Published in final edited form as:

Adv Anat Pathol. 2012 May ; 19(3): 170–180. doi:10.1097/PAP.0b013e318253462f.

New Therapeutic Targets in Soft Tissue Sarcoma

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Abstract

Soft tissue sarcomas are an uncommon and diverse group of more than 50 mesenchymal malignancies. The pathogenesis of many of these is poorly understood, but others have begun to reveal the secrets of their inner workings. With considerable effort over recent years, soft tissue sarcomas have increasingly been classified on the basis of underlying molecular alterations. In turn, this has allowed the development and application of targeted agents in several specific, molecularly defined, sarcoma subtypes. This review will focus the rationale for targeted therapy in sarcoma, with emphasis on the relevance of specific molecular factors and pathways in both translocation-associated sarcomas and in genetically complex tumors. In addition, we will address some of the early successes in sarcoma targeted therapy as well as a few challenges and disappointments in this field. Finally we will discuss several possible opportunities represented by poorly understood, but potentially promising new therapeutic targets, as well as several novel biologic agents currently in preclinical and early phase I/II trials. This will provide the reader with context for understanding the current state this field and a sense of where it may be headed in the coming years.

Keywords

Soft tissue sarcoma; targeted therapy; molecular mechanisms

Introduction

Soft tissue sarcomas are rare tumors showing mesenchymal differentiation that may arise anywhere in the body. They are comprised of a broadly diverse array of subtypes, each with distinct molecular, clinical and behavioral features.¹ Although outcomes vary greatly by sarcoma subtype, current therapies are limited and cure is only attainable by complete surgical resection. Neither radiation nor conventional cytotoxic chemotherapy have had much impact in improving survival, although radiation may prevent local recurrence and

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Disclosures: None

chemotherapy may temporarily delay disease progression.^{2, 3} The need for more effective therapies is highlighted by poor five year sarcoma survivals of approximately 50% -- a rate that has shown little improvement in recent years.⁴

Fortunately, the past few decades have led to a vastly improved understanding of the molecular underpinnings of sarcomagenesis. It is hoped that these new insights will lead to the development of more effective therapies against driving events in these tumors. In part because of their rarity, sarcomas may represent ideal targets for the discovery of new treatments. Rare, or orphan diseases can be defined as diseases affecting fewer than 0.07% of the population (<200,000 people) in the US and less than 0.05% of the population in Europe.⁵ While advances in treatments for the most common cancers have occurred only incrementally over the past 20 years, dramatic successes in therapy have been seen disproportionately in orphan diseases, including some sarcomas. The Orphan Drug Act, introduced in 1982, was enacted to provide incentives to encourage research into therapies for rare diseases.⁶ Since then, over 300 agents have been approved for orphan diseases and nearly a thousand more are in development.⁷ These rapid advances may not only be due to financial incentives, but rather to the underlying biology of rare tumors.⁸ That is, many rare tumors may be driven by a single genetic event, and rely on this aberration to survive (oncogenic addiction), whereas more common malignancies are driven by a wider array of molecular events.⁸

This theory has been borne out in some sarcomas, most notably gastrointestinal stromal tumor (GIST) and dermatofibrosarcoma protuberans (DFSP), in which targeted agents have had a high degree of treatment success. However, in other sarcoma types, the molecular events underlying sarcomagenesis are more complex, and determining the most effective targeted strategies remains a work in progress. This review will highlight some of the recent advances in understanding and targeting the molecular pathways involved in sarcomagenesis.

Current Diagnosis and Management of Sarcoma

Sarcomas are a diverse group of tumors, not only because of the wide array of histologic subtypes, but also due to their varied clinical courses and expected prognoses. A correct diagnosis is critical, not just in separating the benign from the malignant tumors, but also to predict the behavior of malignant tumors and to select appropriate therapeutic options. Ideally, diagnosis of sarcoma should be performed by a pathologist familiar not only with the natural history and morphologic features of these tumors, but also with their genetics and molecular biology. Molecular findings should always be interpreted within the context of clinical, histologic, and immunohistochemical data. Appropriate treatment then requires a multidisciplinary collaboration between the pathologist, radiologists, medical, surgical, and radiation oncologists, and orthopedic oncologists, among others. While much of current treatment relies on complete surgical resection, with or without adjuvant radiation or conventional chemotherapy, effective options for metastatic disease are limited, and there is now an increasing demand for more effective targeted therapies. As diverse as sarcomas are, so too are the types of molecularly targeted agents under development and investigation. An understanding of the mechanisms and relevance of these agents is grounded in the underlying pathology and molecular pathogenesis of sarcoma. As the custodians and curators of tumor diagnostic specimens buttressed by a thorough appreciation of pathogenesis, pathologists play a critical and central role in the clinical demonstration of the molecular determinates used to inform rational therapeutic decisions. To maintain the traditional value-added role of pathology as the provider of information informing therapeutic approaches, as a discipline pathologists must embrace and become conversant with the relevant molecular pathways involved in sarcomagenesis and the application of this

information to clinical management. To ignore this emerging revolution threatens to undermine the central role pathologists in the therapeutic value chain.

Sarcoma Molecular Pathogenesis

Sarcomas may be broadly classified by genomic events underlying their development as 1) those with specific translocations or gene amplification, 2) those with defining oncogenic mutations and 3) those with complex genomic rearrangements. Each class contains diverse tumors with a wide array of clinical, histological and molecular characteristics.

Translocation-Associated Sarcomas

Specific, recurrent translocations have thus far been identified in 22 types of benign and malignant bone and soft tissue neoplasms, including 19 soft tissue sarcomas (including 4 tumors of at least intermediate (rarely metastasizing) biologic potential).⁹ Overall, translocation sarcomas are currently thought to account for 20-30% of all sarcomas,^{10, 11} and this number continues to swell with new discoveries of recurrent translocations in additional tumor types. Typically, recurrent translocations in sarcoma result in chimeric fusion genes which function as transcription factors, as is epitomized by the *EWSR1-FLII* fusion gene in Ewing sarcoma. Less frequently, translocation results in overexpression or constitutive activation of a growth factor receptor tyrosine kinase (RTK) or other chimeric growth factor signaling protein, as is seen in DFSP, in which wild types *PDGFB* is overexpressed under the *COL1A1* promoter, and inflammatory myofibroblastic tumor (IMT) in which *ALK* fusion partners promote dimerization of the ALK tyrosine kinase thereby rendering it constitutively active.^{12, 13}

Amplification-Associated Sarcomas

Recurrent amplifications have only been identified in a few soft tissue sarcomas, most notably well-differentiated/ dedifferentiated liposarcoma, in which amplification of chromosome 12q13-15, including *HDM2* (*MDM2*) and *CDK4* is characteristic.¹⁴ Hdm2 functions as an inhibitor of p53. Accordingly, amplification and subsequent overexpression of this chromosomal locus results in inhibition of p53-dependent cell-cycle arrest and apoptosis. Cdk4 is a cell cycle regulator, and over expression of this factor promotes proliferation, while other gene loci within this interval may also have pro-oncogenic effects.

Amplification of *MYC* has been described in secondary (radiation-induced) angiosarcoma,¹⁵ and may be seen sporadically in other sarcomas.¹⁶⁻¹⁸ Myc is a proto-oncogenic transcription factor, which can act as either a transactivator or repressor, and has been reported to be involved in a variety of human malignancies.^{19, 20}

Targeted Therapy Against Fusion Genes

Translocation sarcomas have not yet proven to be as amenable to targeted therapy as had once been hoped, despite a greatly improved understanding of the mechanisms by which chimeric fusion genes promote sarcomagenesis. The few cases in which fusion genes have been successfully targeted are those in which the transgene results in overexpression or constitutive activation of a growth factor or growth factor receptor. The prototype for this class of sarcomas is DFSP, in which the growth factor *PDGFB* is fused to the promoter of the constitutively expressed *COL1A1* encoding a collagen.²¹ Here, inhibition of PDGFR by the RTK- inhibitor (TKI) imatinib mesylate has been shown to dramatically reduce tumor size in previously unresectable cases.²²

IMT represents another potential target for TKI therapy. About 50% of IMT are characterized by translocations involving the *ALK* receptor tyrosine kinase, which result in

Alk overexpression,²³ and/or aberrant localization to the intracellular compartment where receptor dimerization and constitutive activation occur.^{24, 25} Agents currently under investigation for *ALK* rearrangement-positive IMT include crizotinib, a dual Alk/Met inhibitor, which has shown anecdotal benefits in *ALK*-positive but not *ALK*-negative IMT in early phase I trials.²⁶

Another example of successful targeting of a fusion gene is in the locally aggressive diffuse-type tenosynovial giant cell tumor (pigmented villonodular synovitis). Here, constitutive expression of colony stimulating factor-1 (*CSF-1*) under the control of the *COL6A3* collagen promoter in neoplastic cells acts to recruit large numbers of inflammatory and histiocytic cells, including the eponymous multinucleated giant cells.^{27, 28} Targeted inhibition of CSF1R with imatinib has shown promising results in early studies.²⁹ While it remains unclear if this therapy has autocrine, paracrine or combined effects on the proliferation of the neoplastic cells themselves, the reduction in overall tumor size and cellularity from blocking the recruitment of inflammatory cells may result in improved surgical resectability as well as symptomatic relief.²⁹

Success targeting chimeric transcription factors has been more elusive. Myxoid liposarcomas are characterized by a fusion between *FUS* and *DDIT3*. This fusion oncoprotein is reported to both promote proliferation and arrest cells in a primitive pre-adipocyte stage of development.^{30, 31} While it is not a specific targeted agent in the conventional sense, one study has suggested that trabectedin may directly interact with the *FUS-DDIT3* fusion protein and inhibit its ability to bind to DNA promoter sequences.³² Trabectedin has shown some promise as an adjuvant therapy in myxoid liposarcoma.³³

Another approach is to inhibit downstream factors upregulated by fusion genes. Several chimeric transcription factors, including *PAX3-FOXO1A* and those involving *EWSR1* or *FUS* fusion genes, among others, upregulate expression of growth factors, including *IGF1R*, *PDGFR* and *MET* hepatocyte growth factor receptor,³⁴⁻⁴² which may be targeted by various TKIs. Early preclinical studies have shown some response to MET-inhibitors in alveolar soft part sarcoma,⁴³ and clear cell sarcomas,⁴⁴ while inhibition of IGF1R may be beneficial in Ewing sarcoma.⁴⁵⁻⁴⁷ Similarly, the *SS18-SSX* fusion oncoprotein seen in synovial sarcoma upregulates *FGF*, which activates the Ras pathway and promotes proliferation.^{48, 49} Preclinical studies have suggested that targeted therapy against FGFR may inhibit growth of synovial sarcoma.⁴⁹

It has also been postulated that translocation variants in sarcoma may be predictive of patient outcome, however, the encouraging results of early studies in alveolar rhabdomyosarcoma, Ewing sarcoma and synovial sarcoma have not been clearly validated in more recent reports.⁵⁰⁻⁵⁷

Sarcomas with Driving Oncogenic Mutations

Several sarcomas have been identified in which tumorigenesis is primarily driven by single activating gene mutations. The exemplar of this class of sarcoma is GIST, which represents one of the earliest great success stories of targeted therapy. GISTs require activating mutations in the *KIT* receptor tyrosine kinase, or less frequently, in *PDGFRA* for tumor proliferation.^{58, 59} The majority of cases have mutations in exon 11 of *KIT*, and respond dramatically to imatinib mesylate therapy, with up to 80% of patients demonstrating at least partial response, while mutations in exon 9 or *PDGFRA* render tumors resistant.^{58, 59} These tumors illustrate the concept of oncogenic addiction, in which activation of a single pathway is required for tumorigenesis. Unfortunately, GIST frequently develop secondary resistance to imatinib, which in many cases is due to additional activating mutations in *KIT* or to mutations in *PDGFRA*.⁶⁰ In these cases, second line therapy with a different TKI, such as

sunitinib, have shown some benefits in delaying tumor progression.⁶¹ Agents directed against PDGFR- α are also in development.⁶² Ultimately, however, patients are rarely cured with TKI therapy alone.⁶¹

Sarcomas with Complex Karyotypes

The largest category of sarcoma includes those with complex cytogenetic alterations. This class is primarily comprised of higher grade spindle cell and pleomorphic sarcomas, such as leiomyosarcoma, undifferentiated pleomorphic sarcoma/ malignant fibrous histiocytoma (UPS/MFH) and angiosarcoma.⁶³

Complex karyotype sarcomas are thought to occur as a result of genomic instability and failure of DNA repair and maintenance mechanisms. One pathway by which this may occur is by telomere loss. Telomeres are responsible for maintaining the integrity of chromosome ends, however, these sequences themselves shorten within each mitotic cycle and are prone to breakage under stress conditions or uncontrolled proliferation. While in normal cells, telomere shortening results in cellular senescence, in tumor cells which have circumvented normal cell cycle controls, division continues to occur, with disastrous consequences for chromosomes. Telomere loss allows sticky ends of chromosomes to bind to nearby strands of DNA, inducing a cycle of unregulated fusion and breakage, with the end result of bizarre chromosomal inversions, amplifications, duplications, and translocations which characterize high grade malignancies.⁶⁴ Genomic rearrangements may also occur as a result of a single catastrophic event, known as chromothripsis, which appears to occur in at least 2-3% of all cancers, and is suggested to occur in up to 25% of malignant bone tumors.⁶⁵

Paradoxically, some sarcomas, including Ewing sarcoma, UPS/MFH and liposarcoma, actually have increased telomerase activity, despite prior genomic rearrangement.⁶⁶⁻⁶⁸ High telomerase activity or the presence of alternative lengthening of telomeres is associated with poor prognosis.^{66, 67} It is thought, therefore, that targeting of telomere maintenance mechanisms may be an effective therapy in selected mesenchymal tumors.⁶⁹

Regardless of the underlying chromosomal events, complex karyotype sarcomas, like carcinomas, seem to develop mutations or activating events in particular cell survival or proliferation pathways, including cell-cycle checkpoints, apoptosis, stress response, and metabolic/proliferative pathways.^{70, 71} While no single event may be seen in all tumors, understanding which pathways are the most frequently dysregulated, and at what step in signaling, may narrow the options for the best potential drug-targets (Figure 1).

Targets in Transcriptional Regulation

Regulation of DNA transcription and replication involves epigenetic modification of both DNA (via promoter methylation) and DNA-associated proteins such as histones (via acetylation and other post-translational modifications). In the acetylated state, chromatin assumes an open configuration which allows transcription factors access to DNA. De-acetylation of histones by histone deacetylases (HDACs) causes histones to form tightly wound spindles, rendering associated DNA into a compact, inactive state unsuitable for transcription.⁷² Regulation of acetylation and deacetylation of non-histone factors by histone acetylases and HDACs may also play a role in protein stability, cell signaling and protein cellular localization and function.⁷² While the epigenetic mechanisms by which HDAC overexpression functions in tumorigenesis are not fully elucidated, HDAC inhibitors have shown promise in preclinical studies of MPNST,^{73, 74} Ewing sarcoma⁷⁵ synovial sarcoma,⁷⁶ fibrosarcoma,⁷⁷ GIST,⁷⁸ uterine sarcomas,⁷⁹ and dedifferentiated liposarcoma.⁸⁰ Currently, HDACis are in phase I/II trials for sarcoma and other diseases as single agents and in combinations.

Direct epigenetic regulation of DNA involves transcriptional silencing via promoter hypermethylation. Hypermethylation of regulatory genes has been identified in a variety of sarcomas compared to non-neoplastic tissue.⁸¹ In particular, methylation of *MGMT*, a DNA repair factor, has been reported to be associated with aggressive tumors.⁸¹ In addition, hypermethylation of *CEBPA*, a transcription factor involved in adipocytic differentiation, has been recently identified in 24% of dedifferentiated liposarcoma. Moreover, demethylation of *CEBPA* in dedifferentiated liposarcoma cell lines and xenografts resulted in growth inhibition.⁸⁰ Thus, DNA methyltransferases (DNMT), which are required to maintain the hypermethylated state, may represent therapeutic targets in selected sarcomas. As with HDACs, however, we do not know if arbitrary alterations in DNA or DNA binding proteins will be helpful or harmful until human clinical trials are performed.

Targets in Mitotic Machinery

Aurora kinases (AurKs) are a family of serine/threonine kinases required for progressive stages of mitosis and cell division. AurKs are involved in centrosome duplication, spindle formation, alignment of chromosomes on the mitotic spindle, progress through mitotic checkpoints and cytokinesis.⁸² Dysregulation of AurKs has been reported in a variety of carcinomas,⁸² but little data is available on their role in mesenchymal neoplasms. Nevertheless, targeted therapy against AurK A and B has shown encouraging anti-tumor effects in *in vitro* and xenograft studies using Ewing sarcoma-derived cell lines, which appear to overexpress both AurK A and B.⁸³⁻⁸⁵ These preclinical results suggest that aurora kinases may be candidate targets for directed therapy in some sarcomas.

Kinesins are another critical component of the mitotic spindle. Kinesins are microtubule dependent motor proteins with ATPase activity, which function in cell division and cellular transport. Kinesins are involved in nearly all aspects of cell division, from chromosome condensation and segregation, to spindle assembly and chromosome positioning, to cytokinesis.⁸⁶ Altered expression of kinesins is seen in a variety of cancers, and has been associated with tumor progression.⁸⁶ Although little is understood about the role and regulation of kinesins in sarcoma, kinesin inhibitors have shown activity in some sarcoma xenografts,⁸⁷ and are currently in phase I trials.⁸⁸

Targets in Cell Survival and Stress Response

Apoptosis and Cell Death

Several mechanisms exist by which sarcoma cells escape programmed cell death. The intrinsic p53 pathway is one of the most frequently inactivated pathways in sarcoma, *TP53* is commonly mutated (inactivated) in complex karyotype sarcomas, including pleomorphic liposarcoma, leiomyosarcoma, and UPS/MFH.⁸⁹ Alternatively, p53 activity, stability or subcellular localization may be dysregulated. *HDM2*, whose protein product binds to and inactivates p53 protein, is amplified in some soft tissue sarcoma, including atypical lipomatous tumor/well differentiated liposarcoma and in some bone tumors such as low grade parosteal and intramedullary osteosarcoma.⁹⁰⁻⁹² Effects of p53 on the intrinsic cell-death pathway may also be negated by antiapoptotic regulatory factors, including bcl-2, which is overexpressed in up to 60% of sarcomas.⁶³ Inhibition of p53-mediated apoptosis may protect tumor cells against chemotherapy and radiotherapy-mediated cell death. Accordingly, preliminary studies targeting this pathway using antisense mRNA-mediated knockdown of bcl-2 have shown that loss of bcl-2 may induce apoptosis or sensitize cells to death from conventional chemotherapy.⁹³ Another tactic, which may be useful in well-differentiated/dedifferentiated liposarcoma, involves inhibiting Hdm2-p53 interactions, with agents such as RG7112.⁶²

While the intrinsic cell death pathway responds to internal cellular stimuli, the extrinsic apoptotic pathway is triggered by death receptors located in the cell membrane, and is independent of p53 activation. Binding of Apo2L/TRAIL to the death receptors DR4/TRAIL-R1 or DR5/TRAIL-R2 triggers apoptosis via activation of caspase 8.⁹⁴ Notably, Ewing sarcoma and rhabdomyosarcoma have both been shown to express TRAIL-receptors, potentially rendering them susceptible to TRAIL-mediated cell death.⁹⁵ Moreover, recent studies with recombinant TRAIL, both in the laboratory and in phase I trials, have indicated a possible role for addition of TRAIL to chemotherapy regimens in some tumors, including chondrosarcoma, which is otherwise notoriously chemoresistant.⁹⁶

Autophagy is another survival process known to play a role in sarcomagenesis. Autophagy is a cellular mechanism used to dispose of damaged organelles and reprocess proteins,⁹⁷ which may either promote apoptosis or rescue of damaged cells, depending on cellular circumstances. In damaged cells or those which are rapidly proliferating, autophagy enables cells to optimize nutrient usage and streamline cellular machinery, and may also degrade depolarized mitochondria that might activate apoptosis.⁹⁷ In *in vitro* studies of malignant peripheral nerve sheath tumors (MPNSTs) treated with HDACis, autophagy has been shown to promote tumor survival,^{73, 74} and inhibition of autophagy results in cell death.⁷³ In GIST, which are normally resistant to apoptosis, inhibition of autophagy potentiates the effects of imatinib, leading to increased cell death.⁹⁸ Autophagy blockade, in concert with conventional or targeted therapy, may therefore be a useful adjunct therapy.

Cellular response to stress is also mediated by a variety of chaperone and stress-response factors known as heat shock proteins (HSPs). HSPs act as key regulatory factors under conditions of cell stress, and may be pro or anti-apoptotic. For instance, HSP27 and HSP70 are both thought to serve antiapoptotic functions and may play a role in tumorigenesis.⁹⁹ HSPs, in their role as intracellular chaperones may protect other factors from degradation, and may also interact with both HDACs and non-histone deacetylases.⁷² HSP90, in particular, has been reported to regulate stability of oncogenic KIT,¹⁰⁰ and agents targeting HSP90 (IPI-504) showed promise in early studies.^{78, 100} However, in phase III studies, IPI-504 did not show a clinical effect in TKI-resistant GISTs.¹⁰¹ Another HSP90 inhibitor, STA-9090, is currently under investigation.¹⁰¹

Targetable Proliferation Pathways

Sarcomas appear to be heavily reliant upon growth factor signaling for proliferation and survival. Despite this, high grade sarcomas have thus far shown little response to single agent therapy with TKIs,^{93, 102} although, as discussed above, a larger role may be seen for TKI therapy in translocation-associated sarcomas. Ultimately, because of redundancy and interconnectedness of survival and proliferation pathways, effective targeting may require simultaneous targeting of multiple pathways.

Cell Cycle Regulation

Dysregulated tumor proliferation requires abolition of normal cell-cycle checkpoints. The *RB1* tumor suppressor gene functions as a G1/S phase checkpoint. Both inactivation of Rb or loss of p16INK4a, required to maintain activation of Rb, are frequently observed in UPS/MFH, while loss of *RB1* is seen in leiomyosarcoma, malignant peripheral nerve sheath tumor (MPNST), and osteosarcoma.^{63, 91} Loss of p16INK4a is also associated with tumor progression. Attempts to target this pathway via inhibition of the pro-cell-cycle progression factors CDK4 and 6, have thus far been unsuccessful.¹⁰³

Proliferation and Survival

Cellular proliferation and survival often involve signaling through the interconnected Ras and Akt pathways. The Akt pathway is normally activated by a growth factor binding to a RTK, but may also be activated by downstream events, including activating mutations in *PIK3CA*, as is seen in myxoid/round cell liposarcoma,^{104, 105} or by loss of the inhibitor PTEN, which has been reported in leiomyosarcoma, and is associated with aggressive behavior.^{63, 91} PI3K activation in turn leads to activation of Akt, and subsequently, the mTOR complexes (mTORC) 1 and 2, which regulates protein translation and other cellular processes.

A variety of RTK are overexpressed or constitutively activated in sarcoma, both in translocation associated sarcomas, as discussed above, and in karyotypically complex tumors. Because RTKs and their ligand growth factors are so frequently overexpressed in sarcomas, they are thought to represent attractive therapeutic targets. A wide array of TKIs with varying receptor specificity have been developed in recent years, although, thus far, the response rates in non-translocation sarcomas have been mixed.^{93, 102} For example, while EGFR is overexpressed in approximately 60% of soft tissue sarcomas, especially in sarcomas with complex karyotypes (eg, UPS/MFH, myxofibrosarcoma, MPNST, and leiomyosarcoma), as well as in synovial sarcoma,¹⁰⁶ one phase II study of gefitinib, a specific TKI against EGFR, had poor results, with best response being stable disease only in 10/46 patients.¹⁰⁷ In contrast, in preclinical studies of epithelioid sarcoma, which have been shown to overexpress EGFR,¹⁰⁸ erlotinib-induced inhibition of EGFR alone was cytostatic, while combined inhibition of EGFR with mTOR had synergistic effects on growth inhibition.¹⁰⁹

IGF-1R is also frequently overexpressed in both bone and soft tissue sarcomas, and has been reported to be associated with aggressive behavior in synovial sarcoma and alveolar rhabdomyosarcoma.¹¹⁰ Nevertheless, in sarcomas other than Ewing sarcoma, IGF-1R inhibition has not yet shown promising results.¹¹¹ The RTK *MET* has been shown to be activated in MPNST, and in preclinical studies, inhibition of MET with the targeted agent XL184 led to reduction of metastatic potential.¹¹² However, as yet, this agent has not entered the clinical testing stage for sarcoma. Minor activity of MET inhibitor ARQ197 was reported in abstract form in 2009 and it is unclear if future studies will be pursued examining this agent.¹¹³

One difficulty in targeting the Akt pathway via RTKs is that one tumor may express multiple RTKs and growth factors, and PI3K may also be activated by Ras signaling. Agents have been developed against the downstream effector mTOR with varying specificities against mTORC1 and mTORC2. One difficulty with mTORC1-inhibitors such as sirolimus and temsirolimus is that blockade of mTOR often leads to paradoxical increase in PI3K and Akt activity.^{114, 115} This homeostatic mechanism may be one reason there have not been more overt responses in cancer, including sarcomas, to mTOR inhibitors, with the largest phase II study of mTOR inhibitors in metastatic or unresectable soft tissue sarcoma demonstrating only a 2% RECIST response rate.¹¹⁶ To circumvent these homeostatic effects, newer agents in development include dual mTOR and PI3K inhibitors, as well as Akt-inhibitors. It is hoped that elucidation of the mechanisms by which mTOR inhibitors function in sensitive tumors, such as PEComas,¹¹⁷ will lead to improved usage of this family of agents.

The Ras pathway is involved in cell proliferation, survival, differentiation and angiogenesis as well as in motility and invasion, and may cross-activate the Akt pathway via PI3K. Activating *RAS* mutations have been found in leiomyosarcoma and UPS/MFH,⁹¹ and activation of downstream factors MEK and ERK have been described in UPS/MFH,¹¹⁸ and

osteosarcoma,¹¹⁹ among others. *BRAF*, an intermediary in the Ras pathway, has been shown to be mutated in a minority of GIST lacking *KIT* or *PDGFRA* mutations.¹²⁰ V600E mutant B-raf, as found in a tiny subset of GIST, may be targeted by vemurafenib, which has not been well-studied in sarcoma. B-raf is at least partially inhibited by sorafenib, which also has effects on multiple RTKs. Sorafenib has shown antitumor effects *in vitro* in MPNST, synovial sarcoma and chondrosarcoma via the Ras pathway,¹²¹⁻¹²³ as well as in desmoid tumors,¹²⁴ and showed minor activity against angiosarcoma in a phase II trial.^{93, 125} The data with vemurafenib in V600E mutant melanoma indicate the importance of the specificity of the inhibitor for the driving mutant kinase.¹²⁶ However, the relatively short time to resistance to RAF inhibitors has led many researchers to pursue other inhibitors of the Ras pathway, including agents targeting MEK and ERK. MEK inhibitors have shown promise in preclinical studies,^{119, 127} and several are in phase I/II trials.

Angiogenesis

Recruitment of new vessels is required for tumors to both grow and metastasize. Thus, targeting of proangiogenic growth factors has long been heralded as a potential therapy for sarcoma, and other tumor types. In addition to their roles in autocrine and paracrine proliferation pathways, VEGF and VEGFR have specific proangiogenic effects and are thought to be required for recruitment of new vessels into a growing tumor.^{128, 129} VEGF is overexpressed in about a quarter of all soft tissue sarcoma, including epithelioid sarcoma, alveolar soft part sarcoma, UPS/MFH, DFSP, and leiomyosarcoma,¹²⁸ and has been associated with high metastatic potential.^{91, 128} VEGF and VEGFR may be targeted by a variety of drugs, including bevacizumab, sunitinib, and pazopanib. Unfortunately, in a number of phase I and II trials, responses to these agents have proven mixed, at best, even in vascular malignancies such as angiosarcoma and hemangioendothelioma.^{102, 125} Others have reported that bevacizumab in combination with doxorubicin provided at least stable disease for 11/17 patients with metastatic sarcoma, but with significant cardiac toxicity for some.¹³⁰ Thus far, single agent therapy targeting VEGFR does not appear promising, likely due to the complex, multistep nature of angiogenesis induction.

Other pro-angiogenic factors that may prove targetable include basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and transforming growth factor alpha (TGF- α).⁹¹ Conversely, thrombospondin-1, an inhibitor of VEGF, VEGFR and IL-8, has been reported to be decreased in some sarcomas.^{131, 132} ABT-510, a thrombospondin-1 mimetic, has been assessed in a phase II trial, with about half of the treated tumors showing stabilization as best response.¹³¹

Other targets

The Notch family of receptors has been implicated in control of differentiation. Preclinical studies have suggested that inhibition of Notch may reduce the invasiveness of both osteosarcoma and rhabdomyosarcoma,^{133, 134} and promote differentiation of rhabdomyosarcoma.^{135, 136} However, the effects of Notch signaling appear to be tumor type-specific, as Notch family members may act as either oncogenes or tumor suppressors, and activation of Notch in Ewing sarcoma cell lines led to growth inhibition.¹³⁷ The gamma secretase inhibitor RO4929097 which blocks notch signaling by preventing cleavage of the activated intracellular domain of Notch from the transmembrane domain is currently in phase I/II trials. It remains to be seen, however if this therapy will have pro- or anti-proliferative effects in a population of unselected tumors.

Another pathway reported to be upregulated in some sarcomas is the hedgehog pathway, which normally plays a critical role in embryogenesis. Activation of downstream targets of the hedgehog pathway has been reported in embryonal rhabdomyosarcomas.^{138, 139}

Moreover, inhibition of hedgehog pathway signaling reduced proliferation of embryonal rhabdomyosarcoma cell lines.¹⁴⁰ These findings are the basis of a phase I/II trial combining the hedgehog signaling inhibitor GDC-0449 with a notch signaling inhibitor in metastatic sarcoma.

Conclusion

Targeted therapy, i.e. agents beyond standard cytotoxic chemotherapy agents, offers the hope for improved treatment of this heterogeneous and difficult to manage group of malignancies. While some sarcoma subtypes have responded brilliantly, other common diagnoses such as leiomyosarcoma and UPS have largely failed to respond to the available portfolio of agents to date. Fortunately, an increasing number of agents are under investigation, primarily in phase I/II trials (Table 1), inhibiting any number of cellular kinases and other processes responsible for tumor cell survival. As our understanding of the mechanisms of tumorigenesis and the pathways required for sarcoma survival and metastasis increases, it is hoped that so too will our ability to correctly identify therapeutic targets and develop effective drugs. Future clinical trials will need to more specifically select patients with appropriate molecular alterations for the therapy tested, and examine groups of specific agents to better achieve the goal of truly personalized treatment for sarcoma.

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Figure 1. Molecular pathways involved in sarcomagenesis. Green indicates pro-tumorigenic factors that are activated or overexpressed in sarcoma. Red indicates tumor suppressors that may be inactivated in sarcoma.

Table 1

Selected approved and investigational targeted agents in sarcoma

Agent	Status	Specificity
Tyrosine Kinase Inhibitors		
Imatinib mesylate	FDA approved for GIST, DFSP	kit, abl, PDGFR
Sunitinib	FDA approved GIST (2 nd line)	Multiple tyrosine kinases: PDGFR, kit, RET, CSF-1R, Flt3, VEGFR
Sorafenib	Phase II ^{125, 141}	Multiple kinases: kit, VEGFR, PDGFR, raf
Gefitinib	Phase II ¹⁰⁷	EGFR
R1507	Phase I/II ¹⁴²	IGF-1R
Figitumumab	Phase I ¹⁴³	IGF-1R
Crizotinib	Phase I ²⁶	Alk/ Met
ARQ197	Phase II ¹¹³	Met
mTORC1 Inhibitors		
Sirolimus	Phase II ¹⁴⁴	mTORC1
Temsirolimus	Phase II ¹⁴⁵	mTORC1
Ridaforolimus (deferolimus)	Phase II/III ¹¹⁶	mTORC1
Everolimus	Phase I/II ¹⁴³	mTORC1
mTORC catalytic domain inhibitors		
AZD8055	Preclinical/Phase I ¹⁴⁶	mTORC1/mTORC2
PI3K Inhibitors		
GSK1059615	Phase I ¹⁴⁷	PI3K
Dual mTOR/PI3K Inhibitors		
BEZ235	Phase I ¹⁴⁸	PI3K class I, mTOR
AKT Inhibitors		
MK2206	Phase I ¹⁴⁹	Akt
Ras Pathway Inhibitors		
Selumetinib	Phase II ¹⁵⁰	Mek
Anti-Angiogenic Agents		
Bevacizumab	Phase II ^{130, 151}	VEGFR
Pazopanib	Phase II ¹⁵²	VEGFR, PDGFR, kit,
Cediranib	Phase I ¹⁵³	VEGFR
Brivanib	Phase II ¹⁵⁴	VEGFR, FGFR
ABT-510	Phase II ¹³¹	Thrombospondin mimetic
Pro-Apoptotic Agents		
RG7112 (RO5045337)	Phase I/II ¹⁵⁵	Hdm2-p53 interactions
Dulamnermin (r-hu anti-Apo2/TRAIL)	Phase I ⁹⁶	TRAIL-R
Oblimersen (G3139)	Phase I ¹⁵⁶	Bcl-2 (antisense oligonucleotide)
Cell Cycle Progression/Proliferation Inhibitors		

Agent	Status	Specificity
PD0332991	Phase I/II ¹⁵⁷	Cdk4
Ispinesib	Phase I ^{87, 88}	Kinesin
MLN8054, MLN8237	Preclinical/Phase I ¹²⁶	AurkA
Epigenetic Modifier Inhibitors		
Panobinostat	Phase II ¹⁵⁸	HDAC
Vorinostat	Phase II ¹⁵⁹	HDAC
Azacytidine	Phase I/II ¹⁶⁰	DNA methyltransferase