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. 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%). 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.

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. Beyond TNM stage and histologic grade, additional prognostic factors include surgical margins, age, anatomic site, and histologic subtype.
According to the World Health Organization classification, over 100 distinct histological subtypes have been categorized. 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 approaches.

**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.
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. 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. 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. Surgery is the standard of care for LPS, but recurrence is common and resistance to chemotherapeutics underscores a critical need

### Table 1: Soft-tissue sarcoma genomic landscape

<table>
<thead>
<tr>
<th>Sarcoma type/sub-type</th>
<th>Gene/chromosome alteration</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPS (WDLS, DDLS)</strong></td>
<td>HMGA2 amplification</td>
<td>76%</td>
</tr>
<tr>
<td></td>
<td>MDM2 amplification</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>CDK4 amplification</td>
<td>95%</td>
</tr>
<tr>
<td><strong>LPS (MLS)</strong></td>
<td>13q21–13q32 amplifications</td>
<td>24%</td>
</tr>
<tr>
<td></td>
<td>Telomerase reactivation</td>
<td>69%</td>
</tr>
<tr>
<td></td>
<td>Telomerase reactivation</td>
<td>39%</td>
</tr>
<tr>
<td></td>
<td>C22B7 TERT mutation</td>
<td>74%</td>
</tr>
<tr>
<td><strong>AS</strong></td>
<td>TP53 mutation</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>8q24.21 amplification</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>10p12.33 amplification</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>5q35.3 amplification</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>VEGF overexpression</td>
<td>21%–25%</td>
</tr>
<tr>
<td></td>
<td>Inactivating PTPRb</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>Likely activating PLCG1</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>PIK3CA mutations</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>FLT4 mutations</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>H1KINRAS mutations</td>
<td>13%</td>
</tr>
<tr>
<td><strong>LMS</strong></td>
<td>Genomic imbalances</td>
<td>88%</td>
</tr>
<tr>
<td></td>
<td>Aberrant chromosome numbers and structures</td>
<td>60%</td>
</tr>
<tr>
<td></td>
<td>Promoter hypermethylation of RASSF1A</td>
<td>39%</td>
</tr>
<tr>
<td><strong>LMS (ULMS)</strong></td>
<td>X-chromosome gains</td>
<td>48%</td>
</tr>
<tr>
<td></td>
<td>10q chromosome region loss</td>
<td>62%</td>
</tr>
<tr>
<td></td>
<td>13q chromosome region loss</td>
<td>Most</td>
</tr>
<tr>
<td><strong>RMS</strong></td>
<td>Ras pathway mutations</td>
<td>35%–45%</td>
</tr>
<tr>
<td></td>
<td>TP53 mutations</td>
<td>5%–22%</td>
</tr>
<tr>
<td></td>
<td>MDM2 amplification</td>
<td>10%–17%</td>
</tr>
<tr>
<td><strong>RMS (ERMS)</strong></td>
<td>CDKN2A/B focal deletion</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>FGFR4 activating mutations</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>NF1 locus deletions</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td>Ras family activating mutations</td>
<td>12%–42%</td>
</tr>
<tr>
<td></td>
<td>High GLI1 expression</td>
<td>21%</td>
</tr>
<tr>
<td></td>
<td>FGFR4 mutations</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>PIK3CA mutations</td>
<td>5%</td>
</tr>
<tr>
<td><strong>RMS (ARMS)</strong></td>
<td>PAX3–FOXO1 gene fusions</td>
<td>55%</td>
</tr>
<tr>
<td></td>
<td>PAX7–FOXO1 gene fusions</td>
<td>22%</td>
</tr>
<tr>
<td><strong>ES</strong></td>
<td>EWS–FLI1 translocation</td>
<td>Characteristic</td>
</tr>
<tr>
<td><strong>SS</strong></td>
<td>SYT–SSX1 translocation fusion</td>
<td>Characteristic</td>
</tr>
<tr>
<td><strong>Monophasic SS</strong></td>
<td>Genetic aberrations</td>
<td>78%</td>
</tr>
<tr>
<td><strong>Poorly differentiated SS</strong></td>
<td>Genetic aberrations</td>
<td>5%</td>
</tr>
<tr>
<td><strong>Biphasic SS</strong></td>
<td>Genetic aberrations</td>
<td>16%</td>
</tr>
<tr>
<td></td>
<td>Overexpression: KRT5, KRT7, KRT8, KRT14</td>
<td>Preferentially expressed in biphasic samples</td>
</tr>
<tr>
<td></td>
<td>Overexpression: EST, ELF3</td>
<td>Preferentially expressed in biphasic samples</td>
</tr>
</tbody>
</table>

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

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).

WDLS and DDLS occur either in the retroperitoneal region or in the extremities. Patients with retroperitoneal WDLS or DDLS have higher rates of local recurrence and disease-related deaths than those with extremity tumors. 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.

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

<table>
<thead>
<tr>
<th>Target(s)</th>
<th>Therapeutic</th>
<th>Status</th>
<th>Trial ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS CDK4/6</td>
<td>Palbociclib (PD0332991)</td>
<td>Phase II trial completed (2017)</td>
<td>NCT01209598</td>
</tr>
<tr>
<td>LPS VEGFR and PDGFR</td>
<td>Pazopanib</td>
<td>Phase II trial completed (2017)</td>
<td>NCT01506596</td>
</tr>
<tr>
<td>LPS (MLS) NY-ESO1</td>
<td>CAR T cells</td>
<td>Phase II trial ongoing</td>
<td>NCT02992743</td>
</tr>
<tr>
<td>LPS (MLS), SS Class I MHC expression</td>
<td>Recombinant IFNγ</td>
<td>Pilot study ongoing</td>
<td>NCT01957709</td>
</tr>
<tr>
<td>LPS (DDS), LMS mTOR and CDK4/6</td>
<td>Everolimus + ribociclib</td>
<td>Phase II trial ongoing</td>
<td>NCT03114527</td>
</tr>
<tr>
<td>LPS, LMS PDL1 and mTOR</td>
<td>Avelumab (PDL1 mAb) + trabectedin</td>
<td>Phase II trial ongoing</td>
<td>NCT030743</td>
</tr>
<tr>
<td>LPS, ES, AS (UPS) PDI and mTOR</td>
<td>Nivolumab (PDL1 mAb) + AB1009 (mTOR inhibitor)</td>
<td>Phase II trial ongoing</td>
<td>NCT0319017</td>
</tr>
<tr>
<td>AVEGBevacizumab (VEGF mAb)</td>
<td>Phase II trial ongoing</td>
<td>NCT00288015</td>
<td></td>
</tr>
<tr>
<td>LMS, SS EGFR</td>
<td>Anlotinib (AL3818)</td>
<td>Phase III trial ongoing</td>
<td>NCT03016819</td>
</tr>
<tr>
<td>RMS NY-ESO1, MAGEA4, PRAME, survivin, and SSX</td>
<td>TAA-specific CTLs</td>
<td>Phase I trial ongoing</td>
<td>NCT02239861</td>
</tr>
<tr>
<td>RMS, ES Immunomodulated lysis</td>
<td>Recombinant vaccinia GM-CSF (JX594)</td>
<td>Phase I trial completed</td>
<td>NCT01169584</td>
</tr>
<tr>
<td>RMS, SS CD56 and tubulin</td>
<td>Lorvotuzumab mertansine</td>
<td>Phase II trial ongoing</td>
<td>NCT02452554</td>
</tr>
<tr>
<td>ES EWS-FLI1</td>
<td>TK216</td>
<td>Phase I trial ongoing</td>
<td>NCT02657005</td>
</tr>
<tr>
<td>SS G6PD</td>
<td>DHEA</td>
<td>Phase II trial ongoing</td>
<td>NCT02683148</td>
</tr>
<tr>
<td>SS mTOR, c-KIT, and PDGFR</td>
<td>Everolimus + imatinib mesylate</td>
<td>Phase II trial completed</td>
<td>NCT01281865</td>
</tr>
<tr>
<td>SS NY-ESO1-expressing tumor cells</td>
<td>Autologous dendritic cells loaded with allogeneic tumor lysate expressing NY-ESO1</td>
<td>Phase I/II ongoing</td>
<td>NCT01883518</td>
</tr>
<tr>
<td>Advanced STS postchemotherapy VEGF1 and VEGF2, VEGF3, PDGFR, and c-KIT</td>
<td>Pazopanib</td>
<td>FDA-approved (2012)</td>
<td>—</td>
</tr>
<tr>
<td>Several Immunomodulated lysis</td>
<td>HSV1716</td>
<td>Phase I trial ongoing</td>
<td>NCT00931931</td>
</tr>
<tr>
<td>Several Tubulin and mitotic spindle</td>
<td>Erlotinib</td>
<td>FDA-approved (2015)</td>
<td>—</td>
</tr>
<tr>
<td>Several DNA repair</td>
<td>Trabectedin</td>
<td>FDA-approved (2016)</td>
<td>—</td>
</tr>
<tr>
<td>Several PDI and CTLA4</td>
<td>Nivolumab ± ipilimumab (CTLA4 mAb)</td>
<td>Phase II trial ongoing</td>
<td>NCT02500797</td>
</tr>
<tr>
<td>Several mTOR</td>
<td>Ridaforolimus</td>
<td>Phase II trial completed (2015)</td>
<td>NCT00112372</td>
</tr>
<tr>
<td>Several VEGFR, PDGFR, and DNA replication</td>
<td>Pazopanib + topotecan</td>
<td>Phase II trial ongoing</td>
<td>NCT02357810</td>
</tr>
<tr>
<td>Several Topoisomerase 2 and PDGFR</td>
<td>Dexrazoxane + doxorubicin + olaratumab</td>
<td>Phase II trial ongoing</td>
<td>NCT025843</td>
</tr>
<tr>
<td>Several Raf, VEGFR1, VEGFR2, VEGFR3, PDGFR B, and c-KIT</td>
<td>Sorafenib</td>
<td>Phase II trial completed</td>
<td>NCT00317620</td>
</tr>
<tr>
<td>Several PDL1 and NY-ESO1</td>
<td>Atezolizumab (PDL1 mAb) + CMB305</td>
<td>Phase II trial ongoing</td>
<td>NCT026099</td>
</tr>
<tr>
<td>Several Histone-lysine methyltransferase EZH2</td>
<td>Tazemetostat</td>
<td>Phase II trial ongoing</td>
<td>NCT02601950</td>
</tr>
</tbody>
</table>

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

**Myxoid liposarcoma**

MLS and round-cell LPS constitute 30% of all LPS.\textsuperscript{14} These subtypes frequently occur in the lower extremities,\textsuperscript{14} with metastases observed commonly in the lungs, soft tissue, and bones.\textsuperscript{13} Histologically, these cancers consist of round to oval metastases observed commonly in the lungs, soft tissue, and bones.\textsuperscript{13} 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.\textsuperscript{13–15,17–19} This causes amplification of such genes as \textit{HMGAA2}, \textit{MDM2}, and \textit{CDK4}.\textsuperscript{13–15,17–19} The occurrence of both \textit{CDK4} and \textit{MDM2} amplification is associated with higher local recurrence rates (47% versus 12.5% in \textit{MDM2}-exclusive amplifications).\textsuperscript{20}

Although WDLS is locally aggressive, it has relatively little metastatic potential\textsuperscript{12} and exhibits fewer copy number aberrations (CNAs) relative to DDLS: 5.7% in WDLS versus 21% in DDLS.\textsuperscript{10,21} Of the 11 chromosomes amplified in WDLS, the most frequent is 12q13–12q15, found in 95% of cases with \textit{CDK4} amplifications, 87% with \textit{MDM2} amplifications, and 76% with \textit{HMGAA2} amplifications.\textsuperscript{10} Progression from WDLS to DDLS involves additional genomic alterations\textsuperscript{48} and importantly the downregulation of adipocyte differentiation programs.\textsuperscript{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.\textsuperscript{18}

A major element of dedifferentiation from WDLS to DDLS is the loss or downregulation of adipogenesis.\textsuperscript{10,21–24} Adipocyte-metabolic genes such as \textit{LIPE},\textsuperscript{10,21,23} \textit{PLIN},\textsuperscript{23} and \textit{PLIN2},\textsuperscript{21} among others, are also uniquely absent in DDLS,\textsuperscript{23} thus displaying a distinctive genomic landscape with global suppression of adipogenesis. Expression of genes related to apoptosis (\textit{BAX}, \textit{BIRC5}, \textit{SULF1}), cytoskeleton arrangement and maintenance (\textit{CTNNB1}, \textit{MARKS}, \textit{TMP4}, \textit{PLEC}), Ras-related genes (\textit{RAB23}, \textit{HRASLS3}, \textit{RAB20}), transcription factors (\textit{TLE4}, \textit{FOXF2}, \textit{SOX11}), and cell-cycle control (\textit{MAPK1}, \textit{CDC2}, \textit{CCNB2}) are differentially expressed between DDLS and WDLS.\textsuperscript{23}

**Pleomorphic liposarcoma**

PLS comprises 5% of all LPS, making it the rarest subtype.\textsuperscript{14} Along with DDLS, PLS is also aggressive with metastatic potential and associated with increased disease-related deaths.\textsuperscript{9} These high-grade tumors arise frequently in the retroperitoneum\textsuperscript{9} and the lower extremities, and have a high risk of recurrence.\textsuperscript{14} PLS has unusual histological features\textsuperscript{9} similar to malignant fibrous histiocytoma, but with the presence of adipose differentiation.\textsuperscript{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.\textsuperscript{13–15,17–19} This causes amplification of such genes as \textit{HMGAA2}, \textit{MDM2}, and \textit{CDK4}.\textsuperscript{13–15,17–19} The occurrence of both \textit{CDK4} and \textit{MDM2} amplification is associated with higher local recurrence rates (47% versus 12.5% in \textit{MDM2}-exclusive amplifications).\textsuperscript{20}

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**Genomic characteristics of MLS**

Interestingly, MLS displays very few genomic imbalances and in particular lacks high amplifications commonly observed in the other subtypes.\textsuperscript{14,25} MLS is characterized by the presence of a unique reciprocal translocation of bands 13q, which encodes for \textit{CHOP}, and p11, which encodes for \textit{TLS}/\textit{FUS} on chromosomes 12 and 16, respectively.\textsuperscript{3,24,25} 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.\textsuperscript{9} Amplifications of 13q, specifically 13q21–13q31 and 13q32, are also observed frequently in MLS and are associated with poor overall survival.\textsuperscript{25} Telomerase reactivation is moderate in MLS (39%),\textsuperscript{19} but the \textit{TERT} promoter mutation C228T occurs commonly in MLS cases (74%).\textsuperscript{26}

**Genomic characteristics of PLS**

PLS is distinguished in having the most chromosome imbalances,\textsuperscript{14,16,25} with more gains and deletions of chromosome regions than any other LPS subtype, occurring on all chromosomes.\textsuperscript{25} Unlike MLS, PLS has not been associated with any translocations,\textsuperscript{9,27} instead, frequent CNA amplifications occur in a number of chromosome regions.\textsuperscript{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.\textsuperscript{25} PLS displays differentially high amplifications of \textit{CCND1} and similarly high amplification of \textit{CCND2}, \textit{MYB}, \textit{MDM2}, \textit{GLI1}, and \textit{CDK4} to DDLS.\textsuperscript{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. About 47% of patients with DDLS treated with eribulin showed complete or partial response or stable disease. Approximately 45% of patients with other LPS subtypes (eg, PLS and MLS) showed stable disease. 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. Overall survival was improved in LPS patients treated with eribulin compared to dacarbazine.

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. 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 NF1.

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, 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. 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. 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. 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. A similar trial with pazopanib is ongoing that includes low-grade subtypes, such as WDLS. 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.

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. 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. These promising findings led to a Phase III clinical trial. 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.

Liposarcoma: immunotherapy
DDLS and PLS
Currently, two immunotherapeutic drug candidates are in clinical trials for these two high-grade and aggressive subtypes of LPS. Both trials involve monoclonal antibodies against the immunotargets PD-L1 and CTLA4, namely nivolumab and ipilimumab. One trial is investigating the potential neoadjuvant effect of these two drugs in patients with surgically resectable tumors. The other trial will examine the efficacy of nivolumab with or without ipilimumab in patients with unresectable or metastatic disease.

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\(\gamma\) 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-PDGFRA 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 affects 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
samples, as it is an early but often necessary event, induced angiosarcoma can be observed in up to 100% of clear upregulation at the transcriptional level. Moreover, showed that in 42 angiosarcoma tumor samples, 60% overexpression in angiosarcoma is well documented. Furthermore, MYC gene amplification is typically, whereas atypical vascular lesions have rarer MYC amplifications. Furthermore, MYC gene amplification is typically, but not always, related to Myc protein overexpression, suggesting an alternative potential regulatory pathway of MYC expression, such as epigenetic control. 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.

**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. Although high VEGF expression correlates significantly with increased tumor grade in various sarcomas, increased VEGF serum levels are related to worse prognoses, particularly in LMS patients, although the use of VEGF as an independent predictor of clinical outcome as a whole remains controversial. 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%). 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. 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. 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. 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. More specifically, 10 of 39 (26%) samples had inactivating PTPRB mutations, whereas 3 of 34 (9%) samples likely had activating PLCG1 mutations. PTPRB is a tyrosine phosphatase that inhibits angiogenesis by negatively regulating VGF tyrosine kinases, including VEGFR2, and can often be truncated in angiosarcoma, which may contribute to disease pathogenesis. In vitro models have shown that inhibition of PTPRB increases angiogenesis, 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 autoinhibitory cSH2 domain of the protein. 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. 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.

**Angiosarcoma: targeted therapeutics**

**Tyrosine kinase inhibitors**

Cytotoxic chemotherapy drugs like ifosfamide, anthracyclines, and taxanes are currently used in treating angiosarcoma. 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.

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 effectiveness. Therefore, these therapeutics may provide a platform for the development of novel therapeutic strategies for angiosarcoma.
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 preoperative radiotherapy followed by resection of the tumor bed in two cases of angiosarcoma of the nose was very well-tolerated and had an antitumor activity in both cases of angiosarcoma.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).86,87 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.96 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, ESRI, 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, and 60% of them have aberrant chromosome numbers and structures. More aberrations are reported in higher grade tumors than in lower grade ones. LMS tumors have pleomorphic histology, absence of CD44 variant 3, CD34, fibrous histiocytoma, and is associated with poor prognosis. Tumor suppressor RASSF1A is a frequent genomic abnormality as mentioned earlier, loss of 13q, which houses the tumor suppressor 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. This was accompanied by rapid onset and an 80% increase in incidence of LMS as early as 2 months after birth. Interestingly, there was no appearance of ULMS, suggesting an altered molecular pathogenesis. 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.

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, and the ULMS subtype is resistant to chemotherapy, 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. 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. Shan et al illustrated the efficacy of an AurKA inhibitor, MK5108, in a mouse model of LMS in which treatment decreased tumor size by 80% and increased life span, with widespread smooth muscle hyperplasia, mainly in the blood vessels, and urinary and intestinal tracts as early as 2 months after treatment.

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. One group is investigating the potential therapeutic benefits of simultaneous AurKA and mTOR inhibition 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.\textsuperscript{97} Interestingly, LMS subtype II shows overexpression of both \textit{MTOR} and \textit{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.\textsuperscript{99} LMS patients with strong ROR2 staining have worse 5-year disease-specific survival than those with weak or undetectable ROR2 staining.\textsuperscript{99} It is noteworthy that ROR2 expression is consistent between primary and metastatic LMS tumors, which might enable a common treatment for both.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{101} Specifically, this population and differentiated tumor populations have shown high activation of EGFR.\textsuperscript{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.\textsuperscript{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**

\textit{PSMB9/LMP2} encodes a subunit of the proteasome that is involved in antigen processing and frequently genetically altered in ULMS.\textsuperscript{90} \textit{LMP2} loss is observed in 85\% of ULMS cases,\textsuperscript{90} and over 30\% of samples have essential mutations in \textit{LMP2}.\textsuperscript{90} Female mice with mutated copies of \textit{LMP2} spontaneously develop ULMS by 14 months, with 40\% prevalence.\textsuperscript{102} One group showed that inoculation of ULMS cells expressing exogenous LMP2 led to a reduction in tumor growth with no toxicity.\textsuperscript{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.\textsuperscript{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.\textsuperscript{104} Since the c-Met protein is overexpressed in LMS,\textsuperscript{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,\textsuperscript{105} which significantly decreased tumor growth when used to treat LMS in tumor-bearing mice.\textsuperscript{104} There have been several completed clinical trials for AMG102 in other nonsarcoma cancers,\textsuperscript{106–109} one, in particular, showed promising results when combined with epirubicin, cisplatin, and capecitabine for gastric and esophagealgastric cancers.\textsuperscript{106} Another clinical trial is ongoing for squamous-cell lung carcinoma,\textsuperscript{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.\textsuperscript{112} A number of LMS tumors are positive for PD1 expression, and two clinical trials are investigating the effect of pembrolizumab alone\textsuperscript{113} or in combination with the immunosuppressor cyclophosphamide\textsuperscript{114} 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.\textsuperscript{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.\textsuperscript{112} However, the response to pembrolizumab was dramatically different in different individual tumors in a single ULMS patient.\textsuperscript{116} Molecular analysis suggested that loss of \textit{PTEN} in LMS may correspond to resistance to PD1 inhibition.\textsuperscript{116} Since \textit{PTEN} loss or its equivalence is a frequent genetic alteration in LMS,\textsuperscript{88,94,95} other immunotherapeutic
strategies may be considered for patients with these tumors. Overall, immunocheckpoint 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. LMS has also shown high infiltration of tumor-associated macrophages, which can promote cancer-cell aggressiveness, and patients have shown significantly poorer prognoses. 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. In vitro cell-based studies demonstrated phagocytosis of LMS cells by macrophages when treated with an anti-CD47 antibody. 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. Currently, four different anti-CD47 antibodies are undergoing clinical trials for use in hematological and solid cancers. 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. They represent 3%-4% of all childhood cancers and are the most common childhood and adolescent STS, accounting for 40% of pediatric STS. 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%). 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.

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. Additional genetic risk factors include neurofibromatosis type 1, Li–Fraumeni syndrome, Beckwith–Wiedemann syndrome, hereditary retinoblastoma, nevoid basal-cell carcinoma syndrome, Rubinstein–Taybi syndrome, and Costello syndrome. Though advancements in multimodal chemotherapy have shown large increases in patient survival, toxicity remains an issue, and the 5-year survival rate for metastatic disease remains at 30%, 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, whereas alveolar RMS (ARMS) accounts for ~20% of cases. 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. 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.

Five-year survival rates for RMS vary depending on the risk group and subtype, but the overall ERMS 5-year survival rate is 73.4%, whereas the ARMS 5-year survival rate is 47.8%. In RMS, the overall somatic mutation burden is relatively low, but various chromosomal alterations are key and could serve either as prognostic indicators or as targets for therapy.

Rhabdomyosarcoma: genomic landscape
In RMS, the overall somatic mutation burden is relatively low, 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. PAX3–FOXO1 gene fusions have been detected in 55% of ARMS patients, whereas PAX7–FOXO1 gene fusions were found in 22% of ARMS patients. 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.\textsuperscript{135,136} though PAX3–FOXO1-specific targets, such as PDGFR, have also been shown to be upregulated.\textsuperscript{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.\textsuperscript{138} 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,\textsuperscript{135,138} suggesting the need for coexisting alterations. Potential cooperating events seem to include dysregulation of the Hippo pathway\textsuperscript{139} and PAX3–FOXO1 bypassing cellular senescence by cooperating with loss of INK4a.\textsuperscript{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.\textsuperscript{141,142}

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.\textsuperscript{134} The presence or absence of these PAX3/7–FOXO1 fusions has been used to subcategorize RMS more accurately as a whole,\textsuperscript{141,142} since the prognosis and molecular profiling for fusion-absent ARMS patients are nearly indistinguishable from those of ERMS patients, despite their histological differences.\textsuperscript{141} ARMS samples that were fusion absent also had significantly more somatic mutations than those that were fusion present,\textsuperscript{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,\textsuperscript{132} which is associated with worse overall survival.\textsuperscript{142}

**Embryonic rhabdomyosarcoma**

ERMS most characteristically shows allelic loss at chromosome 11p15.5,\textsuperscript{134} 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,\textsuperscript{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.\textsuperscript{144} 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;\textsuperscript{144,145} and translocations in the 1p11–1q11 region.\textsuperscript{146} Moreover, studies have shown that 35% (5 of 14) of ERMS samples contain mutant NRAS or KRAS genes,\textsuperscript{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.\textsuperscript{132} Additional mutations or gene amplifications have also been shown in TMP53, MDM2, CDKN2A, GLI1, CTNNB1, and PTPN11.\textsuperscript{124,132,148–150} Though p53 expression has been shown in ample RMS,\textsuperscript{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,\textsuperscript{151} but Taylor et al reported that only 5% (1 of 20) of tumor samples showed P53 mutations.\textsuperscript{152} Despite these differences, neither source reported a correlation between P53 mutation status and prognosis.\textsuperscript{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 samples\textsuperscript{127} and Taylor et al reporting 10% (2 of 20).\textsuperscript{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.\textsuperscript{144} Similarly, Pressey et al showed that high expression of GLI1 was present in 21% (15 of 70) of ERMS tumors,\textsuperscript{144} 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.\textsuperscript{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.\textsuperscript{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. 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, alternative targeted therapies also show promise. One study showed RMS cell lines were sensitive to an IGF1 receptor small-molecule inhibitor. 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. Guenther et al. used the dual PI3K–mTOR inhibitor P1103 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. Renshaw et al. inhibited the same pathways, but used 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.

Rhabdomyosarcoma: immunotherapy
The oncogenic protein Pax3–FoxO1 plays a role in the development of RMS and promotes an immunosuppressive tumor microenvironment 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. 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. 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. A second-generation clinical trial, which enrolled similar participants, modified the regimen to induce a greater immunorespose and antitumor effect. 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 immunostimulatator added was IL7, as opposed to IL2. The regimen was tolerated well, with no treatment-related high-grade adverse effects. 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%. 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.

Oncolytic viruses in the treatment of RMS
Two trials are investigating the effectiveness of two different oncolytic viruses in the treatment of RMS. 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. The other trial, ongoing, is examining the antitumor effect of a herpes virus.

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. 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. The five antigens being tested are SSX, survivin, NY-ESO1, MAGEA4, and PRAME. 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.

Ewing’s sarcoma
ES is a neuroectodermal-related malignancy of the bone and soft tissue. ES occurs in children and young adults, with higher frequency in males. Frequent primary ES sites include the paravertebral region, the chest wall, and the
lower extremities. 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. However, 30%–50% of those with localized disease will experience relapse within 3 years. 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. The hallmark of ES is the translocation fusion between the chromosome regions of the EWS–ETS family of transcription factors. 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. The extent of differentiation is not a significant prognostic factor for patients with Ewing family tumors.

**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. ES tumors arise from embryonic osteochondrogenic progenitors that positively express ERG, GDF5, and PTHLH. ES and the Ewing family of tumors are characterized by the translocation of EWS and the ETS family of transcription factors, mainly in FLI1 (≥85% of cases) and ERG (10% of cases), and even some rarer fusions with ETV1, EIAF, and FEV (1%–5%). There are four structural variants of EWS–ERG fusion transcripts and up to 18 possible variations of EWS–FLI1 transcripts, with the most common being types I and II. Previously, type I showed significantly higher median overall survival compared to other transcript types (9 versus 2 years), but with recent treatment advancements for ES this disparity is now equalized. There are very few mutated genes observed in ES related to signaling pathways and chromatin-modifying genes. Infrequently but consistently, aberrations are detected in three genes: STAG2 (15%–17%), CDKN2A (12%–22%), and TP53 (6%–7%). STAG2 and CDKN2A mutations are mutually exclusive and observed in primary tumors, as well as cell lines. Patients carrying STAG2 or TP53 mutations or both have much lower survival rates. 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.

**ES: genomic landscape**

The translocation fusion between EWS and ETS family members produces a potent oncogenic transcription factor capable of inducing tumorigenesis through increased cell viability and proliferation, apoptosis inhibition, metabolic changes to favor biosynthesis, and subsequent cell division. EWS–ETS regulates cell proliferation and anchorage-independent growth in ES cells, but not in a non-ES cell line. It also regulates cell viability through LRWD1, which plays a role in stabilizing the origin recognition complex required for precise DNA replication. 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. 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. 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. 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.

Metastasis is a crucial factor leading to mortality in ES. Recently, Choo et al demonstrated the importance of TWIST1, a transcription factor involved in early development, in ES metastasis. 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. Another recent discovery was the oncogenic potential of KDM3A, a histone demethylase, in ES. KDM3A silencing in vitro showed a 50% decrease in migration and invasion and a tenfold decrease in metastatic burden compared to controls (in mice). 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. 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, 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–FLI1 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–FLI1, 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–FLI1 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–FLI1 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–FLI1-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.\(^{196}\) A clinical trial with this combination has indeed begun, but results have not yet been posted.\(^{196}\)

IGF1R 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–FLI1 can directly bind to the promoter and represses the expression of a regulator of the IGF-signaling pathway, IGFBP3, which is a tumor supressor that disrupts interaction between the receptor IGF1R 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 IGF1R-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 IGF1R 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 pathways\(^{205}\) or in combination with other chemotherapeutic drugs.\(^{206}\)

ES: immunotherapy

Breakthroughs in immunobased treatments, notably with regard to PD-L1/PD1-inhibiting antibodies, have led to successful treatments in several cancers. Recently, the FDA approved a fourth PD-L1-specific antibody – durvalumab. Following are some of the major advances in immunotherapy for the treatment of ES.

Immunoblockage: PD-L1 status and efficacy

Recently, Spurny et al discovered a lack of PD-L1 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 ES210,211 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.211 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 \textit{SYT–SSX} is a now a key characteristic of SS, with \textit{SYT–SSX}1 found primarily in biphasic SS, but in rare cases also in monophasic SS, whereas \textit{SYT–SSX}2 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 \textit{SYT–SSX}1 have better survival outcomes compared to those with the \textit{SYT–SSX}2 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 \textit{ERBB}2 and \textit{IGFBP}2 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 \(\alpha\)-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 \textit{MDM}2, \textit{MSH}2, \textit{KCNK}12, \textit{DCC}, \textit{CDK}2, \textit{ERBB}3, \textit{SAS}, and \textit{CDK}4.229 Conversely, the most frequent losses occur on chromosomes 3 and 13,230 with common losses of \textit{HRAS}, \textit{RASSF}1, and \textit{CCND}1.229 Over 50% of SS cases show positive expression for EGFR, over 40% express \textit{SALL}2, 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 some commonly upregulated genes in SS to be expressed in the epithelial region of the biphasic subtype, suggesting the potential origin of SS was derived from neural crest cells.231

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\(\beta\) 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\(\alpha\).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

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also had regressed lung metastases. The treatment was well tolerated, leading to the initiation of other similar clinical trials looking into the effectiveness of NY-ESO1 in immunotherapy. One currently active clinical trial is examining the efficacy of combining atezolizumab, a PD-L1 inhibitor, with a dendritic cell-specific vector containing the gene for NY-ESO1. Another clinical trial took a different approach in that allogeneic tumor lysate expression using NY-ESO1 was used to prime patients’ autologous dendritic cells. 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. 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. Also, EZH2 is an important histone methyltransferase and, when overexpressed in STS samples, is correlated with greater tumor size, metastases, and poor progression. In particular, EZH2 mediates the expression of the tumor suppressor ERG1 via SYT-SSX in SS. Also, elevated levels of EZH2 are found in RMS and ES. 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.

**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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