Translating Gene Expression Into Clinical Care: Sarcomas As a Paradigm

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**ABSTRACT**

Whereas most solid tumors are characterized by considerable genetic instability and molecular heterogeneity, sarcomas include many subtypes with very specific underlying molecular events driving oncogenesis. Gene expression profiling and other modern techniques have consequently had particular success in identifying the critical biologic pathways active in specific sarcomas, yielding insights which can be translated into useful diagnostic biomarkers. Public availability of data sets and new sequencing-based technologies will accelerate this process. Molecular studies have also identified oncogenic pathways of particular importance in sarcomas which can be targeted by investigational drugs. Examples include histone deacetylases in translocation-associated sarcomas of young adults, Akt/mammalian target of rapamycin in pleomorphic sarcomas, and macrophage colony-stimulating factor in tenosynovial giant cell tumor. Despite challenges in organization and accrual, future clinical trials of sarcomas need to be designed that take into account specific molecular subtypes as distinct diseases.

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**SARCOMAS: CLINICAL FEATURES**

Soft tissue sarcomas are a fascinating group of diseases, including many entities with distinct underlying molecular events that appear to drive their oncogenesis. These tumors arise in the mesenchymal tissues that make up the bulk of body mass, including skeletal and smooth muscle, fat, vessels, peripheral nerves, tendons, ligaments, joints, and other fibrous connective tissues. Traditionally, these diseases have been defined and named based on histologically evident lines of differentiation, resulting in more than 50 different subtypes. Whereas sarcomas are actually common among the cancers occurring in most mammals, they are relatively rare in humans, accounting for only approximately 1% of malignancies. Consequently, the individual sarcoma subtypes are each very rare. Diagnosis can be quite challenging, as related entities often display subtly distinct spindle cell growth patterns that require subspecialty expertise and/or molecular studies for confident assignment. This topic has remained an active area of research among pathologists who through assembly of larger collections from referral practices, astute morphologic investigations, and the use of ancillary techniques such as immunohistochemistry have been quite successful in defining reproducible and distinct diagnostic subtypes. In many cases, a morphologically defined entity has only much later been confirmed to have a distinct molecular oncogenesis, such as for Ewing sarcoma, first recognized by James Ewing in 1921, and found to have a consistent underlying translocation six decades later. In other cases, a distinctive pathologic entity, such as gastrointestinal stromal tumor (GIST) led to the discovery of a novel treatment paradigm.

The mainstay of sarcoma treatment is wide local excision with neoadjuvant or adjuvant radiation therapy. The location of these tumors is frequently in large limb compartments away from organs, facilitating aggressive attempts to achieve local control, with more than 90% success rates in modern practice. However, systemic chemotherapy provides only limited benefit, excepting a few subtypes that mainly afflict children and young adults (eg, osteosarcoma, rhabdomyosarcoma, Ewing sarcoma). The rarity of individual sarcoma subtypes has led to many clinical trials combining patients with soft tissue sarcoma in order to achieve statistical power. However, as molecular studies clearly show diverse mechanisms of disease, such an approach hampers the identification of targeted agents that could be of benefit in specific sarcoma subtypes.
It is difficult, and sometimes counterproductive, to attempt to group together unrelated types of sarcomas. However, as others have noted, there is a correlation between tumor cell pleomorphism and genomic complexity, borne out by increasingly detailed molecular observations. These extend from karyotype studies to gene expression profiling and array comparative genomic hybridization (aCGH), and have highlighted specific genetic events associated with some diagnoses, particularly among subtypes affecting young patients and/or with nonpleomorphic histology.

Synthesizing our current knowledge of histology, clinical features, and specific molecular events that define tumor subtypes, we can recognize four groups of sarcomas, examples of which are shown in Figure 1. Group 1 sarcomas have nonpleomorphic histology and known pathognomonic molecular events (eg, GIST with activating KIT mutations, dermatofibrosarcoma protubersans and pigmented villonodular synovitis where translocations fuse collagen promoters to growth factors, and sarcomas bearing fusion transcription factor translocations including Ewing family tumors). Group 2 sarcomas affect younger patients and generally have nonpleomorphic histology and karyotypes of limited complexity, but pathognomonic molecular events, which are likely to exist, have yet to be identified. Group 3 sarcomas occur in adult populations and show pleomorphic histology, but on a background of complex changes do include consistently identified molecular events (dedifferentiated liposarcoma with CDK4/MDM2 amplifications, malignant peripheral nerve sheath tumor with NF1 deletions, myxoinflammatory fibroblastic sarcoma with recently recognized t(1;10) and 3p amplifications). Finally, group 4 sarcomas, the group most common in adult populations, have complex karyotypes, pleomorphic histology, and lack consistently identifiable molecular events (eg, undifferentiated pleomorphic sarcoma/malignant fibrous histiocytoma, leiomyosarcoma, pleomorphic lipo- and rhabdomyosarcomas, angiosarcoma, osteosarcoma, myxofibrosarcoma, myofibroblastic sarcoma).

This classification can act as a road map for the diagnostic and therapeutic challenges we currently face for sarcomas. For at least some of the group 1 tumors, drugs targeting the pathognomonic gene target are already in clinical use. The oncogenic pathways involved are highly represented in gene expression profiling studies; for example, KIT and regulatory genes involved in KIT signaling are prominent in the GIST-specific gene cluster. Further improving outcome in these tumors depends on incremental changes to existing drugs or on identifying even better tools to attack the tumor-specific molecular changes. For other tumors in group 1, the genetic events are well-known but targeted treatments are only now being assessed. Gene expression profiling has contributed significantly to the understanding of these tumors. A number of large sarcoma gene expression studies that have included GIST have shown that these tumors have relatively homogenous gene expression patterns compared to other studies that have included GIST have shown that these tumors have.

**Targeted Therapy for Sarcomas, below).** Other gene expression studies on Ewing family tumors and osteosarcomas have identified poor prognosis signatures. While these and similar prognostic classifiers do not in and of themselves identify new therapies, they may ultimately help to tailor treatment to the disease.

Group 2 tumors may well prove amenable to similar strategies, but the underlying molecular events must first be better characterized. Knowledge about such tumors is likely to change rapidly. For example, Yang et al recently used high-resolution aCGH to identify common duplications of the T (brachyury) gene in 6q27 in four families with familial chordoma. Validation on larger patient series, with additional functional support, could move this tumor type into group 1.

This translational approach is less encouraging for the more pleomorphic, complex sarcomas. As with most carcinomas, the tumors in groups 3 and 4 are characterized by significant genetic heterogeneity. Advances in understanding and treating these tumors will accordingly require the type of large detailed studies that are being performed on carcinomas. An example of this is seen in the study of leiomyosarcoma. There have been a number of small studies that have looked at leiomyosarcoma gene expression using DNA microarrays, but these have been hampered by considerable variation in expression from tumor to tumor. However, there have been some larger studies suggesting that there is a relatively well-defined subgroup of leiomyosarcomas. Francis et al generated expression profiles from 40 cases and found a group of 11 which shared consistent features. In a more recent study, we found a similar muscle-enriched leiomyosarcoma gene cluster in a data set of 51 cases and in the retrospective analysis of a published data set. These findings are supported by array CGH identifying specific copy number changes, correlated with gene expression profiling and immunohistochemistry, identifying markers associated with improved survival. Therefore, consistent molecular subtypes are likely to exist within the class of leiomyosarcomas.

It is possible that in some cases, leiomyosarcoma or other forms of mesenchymal differentiation represent an acquired phenotype. The histopathologic impression of smooth muscle differentiation may be due to a convergence of smooth muscle features rather than development from a smooth muscle precursor. Genetic events such as MYOCD amplification could lead to the acquisition of smooth muscle features. As we learn more about the signaling pathways and molecular features of these high-grade sarcomas, we may find that classifying pleomorphic sarcomas into specific subtypes using histology and limited immunohistochemistry does not correlate with specific biologic entities. Therefore, we may be engaging in a classification exercise that reflects the effects of a few genes profoundly altering morphology, but which do not actually impact on tumor oncogenesis, prognosis, or treatment in a clinically important fashion.

One important lesson that can be drawn from the sarcoma expression profiling studies published to date, and relevant to all tumor types, is that public presentation and access to primary data sets critically accelerates scientific advancement in the field. A list of major published sarcoma gene expression profiling studies, with links to the primary data where available, is presented in Table 1. For example, published data sets containing synovial sarcoma expression profiles proved important to validate and characterize the world’s first synovial sarcoma mouse model, whereas potentially important data sets published without concurrent access to primary data were not able to contribute. Sarcoma primary expression
data can find surprising uses, such as contributing to the development of translocation-mining algorithms that identified the karyotypically cryptic TMPRSS2-ERG translocations present in a majority of prostate cancers. Access to primary data is critical for functional studies and identification of relevant drug targets, to confirm reproducibility of findings, to allow combinations of multiple data sets and tumor type comparisons that generate novel observations, and to allow application of new bioinformatics methodology. Despite
Obvious advantages, published standardization of data format, and the availability of curated public repositories such as the National Center for Biotechnology Information’s GEO resource, many data sets have still not been made publicly available.

**TRANSLATING GENE EXPRESSION PROFILING INTO NEW DIAGNOSTIC TOOLS**

Perhaps the most straightforward application of gene expression into clinical care is at the level of diagnostics, an area where public accessibility to primary data has proven to be very helpful. Although sarcoma multigene diagnostic signatures have been published, these have not found clinical application. Gene expression profiling is not well-suited as a clinical diagnostic tool, as it is relatively expensive, technically demanding, and requires special tissue handling protocols for optimal results (eg, freezing within 30 minutes of surgical hemostasis, creating major difficulties for hospital laboratory workflow). For these reasons, techniques that can be applied to standard formalin-fixed, paraffin-embedded pathology laboratory tissue blocks are much more readily applicable to clinical practice. RNA-based methodologies including quantitative real-time polymerase chain reaction, cDNA-mediated annealing, selection, extension, and ligation (DASL), quantitative nucleic acid protection, and nonamplified color-coded probe pair assays are in development as clinical tools for generating focused, diagnostic expression profiles from paraffin-embedded samples. Immunohistochemistry remains the main ancillary molecular diagnostic test employed in pathology laboratories, although it is not truly quantitative. Immunohistochemistry and in situ hybridization do however provide a direct morphologic context at the individual cell level, and are particularly useful for fine needle aspirates and core biopsies where very little tumor tissue is obtained. By detecting protein expression, immunohistochemistry also has advantages for clinical validation of drug targets.

A straightforward method to translate expression profiles into clinical use is thus to interrogate published data for genes that discriminate histologically related diagnoses, and test antibodies against the encoded proteins on surgical pathology specimens. GIST were among the first sarcoma expression profile data sets to be publicly released, and revealed the gene for protein kinase C-theta to be much more highly expressed than in leiomyosarcomas. This finding was independently confirmed at the immunohistochemical level and shown to be clinical useful by virtue of detecting c-kit negative variants of GIST. Tissue microarrays complement gene expression profiles and provide an excellent validation platform on large numbers of cases representing a spectrum of differential diagnoses and/or clinical outcomes. The combination of gene and tissue microarrays has led to the identification of apolipoprotein D as a diagnostic marker for dermatofibrosarcoma protuberans, DOG1/TMEM16A for GIST, and TLE1 for synovial sarcomas.

### Table 1. Sarcoma Profiling Studies, Intermediate to Large Sizes (n > 40)

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year</th>
<th>Platform</th>
<th>Sample Size</th>
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<th>Sarcoma Subtypes</th>
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<td>77</td>
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<td>49</td>
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<td>Lee40</td>
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<td>smd.stanford.edu</td>
<td>Leiomysarcoma (6 other)</td>
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<td>Henderson49</td>
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<td>96</td>
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<td>Detwiler50</td>
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<td>Morgan51</td>
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<td>Segal52</td>
<td>2004</td>
<td>U95A</td>
<td>81</td>
<td>Article supplement</td>
<td>Clear cell, melanoma, other</td>
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<td>Linn53</td>
<td>2003</td>
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<td>53</td>
<td>GSE3930</td>
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<td>Segal54</td>
<td>2003</td>
<td>U95A</td>
<td>51</td>
<td>Supplement (broken link)</td>
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</tr>
<tr>
<td>Nagayama55</td>
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<td>NA</td>
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</tr>
<tr>
<td>Nielsen56</td>
<td>2002</td>
<td>22K, 42K cDNA</td>
<td>46</td>
<td>GSE3443</td>
<td>6 subtypes</td>
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</table>

Abbreviations: E-MEXP, array express accession numbers; GSE, gene expression omnibus; NA, not available.

*Online query (no data access).
†Xenograft data available.
‡Partial data set released.

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Fusion Transcription Factors

Whereas published morphologic, karyotypic, genetic, and expression profile research has led to significant advances in sarcoma diagnosis, with the exception of GIST the same cannot yet be said for therapy. Among the well-defined molecular events underlying sarcoma biology, chromosomal translocations creating a fusion transcription factor are the most common (Table 2).69–91 However, drugs which directly inhibit these oncoproteins are not available. Instead, there is an immediate need to determine the molecular pathways already exist.

In synovial sarcoma, the most common of the translocation-associated soft tissue sarcomas in adult populations, fusion oncprotein effects are clearly mediated by chromatin structure.92 Although neither the SS18 nor the SSX fusion partner proteins contain a direct DNA binding domain, SSX represses transcription of reporter genes.93 SSX and SS18-SSX colocalize with components of the repressive Polycomb complex, and associate with condensed chromatin and epigenetically silenced genes.94 An initial event in Polycomb-mediated gene silencing involves histone deacetylation,95,96 and protein-protein interactions of histone deacetylase components have been demonstrated not only with the transcriptional corepressor TLE197 (originally recognized in synovial sarcoma by expression profiling55), but also with the SS18 component of the oncoprotein itself98 (Fig 2). The tumor suppressor gene EGR1 is a direct target of the synovial sarcoma oncoprotein (again, originally recognized with the aid of expression profiling99), where the EGR1 promoter displays histone hypoacetylation, Polycomb repressor complex binding and histone H3 K27 methylation of its promoter,177 repressing EGR1 transcription in synovial sarcoma.99 Chromatin immunoprecipitation assays show that treatment of synovial sarcoma with histone deacetylase inhibitors reverses these changes at the EGR1 promoter; in preclinical models, synovial sarcoma cell death follows.100

The disease most similar to synovial sarcoma, in terms of molecular biology, is endometrial stromal sarcoma. In this cancer of adult women, the fusion oncprotein partners (JAZF1-JJAZ1 or their variants) interact with Polycomb components, histone deactylase 2 (HDAC2) expression is high, and the HDAC inhibitors valproate and SAHA induce endometrial stromal sarcoma cell death.101,102 Further support for the hypothesis that abnormal gene repression is key to the oncogenesis of related translocation-associated sarcomas afflicting

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**Table 2.** Fusion Transcription Factor Oncogenes in Sarcomas

<table>
<thead>
<tr>
<th>Disease</th>
<th>5′ Partner</th>
<th>3′ Partner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing sarcoma</td>
<td>EWSR1 (or FUS)</td>
<td>FLI (or ERG, ETVI, E1A, FEV)</td>
</tr>
<tr>
<td>Clear cell sarcoma</td>
<td>EWSR1</td>
<td>ATF (or CREB)</td>
</tr>
<tr>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>EWSR1 (or TAF2)</td>
<td>NFAZ2CHN</td>
</tr>
<tr>
<td>Desmoplastic small round cell tumor</td>
<td>EWSR1</td>
<td>WT (or JAZF1)</td>
</tr>
<tr>
<td>Myxoid liposarcoma/round cell liposarcoma</td>
<td>FUS (or EWSR)</td>
<td>DDI3 (or TLE)</td>
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<tr>
<td>Low-grade fibromyxoid sarcoma</td>
<td>FUS</td>
<td>CREB2 (or ASPL)</td>
</tr>
<tr>
<td>Alveolar rhabdomyosarcoma</td>
<td>PAX2 (or PAX7)</td>
<td>FKHR (or NCOAT)</td>
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<tr>
<td>Alveolar soft part sarcoma</td>
<td>ASP (or EWSR)</td>
<td>TFE (or JAZF1)</td>
</tr>
<tr>
<td>Endometrial stromal sarcoma</td>
<td>JAZF1 (or EPC1)</td>
<td>JAZ1/SUZ1 (or PHF1)</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>SYT/SS1 (or SS18L)</td>
<td>SSX (or SSX, SSX2)</td>
</tr>
</tbody>
</table>

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**TARGETED THERAPY FOR SARCOMAS**

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**Fig 2.** SS18/SSX recruits repressive complexes (red) to promoters of target genes. Trimethyl (me3) and acetyl (ac) modifications are shown for lysines 4 and 27 of histone 3 (H3K4, H3K27). A bivalent chromatin structure is shown (H3K4me3/ H3K27me3), as found at the EGR1 promoter. HAT, histone acetyltransferase; HDAC, histone deacetylase; TF, transcription factor; PRC1/PRC2, polycomb repressive complex 1/2.
young adults comes from recent work on Ewing sarcoma. Expression of the EWS-FLI1 fusion oncoprotein in Ewing sarcoma is associated with more genes being down- than upregulated. Studies employing gene expression profiling have demonstrated that at least two critical repressors of transcription and mesenchymal differentiation are among the most highly induced targets of EWS-FLI1: EZH2 (the histone methylase effector of Polycomb repression) and NKK2.2 (which in association with TLE1 is a key effector of oncogenesis in Ewing sarcoma); in both cases their effects can be reversed with histone deacetylase inhibitors. The HDAC inhibitors sodium butyrate, trichostatin A, vorinostat, and MS-275 are all effective in killing Ewing sarcoma cells, and romedepsin/FK228 has antitumor activity in xenograft models. In vitro efficacy of these agents has been demonstrated in several other types of fusion transcription factor-associated sarcomas. Of note, the TMPRSS2-ERG translocation, found in many prostate carcinomas, includes one of the same 3’ fusion transcription factor components found in Ewing sarcoma. Several clinical trials of HDAC inhibitors are underway in prostate carcinoma, and indeed HDAC inhibitors may have therapeutic activity in many types of cancer. Nevertheless, translocation-associated sarcomas may be the best subject for investigations of HDAC inhibitor efficacy in solid tumors, as the preclinical evidence for specific actions to reverse specific oncogenic events is strong. A phase II investigational new drug trial of the HDAC inhibitor SB939 in translocation-associated sarcomas is planned in Canada (National Cancer Institute of Canada Clinical Trials Group IND.200), and a phase I/II trial of belinostat in sarcomas is nearing completion in Denmark and the United Kingdom (NCT00878800).

**IGF/Akt/Mammalian Target of Rapamycin Pathway**

Activation of the Akt/mammalian target of rapamycin (mTOR) pathway, leading to apoptosis resistance and persistent growth independent of nutritional supply, is one of the most important oncogenic pathways in human cancer, and appears to be of particular importance in sarcomas. This pathway is activated by growth factors, particularly insulin-like growth factor (IGF), via PI3K, and is tightly regulated by the critical tumor suppressor phosphatase and tensin homolog (PTEN). PTEN conditional knockout mice develop leiomyosarcomas with high penetrance and rapid onset, and expression profiling studies find tonic activation of the IGF/Akt/mTOR pathway to be common in primary human pleomorphic sarcomas. Akt/mTOR inhibitors are effective against group 4 sarcomas in vitro and in mouse models, and promising response rates have been reported with the mTOR inhibitor AP23573 (deforolimus) in phase I and II (NCT00903080) studies of advanced sarcomas; a phase III trial is now open (NCT00538239). The IGF/Akt/mTOR pathway is also clearly important in group 1 sarcomas, including Ewing sarcoma; dramatic albeit temporary responses to antibodies blocking IGF receptor 1 have been described and a phase II clinical trial (SARC011, NCT00642941) has recently closed with results expected in the near future. As the best clinical agents (in terms of pharmacokinetics and toxicities) are becoming more clear, additional clinical trials are expected.

**CSF1 Inhibitors**

Recent advances in the understanding of the biology of pigmented villonodular synovitis (PVNS), an aggressive form of tenosynovial giant cell tumor based in large joints, provide a good example of how expression profiling can simultaneously lead to biologic insight and the identification of a novel clinical treatment. Although malignant transformation of these soft tissue tumors is rare, multiple local recurrences are common and can lead to severe joint disease. Using a combination of gene expression profiling and tissue microarray analysis, CSF1 and CSF1R were found to be highly expressed in these tumors. As a common breakpoint has been previously identified at 1p13, these new findings helped pinpoint the break to the CSF1 locus. Tenosynovial giant cell tumor cells secrete high levels of CSF1 (macrophage colony-stimulating factor) and this event recruits CSF1R-expressing macrophages, such that the cells with the translocation constitute only a small minority (5%) of the tumor cell population while the majority of the cells in the lesions are reactive, non-neoplastic macrophages. Neither specific clonal break points nor transcripts demonstrating fusion of the most commonly translocated gene partners COL6A3 and CSF1 have been consistently detectable, possibly because the translocation involves a promoter donation leading to overexpression of full length CSF1 and does not represent an exon fusion. A case report has demonstrated a complete clinical response to small molecule inhibitors of CSF1R, with relapse of the tumor occurring on discontinuation of the drug. This result suggests that CSF1 inhibitors are working testing in severe PVNS. Whereas surgery plus radiation therapy can often give complete local control in the joint space, the use of surgery in this setting may still lead to joint damage. Combining a pathway inhibitor (cytoreductive agent) with a cytotoxic agent (radiation therapy) may be as effective, and would be more easily applicable than surgery in multiply recurrent, aggressive cases.

Studies in leiomyosarcoma have shown that macrophages and the macrophage response to CSF1 may play a wider role in soft tissue sarcomas. These studies began with the realization that the gene expression signatures of tenosynovial giant cell tumors/PVNS were largely representative of the macrophage response to CSF1 expression. By looking for this gene signature in other sarcomas, it was evident that some expressed this signature (albeit at a lower intensity than in PVNS). Leiomyosarcomas, in particular, demonstrate considerable heterogeneity for this signature. In a series of studies that looked at macrophage numbers, the expression of CSF1 response genes by expression profiling and by immunohistochemistry was associated with a worse clinical outcome among leiomyosarcomas. In a tissue microarray study of 149 cases, an increase in CD163- or CD68-positive macrophages within the tumor correlates with poor outcome in soft tissue leiomyosarcomas; in the presence of dense, moderate, or sparse CD163- or CD68-positive macrophages these tumors had 5-year disease-specific survival rates of 40%, 70%, and 100%, respectively (P = .002). For gynecologic leiomyosarcomas, the coordinate expression of four CSF1 markers was the only independent prognosticator in multivariate analysis (hazard ratio, 4.2; P = .03). These findings are particularly interesting in that they concern a sarcoma of complex karyotype (group 4), for which there exist few prognostic factors beyond conventional clinicopathologic staging. Moreover, these findings suggest a therapeutic opportunity in leiomyosarcomas with CSF1 inhibitors targeting the macrophage component, which has had preliminary success in other tumors.
Expression profiling and other molecular techniques have revealed characteristic and often unique molecular changes in sarcomas, and gene expression patterns among sarcoma subtypes are often vastly different. Logically, the design of clinical trials should take this into account, as it is not reasonable to assume that these different sarcoma types share enough biology to have similar responses to increasingly sophisticated drug treatments. Unfortunately, most clinical trials in the field to date have lumped together different soft tissue sarcoma entities, in large part to accelerate accrual of a statistically powered sample. This approach has likely impeded the identification of effective therapies for individual subtypes. Thus, there is a clear and urgent need for molecular subtype–specific soft tissue sarcoma trials. It has been, of course, quite difficult to find enough patients with specific tumor types to conduct such trials; interinstitutional cooperation is clearly required, but many of the large cooperative cancer groups in the United States do not even have active sarcoma committees. One possible solution involves patient support groups specific for sarcoma subtypes, formed in part due to the larger sense of community that the Internet generates. These groups have organized for support and in some cases to fund research, but also are keen to help spread knowledge of available clinical trials and to promote recruitment.

The gene microarray platform has been employed to profile the main types of sarcomas, and finer resolutions with larger tumor sets are still being generated. These technologic advancements can add their own functionality; for example, very high density arrays can profile each exon of each gene and allow detection of splice variants and fusion transcripts. The bottleneck of these techniques is now mostly centered on interpretation and functional analysis of the data, for which more fundamental information is required (eg, relating to transcriptional regulatory circuits and to the interaction of tumors with host cells and the microenvironment). Some of this knowledge may come from a mixture of observational research, as much of sarcoma gene profiling work is right now, and experimental studies. The profiling of sarcoma cell lines combined with experiments manipulating expression of critical genes will provide insights into how changes in transcript levels reflect dynamic cell processes like receptor signaling.

The rapidly developing field of high throughput sequencing is expected to allow for an even richer understanding of sarcoma molecular biology. A variety of new applications are emerging such as deep sequencing, RNA sequencing for expression profiling (RNA-seq), and chromatin immunoprecipitation sequencing (theoretically an excellent method for identifying the targets of fusion transcription factor oncoproteins). Indeed, though microarrays are on the verge of widespread clinical use, they may soon be rendered obsolete by these newer sequencing methods. We will be able to correlate gene expression with transcription factor binding, while observing the impact of SNPs and gene mutations. Even as we are developing methods to interpret the data, these new applications have already had an impact in revealing the biology of related tumor types. Shah et al124 were able to use high throughput sequencing to identify the causative mutation in a mesenchymal malignancy of the ovary (FOXL2 in granulosa cell tumors) by sequencing the transcriptome of just four frozen tumor specimens. They were successful in part because this tumor type is genetically stable, as reflected by nonpleomorphic histology, yet no causative molecular event had been identified with previous technologies such as karyotype analysis. Many sarcomas have similar characteristics (group 2, Fig 1), and by identifying underlying causative molecular events this approach could naturally lead to the identification of specific targeted therapies (as in GIST125). Thus, the biology of sarcomas suggests that they can represent a paradigm among solid tumors for the translation of gene expression into clinical care.

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