Introduction

Soft tissue sarcoma (STS) encompasses a range of over 40 histologic diagnoses with varying biology, from translocation-defined entities to diseases with complex karyotypes. Roughly 10,000 new cases are diagnosed in the United States per year across all ages, comprising 1% of adult cancers and 7% of childhood cancers. While localized resected disease can often be cured, controversy exists regarding optimal management of tumors in younger patients with high-grade disease or larger tumors. Prognosis is significantly worse when tumors cannot be controlled by surgery and radiation therapy or in the setting of metastatic disease, and chemotherapy is the primary modality of treatment. Because of the poor overall survival of 20% to 25% at 2 years, as well as the short median survival of 12 months and the rarity of these tumors, there is a need for novel approaches for therapy. Due to the biologic diversity, it is unlikely that a single agent or a combination of agents would be successful across the spectrum of diagnoses.

Chemotherapy in STS

Historically, doxorubicin-based therapy has been the standard first-line agent in the treatment of STS when chemotherapy is indicated, typically in metastatic or unresectable disease. While combination therapy, mainly with ifosfamide, has improved response rates, toxicities have increased with no clear corresponding benefit in overall survival. Response rates to front-line chemotherapy vary, ranging from 10% to 46% with single agents and with combinations of chemotherapy. Confounding this even further is the spectrum of response rates

Clinically validated predictive tests for systemic therapy response in soft tissue sarcoma are still being sought.
between histologies. In addition, patient age affects analyses; improved responses in younger patients have been reported. Finally, specific subtypes may benefit from agents outside the typical approach of anthracyclines and alkylators (eg, taxanes for angiosarcoma and trabectedin for myxoid/round cell liposarcomas). Tyrosine kinase inhibitors have revolutionized the management of gastrointestinal stromal tumors (GISTs), a subtype of STS, showing that significant clinical benefit is possible among the diagnoses currently categorized as STS if the correct therapy can be matched to the tumor biology. Interpretation between trials is difficult in part due to the heterogeneity of diagnoses and ages among cohorts and has contributed to conflicting results between trials. Neither a systematic method to guide chemotherapy for an individual patient nor a clear national standard of care has yet been developed, thus necessarily leading to a multifactorial, patient-specific analysis to determine whether or not to offer therapy. This is particularly complicated in second-line therapies, where many agents are available with similar low response rates, and the short median survival of these patients does not always allow for multiple treatment attempts. Response rates are so low in the second-line setting that progression-free survival (PFS) has become the standard endpoint in clinical trials, with PFS at 3 months above 40% demonstrating activity and below 20% demonstrating inactivity.

Ifofosamide is a standard second-line agent, with dose-dependent activity ranging from 10% to 40% with particular activity in synovial cell sarcoma. Gemcitabine and docetaxel combination chemotherapy has demonstrated particularly good response rates in leiomyosarcoma, especially uterine leiomyosarcoma, and has become front-line therapy in our institution for this malignancy. Response rates of 16% to 43% have been reported in STS series. Vinorelbine has particular activity in rhabdomyosarcoma, having been studied particularly in the pediatric population; it also has a 6% response rate in heavily pretreated STS.

Many agents beyond the scope of this article are being explored in STS. These include agents with a wide range of activity, such as cytotoxic and targeted agents. Trabectedin and ifosfamide derivatives are currently being studied in phase III studies, and mTOR inhibitors have been studied in maintenance, with approval pending. Insulin-like growth factor (IGF) pathway inhibitors, proteasome inhibitors, cell cycle and growth modulators, kinase inhibitors and others are being explored in earlier phase trials, at times with corresponding biomarker development. The vast numbers of available agents and numerous histologic diagnoses complicate study design, which can lead to overall negative conclusions and also overlook clinical benefit in a particular subtype of STS. A means to enrich responders and exclude nonresponders in early-phase trials for STS is needed.

Assay-Guided Therapy

Prospective determination of antibiotic sensitivity and resistance has been the standard of care in infectious diseases for many years. In contrast, due to the lack of reproducible and predictive assays, treatment protocols of cancer patients have been designed according to tumor histology rather than to the tumor’s sensitivity to a given chemotherapeutic agent. Therefore, development of novel drug sensitivity studies to predict patient response before initiation of therapy would help to design the most efficient therapeutic regimen for individual patients while sparing others who are not likely to benefit from the given therapy.

A formal analysis conducted by both the American Society of Clinical Oncology and the Blue Cross and Blue Shield Association’s Technology Evaluation Center concluded that predictive assays were not ready for routine clinical use. However, both studies noted that versions of these assays that meet specific parameters might improve prediction of patient response to therapy. Reasons for specific problems with previous assays included the low percentage of successfully completed assays that yielded clinically useful results, rare interpretations that differed from standard of care, trial design that did not include choices among several treatment options, technical complexity that made application beyond a single laboratory/institution unlikely, and the long time needed to study completion, thus delaying therapy. Minimum criteria that have been proposed for a successful assay include standardizing materials, testing a range of concentrations to provide a dose response curve, applying simple techniques with the possibility of automation, and measuring cell survival with a clear interpretation. Rather than calling for an end to predictive assays, the authors recognized that the need for such an assay has even more appeal as new agents become available. Making rational choices between increasing numbers of regimens is not possible beyond matching a particular histology to a regimen. A method of enhancing responders on a given regimen or excluding nonresponders would be of great benefit to sarcoma patients. These studies concluded that future assays should be studied in the context of a clinical trial, particularly for diseases with a short median survival, when the window for multiple, sequential therapeutic regimens is narrow and when performance status can rapidly decline and thus preclude additional therapies. The importance of a predictive assay would be even more appealing when there is a choice among multiple agents with relatively low percentages of activity, as is the case with STS.

Despite substantial progress made in recent years for other malignant diagnoses, no clinically validated tests are currently available to predict the efficacy of a given agent for an individual patient with STS other than GIST. Although there is consensus supporting the need to develop and integrate the evaluation of predictive
biomarkers in clinical trials, the practical application of such an approach is still lacking.33

**Assay-Guided Therapy Trials and Retrospective Analyses**

A search of PubMed literature was conducted that included the following terms and combinations thereof: “drug screening assays, antitumor,” “predictive assay,” “ex vivo,” “cancer prediction,” “individualized medicine,” “chemosensitive,” “chemoresistant,” “in vitro,” “biomarker,” “prediction,” “cancer,” “prognosis,” “clinical trial,” and “sarcoma.” Articles were chosen based on review of the abstract and English language. In the following, we present a spectrum of methods and highlight the strengths and limitations of each method. Clinical trials are emphasized, particularly in sarcoma, though these are limited (Table 1).34-39

**Cell Viability Assays**

The concept of utilizing tumor cells exposed to chemotherapeutic agents and extrapolating the effects to in vivo disease is as old as modern chemotherapy. Growth inhibition or cell death has been used in previous iterations of assays of sensitivity to conventional chemotherapeutic agents.40-44 However, due to poor tumor growth under assay conditions, methods that are labor-intensive and time-consuming, and the use of uncertain criteria for defining “sensitivity” or “resistance,” these assays have not yet gained wide clinical acceptance. Several methods have been explored using the cell-based platform.

The human tumor cloning assay (HTCA) and capillary cloning system (CCS) utilize tumor cell proliferation, measured as the number of colony-forming units in solid media over a period of 2 to 4 weeks.57-45 While these assays measure tumor cell growth, they remove the tumor microenvironment and the three-dimensionality of an in situ tumor. The techniques are also labor-intensive, taking weeks to complete. They have been studied in a relatively large number of patients and histologic subtypes. One trial reported a 64% rate of successful assay completion.37 In this trial, a range of malignancies had successfully completed assays, and those treated according to the assay results had an overall response rate of 25%. Patients placed on empiric therapy due to an unsuccessful assay had a response rate of 14%. Of the 470 patients, 14 (3%) had sarcoma.37

The above assays measure tumor cell growth but do not take into account tumor cells that are alive but not dividing. This important distinction, which can be clinically relevant, is incorporated into the differential staining cytotoxicity (DiSC) assay. This assay uses cells cultured from the primary tumor and then counts live cells after incubation with agents for 4 to 6 days. Modified versions of this test have been used in lung cancer trials in the past with a trend toward improved response-based assay results. Multiple trial designs, including selections of combinations of therapy, have been attempted.36,46,47 A series that included limited-stage small cell lung cancer patients demonstrated that the biopsy needed for the test

### Table 1. — Selected Studies Evaluating Predictive Assays in Cancer Therapy

<table>
<thead>
<tr>
<th>Study/Assay</th>
<th>Method Overview</th>
<th>Successful Assay</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP34</td>
<td>Multicenter study of metastatic melanoma patients</td>
<td>98% successful; 71% with successful assay received therapy</td>
<td>Response 36.4% in chemosensitive patients vs 16.1% in chemoresistant patients; overall survival 14.6 mos in chemosensitive patients vs 7.4 mos in chemoresistant patients</td>
</tr>
<tr>
<td>SRCA35</td>
<td>Primary tumor cells implanted under renal capsule</td>
<td>90% tumor growth in xenograft and assessable after chemotherapeutic therapy</td>
<td>11 patients received a tested combination of chemotherapy; 6 of 7 with relapse had corresponding nonresponder status of xenograft</td>
</tr>
<tr>
<td>DiSC36</td>
<td>Dye exclusion study, cells treated at reference range, 10% of reference range and 100% of reference range. Sensitivity &lt; 50% of control in individual drug assay (7 drugs tested); 3-drug combination starting at week 13 from among 13 combinations proven active in small cell lung cancer</td>
<td>44% of assays completed in 12-wk period</td>
<td>All patients had a complete or partial response to first 12 wks of standard radiation therapy and chemotherapy; among group of 8 who received assay-guided therapy, median survival was 38.5 vs 17.5 mos</td>
</tr>
<tr>
<td>HTCA37,38</td>
<td>Cells from primary tumor grown in vitro in the presence of chemotherapeutic assay and colony counts done</td>
<td>Multiple trials: 45% to 64% successful 470 patients on single trial</td>
<td>Reported higher response rates but nonrandomized</td>
</tr>
<tr>
<td>ATP39</td>
<td>Extremity sarcomas were minced, enzymatically digested tumors plated in 96 well plates, and tested against a panel of chemotherapy</td>
<td>100% successful</td>
<td>No clinical data available</td>
</tr>
</tbody>
</table>

**ATP = adenosine 5’-triphosphate, SRCA = subrenal capsule assay, DiSC = differential staining cytotoxicity, HTCA = human tumor clonogenic assay.**
did not delay initial chemotherapy and could be used to guide the second 12 weeks of therapy. In addition, in 44% of patients, the median survival was 38.5 months vs 17.5 months in the standard care cohort. While the assay tested seven different agents as monotherapy, the patients were treated with a three-drug combination of assay-active agents that had proven efficacy in this disease type.56

While the above-described assays may eventually be automated, this technology does not currently exist. Higher-volume analyses using MTT and adenosine 5′-triphosphate (ATP)-based chemiluminescence assays have utilized microplate readers to measure chemical surrogates of cell survival or proliferation. These have been investigated clinically in a variety of tumor types and are readily automated.48 Whether they accurately correlate to cell survival or number is a matter of ongoing debate. Nevertheless, a multicenter melanoma study demonstrated an overall survival advantage in chemosensitive patients of 14.6 months compared to 7.4 months in chemoresistant patients.51 In this study, combination cytotoxic chemotherapy was analyzed in the ATP-based assay. The authors concluded that further optimization regarding in vitro drug dosages and combinations tested may need to be explored, though this strategy is currently being tested in the phase III setting through the Dermatologic Cooperative Oncology Group.34 Importantly, this trial used combinations not routinely used in melanoma and found higher response rates than those reported in historical data. Feasibility of chemosensitivity testing with an ATP-based assay in an exclusive sarcoma population was performed showing that results can be obtained. Unfortunately, no clinical response data was included in the published study.39

**Genome-Wide Microarray Assays**

Genome-wide microarray assays allow for a snapshot of the transcribed genes and level of transcript in a given tumor. These assays have been used to explore aberrant pathways and to define signatures of tumor subtypes typically within a given histology. Genome-wide microarray assays have been investigated retrospectively due to the cost and time necessary to analyze the large volume of data. Recent reports in bone sarcomas have demonstrated that enriched subsets of genes produce reliable predictive signatures compared to clinical history. This was validated prospectively in 1 case, compared to histologic tumor necrosis in 8 of 8 patients.49-51 Ongoing studies in sarcoma appear to be underway comparing gene signatures to outcome based on observed abstracts at meetings; however, publications remain limited to biology without clinical outcomes data.52 With large data sets and numbers of genes upregulated and downregulated in a given experiment, it is often difficult to sort out the relative contributions of the individual genes. Furthermore, the data is not necessarily correlated with protein expression and is unable to identify protein modifications that may be important in the pathogenesis of a given tumor. Inherent biases built into the methodologies also complicate the interpretation of results.53

**Protein Assays**

Putative markers of chemotherapy resistance including efflux pumps, enhanced DNA repair, mutated target proteins, and variation of metabolizing enzymes have been thoroughly investigated with varying results. Examples of single proteins with prognostic and therapeutic importance can be readily found in cancer literature, with a notable example being hormone receptors in breast cancer. However, markers that affect therapeutic decision making have yet to be developed in non-GIST STS. Examples of clinical trials in sarcoma include investigating the efflux pump P-glycoprotein (P-gp) and O6-methylguanine-DNA-methyltransferase (MGMT). P-gp levels were assessed by immunohistochemistry in an osteosarcoma clinical trial that included P-gp substrates doxorubicin and methotrexate in the treatment of all patients. There was no difference in overall or event-free survival in this study based on P-gp expression.54 MGMT, which repairs DNA damage due to methylation and is inhibited by O6-benzylguanine, varied considerably in the blood of patients with STS, and targeted inhibition of MGMT did not produce any objective responses.55

**Xenograft Assays**

Patient-derived mouse xenograft models allow for recapitulation of three-dimensional tumor architecture and incorporate aspects of tumor stroma. Two major limitations of animal studies are cost and labor intensity. While sarcoma xenografts have been generated, a comparison of xenograft response to clinical outcome data has not yet been published. One group reported establishment of a series of xenografts that were treated with conventional cytotoxic agents and analyzed for biologic markers of resistance including MDR1, topoisomerase IIα, and glutathione S-transferase. This panel showed low responses to conventional agents and did not have a strong correlation between selected gene markers and chemotherapeutic effect.56

Xenografts have been used recently in non–small cell lung cancer (NSCLC) to explore the chemosensitivity of selected agents or combinations. The subrenal capsule assay uses subcapsular renal transplants of human tumors into murine xenografts treated with various chemotherapies in this disease, with a recently published report establishing a series of xenografts and treating them with three chemotherapeutic combinations.35 A 90% engraftment rate was achieved, and about half of the tumors were tested against all three chemotherapeutic regimens. The xenografts resembled the primary tumor histologically. Almost a third of the tumors were not sensitive to any of the tested combinations. Perhaps most importantly, a series of 11 patients received one
of the tested combinations (vinorelbine and cisplatin). Seven of these patients recurred, 6 with corresponding nonresponsive xenografts. One xenograft was sensitive to the combination and recurred. Results from this assay took 6 to 8 weeks. The authors concluded that there is a correlation between the assay results and clinical data in the recurrence group and that xenograft-based testing may help identify new active combinations in NSCLC.35

Summary of Predictive Assay Approaches

Individual gene tests, translocation analysis, and protein tests are currently widely used in breast cancer, leukemia, and other malignancies to tailor therapy. For example, FLT3 testing modifies prognosis for acute myelogenous leukemia patients, and therapy with a targeted tyrosine kinase inhibitor molecule or a lower threshold for allogeneic transplantation is being explored in clinical trials at this time. Similarly, the BCR-ABL translocation is used to guide therapy, also with a tyrosine kinase inhibitor in chronic myelogenous leukemia and childhood acute lymphoblastic leukemia. However, no cell-based assays or genome-wide analyses are currently used for the selection of chemotherapy as the accepted standard of care. In non-GIST STS, histology and clinical features are often used to guide therapy, but predictive assays are currently not routinely used, if at all. [18F]fluorodeoxy-D-glucose positron emission tomography (FDG-PET) scans are being evaluated in sarcomas to determine the extent of disease and residual disease. One study of 46 patients with STS showed that baseline PET intensity and metabolic response were predictors of relapse-free survival, and a combination of baseline signal intensity and metabolic response was a robust predictor of overall survival.57 A recent study of high-grade bone sarcomas showed good negative correlation of PET avidity changes after one cycle of chemotherapy with tumor necrosis, with a threshold signal reduction of 60% after one cycle of chemotherapy.58 However, a sarcoma cohort including intermediate- and high-grade tumors was more complex, with lower sensitivity and specificity.59 A recent task force report has no recommendation on the routine use of a PET scan during therapy to follow response or to predict response in sarcomas.60

Many STS clinical trials have compared cytotoxic agents alone or in combination with a spectrum of response rates. While clinical data have led to some insight in matching tumor types that tend to have better response rates, a mechanism-based analysis of tumor tissue treated with chemotherapy is lacking. Doxorubicin has remained the standard of care for 35 years.6 It is not always clear how cytotoxic agents, which damage DNA or inhibit critical pathways in cells, induce a response. While mechanisms of action of these agents are postulated, biomarkers of efficacy or resistance remain largely unknown. Markers of DNA damage, apoptosis, autophagy, and cell cycle arrest have been identified, though these pathways are complex, interconnected, and often redundant (Table 2).

Traditional approaches to the preclinical investigation of novel cancer therapies rely on the use of established human cancer cell lines. These cell lines are maintained in vitro in serum-based growth media, and their responses to experimental cancer therapeutic agents are assessed by studying growth and apoptosis in vitro or in vivo. Prolonged culture of human cancer cells in serum and on tissue culture plastic results in cell lines that may not be

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP</td>
<td>Posttranscriptional modification of cellular proteins through poly(ADP-ribose)polymerase; this protein is cleaved by caspase-3 during apoptosis</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>RB</td>
<td>Tumor suppressor that interacts with E2F family of proteins and halts G1/S transition when hypophosphorylated</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>Member of a family of proenzymes that signal apoptosis though cleavage of other family members</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Gamma-H2AX</td>
<td>Specific phosphorylation of a histone in response to double-stranded DNA breaks</td>
<td>DNA damage</td>
</tr>
<tr>
<td>Chk1 and Chk2</td>
<td>Key signal transducer kinases that are activated in response to diverse genotoxic insults to transmit the checkpoint signals from the proximal checkpoint kinases</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>Cdc2</td>
<td>A cell division control protein, also known as cyclin-dependent kinase 1 (Cdk1) or p34/cdk1, plays a key role in the G2 to M phase transition</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>Cdc25</td>
<td>Family of dual-specificity phosphatases that positively regulate the cell division cycle by activating cyclin-dependent protein kinases</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen (PCNA) involved in DNA synthesis and postreplication repair</td>
<td>Cell proliferation</td>
</tr>
<tr>
<td>LC3</td>
<td>Microtubule associated protein that interaction between microtubules and the cytoskeleton activated during autophagy</td>
<td>Autophagy</td>
</tr>
</tbody>
</table>
representative of the parent tumor. Such differences are of concern in the study of basic cancer biology and are fundamental to our approach in studying this disease. In particular, culture selection in cell lines may disturb the in vitro relationship between the cancer stem cell and its progeny, and it also removes tumor-stromal interactions that are essential to the three-dimensional biology of solid tumors in vivo. To investigate novel therapeutic and diagnostic strategies with greater accuracy, new preclinical strategies such as biomarker assessments are needed to assess anticancer therapies in order to determine if patients are likely to benefit from a given therapy.

We propose that future STS studies should involve evaluations of molecular pathways in primary sarcoma tissue. There must be a focus on the biology of the tumor in addition to the histology. Treatment with clinically relevant agents in short-term ex vivo assays and biomarker analysis with corresponding in vivo xenograft or clinical outcomes data would help establish patterns or proteins that may provide information regarding tumor sensitivity or resistance to a given treatment (Figure). Predictive markers can serve as the basis for inclusion or exclusion for clinical trials that can incorporate targeted agents in addition to a cytotoxic therapy. This may eventually lead to combinations of medications that can improve response rates by exploiting signaling or biologic abnormalities in malignant cells.

**Conclusions**

The goals of predictive assays or biomarkers are to match efficacious chemotherapy to an individual patient and to exclude chemotherapy that would cause toxicity without benefit. Diseases with a poor prognosis, genetic heterogeneity, many therapeutic options, and a poor response rate to traditional chemotherapy provide the rationale for predictive studies. A quick, reproducible, patient-specific assay that could test many single agents and combinations based on the mechanisms of the therapeutic agents would be ideal. In the setting of conventional cytotoxic agents, biomarkers of biological efficacy are not always known, though molecular markers of DNA damage, cell cycle arrest, apoptosis, autophagy, and necrosis may provide predictive data.

Markers of tumor response to cytotoxic chemotherapy have been explored in breast and colon cancer. While the results for colon cancer are controversial and mixed, markers in breast cancer such as topoisomerase IIα and erbB2 show good correlation to response, and ABCB1 (P-gp) and BCL-2 overexpression show good correlation to resistance. A clinical trial similar to that shown in the Figure could be conducted to assess the positive and negative predictive value and match patients to chemotherapy to determine if this can improve response rates, progression-free survival, and overall survival.

Figure. — Ex vivo assay schema. This theoretical trial design incorporates an ex vivo assay strategy for metastatic or unresectable STS patients and would allow for prospective assessment of molecular markers, calculation of positive and negative predictive value to first-line therapy, and match chemotherapy in the second line based on ex vivo assay response. While first-line therapy would be anthracycline-based, second-line therapies could consist of cytotoxic agents or combinations with established efficacy or with potential, utilizing the assay to match the patient to therapy. SD = stable disease, PR = partial response, CR = complete response, PD = progressive disease.
References


