

# Role of Molecular Profiling in Soft Tissue Sarcoma

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## Abstract

Diagnosis and treatment of soft tissue sarcoma (STS) is a particularly daunting task, largely due to the profound heterogeneity that characterizes these malignancies. Molecular profiling has emerged as a useful tool to confirm histologic diagnoses and more accurately classify these malignancies. Recent large-scale, multiplatform analyses have begun the work of establishing a more complete understanding of molecular profiling in STS subtypes and to identify new molecular alterations that may guide the development of novel targeted therapies. This review provides a brief and general overview of the role that molecular profiling has in STS, highlighting select sarcoma subtypes that are notable for recent developments. The role of molecular profiling as it relates to diagnostic strategies is discussed, along with ways that molecular profiling may provide guidance for potential therapeutic interventions.

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**S**oft tissue sarcoma (STS) is a diverse category of >50 distinct malignancies that pose many diagnostic and treatment-related challenges due to their remarkable heterogeneity. Molecular profiling has emerged as a useful tool for the mechanistic understanding, diagnosis, and therapeutics of STS, and continues to evolve at an exceptional pace. “Molecular profiling” often refers to in-depth analysis of DNA and RNA sequencing, as well as protein expression, which is the accepted definition used to frame this current review. In routine practice, fluorescence in situ hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR) are used to detect tumor-specific alterations, such as translocations, gene fusions, and amplifications. Immunohistochemistry (IHC) is also commonly used to detect loss or overexpression of proteins of interest (eg, DOG1 in gastrointestinal stromal tumors [GISTs]). Next-generation sequencing (NGS) of either DNA or RNA and gene expression profiling through microarray are being used as research tools to subclassify STS and identify therapeutic targets. The role of these newer techniques

in the daily clinical care of patients with STS is evolving and mostly remains investigational.

## Strategies for Diagnosis and Classification

Traditionally, STSs have been diagnosed histologically through evaluating tumor grade and identifying tissue of origin. This pathologic diagnosis via light microscopy remains the gold standard. Molecular profiling, then, is often ordered as a companion test, especially when histologically based diagnoses are challenging. GEN-SARC<sup>1</sup> was a prospective observational study in which 384 patients had their tumors initially reviewed by expert pathologists solely based on histology and standard-of-care IHC. Tumors were then rereviewed based on results from various molecular tests, including FISH, array comparative genomic hybridization, and RT-PCR. Of the 384 sarcomas, 53 (13.8%) required diagnosis modification after the molecular results were available. Of the initial 43% of tumors for which diagnosis was certain at initial expert pathology review, 94% were confirmed molecularly.

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## Molecular Profiling in Soft Tissue Sarcoma

Several large-scale studies have used multiplatform profiling technologies to examine STS and create detailed maps of molecular and biological markers across sarcoma subtypes (Table 1). It is evident that in general STSs have a low mutational burden, with *TP53*, *ATRX*, *RBI*, and *BRCA2* representing some of the more commonly mutated genes across subtypes. More characteristically, STS is delineated by copy-number changes, chromosomal losses, or gene fusions.<sup>1-8</sup>

The Cancer Genome Atlas (TCGA) Research Network recently published data from a multiplatform analysis of 206 adult STSs,<sup>5</sup> which consisted predominantly of leiomyosarcoma (LMS), dedifferentiated liposarcoma, and undifferentiated pleomorphic sarcoma (UPS), but also included myxofibrosarcoma (MFS), synovial sarcoma, and malignant peripheral nerve sheath tumors (MPNSTs). The complex genomic STSs were found to have more somatic copy-number alterations compared with other

**Table 1. Selected Large-Scale Analyses of Soft Tissue Sarcomas**

Study	N	Sarcoma Subtypes	Techniques Used	Key Alterations	Special Comments
Italiano et al, <sup>1</sup> 2016	384	6 (soft tissue)	IHC, aCGH, RT-PCR, FISH, qPCR	<i>TP53</i> , <i>RBI</i> , <i>PIK3CA</i> , and <i>CDKN2A</i>	Molecular profiling is necessary for accurate diagnosis. 53/384 (13.8%) of diagnoses required modification from histological diagnosis alone based on molecular findings
Movva et al, <sup>2</sup> 2015	2,539	61 (bone and soft tissue)	IHC, FISH/CISH, NGS, Sanger sequence	<i>TP53</i> (26.3%) and <i>BRCA2</i> (17.6%) mutations	DNA mutations identified in 47% of samples
Gounder et al, <sup>4</sup> 2017	5,749	56 (bone and soft tissue)	NGS	<i>RBI</i> (15.8%) <i>CDKN2A</i> (17.2%) <i>CDKN2B</i> (13.0%) <i>CDK4</i> (9.8%) <i>MDM2</i> (9.8%) <i>ATRX</i> (8.7%) <i>KIT</i> (7.4%)	Tumor mutational burden was 2.5/Mb (0-328)
The Cancer Genome Atlas Research Network, <sup>5</sup> 2017	206	6 (soft tissue)	Whole-exome and genome sequencing, DNA methylation, DNA copy number, telomere length, mRNA expression and microRNA-based cluster analysis, RPPA analysis	<i>TP53</i> , <i>ATRX</i> , and <i>RBI</i> mutations	Low somatic mutation burden (average 1.06/Mb); more copy number alterations vs other tumors; immune microenvironment signatures
Italiano et al, <sup>6</sup> 2017	587	Multiple	NGS	<i>TP53</i> (34.7%) <i>ATRX</i> (9.1%) <i>RBI</i> (8.4%) <i>KMT2D</i> (5.8%) <i>NF1</i> (5.3%) <i>ATM</i> (5.1%) <i>PIK3CA</i> (4.9%) <i>ERBB4</i> (4.2%) <i>PTEN</i> (4%) <i>ARID1A</i> (3.7%)	Most frequently amplified genes were <i>MDM2</i> (20%), <i>CDK4</i> (16.7%), <i>GL1</i> , <i>MAP2KA</i> , and <i>TERT</i> (3.2% for each); most frequently deleted were <i>RBI</i> (12.7%), <i>CDKN2A</i> (10.3%), <i>CDKN2B</i> (9.7%), <i>TP53</i> (9.5%), <i>PTEN</i> (8.5%); 92.5% of patients had at least one targetable mutation, copy number alteration, and/or fusion gene
Barretina et al, <sup>8</sup> 2010	207	7 (soft tissue)	DNA sequencing, copy number analysis, mRNA expression profiling	<i>KIT</i> (23% of GIST); <i>PIK3CA</i> (18% of MRC); <i>TP53</i> (16.7% of pleomorphic sarcomas); <i>NF1</i> (10.5% of MFS and 8.3% of pleomorphic sarcomas)	90% amplification of chromosome 12q in dedifferentiated liposarcoma

Abbreviations: aCGH, array-comparative genomic hybridization; CISH, chromogenic in-situ hybridization; FISH, fluorescence in-situ hybridization; GIST, gastrointestinal stromal tumor; IHC, immunohistochemistry; MFS, myxofibrosarcoma; MRC, myxoid/round cell liposarcoma; NGS, next-generation sequencing; qPCR, quantitative polymerase chain reaction; RPPA, reverse phase protein array; RT-PCR, reverse-transcription polymerase chain reaction.

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TCGA tumor types, and the overall somatic mutation burden was low, averaging only 1.06 per Mb. In this and other series, molecular profiling has also been used to subclassify more common STS, such as LMS, either biologically or prognostically.<sup>1,9-11</sup>

A broad subset of STSs remains classified under the nonspecific umbrellas of UPS or sarcomas not otherwise specified (NOS). Using microarray data, Konstantinopoulos et al<sup>12</sup> reclassified 76 malignant fibrous histiocytomas (MFHs) and 10 NOS tumors using a 170-gene signature. Although the term “MFH” has since been abandoned, 3 of the 10 NOS tumors were predicted to be liposarcoma, with 3 predicted as LMS and 1 as fibrosarcoma. After unsupervised hierarchical clustering, 6 of the 10 NOS samples (60%) were found clustered with their predicted subtype, providing support for molecular profiling in reclassification of these heterogeneous categories. Others have performed similar work,<sup>13-15</sup> and future studies may continue to more appropriately classify these undifferentiated tumors. Interestingly, in the TCGA data set,<sup>5</sup> MFS, which was more recently separated from UPS on clinicopathologic grounds, had a very similar molecular profile to UPS, suggesting that UPS and MFS may exist instead as a spectrum of the same disease. The investigators<sup>5</sup> postulated that the similarities may have been due to the fact that approximately 65% of the samples were nonclassic MFS, including high-grade epithelioid tumors. The role that molecular profiling has played in the diagnosis and classification of selected STS subtypes is discussed herein.

### Small Blue Round Cell Tumors

Undifferentiated round cell sarcomas, although lacking the characteristic *EWS-ETS* translocation of Ewing sarcoma, have in general been treated as such. Recently, the identification of CIC and BCOR fusion proteins has shown promise in helping to unify these Ewing-like small blue round cell tumors (SBRCTs).

Selecting from a population of patients with *EWSR1*- and *FUS*-negative SBRCTs, Antonescu et al<sup>16</sup> identified a study cohort of 115 patients over a 20-year history with a CIC gene break-apart signal; 57% were positive for fusion to *DUX4* genes, either at 4q35 (35%) or 10q26 (22%). In this cohort, CIC-rearranged tumors predominantly arose from the soft tissue (86%). Compared with an Ewing sarcoma cohort, these tumors had a higher metastasis

rate and patients were older and had a significantly reduced overall survival (43% vs 79% at 5 years), matched for stage and age ( $P=.002$ ). Other studies and case reports have also noted a connection between CIC-fusion and undifferentiated Ewing-like round cell sarcomas.<sup>17-19</sup> Importantly, another study demonstrated that CIC break-apart FISH assays can miss a significant minority of CIC-*DUX4* SBRCTs (estimated as up to 14%). RNA sequencing revealed CIC-fusion transcripts in these cases, reinforcing that FISH analysis should be used cautiously as only an adjunct to morphologic and IHC assessment, which remain the diagnostic standards.<sup>20</sup>

Another subset of Ewing-like SBRCTs consists of a fusion protein between BCOR and CCNB3. Although most of these sarcomas harboring the BCOR-CCNB3 gene fusion arise in bone, a subset are soft tissue tumors. BCOR-CCNB3 fusions have been found in various series of undifferentiated, unclassified sarcomas, including those from soft tissue, with a predilection for male patients.<sup>21,22</sup> Other BCOR fusion partners include *MAML3* and *ZC3H7B*, and they have again been detected predominantly in male patients with both bone and soft tissue tumors, appearing to have an aggressive clinical course.<sup>23</sup>

### Gastrointestinal Stromal Tumors

Among STSs, GISTs are often used as the archetypes for the utility of molecular profiling. Historically, limited panels using a small number of genes have been used. It has already been described at length that the gain-of-function mutation in the oncogenic receptor tyrosine kinase protein “KIT” is seen in >80% of GISTs and serves as a universal therapeutic target in KIT-containing GISTs.<sup>24-26</sup>

Within the remaining approximately 15% of KIT/PDGFR $\alpha$  wild-type GISTs (WT-GISTs), however, several new subtypes have been characterized, including *SDH*, *NF1*, and *BRAF*/*k-RAS*-associated GIST.<sup>27,28</sup> *SDH*-deficient GISTs constitute >80% of KIT/PDGFR $\alpha$  WT-GISTs<sup>29</sup> and can occur either as part of a syndrome (eg, Carney triad) or sporadically. These GISTs are epithelioid or mixed in morphology and appear to be poorly responsive to tyrosine kinase inhibitor therapies.<sup>30</sup> *NF1*-associated GISTs tend to be multifocal with spindle-cell morphology and are typically less responsive to imatinib therapy.<sup>31</sup> Finally, up to 13% of WT-GISTs have been found to have mutations in exon 15 of *BRAF*. These also

have a spindle cell morphology, and are driven by a substitution of V600E on exon 15, which activates the kinase domain of *BRAF* and results in KIT-independent growth. This substitution is thought to mediate the imatinib-resistance commonly seen in these *BRAF/k-RAS*-associated GISTs.<sup>28,32–35</sup>

There remains a small subset of GISTs lacking the aforementioned canonical mutations, which are often referred to as “quadruple-WT-GISTs” (qWT-GISTs). Molecular profiling has allowed for further characterization of these qWT-GISTs, however, and some have argued that “WT-GIST” is a misnomer, because recent analyses have identified at least 9 other distinct driver mutations.<sup>36</sup> Newly identified alterations in these so-called qWT-GISTs include neurotrophic tropomyosin receptor and cyclin-dependent kinases (NTRK and CDK6), the ETS-transcription factor ERG, and fibroblast growth factor receptor FGFR1, along with LTK, PARK2, SUFU, and others.<sup>28,29,37</sup>

The identification of an *NTRK* alteration in qWT-GISTs is particularly important because of new molecularly directed therapies that are currently under investigation. The *NTRK* gene fusion, in this case, of *ETV6-NTRK3* results in irreversible activation of IGF1R with sustained cell survival through the Ras-Erk1/2 and PI3K-Akt pathways and serves as a potential therapeutic target for this GIST subtype with NTRK inhibitors, such as larotrectinib or entrectinib.<sup>37,38</sup>

### Epithelioid Sarcoma

Both the distal and proximal types of epithelioid sarcoma have been shown to exhibit alterations in the *SMARCB1/INI-1* gene on chromosome 22q.<sup>39,40</sup> It appears that deletion of the tumor suppressor *SMARCB1/INI-1* occurs in 45% to 90% of cases of epithelioid sarcoma, which results in a loss of the functional protein SMARCB1/INI-1 in the SWI/SNF complex.<sup>39,41,42</sup> INI-1 is a component of SMARCB1, which is a subunit of the SWI/SNF complex, which in turn serves as an ATP-dependent agent of chromatin remodeling and activates gene transcription through epigenetic changes.<sup>43</sup> Loss of INI-1 expression by IHC has been seen in >90% of epithelioid sarcomas<sup>40</sup> and IHC demonstration of INI-1 loss is considered a molecular hallmark of diagnosis in epithelioid sarcoma.

Importantly, INI-1 loss is not unique to epithelioid sarcoma. A loss of SMARCB1/INI-1 expression has also been shown in malignant rhabdoid tumors,

as well as renal medullary carcinomas, epithelioid MPNSTs, and others.<sup>43–45</sup> This lends further support for the importance of clinical and histologic assessment as a cornerstone of the diagnostic process.

### Strategies for Therapy

The utility of molecular profiling to direct therapy in patients with refractory disease has been studied extensively. In a recent series of 107 patients whose tumors were interrogated on a 405-gene panel for DNA and 265-gene panel for RNA, 57% had at least one treatment-linked alteration. Notably, NGS changed the initial diagnosis and resulting treatments in 5% of patients (eg, LMS to liposarcoma).<sup>4</sup> In another study of 102 patients who had profiling on a 236- or 315-gene panel, 61% were considered to have an actionable mutation and 16% were able to be treated with targeted therapy.<sup>46</sup> It is important to note that “actionable” in this particular study was loosely defined as “any gene alteration that is either directly targeted or a pathway component of a directly targeted gene by an approved or investigational drug.” Nonetheless, there are ongoing developments to use information from molecular profiling to direct new and investigational therapies (see next section), and results from recent molecular-based trials are highlighted in Table 2. Several specific STSs have been selected for further discussion.

### CDK4 Inhibitors in Liposarcoma

Liposarcoma, one of the most common STSs, is classified into several types: well-differentiated/dedifferentiated, myxoid/round cell, and pleomorphic liposarcoma. Although the treatment strategy for all of these is typically surgical, finding a successful targeted therapy for well-differentiated/dedifferentiated liposarcoma is of particular interest in the setting of recurrent disease.<sup>47</sup>

From a molecular standpoint, both well-differentiated and dedifferentiated liposarcoma are characterized by amplification of regions of chromosome 12q13–15 containing the *MDM2* and *CDK4* genes, demonstrated in >90% of cases.<sup>48,49</sup> Palbociclib, ribociclib, and abemaciclib are cyclin-dependent kinase inhibitors that are FDA-approved for postmenopausal patients with metastatic breast cancer.<sup>50</sup> As such, naturally, their utility in the treatment of liposarcoma has been explored.

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**Table 2. Selected Studies With Molecular Profiling as a Guide for Therapy**

Study	N	Sarcoma Subtypes	Molecular Target	Targeted Therapy	Results
Groisberg et al, <sup>46</sup> 2017	102	Multiple	Multiple	Multiple	61% with potentially actionable alteration. Of the entire cohort, 58% chose to participate in a trial; 16% received matched therapy and 50% of these had SD
Dickson et al, <sup>52</sup> 2016	60	Well-differentiated, dedifferentiated LPS	<i>CDK4</i>	Palbociclib	CR (n=1), PR (n=1); 57.2% PFS at 12 weeks
Ray-Coquard et al, <sup>53</sup> 2012	20	Well-differentiated, dedifferentiated LPS	<i>MDM2</i>	RG7112 (MDM2-inhibitor)	PR (n=1), SD (n=14), PD (n=5, all dedifferentiated LPS) prior to surgical resection
Gounder et al, <sup>59</sup> 2017	31	Epithelioid sarcoma	<i>EZH2</i>	Tazemetostat	PR in 4 patients, SD ≥32 weeks in 6 patients
Schoffski et al, <sup>60</sup> 2017	33	Synovial sarcoma	<i>EZH2</i>	Tazemetostat	SD in 10 patients (30%), lasting ≥16 weeks in 5 patients (15%)
Hyman et al, <sup>64</sup> 2017	55	TRK fusion-positive tumors, including sarcomas	<i>NTRK1</i> (25) <i>NTRK2</i> (1) <i>NTRK3</i> (29)	Larotrectinib	Patient with GIST with ongoing PR at 4 months; other STS-specific data unknown

Abbreviations: CR, complete response; GIST, gastrointestinal stromal tumor; LPS, liposarcoma; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; STS, soft tissue sarcoma.

Two phase II, nonrandomized, open-label clinical trials have demonstrated significant and favorable responses to *CDK4* inhibition. In the original study,<sup>51</sup> 30 patients were treated with palbociclib. Of the tumors screened, 92% demonstrated *CDK4* amplification. Of the 29 evaluable patients, 19 were progression-free at 12 weeks (66%), which compared favorably with an expected progression-free survival (PFS) rate of 40%. Median PFS was 17.9 weeks. In a follow-up study using an alternate dosing schedule, there was a 12-week PFS rate of 57.2% and a median PFS of 17.9 weeks in the 57 evaluable patients.<sup>51,52</sup> These are among the first trials to date to specifically use targeted molecular therapy in liposarcoma.

The initial trials of *MDM2* antagonists were beleaguered by severe toxicities, but showed a favorable effect on tumor progression.<sup>53</sup> Further research in vitro and in xenograft models have shown continued promise and a potential modulatory effect of *MDM2* on *CDK4* and synergy with *PI3k/mTOR* pathways.<sup>54,55</sup> Attempts to make these agents more tolerable are underway.<sup>46</sup>

### **EZH2 Inhibitors in Epithelioid Sarcoma and Synovial Sarcoma**

*EZH2* inhibition has shown promise as a new potential molecular target for therapy. *EZH2* is the catalytic subunit of the polycomb repressive complex 2

(*PRC2*), a multiprotein complex responsible for the methylation of chromatin and repression of gene expression. This complex antagonizes the work of *SWI/SNF*, which is often rendered ineffective by alterations in the *SMARCB1* subunit, as previously discussed.<sup>56</sup> Recent in vivo and xenograft studies have supported the theory that *EZH2*-mediated activity of *PRC2* may be an attractive therapeutic target, demonstrating inhibition of cell growth and migration in synovial sarcoma lines to *EZH2* inhibition.<sup>57,58</sup> Clinical trials have now begun, and preliminary data for tazemetostat, a potent and selective *EZH2* inhibitor, has shown guarded promise.

Gounder et al<sup>59</sup> recently released preliminary data from a phase II multicenter study of tazemetostat in adults with *INI1*-negative sarcoma (ClinicalTrials.gov identifier: NCT02601950). Results from participants with epithelioid sarcoma have been notably positive. Although the final data are still pending, of the 31 patients with a median of one prior systemic therapy exposure, 4 (13%) have shown a partial response and 6 (32%) have exhibited stable disease at ≥32 weeks.

Conversely, Schoffski et al<sup>60</sup> presented less promising preliminary data from the synovial sarcoma cohort in the same trial: of the 33 patients treated with tazemetostat, although 30% exhibited stable disease at 16 weeks, it lasted >16 weeks in only 15%. No ob-

jective responses were seen in the synovial sarcoma cohort, and therefore tazemetostat was not considered to be clinically active in this cohort. Synovial sarcoma is defined by a specific chromosomal translocation resulting in an SS18-SSX fusion oncogene. This fusion protein competes with SMARCB1 for incorporation into the SWI/SNF complex assembly, which is thought to promote oncogenesis through functional instability of the SWI/SNF complex.<sup>61,62</sup> A recent report showed that recruitment of SWI/SNF complexes to polycomb-silenced chromatin leads to polycomb eviction and loss of histone-association with transcriptional repression.<sup>63</sup> SWI/SNF complexes without SMARCB1, as seen in epithelioid sarcoma, cannot evict polycomb, which leaves the repressive modifications in place. However, in tumors like synovial sarcoma, the SWI/SNF complex may be able to evict polycomb because of the incorporation of SS18-SSX in the SWI/SNF complex. This may lead to a loss of the repressive histone modifications and, in turn, activate expression of the *SOX2* gene and drive proliferation. This model may explain why no patients with synovial sarcoma derived a response from tazemetostat. In both the synovial sarcoma and epithelioid sarcoma cohorts, the rate of grade  $\geq 3$  adverse events was low and there were no treatment discontinuations due to adverse events.

### ***NTRK3* as a New Target in WT-GIST and Other STSs**

*NTRK3* has recently been identified as a therapeutic target in qWT-GISTs and other STSs.<sup>37</sup> The Trk receptor family includes 3 transmembrane protein receptors that are encoded by the *NTRK1*, *NTRK2*, and *NTRK3* genes. Alterations in *NTRK* genes occur in a variety of tumors, albeit in most at a low frequency, and lead to a constitutively activated kinase that confers oncogenic potential. Larotrectinib, a selective pan-TRK inhibitor, has been recently studied in phase I trials. A review of the data sets for 3 of these studies included 10 patients with sarcoma and 2 with GIST. *NTRK* alterations were detected across the studies using NGS, FISH, and IHC. Sarcoma subtypes included infantile fibrosarcoma, sarcoma NOS, and MPNST. Responses were seen across tumor types regardless of *NTRK* gene type or fusion partner, with an overall objective response rate of 78%, including a patient with GIST who had an ongoing partial response at 4 months.<sup>64</sup> The most

common treatment-emergent adverse events were fatigue, dizziness, and nausea.

### **Predictive Biomarkers for Immune Checkpoint Inhibition**

Immune checkpoint inhibitors such as anti-PD-1, anti-PD-L1, and anti-CTLA-4 antibodies have recently gained interest as potential therapeutic options in STS. Results have been mixed overall, with some response noted in UPS and dedifferentiated liposarcoma with the anti-PD-1 antibody pembrolizumab,<sup>65</sup> but not in uterine LMS using another PD-1 inhibitor, nivolumab,<sup>66</sup> or in synovial sarcoma using the anti-CTLA-4 antibody ipilimumab.<sup>67</sup> The exact factors that mediate activity in some STSs versus others are still under investigation, but the differing immune microenvironment of sarcoma subtypes may play a role. Pollack et al<sup>68</sup> recently found that high-grade sarcomas such as UPS had higher levels of genes related to antigen expression, high TCR clonality, and high PD-1 and PD-L1 expression. Contrary to the poor clinical activity of nivolumab in LMS, the specimens analyzed in this series also appeared to fit the pattern of an inflammatory tumor type similar to UPS.

Many studies have examined the expression of PD-1 and PD-L1 in sarcoma subtypes.<sup>2,69,70</sup> However, expression through IHC has been inconsistent across studies, likely due to variability in antibodies and cutoff points.<sup>71</sup> Indeed, in the study of pembrolizumab in STS, PD-L1 expression was positive at the 1% threshold in only 3 pretreatment samples (4%).<sup>65</sup> Notably, all 3 patients had UPS; however, reliable correlations between response and PD-L1 expression cannot be made in this series. It has been proposed that high mutational burden identified through NGS may serve as a positive predictive factor for immune checkpoint inhibition<sup>71</sup> based on the experience in other malignancies, such as melanoma<sup>72</sup> and non-small cell lung cancer.<sup>73</sup> The field of NGS in STS, however, is still developing and mutational burden as a predictor of immunotherapy response has not yet been investigated.

### **Implications for the Future**

Molecular profiling in STS, with its implications for diagnosis and therapeutic interventions, continues

to progress rapidly. These techniques are becoming increasingly important in confirmation of diagnosis and sarcoma subtype refinement. Large genomic databases continue to investigate these rare diseases and identify new potential treatment approaches, such as newer generation TORC1/TORC2 inhibitors in LMS.<sup>5</sup> Genomic biomarkers for response to immunotherapy in STS will also be helpful as

the field continues to move forward. Other ongoing initiatives include the MULTISARC phase III precision medicine trial,<sup>6</sup> in which patients with doxorubicin-refractory STS will be randomized to standard-of-care treatment or treatment based on genomic profiling. Diagnosis and treatment of sarcoma is evolving rapidly, and an intimate knowledge of molecular profiling is essential to the understanding and further development of the field.

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