name from its microscopic similarity and proximity to the synovium, which is a specialized connective tissue that lines synovial joints, but in reality the development of tumor cells is not necessarily of synovial origin. While it is an STS typically found in the arms or legs and usually close to tendon sheaths and joint capsules, it can also occur in other locations, such as the heart, brain, and prostate. SS accounts for ~5%–10% of all STS²¹⁶ and 10%–20% of STS in adolescents and young adults;^{217,218} the median age of diagnosis is 35 years, though ages can range 5–85 years.^{217,219} Current standard of treatment includes surgery and radiotherapy, with SS displaying some sensitivity to chemotherapeutic agents like anthracyclines and ifosfamide.²¹⁷ Overall outcomes are poorer in adults, with the 5-year cancer-specific survival rate being 83% for children and adolescents, but only 62% in adults; even among adolescents and children, younger patients have better outcomes.²²⁰ The extremities are the most common site of tumor origin, accounting for ~70% of cases;²²⁰ ~50% of patients exhibit metastatic disease, with 74%–81% of those experiencing metastasis to the lungs.^{216,220} Smaller tumors and those restricted to the extremities portend better outcomes.²²⁰ Despite differences in survival outcomes among varying ages, histological features of SS between children and adults seem to be identical.²²⁰

Synovial sarcoma: subtypes

Although its cellular origin is unclear (its name is counterintuitive, because SS actually may not be of synovial origin),^{216,221} SS is generally divided into three histological subtypes: monophasic, biphasic, and poorly differentiated.^{216,220,221} Monophasic SS is characterized by the presence of spindle cells and the absence or near-absence of glandular epithelial cells, whereas biphasic SS has equal presence of both spindle cells and glandular epithelial cells.^{216,221} In addition, monophasic SS displays fibrous and sarcomatous cells that are relatively uniform and small and form sheets. In contrast, biphasic SS presents with an epithelial appearance. Furthermore, poorly differentiated SS shows similarities to the small round cells found in ES.^{216,221} Another characteristic of SS is the unique chromosomal translocation (t[X;18]), which results in fusion of the *SYT* gene to the *SSX1*, *SSX2*, or, on rare occasions, the *SSX4* gene.^{217,220,222} A cytogenetic approach that makes use of reverse-transcription polymerase chain reactions can help to differentiate the monophasic and biphasic forms.

Synovial sarcoma: genomic landscape

Initially, SS was categorized in the miscellaneous soft-tissue tumor group by the World Health Organization, due to its unknown origin, despite the misleading name, as it bears no resemblance to synovial cells.^{223,224} The discovery of the translocation fusion *SYT-SSX* is now a key characteristic of SS, with *SYT-SSX1* found primarily in biphasic SS, but in rare cases also in monophasic SS, whereas *SYT-SSX2* is found only in the monophasic subtype.^{223,225–228} Those patients with the biphasic subtype appear to have longer survival than those with the monophasic counterpart, but more data are needed to corroborate these findings.²²⁹ Similarly, those with *SYT-SSX1* have better survival outcomes compared to those with the *SYT-SSX2* fusion product.²²⁹ Overall, 55% of all SS cases showed changes in the DNA CN and chromosome arms.²³⁰ There is a higher frequency of genetic aberrations in monophasic subtypes (78% of cases) compared to only 16% and 5% of biphasic and poorly differentiated SS, respectively.²³⁰ *ERBB2* and *IGFBP2* are genes highly expressed in the epithelial region of the biphasic subtype, potentially indicating the involvement of these genes in epithelial differentiation programs in the biphasic subtypes.²³¹ Additionally, E-cadherin and α -catenin are preserved in the epithelial components of the biphasic subtype and are correlated with longer survival rates.²³² Expression of E-cadherin is also associated with low mitotic rates.²³²

The most frequent gains occur on chromosomes 2, 8, and 12,²³⁰ and in particular comprise *MDM2*, *MSH2*, *KCNK12*, *DCC*, *CDK2*, *ERBB3*, *SAS*, and *CDK4*.²²⁹ Conversely, the most frequent losses occur on chromosomes 3 and 13,²³⁰ with common losses of *HRAS*, *RASSF1*, and *CCND1*.²²⁹ Over 50% of SS cases show positive expression for EGFR, over 40% express SALL2, and the majority show the presence of Bcl2 (91%), pancytokeratin (77%), EMA (75%), and cytokeratin 7.²³³ Using a cDNA microarray, Nagayama et al discovered that SS clustered together with another STS type: malignant peripheral nerve sheath tumors (MPNSTs).²²³ However, Terry et al later showed that TLE staining can differentiate SS from MPNST: >90% of SS samples expressed TLE, but <5% of MPNSTs stained positively for TLE.²³⁴ Nagayama et al also discovered some commonly upregulated genes in SS to be crucial for neural crest development, migration, and differentiation, suggesting the potential origin of SS was derived from neural crest cells.²²³

Synovial sarcoma: targeted therapeutics

Several targeted therapies are currently being tested in clinical trials.^{235–238} One trial that is currently recruiting patients

is examining the efficacy of inhibiting glucose-6-phosphate dehydrogenase, which is crucial for tumorigenesis of SS.²³⁵ Two clinical trials, one for pediatric patients and the other for adults, are investigating the effectiveness of inhibiting a polycomb group protein – EZH2.^{236,237} Another trial is investigating the effect of inhibiting the angiogenic receptors VEGFR2 and VEGFR3.²³⁸ Several clinical trials are also examining combination regimens.^{239,240} One investigated the combination of an mTOR inhibitor, everolimus, and a multiple TKR inhibitor (c-Kit and PDGFR) – imatinib. The findings were quite promising: of nine participants analyzed, five showed stable disease and four had progression, for a response rate of 55%.²³⁹ A trial of dacarbazine, a chemotherapeutic agent, together with sorafenib, an Raf/VEGFR2/PDGFR β inhibitor, resulted in a response rate of >70%: 5 patients had partial responses, 22 had stable disease, and 8 had disease progression, with one death due to adverse effects.²⁴⁰

Synovial sarcoma: immunotherapy

The use of the SYT-SSX fusion product as a target for immunotherapy in SS has shown some therapeutic benefit.^{241–243} Suminoe et al showed that vaccination of dendritic cells armed with the SYT-SSX peptide was well tolerated, with no adverse effects in one patient whose disease had been stable for 2 months.²⁴² Similarly, though using only the fusion peptide, Kawaguchi et al also demonstrated a patient's tolerance to the vaccine. The patient had no disease progression, along with a decrease in circulating cytotoxic T lymphocytes, suggesting these were potentially localized at the site of the tumor, though no biopsy was performed.²⁴¹ Kawaguchi et al further demonstrated the efficacy of the fusion peptide vaccine in conjunction with the administration of an immunostimulant – IFN α .²⁴³ Half the patient cohort had stable disease, as opposed to only 11% of patients in the vaccine-only arm.²⁴³ One caveat of these clinical trials is small samples, owing to the rarity of this type of sarcoma, making it difficult to see the full potential in utilizing the fusion peptide as a therapeutic target.

NY-ESO1 is a tumor antigen belonging to the testis family that is highly expressed in malignant tissues and in the testis, but not in other normal tissues.^{244,245} Over half of SS patients express NY-ESO1,²⁴⁴ and because it is immunogenic,^{246–248} it is an attractive target for immunotherapy. In a first-ever clinical trial looking into the effect of administering NY-ESO1 receptor-expressing autologous T cells in patients with metastatic SS, Robbins et al reported a response rate of >60%.²⁴⁵ Four of six patients showed partial responses, with the longest being 18 months, and several individuals

also had regressed lung metastases.²⁴⁵ The treatment was well tolerated, leading to the initiation of other similar clinical trials looking into the effectiveness of NY-ESO1 in immunotherapy.^{249–251} One currently active clinical trial is examining the efficacy of combining atezolizumab, a PDL1 inhibitor, with a dendritic cell-specific vector containing the gene for NY-ESO1.²⁵² Another clinical trial took a different approach in that allogeneic tumor lysate expression using NY-ESO1 was used to prime patients' autologous dendritic cells.²⁵³ Upon completion, and depending upon the results, these investigational new therapies could be used in the clinic as treatment measures for SS patients.

Epigenetic changes in STS clinical samples

Epigenetic regulators are more recently identified therapeutic targets for STS, and small-molecule inhibitors that inhibit them could prove to be useful in treating the disease. In particular, such regulators as DNA methyltransferases, histone deacetylases (HDACs), and the histone-modifying enzyme EZH2 have been implicated in the pathogenesis of several types of STS.²⁵⁴ Kawaguchi et al²⁵⁵ used clinical samples to demonstrate that the promoter methylation status of tumor-related genes could have an association with the pathogenesis of STS. Currently, however, no DNA methylation inhibitors have been tested for use in STS.

Histone modifications like phosphorylation, acetylation, methylation, and ubiquitination are crucial in the regulation of genes, including oncogenes and tumor suppressors. In particular, acetylation changes modulated by HDAC occur in several malignancies, including STS. Preclinical treatment with PCI24781, an HDAC inhibitor, has been shown to inhibit growth and cause apoptosis in several STS cell lines, including SS and uterine sarcoma.²⁵⁴ Also, EZH2 is an important histone methyltransferase and, when overexpressed in STS samples, is correlated with greater tumor size, metastases, and poor progression.²⁵⁶ In particular, EZH2 mediates the expression of the tumor suppressor *ERG1* via SYT-SSX in SS.²⁵⁶ Also, elevated levels of EZH2 are found in RMS and ES.²⁵⁷ As such, EZH2 inhibition might prove successful as a therapeutic approach for STS. Currently, the EZH2 inhibitor tazemetostat is being tested in a Phase II multicenter clinical trial for patients with SS.²⁵⁷

Conclusion

Given the genetic and histological diversity of this large family of cancers, the management of adult STS calls for a multidisciplinary approach to achieve optimal outcomes.

Over the past 30 years, our knowledge of STS biology has progressed very little when compared with other malignancies. This trend has slowly started to change, and additional developments could lead to new therapeutic strategies and treatment options. Several clinical investigations of therapies outlined in this review for STS are under way, and these summarized in Table 2.

Disclosure

The authors report no conflicts of interest in this work.

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