Managing sarcoma: where have we come from and where are we going?

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Abstract: Sarcomas are a heterogeneous group of neoplasms of mesenchymal origin. Approximately 80% arise from soft tissue and 20% originate from bone. To date more than 100 sarcoma subtypes have been identified and they vary in molecular characteristics, pathology, clinical presentation and response to treatment. While sarcomas represent <1% of adult cancers, they account for approximately 21% of paediatric malignancies and thus pose some of the greatest risks of mortality and morbidity in children and young adults. Metastases occur in one-third of all patients and approximately 10–20% of sarcomas recur locally. Surgery in combination with preoperative and postoperative therapies is the primary treatment for localized sarcoma tumours and is the most promising curative possibility. Metastasized sarcomas, on the other hand, are treated primarily with single-agent or combination chemotherapy, but this rarely leads to a complete and robust response and often becomes a palliative form of treatment. The heterogeneity of sarcomas results in variable responses to current generalized treatment strategies. In light of this and the lack of curative strategies for metastatic and unresectable sarcomas, there is a need for novel subtype-specific treatment strategies. With the more recent understanding of the molecular mechanisms underlying the pathogenesis of some of these tumours, the treatment of sarcoma subtypes with targeted therapies is a rapidly evolving field. This review discusses the current management of sarcomas as well as promising new therapies that are currently underway in clinical trials.

Keywords: chemotherapy, current management, new therapies, radiation therapy, sarcoma, surgery, targeted therapies

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Introduction
Sarcomas are highly diverse mesenchymal malignancies of the bone, cartilage, muscle, peripheral nerves and adipose or fibrous connective tissues.1 Although the ultimate cells of origin of sarcoma subtypes remain unclear, there is increasing evidence that they arise de novo from mesenchymal pluripotent stem cells.2,3 An extension of this theory would be that alteration(s) in mesenchymal stem cell genetics can give rise to several sarcomas, including osteosarcoma, Ewing’s sarcoma, synovial sarcoma, chondrosarcoma, rhabdomyosarcoma, fibrosarcoma and liposarcoma.4 At present, sarcoma classification is based on the tissue type it resembles. This form of classification is, however, challenging for sarcoma subtypes that do not show any clear similarities to normal tissue, such as clear cell and epithelioid sarcomas. More recently, classifications have been revised to include molecular features and genetic profiles of sarcomas.5–7 From a molecular point of view, sarcomas may be broadly classified into two types: (1) sarcomas with simple karyotypes characterized by chromosomal translocations or specific mutations; and (2) sarcomas with complex aneuploidy karyotypes, consisting of numerous losses, gains and amplifications.8 Approximately 15–20% of sarcomas fall into the simple karyotype subgroup, while the vast majority fall into the complex karyotype subgroup.9,10 There is, however, room for improvement in the identification of biomarkers for sarcoma subtypes and determination of
optimal subtype-specific treatment strategies. The need for alternative treatments such as targeted therapies and immunotherapies is underscored by observations that several sarcoma subtypes are poorly responsive to chemotherapy and radiation.

This review will focus on the current treatment strategies for sarcoma and the emerging field of targeted therapies for sarcoma.

**Local control in sarcoma**

**Surgery**

Complete excision, when possible, is the standard therapy in the management of most subtypes of localized soft tissue sarcoma, and when possible, this confers the greatest possibility of cure. In order to adequately ensure removal of all tumour microsatellites, the tumour must be resected with a perimeter of healthy tissue.11 This lowers the risk of local recurrence and allows for better prognosis. Surgical removal of tumours takes into account limb and function-sparing while accomplishing suitable biological margins.12 The width of margin clearance necessary is subject to controversy and is affected predominantly by the anatomical location of the tumour and its size at presentation. Tumours located in the retroperitoneum, for example, cannot be removed with the same excisional margin as similar neoplasms arising from the thigh or buttock.13 What is unequivocal is that a resection with a microscopically involved margin carries a higher risk of recurrence than another where the margins are clear, even if by a few millimetres. In pursuit of clear margins, surgical resections require aggressive removal of involved tissue and sometimes reconstruction with grafts is necessary. Surgery for sarcoma carries major morbidity and is best performed in centres familiar with sarcoma management and with access to multimodality treatment. In addition, the outcome of patients treated at specialist sarcoma centres is better than patients treated at generalist units.14

The current gold standard for the treatment of bone sarcomas is limb salvage surgery with the aim of preserving a limb with sufficient functionality and without compromising the patient’s overall survival. It implicates clear-margin resection of the tumour followed by reconstruction of the bone defect with endoprosthetics, allografts or autografts.15 Surgery used in combination with chemotherapy increases the overall survival and progression-free survival significantly.16,17 Even though localized sarcomas have a high cure rate with surgery, when they recur and/or metastasize they have a poor prognosis with a median survival of approximately 12 months.18–20

**Radiation therapy**

Radiation is frequently used to treat soft tissue sarcoma of the extremity. However, although neoadjuvant (before surgery) and adjuvant (after surgery) radiation significantly improve local control of non-metastatic low-grade and high-grade sarcomas of the extremity, in many studies there is no statistically significant benefit for overall survival.21 In this group of patients, radiotherapy (RT) has been used both neoadjuvantly and adjuvantly, with no difference in progression-free survival between these approaches. However, adjuvant RT is associated with a higher incidence of late normal tissue toxicity. For this reason, neoadjuvant RT is often used as an alternative, despite the increased incidence of wound-healing problems.22 The main advantages of neoadjuvant RT are that target volume definition is easier with the visible tumour in situ, normal tissues are displaced out of the field and doses required are lower than with adjuvant treatment. In addition, the tumour may decrease in size, facilitating resection.20

Soft tissue sarcomas of the retroperitoneum present a different problem. These tumours are frequently very large at presentation and are more difficult to remove surgically than extremity tumours. Local control of these tumours remains a challenge and the role of RT in treating these tumours remains controversial. No large randomized trials exist because of the rarity of these tumours, but results from smaller series are at odds, with some showing no benefit from the addition of RT23 and others showing some benefit in terms of delaying local recurrence24 but little effect on overall survival. Both preoperative and postoperative RT are challenging in this site because of the proximity of the tumour to sensitive intra-abdominal organs. Preoperative RT, especially for tumours with borderline resectability, may be associated with fewer complications.

For bone sarcomas, the role of RT is also not well defined. The most common subtypes of bone sarcoma are osteosarcoma and Ewing’s sarcoma. Both of these are considered systemic diseases and the mainstay of treatment involves...
Chemotherapy and surgery. In osteosarcoma, the role of RT is confined to unresectable tumours, postsurgery in the case of positive margins and palliation. In Ewing’s sarcoma, RT has a greater role to play and may be curative in certain patients where tumours are unresectable, clear margins are not achievable or the tumour is deemed high risk.

Advanced RT techniques such as intensity-modulated radiation therapy (IMRT) and volumetric modulated arc therapy may play a role in the current clinical challenges of radiogenic wounds and associated toxicities, and are becoming mainstream in many departments when treating difficult cases. These techniques use a combination of advanced hardware and software which allow radiation dose to conform to complex tumour shapes. The advantage of this is that the optimal amount of radiation can be directed to the tumour or tumour bed, while sparing normal tissue as much as possible. Sladowska and colleagues and Paumier and colleagues demonstrated that, compared to conventional RT, IMRT improves dose distribution, target coverage and normal tissue sparing in soft tissue sarcomas of the thigh and retroperitoneal sarcoma.

Particle therapy, which uses charged particles such as protons and carbon ions, may also have a role to play in RT for sarcomas. Indeed, there is some evidence to suggest that particle therapy may be a more effective modality in the management of bone and soft tissue sarcomas not eligible for surgical resection, providing good local control and offering a survival advantage without unacceptable morbidity.

Particle therapy utilizes the Bragg peak effect to deliver high-dose radiation to the tumour while minimizing the dose delivered to adjacent normal tissue. Particle therapy is, however, extremely expensive, and not routinely available for all cases.

Systemic control in sarcoma

Chemotherapy

The role of chemotherapy in the management of sarcoma is variable and, in some cases, controversial. Significant benefit is seen in a limited group of chemosensitive sarcoma subtypes, including embryonal and alveolar rhabdomyosarcoma, Ewing’s sarcoma and osteosarcoma, and it is thus an integral part of the management of these sarcomas. Indeed, chemotherapy has drastically improved the long-term survival of these patients and offers possibility of cure, even in some cases with metastatic disease.

There are also examples of more recently described chemotherapies that appear effective in specific sarcoma subtypes; these will be discussed later. Other sarcoma subtypes vary from fairly sensitive to completely chemotherapy-unresponsive; in the majority of these cases, patients with metastatic disease face a dismal prognosis.

Single-agent chemotherapy. Doxorubicin (also known as Adriamycin), epirubicin and ifosfamide are the only single-agent chemotherapeutic drugs that consistently achieve response rates of >20% in metastatic sarcomas. However, the range of activity of these agents varies greatly for different histological subtypes and there is individual variability for drug efficacy.

Doxorubicin and epirubicin are anti-cancer antibiotics that belong to the anthracycline class of drugs. They are a four-membered ring system containing an anthraquinone chromophore and an aminoglycoside. Doxorubicin (Figure 1) was first used as a single-agent chemotherapeutic treatment in the 1970s. Upon intravenous injection, doxorubicin is rapidly taken up into the nucleus of cells, where it binds to DNA with high affinity. It acts by intercalating between DNA base pairs and binding to DNA-associated enzymes, such as topoisomerase enzymes I and II and DNA and RNA polymerases. This induces DNA damage and the cessation of DNA replication and mRNA transcription. Furthermore, cells arrest in G1 and G2 in an attempt to repair the damage, but when the damage is irreparable the apoptotic cell death pathway is triggered. Other actions of doxorubicin include the generation of free radicals, causing additional DNA damage, inhibition of
macromolecule production, DNA unwinding and an increase in alkylation.\textsuperscript{42} The reported response rates of sarcomas to doxorubicin vary significantly, ranging from 10\% to 25\%, with the majority of cases showing a partial response.\textsuperscript{43–48} In osteosarcoma, studies have demonstrated that cells are sensitized to doxorubicin treatment when autophagy, a process important for cell survival, is inhibited by blocking the high-mobility group box 1 protein (HMGB1).\textsuperscript{49} It is therefore possible that treatment of sarcomas with doxorubicin may be more successful when combined with an inhibitor of autophagy. Adverse effects of doxorubicin include both acute and chronic cardiotoxicity, reversible myelosuppression, alopecia, mucositis, nausea and vomiting.\textsuperscript{50,51}

Multiple trials have compared the effects and responses of doxorubicin and epirubicin in sarcoma treatment. In most cases no clear benefit for one over the other drug was seen. However, patients on the doxorubicin schedule demonstrated worse cardiovascular and haematologic toxicity and hence epirubicin is often favoured over doxorubicin.\textsuperscript{52} While epirubicin (Figure 2) acts in a similar way to doxorubicin, the spatial orientation of the hydroxyl group at the 4′ carbon of the sugar is different and this opposite chirality has been proposed to account for its reduced toxicity.\textsuperscript{52,53}

Ifosfamide (Figure 3) is a nitrogen mustard alkylating agent that terminates proliferating cancer cells by adding alkyl groups to guanine bases in DNA molecules. This inhibits tumour growth because the guanine nucleotides become cross-linked and prevent DNA double-helix strands from uncoiling and replicating.\textsuperscript{54} Ifosfamide consistently shows response rates comparable to doxorubicin and it has a 25\% average response rate among patients who show limited responses on a doxorubicin-based schedule.\textsuperscript{55–63} Unlike doxorubicin, which is administered as a single-day infusion,\textsuperscript{64} ifosfamide is administered intravenously over several days at a time\textsuperscript{65} and usually requires hospital admission. The toxicities associated with ifosfamide differ to those caused by doxorubicin, and include haemorrhagic cystitis, renal tubular acidosis, salt-wasting nephropathy, central nervous system toxicity and usually encephalopathy.\textsuperscript{54,66–68} Ifosfamide shows less evidence of cardiotoxicity, thus rendering it an attractive treatment option.\textsuperscript{58–61,63,69} Ifosfamide-induced urotoxicity can be reduced when it is administered simultaneously with mesna, a thiol compound that binds to the urotoxic ifosfamide metabolite, acrolein, converting it into a nontoxic compound.\textsuperscript{58,70,71}

Other single-agent chemotherapeutic drugs that have shown efficacy in some sarcoma subtypes include gemcitabine and topotecan. Gemcitabine (Figure 4) is a cytotoxic agent that has been tested in clinical trials but efficacy data are conflicting.\textsuperscript{72–75} It is a nucleoside analogue where the hydrogen atoms on the 2′ carbon of deoxycytidine are replaced by fluorine atoms. Gemcitabine arrests tumour growth by replacing cytidine, one of the building blocks of nucleic acids, during DNA replication. As a result, the newly forming DNA strand can no longer be elongated and apoptosis is induced. Gemcitabine also irreversibly inhibits the enzyme ribonucleotide reductase by binding to its active site and preventing the production of deoxyribonucleotides for DNA replication and repair and thus leads to apoptosis.\textsuperscript{76} It is more successful in leiomyosarcoma of uterine and gastrointestinal origin when used in combination with the anti-mitotic chemotherapeutics docetaxel or vinorelbine or the alkylating agent dacarbazine.\textsuperscript{77} Topotecan (Figure 5), a quinoline-based alkaloid extracted from the Asian tree \textit{Camptotheca acuminate}, inhibits topoisomerase-I activity during DNA replication. This causes double-strand breaks as the DNA is not
relieved from torsional strain by topoisomerase-I while replicating. These DNA strand breaks cannot be repaired and apoptosis is triggered. This drug generally demonstrates low activity, but response rates appear to be modest in non-uterine leiomyosarcoma. Treatment response has also been shown for use of this drug in Ewing’s sarcoma and rhabdomyosarcoma.

The taxane agents, paclitaxel and docetaxel, are diterpenes produced by plants of the genus Taxus. Although comparatively inactive as single-agent treatment, taxanes, particularly paclitaxel, appear to show significant response rates in angiosarcoma. This class of drugs functions by disrupting microtubules and thus inhibiting cell division. Other conventional single-agent chemotherapeutic drugs used for the treatment of sarcomas include vinorelbine, methotrexate, dacarbazine, temozolomide, cisplatin and carboplatin, but the response rates for most of them are <20%.

Novel single-agent chemotherapeutics. Trabectedin and eribulin are two novel marine-derived chemotherapeutics which have shown promise for the treatment of leiomyosarcoma and liposarcoma, which together account for approximately 30% of all soft tissue sarcomas. Trabectedin (Figure 6) is a marine-derived alkaloid that is characterized by three fused tetrahydroisoquinoline rings. Two of these rings covalently interact with the minor groove of the DNA double helix and the third ring interacts with nearby nuclear proteins. These chemical interactions stimulate a cascade of events that compromises DNA binding proteins, including several transcription factors and the DNA nucleotide excision repair machinery. This results in double-strand DNA damage followed by a G2/M cell cycle arrest and ultimately apoptosis. Trabectedin also targets the tumour microenvironment by triggering apoptosis in monocytes, including tumour-associated macrophages (TAMs), which are known to promote tumour progression and metastasis. Furthermore, trabectedin inhibits the transcription of pro-inflammatory mediators (cytokines and chemokines), which also play a role in tumour growth and progression.

Two independent phase II clinical trials in 2004 provided initial analysis of trabectedin in advanced sarcoma subtypes refractory to conventional anthracycline/ifosfamide first-line chemotherapy and a median 6-month progression-free survival of 29% and 24% were achieved. The most profound responses were observed in leiomyosarcoma and synovial sarcoma histologies, with 56% and 61% progression arrest of tumour growth respectively. Adverse effects of trabectedin include neutropenia, transaminase elevation, fatigue and emesis. The success of trabectedin in early clinical trials led to its approval by the European Union for advanced soft tissue sarcoma in 2007 and subsequently the drug has been approved in over 70 countries, especially for patients who have failed to respond to standard therapies. A recent phase III clinical trial confirmed that advanced liposarcoma and leiomyosarcoma refractory to doxorubicin and ifosfamide showed a 45% reduction in the risk of disease progression or death when treated with trabectedin in comparison with dacarbazine, which prompted the approval of trabectedin by the US Food and Drug Administration (FDA). The reason(s) why some sarcomas are particularly sensitive to trabectedin is not fully understood. However, it is likely that it interferes with transcription factors that they are addicted to. The myxoid/round cell subtype of liposarcoma is the most sensitive to trabectedin and 95% of these carry a t(12;16) (q13;p11) chromosome translocation which results in
the oncogenic FUS–CHOP fusion-protein. FUS–CHOP functions as a transcription factor that promotes multiple aspects of tumorigenesis, and trabectedin was shown to compete with and displace it from its target promoters.\textsuperscript{106,107}

Recently a phase IIb trial showed that the administration of trabectedin as a first-line chemotherapeutic for advanced soft tissue sarcomas shows no significant improvement in progression-free survival in comparison to doxorubicin, leading to the conclusion that doxorubicin remains the gold standard for first-line treatment of advanced soft tissue sarcomas.\textsuperscript{108} Although trabectedin clearly demonstrates clinical benefit in anthracycline-resistant advanced leiomyosarcoma and synovial sarcoma histologies as a second- or third-line treatment, its benefit in treating other sarcoma histologies warrants further investigation.

Eribulin (Figure 7) is a novel microtubule-targeting chemotherapeutic drug. It was recently approved by the FDA for the treatment of patients with unresectable or metastatic liposarcoma who have received a prior anthracycline-containing chemotherapy regimen.\textsuperscript{109} It is a synthetic analogue of halichondrin B that was originally extracted from the marine sponge \textit{Halichondria Okaida}.\textsuperscript{110} The anti-cancer properties of eribulin are distinct from other tubulin-targeting agents in that it does not affect microtubule shortening but binds to a unique part of tubulin which results in the suppression of microtubule growth and sequestration into non-functional aggregates.\textsuperscript{111–113} In this regard, eribulin has been found to be especially efficacious in patients with tumours harbouring beta-tubulin mutations that are refractory to taxanes.\textsuperscript{114,115} In preclinical models, eribulin was shown to induce an irreversible mitotic arrest, apoptosis and tumour regression in multiple cancer cell lines with a mean IC50 that is 2–4-fold more potent than vinblastine and paclitaxel.\textsuperscript{114,116,117} A broad range of sarcoma cell lines, including liposarcoma, leiomyosarcoma, Ewing’s sarcoma, synovial sarcoma and fibrosarcoma, have been demonstrated to be sensitive to eribulin.\textsuperscript{118} The authors showed that it induced cellular differentiation \textit{in vitro} and reduced tumour formation and vasculature in \textit{in vivo} xenograft models. In a non-randomized phase II study, the EORTC (European Organisation for Research and Treatment of Cancer) Soft Tissue and Bone Sarcoma Group assessed the efficacy and safety of eribulin. This study showed that eribulin exhibited significant anti-tumour activity in metastatic liposarcoma and leiomyosarcoma patients, but not in synovial sarcoma or any other sarcoma subtype.\textsuperscript{119} A phase III study also demonstrated that, compared to dacarbazine, eribulin significantly improves overall survival of patients with refractory leiomyosarcoma and liposarcoma.\textsuperscript{120} The predominant side effects reported for eribulin are neutropenia, anaemia, fatigue, febrile neutropenia, mucositis and sensory neuropathy.\textsuperscript{121}

\textit{Combination chemotherapy}. Combination chemotherapy has been explored extensively and while not always used as a first-line approach to treating patients with metastatic sarcomas, it is an accepted treatment. Most combination chemotherapy regimens include doxorubicin and an alkylating agent.\textsuperscript{122,123} Regimens that appear often in the literature include AIM (doxorubicin, ifosfamide and...
mesna), MAID (mesna, doxorubicin, ifosfamide and dacarbazine) and gemcitabine together with docetaxel, vinorelbine or dacarbazine.\textsuperscript{45,46,124\textendash}129 A variety of bone and soft tissue sarcomas show response to these regimens, with Ewing’s sarcoma and rhabdomyosarcoma showing greater sensitivity to MAID schedules;\textsuperscript{58,126} myxoid liposarcoma, myxofibrosarcoma and synovial sarcoma showing sensitivity to AIM regimens;\textsuperscript{130\textendash}132 and leiomyosarcoma showing better response rates to the gemcitabine-based regimens.\textsuperscript{133} Another combination treatment termed VAC/IE for Ewing’s sarcoma and rhabdomyosarcoma includes vincristine, doxorubicin and cyclophosphamide alternating with ifosfamide and etoposide.\textsuperscript{134,135} This relatively intense chemotherapeutic regimen shows a 16\textendash}46\% overall response with complete responses occurring in approximately 5\textendash}10\% of these sarcomas. About one-third of these complete responders are long-term disease-free survivors.\textsuperscript{126,136\textendash}139

Studies comparing single-agent therapy to combination regimens have failed to provide evidence as to which option provides better overall survival benefit.

Targeted therapies

The development of molecular-targeted therapies for sarcomas is a rapidly evolving field. This therapeutic strategy requires identification of key molecular drivers of sarcomas and recent advances in our understanding of sarcoma biology have led to the identification of several molecular determinants of different sarcoma subtypes (Figure 8). This section will review the most relevant targetable pathways in soft tissue and bone sarcomas, as well as discuss findings from preclinical and clinical trials, which are summarized in Figure 9.

Tyrosine kinase inhibitors

Tyrosine kinase inhibitors have become the most influential targeted therapeutic breakthrough for the treatment of sarcomas. Factors currently targeted in approved treatments include the receptors for the tyrosine kinases c-KIT, platelet-derived growth factor receptor (PDGFR) and vascular endothelial growth factor receptor (VEGFR). Other sarcoma subtype-specific targeted therapies underway include the inhibition of insulin-like growth factor 1 receptor (IGF1R) in Ewing’s sarcoma and MET receptor tyrosine kinase and Src tyrosine-protein kinase in bone sarcomas.

c-KIT, PDGFR and VEGF inhibitors. c-KIT is a class III receptor tyrosine kinase and has been shown to impact on a variety of oncogenic cellular processes such as cell survival, proliferation, differentiation, adhesion and apoptosis by initiating multiple downstream signalling pathways such as the mitogen-activated protein kinases (MAPK), phosphatidylinositol 3-kinase (PI3K) and Janus kinase/signal transducer and activator of transcription (JAK/STAT). PDGF-\(\alpha\) is also a class III receptor tyrosine kinase and a key regulator of mesenchymal cell proliferation and migration.\textsuperscript{140\textendash}142 c-KIT and PDGFR kinases play an important role in the pathogenesis of a number of tumours including gastrointestinal stromal tumours (GISTs). GISTs are the most common mesenchymal tumours of the gastrointestinal tract and are usually resistant to chemo- and radiation therapy. Approximately 95\% of GISTs arising in adults constitutively express active c-KIT; of these patients, 80\% have c-KIT gene mutations which result in ligand-independent constitutive activation of the receptor.\textsuperscript{143,144} This results in uncontrolled cell proliferation and the stimulation of downstream signalling pathways involving PI3K and MAPK.\textsuperscript{145} Sarcomas, like other proliferating malignancies, are also dependent on the formation of new blood vessels (angiogenesis) to support their proliferation, invasion and metastasis.\textsuperscript{146} VEGFR is considered to be one of the most important drivers of angiogenesis and are frequently upregulated in soft tissue sarcomas and are associated with high tumour grade.\textsuperscript{147,148} A study by Zhang and colleagues also showed that overexpression of VEGF-2 in soft tissue sarcoma cell lines resulted in increased tumour vasculature as well as pulmonary metastases in mice.\textsuperscript{149} This section will focus on inhibitors of c-KIT, PDGFR and VEGFR, namely imatinib, sunitinib and pazopanib, which have completed all phases of clinical trials and which have been approved as standard treatment for commercial use by the FDA. These drugs have been shown to inhibit tumour growth with improved response rates and reduced toxicity.\textsuperscript{150\textendash}153

Imatinib was originally synthesized to target the fusion-protein Bcr-Abl for treatment of myelogenous leukaemia, but was subsequently found to also inhibit c-KIT and PDGFR. A study in the United States has demonstrated objective response rates in the range of 50\textendash}70\% for the
treatment of GISTs with imatinib. Imatinib functions by binding in the cleft between the N- and C-terminal domains of c-KIT (Figure 10) which inhibits its association with downstream cell signalling substrates resulting in cell growth arrest and cell death by apoptosis. While most mutations that result in ligand-independent constitutive activation of c-KIT occur in exon 11, there are some less frequent mutations present in exon 9, 13 or 17. These more rare mutations appear to have a different underlying mechanism that results in uncontrolled c-KIT signalling and are less responsive to imatinib treatment. Hirota and colleagues also identified mutations causing constitutively active PDGFRs in a minority of GIST cases which induce cytogenetic changes associated with tumour progression. Similar to the rare c-KIT mutations, these mutations are characterized by insensitivity to imatinib but they are more sensitive to sunitinib, which inhibits multiple receptor tyrosine kinases including c-KIT, PDGFR, VEGFR, RET (rearranged during transfection) and FLT3 (fms-related tyrosine kinase-3). The simultaneous inhibition of these targets results in reduced tumour vascularization and cancer cell death. The ability of sunitinib to target multiple receptors renders it a successful second-line treatment for GIST patients resistant to imatinib. However, this is associated with adverse effects including nausea, diarrhoea, fatigue, hypertension, anorexia, stomatitis, a yellow skin discolouration and hand-foot skin reaction. Based on the success rate of imatinib in GISTS, its therapeutic applications have been extended to other sarcoma subtypes that also exhibit aberrant expression of PDGFR or c-KIT. However, the response rates of these cancers to imatinib have been mostly poor. A recent phase II clinical trial was conducted by the Children’s Oncology Group...
(COG) to test the efficacy of imatinib for the treatment of a variety of paediatric sarcomas with high c-KIT or PDGFR levels. Results showed that out of 48 patients comprising 24 Ewing’s sarcoma, 10 osteosarcoma, 10 desmoplastic small round cell sarcoma and 4 synovial sarcoma cases, only 1 patient showed a partial response to imatinib and the COG concluded that the tyrosine kinases targeted by imatinib are not the molecular drivers of these cancers. It is also possible that these sarcomas escape cell death induced by imatinib by activating alternative signalling pathways such as the PI3K/AKT pathway or through feedback loops. If this is the case then multi-targeted inhibitors may be required. Based on the above reports it is clear, with the exception of most GISTS, that no definitive correlations can be drawn based on the expression levels of PDGFR/KIT and the response to imatinib. Acquired resistance to imatinib has also been reported in some GIST patients during chronic therapy and alternative strategies are therefore required to treat or avert imatinib resistance in these cancers. IMC-3G3 (olaratumab), a humanized anti-PDGFRα monoclonal antibody, showed
promising preclinical efficacy in leiomyosarcoma and osteosarcoma cell lines. Results from a randomized phase Ib/II clinical trial\textsuperscript{164} led to the accelerated approval of olaratumab by the FDA for the treatment of patients with soft tissue sarcoma not amenable to curative treatment with RT or surgery and with a histologic subtype for which anthracycline-containing regime is appropriate.\textsuperscript{165}

Pazopanib is another multi-targeted tyrosine kinase inhibitor that targets several proteins, including VEGFR and PDGFR, which play important roles in angiogenesis, tumour growth and cell survival.\textsuperscript{166} It binds to the ATP-binding site which is located in the cleft between the N- and C-terminal lobes of these receptor tyrosine kinases, rendering them inactive (Figure 11).\textsuperscript{167} Pazopanib has shown promising anti-cancer activity in advanced soft tissue sarcomas with significant responses in leiomyosarcoma and synovial sarcoma.\textsuperscript{168} The outcome of a randomized, double-blind, placebo-controlled phase III trial led to FDA approval of pazopanib for the treatment of advanced soft tissue sarcomas in patients who had received prior chemotherapy.\textsuperscript{153} This, however, excluded patients who received treatment for adipocytic tumours or GISTS as these were unresponsive in the trial. The trial showed benefit across many treatment-resistant metastatic sarcoma subtypes with a significant 3-month median increase in progression-free survival in the treated group versus the placebo group. The most common adverse effects for pazopanib reported were fatigue, diarrhoea, nausea, weight loss and hypertension.\textsuperscript{153,169} The success and approval of this drug lends some evidence to the important role of the VEGFR and related pathways to the growth of diverse sarcoma subtypes.

\textbf{MET, Src, IGFR inhibitors.} The constitutive activation of the MET signalling pathway has been implicated in a wide range of human malignancies, including sarcomas where it promotes cell-matrix dissociation, protease production and consequently invasion and metastasis.\textsuperscript{170,171} MET overexpression correlates with metastasis in osteosarcoma, and the upregulation of MET in primary human bone-derived cells was sufficient to drive their transformation into osteosarcoma cells \textit{in vitro}.\textsuperscript{172,173} The MET inhibitor, crizotinib, inhibits proliferation, survival, invasion and clonogenicity of multiple sarcoma cell lines as well as \textit{in vivo} growth in osteosarcoma bearing mice.\textsuperscript{174–176} At present, a phase II clinical trial [ClinicalTrials.gov identifier: NCT01524926] testing crizotinib in patients \(\geq 15\) years with MET-driven sarcomas and lymphomas is underway.

Src has been identified as an oncoprotein in several human cancers and its role in promoting migration is well established in bone sarcomas.\textsuperscript{177} Indeed, three independent studies have shown that the inhibition of the Src signalling pathway inhibits metastasis of chondrosarcoma cells and the treatment of Ewing's sarcoma cells with the Src inhibitor dasatinib reduced their migratory and invasive ability.\textsuperscript{178–181} Clinically, dasatinib has been tested for the treatment of several solid tumours, including sarcomas, but it has been associated with acute side effects.\textsuperscript{182} Ongoing phase II clinical trials [ClinicalTrials.gov identifier: NCT00788125] are now attempting to further evaluate the use of dasatinib in combination with chemotherapies such as ifosfamide, carboplatin and etoposide.

The IGF signalling pathway promotes cell survival and proliferation by activating the PI3K/AKT/mTOR and Ras/Raf/MAPK pathways.\textsuperscript{183,184} Elevated levels of IGF Receptor-1 (IGFR-1) and its ligands have been observed in Ewing's sarcoma, synovial sarcoma, osteosarcoma and chondrosarcoma, as well as some soft tissue sarcoma subtypes such as leiomyosarcoma and rhabdomyosarcoma, where it correlates with tumour aggression and poor prognosis.\textsuperscript{184–186} IGFR-1 has an established oncogenic role in Ewing’s sarcoma, where it is a direct target gene of the EWS–FLI1 fusion oncoprotein, and it is required for Ewing’s sarcomagenesis.\textsuperscript{187,188} Preclinical data suggest that targeting the IGF
pathway could be a promising molecular therapy for sarcomas. Indeed, humanized monoclonal antibodies targeting IGFR-1 showed promise in phase I and II clinical trials for the treatment of paediatric sarcomas, including osteosarcoma, Ewing’s sarcoma and rhabdomyosarcoma, with a modest clinical benefit in liposarcoma.\textsuperscript{189}–\textsuperscript{191} Unfortunately, most patients who initially respond to IGFR-1 inhibitors develop resistance to the therapy and suffer from relapse or recurrence within several months. Investigations of additional combination/additive treatments are clearly warranted and the factors/pathways responsible for resistance to IGFR-1 inhibitors remain subjects of investigation.\textsuperscript{192}

\textbf{mTOR inhibitors}

Alterations in the mammalian target of rapamycin (mTOR) pathway are commonly associated with sarcoma formation. Therapies inhibiting this pathway are at preclinical and clinical trial phases. mTOR is a protein kinase and downstream effector of the PI3K/AKT pathway in proliferation, cell survival and migration.\textsuperscript{193} Several mTOR inhibitors have been evaluated in single-agent clinical trials, with ridaforolimus being the most extensively studied.\textsuperscript{19} However, while ridaforolimus demonstrated efficacy for the treatment of patients with advanced metastatic sarcoma in phase I and II clinical trials, it did not receive FDA approval because of the results of a larger phase III clinical trial involving 711 patients with advanced sarcoma.\textsuperscript{194}–\textsuperscript{196} In the latter trial, compared to the placebo, tumour progression was only marginally delayed in patients on ridaforolimus and the median benefit of progression-free survival was low (17.7 weeks for ridaforolimus \textit{versus} 14.6 weeks for placebo).\textsuperscript{197} In addition, the mTOR inhibitors, everolimus, sirolimus and temsirolimus have been evaluated in single-agent clinical trials with most of them yielding disappointing results and interpatient variability.\textsuperscript{19} Due to the possibility of compensatory activation of the PI3K/AKT pathway, combination therapies targeting multiple components of the PI3K/AKT/mTOR pathway have been considered, with one possible drawback being increased cytotoxicity to patients.\textsuperscript{193}

\textbf{Inhibitors of the cell cycle}

The cell cycle is regulated at the most basic level by the ordered expression and activation of the family of Ser/Thr cyclin-dependent kinases (CDKs).\textsuperscript{198} As their name implies, the activity of CDKs is dependent on their association with cyclins; when activated, cyclin–CDK complexes drive the cell cycle.\textsuperscript{199} CDK inhibitors (CDKIs) trigger checkpoints which halt the cell cycle and therapeutic agents that inhibit aberrant cell cycle activation are being tested in sarcomas.\textsuperscript{200} Flavopiridol, a non-selective inhibitor of CDK1, 2, 4, 6 and 7 was tested in a phase II clinical trial for the treatment of advanced, metastatic soft tissue sarcomas including fibrosarcoma, liposarcoma, leiomyosarcoma and synovial sarcoma, but no significant responses were observed.\textsuperscript{201} Encouraging results have, however, been observed for palbociclib, a selective CDK4/6 inhibitor, in liposarcoma patients with amplified CDK4. In a recent phase II clinical trial, palbociclib was associated with favourable progression-free survival in patients with well-differentiated or dedifferentiated liposarcoma.\textsuperscript{202} Histone deacetylase (HDAC) inhibitors can induce transcription of key cell cycle regulators including the CDKI p21 which leads to growth arrest and apoptosis in sarcoma cell lines.\textsuperscript{203,204} For example, treatment of chondrosarcoma cell lines with HDAC inhibitors was shown to result in S-phase arrest and transcriptional activation of p21.\textsuperscript{205,206} Based on these and other promising results, HDAC inhibitors are also being investigated in early-phase clinical trials in patients with advanced soft tissue sarcomas.\textsuperscript{207} One emerging phase II study evaluated a single-agent HDAC inhibitor, panabinostat, in patients with translocation-related (myxoid liposarcoma, Ewing’s sarcoma, alveolar soft part sarcoma and synovial sarcoma) and translocation-unrelated (leiomyosarcoma and pleomorphic liposarcoma) soft tissue sarcomas. While this study did not reach its primary endpoint, a subset analysis revealed that six patients with liposarcoma, leiomyosarcoma or Ewing’s sarcoma had prolonged stable disease.\textsuperscript{208}

Mouse double minute 2 homolog (MDM2) is a ubiquitin E3 ligase that mediates the degradation of p53 by the proteasome 26S, and it is frequently amplified and activated in sarcomas.\textsuperscript{209} The inhibition of MDM2 results in increased levels of p53 and consequently the transcriptional activation of, among other p53 targets, CDKIs, leading to cell cycle arrests and/or senescence and cell death by apoptosis.\textsuperscript{210} Numerous therapeutic strategies targeting MDM2 have been developed, including nutlin-3 and RG7112. Nutlin-3 activates the p53 signalling pathway and was shown to lead to major tumour regression in
osteoarcoma xenografts through activation of apoptosis. In a phase I study, sarcoma patients with MDM2-amplified liposarcoma treated with RG7112 showed a significant reduction in tumour growth. 

Aurora kinases (AURKs) constitute a family of serine/threonine kinases that are required for progression through mitosis and cell division. Indeed, they are important in centrosome duplication, spindle formation, alignment of chromosomes on the mitotic spindle, as well as transition through the mitotic checkpoints and cytokinesis. Aberrant expression of AURKA has been implicated in many cancers and contributes to chromosome instability and phosphorylation-mediated ubiquitylation and degradation of the tumour suppressor p53. 

In vitro, inhibition of AURKA by shRNA or chemical inhibitors reduces cell proliferation in multiple sarcoma cell lines; results from a recent phase II clinical trial revealed that alisertib, a small-molecule inhibitor of AURKA, significantly improved prolonged stable disease in angiosarcoma and chondrosarcoma patients.

*Immunotherapy*

Immunotherapy is a treatment designed to harness the ability of the body’s immune system to combat infection or disease to either produce an immune response or to enhance immune resistance to disease. With an advanced understanding of the immune system and cancer immunology, immunotherapy has made a significant impact on the oncology world in the last few years, with promising treatments for diverse malignancies including melanoma, leukaemia, prostate cancer, lung cancer and renal cell carcinoma. Unfortunately, advancements in immunotherapy for the treatment of sarcomas have made limited progress, which can partly be attributed to the rarity and heterogeneity of this cancer type. Recently, however, sarcoma research has turned a corner with numerous immunotherapy research initiatives and multiple early-phase clinical trials underway which include cytokine therapies, adoptive cell therapy, therapeutic cancer vaccines and checkpoint inhibitors/immune modulators. The efficacy of an immune checkpoint inhibitor in sarcomas has only been evaluated by a phase II study that administered ipilimumab, a CTLA-4 inhibitor, to synovial sarcoma patients. However, the study was closed when none of the patients had an objective tumour response. While the checkpoint inhibitor data have been disappointing, it is anticipated that immunotherapies will improve the prognosis of sarcoma patients.

*Mifamurtide*, a novel immunomodulator for the treatment of osteosarcoma. Mifamurtide (muramyl tripeptide phosphatidylethanolamine or MTP-PE) (Figure 12) is an immunomodulator that exhibits anti-cancer activity through activation of monocytes and macrophages. It is a synthetic analogue of the immune stimulatory peptidoglycan motif known as muramyl dipeptide.

![Figure 12. Mifamurtide.](image-url)
(MDP) found in the cell wall of Gram-positive and Gram-negative bacteria. Mifamurtide is encapsulated in liposomes, which favours targeted delivery and enhances the compound’s ability to activate macrophages and monocytes and also reduces the compound’s toxicity. Intra-cellularly, MTP-PE binds to the nucleotide-binding oligomerization domain (Nod) 2 receptor, which is highly expressed in antigen presenting cells. This binding stimulates the production of pro-inflammatory molecules including interleukin (IL)-1, IL-6, IL-8, tumour necrosis factor alpha, nitric oxide and prostaglandins D₂ and E₂. Upregulation of these molecules leads to activation of contact-mediated tumouricidal activity of macrophages and monocytes.

However, there is limited understanding of the cross-talk between osteosarcoma cells, osteoclasts and cells of the immune system and how they may promote tumourigenesis.

In 2009 mifamurtide was approved by the European Medical Agency for the treatment of high-grade non-metastatic resectable osteosarcoma following surgical removal in children, adolescents and young adults. This approval was prompted by the promising data generated from a large phase III randomized prospective intergroup trial. Results showed that intravenous treatment with mifamurtide after complete surgical resection and postoperative multi-agent chemotherapy significantly improved the six-year overall survival from 70% to 78% in patients with newly diagnosed osteosarcoma; patients with metastatic disease showed improvement in five-year overall survival from 40% to 53%. Mifamurtide is generally well tolerated, with reported adverse effects including fever, chills, nausea, headache, fatigue and myalgias. Although mifamurtide is not yet approved in the US, several trials are currently underway to further investigate its efficacy in osteosarcoma.

### Conclusion

Sarcomas continue to present a serious therapeutic challenge, mostly due to the large number of sarcoma subtypes, the heterogeneity within them and their different responses to current treatments. It is anticipated that the identification of the key molecular drivers underlying the various sarcoma subtypes will reveal biomarkers for more reliable diagnosis of sarcomas and lead to the development of more effective targeted therapies.

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### Conflict of interest statement

The authors declare that there is no conflict of interest.

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