

Novel Therapeutic Targets in Soft Tissue Sarcomas

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1. Introduction

Approximately 1% of all malignancies or 10,000 cases diagnosed in North American are sarcomas. Amongst which, soft tissue sarcoma comprises of the majority. Local disease is managed with the use of pre-operative or post-operative radiation and surgery, yielding over 90% local control rate.¹ In the meta-analysis reported by Figueredo et al.,² adjuvant chemotherapy yielded an absolute overall survival benefit of 4% and the benefit was mainly observed in tumours in the extremity, at a deep location, larger than 5 cm or high histological grade. Unfortunately, a recently reported adjuvant trial comparing doxorubicin/ifosfamide combination with observation enrolling patients with such features failed to demonstrate any overall survival benefit.³

Upon follow-up, 50% of localized soft tissue sarcomas will recur either locally, or systemically or both. The benefit of systemic therapy in the recurrent and/or metastatic setting, including doxorubicin, ifsofamide and DTIC, has been shown to be modest with an overall response rate of 10-15%, as single agent, and of 30% in combination at the expense of toxicity without any improvement to the median overall survival rate of 12 months.⁴

In the past 10 years, the most significant advancement has been observed in gastrointestinal stromal sarcoma (GIST), a rare form of gastrointestinal (GI) tract sarcoma. GIST originates from the interstitial cell of Cajal, a pacemaker cell in the muscularis mucosa of the GI tract.^{5,6} It is characterized by the expression of CD117 or c-KIT. Eighty-five to 90% of GIST harbour activating mutation in c-KIT, predominantly at exon 11 (70-75%), followed by exon 9 (10%) and rarely exons 13 and 17 (1-2%). About 5-10% of GISTs have platelet derived growth factor receptor- α mutation, predominantly in exons 12 and 18.⁷⁻¹¹ The rest harbour mutation in neither tyrosine kinase receptors are considered as wild type.^{7,11,12} Imatinib, a tyrosine kinase inhibitor targeting both wild type and mutated c-KIT and PDGFR, as well as ABL kinase and BCR-ABL, was the first therapy licensed in metastatic GIST with or without mutations in C-KIT and PDGFR with an impressive improvement in response (80% response rate as compared to no response for conventional chemotherapy) and a median progression-free survival (PFS) of 2-2.5 years and overall survival (OS) of almost 5 years.¹³⁻¹⁶ More recently, imatinib has been licensed as adjuvant therapy for resected GIST more than 3 cm for 1 year with an improvement in progression-free survival.¹⁷ In the annual meeting of the American Society of Clinical Oncology Meeting in 2011, GISTs with c-KIT expression

and either over 10 cm in diameter or >10 mitotic figures per 50 high power fields were randomized to 1 versus 3 years of adjuvant imatinib treatment. There was not only an improvement in 5-year recurrence-free survival (47.9% versus 65.6%, HR=0.46 and $p<0.001$) but OS (81.7% versus 92%, HR=0.45, $p=0.019$).¹⁸ Upon failure of imatinib, sunitinib has been shown in a randomized controlled trial to improve the median PFS as compared to placebo (24.1 weeks versus 6 weeks, HR=0.33).¹⁹ Unfortunately, recent trials with a more potent c-KIT and PDGFR- α inhibitor, nilotinib, as compared to imatinib in the first-line and as compared to best supportive care in the previously pretreated population failed to provide any PFS or OS benefit.

One of the most important barriers for the management of soft tissue sarcomas is that there are more than 50 histological subtypes with diverse clinical, biological and molecular characteristics. At the molecular level, soft tissue sarcomas can be divided into 2 categories: those with a characteristic chromosomal translocation as in Table 1²⁰⁻²² and those with complex karyotypes, including liposarcomas, except for myxoid/round cell liposarcoma, leiomyosarcoma, malignant nerve sheath tumours, etc. Over the years, significant gain in the knowledge through translational research in soft tissues sarcoma help identifying novel targets for all subtypes or specific subtypes, which has been reflected in design of recent trials in this population.²³ One would hope therapeutic strategies based on molecular or biological characteristic will yield an improvement in the overall survival of patients with metastatic soft tissue sarcomas and possibly in the curative setting.

Despite the impressive improvement in the management and outcome of GIST, resistance to imatinib and sunitinib occurs. Through ongoing translational research, secondary resistance mechanisms, including secondary mutations in c-KIT or PDGFR- α or activation of downstream pathways, have been partially elucidated, which will help developing novel agents alone or in combination with imatinib and/or sunitinib.²⁴

In this chapter, we will focus on the biology of selected targets in soft tissue sarcomas, and their current development in all or certain subtypes of soft tissue sarcomas.

2. Growth signalling pathway

Insulin Growth Factor Receptor (IGF-R)/PI3 Kinase (PI3K)/mTOR pathway

Insulin Growth Factor Receptor and Insulin Receptor

The insulin growth factor pathway consists of 3 ligands, IGF-1, IGF-2 and insulin, IGF binding proteins, and 4 receptors, IGF-1R, IGF-2R, insulin receptor (IR) and hybrid IGF-1R/IR.²⁵⁻²⁷

IGF-1R is a transmembrane receptor tyrosine kinase that comprised of 2 α and 2 β subunits. Upon binding of ligands, with the highest affinity for IGF-1, followed by IGF-2 and the least for insulin, the tyrosine residues in the intracellular domain will be autophosphorylated leading to activation of downstream signalling Raf/MAPK/ERK and c-Raf proliferation pathways and the PI3K/Akt/mTOR survival pathway.²⁶⁻²⁸ Structurally, IGF-1R and IR share 84% homology in the intracellular domain and 100% in the ATP-binding domain, which permits heterodimerization of IGF-1R and IR.²⁶⁻²⁸ Complicating by the fact that IR exists in 2 isoforms, IR-A or fetal IR which is overexpressed in cancer, and IR-B which is present in normal tissues. The 2 isoforms differ by 12 amino acids in exon 11.²⁹ The IR-A isoform has high affinity for IGF-2 and insulin but less so for IGF-1.³⁰⁻³² In the contrary, IGF-2R is a mannose-6-phosphate receptor devoid of any ATP-binding domain, making it a dead

receptor which can modulate the growth stimulation activity of IGF-1R and IR through binding and internalization of IGF-2.³²

At least six different IGFBPs have been identified, which modulate the plasma concentrations of IGF-1 and IGF-2, thus the activation of the receptors in this pathway.²⁵⁻²⁷

Various mechanisms of aberrancy of this pathway have been identified and summarized in Table 2. In short, the fusion protein from the translocation-associated sarcomas binds to either the IGF-2³³⁻³⁶ or IGF-1R promoter,³⁷⁻⁴⁰ leading to pathway activation. Whereas, for those soft tissue sarcomas that harbour complex karyotypes, the pathway is activated through overexpression of ligands, IGF-2,^{36,41,42} IGF-1,⁴² overexpression of IGF-1R and IR,³⁶ activation of downstream PI3K/AKT/mTOR pathway.^{43,44}

Pediatric GISTs are less common to have mutation in c-KIT and PDGFR- α . Agaram et al.⁴⁵ and others reported that as compared to wild-type (WT) GISTs in adult, the transcriptome of WT pediatric GISTs have an increased expression in IGF-1R without amplification.^{46,47} However Tarn et al. reported either overexpression or amplification of IGF-1R in adult WT GISTs.⁴⁸ IGF1-R expression was found to be increased in all 188 patient derived samples with at least moderate expression of IGF1/2 in 115 samples. Elevated IGF-1 or IGF-2 expression is associated with increased mitotic rate and high risk for relapsed in resected samples and elevated IGF-1 level is also found to be related to increased risk for metastatic disease and relapsed disease in the metastatic setting.⁴⁹ Inhibition of IGF1-R by a tyrosine kinase inhibitor leads to apoptosis via the inhibition of the AKT and MAPK.⁴⁸ Synergistic anti-tumor activity is observed when an IGF-1R tyrosine kinase inhibitor is combined with imatinib. Therefore, targeting IGF-1R with or without imatinib is of interest in metastatic WT GISTs.

Currently, there are three different strategies targeting this pathway.

1. Antibody to IGF-1R was the first therapeutic strategy that entered the clinical and current clinical development in sarcoma was summarized in Table 3. These antibodies block the binding of IGF-1 and possibly IGF-2 to IGF-1R. The antibody/IGF-1R complexes will be internalized and then degraded by the proteosome pathway, leading to downregulation of IGF-1R. It has been reported that the activity of these antibody depends on the level of membrane IGF-1R expression.⁵⁰ A number of phase 2 studies in soft tissue sarcomas have been reported and stable disease as best response was the most common.^{51,52} Toxicities include hyperglycemia, headache, skin rash and transient AST/ALT elevation.
2. ATP-mimetic, competitive tyrosine kinase inhibitor which inhibits the activation of IGF-1R homodimer, IR homodimer and IGF-1R/IR heterodimers through IGF-1, IGF-2 and insulin. OSI-906, BMS-754807 and XL-288 are currently in phase I clinical development. To date, no clinical studies in soft tissue sarcomas have been reported.
3. Antibody binds to IGF-1 and IGF-2 leading to inactivation of IGF-1R and hybrid IR/IGF-1R, while sparing the binding of insulin to IR-B, resulting in the absence of hyperglycemia. Recently, a phase I study of MEDI-573 was reported and no hyperglycemia was observed and no response was observed.⁵³

Preclinical studies suggested the level of IGF-1R expression may be a biomarker for clinically efficacy for these antibodies, clinical confirmation remains to be reported. Thus far, there has been no biomarker for response to small molecule reported. Anti-tumour activity in soft tissue sarcoma by targeting the IGF-1R/IR pathway through IGF-1R antibody has been modest. This is possibly related to the fact that most soft tissue sarcoma subtypes have upregulation of IGF-2, which signals through the IGF-1R/IR-A hybrid receptor and is not

blocked by IGF-1R antibody.^{33,34,36,41} Furthermore, some of the soft tissue sarcoma subtypes have pTEN loss, increase in PI3K/Akt mRNA level or activation of IRS-2, circumventing the blockade by the antibody upstream.^{36,41-44} Last but not least, there has been no direct comparison in clinical efficacy amongst these three therapeutic approaches.

PI3K/Akt/mTOR pathway

Growth factor receptor tyrosine kinases activation will lead to recruitment and activation of PI3K to the cytoplasmic side of the cell membrane, which in turn phosphorylates phosphatidylinositol-4,5-phosphate to phosphatidylinositol-3,4,5-phosphate (PIP3). PIP3 is dephosphorylated and inactivated by pTEN, and activates Akt through recruitment to the cell membrane, which in turn activates mTOR-1 directly and indirectly through inhibition of negative regulator of mTOR-1, tuberous sclerosis complex 2. In mammalian cells, there are 2 mTOR complexes, mTOR-1, which is rapalogue sensitive, and mTOR-2, which is rapalogue insensitive. Recently, mTOR-2 is found to activate Akt, leading to mTOR-1 activation.^{54,55}

The PI3K/Akt/mTOR pathway is an ideal anti-cancer target, including soft tissue sarcomas, as it is activated by all the growth factor receptor tyrosine kinases, such as IGF-1R, VEGFR, PDGFR, or by the loss of pTEN or upregulation of PI3K/Akt.^{41,43,44} In turn, the pathway will lead to cell survival and anti-apoptosis through Akt and to cell growth, proliferation, metastasis and angiogenesis through activation of S6-kinase and p70. Preclinical study by Friedrichs et al. reported activation of PI3K/Akt/mTOR in synovial sarcoma cell lines and inhibition of mTOR or PI3K led to tumour regression.⁵⁶ Similarly, inhibition of mTOR by rapamycin led to p53-dependent apoptosis of rhabdomyosarcoma cell lines.⁵⁷ Wan et al. found that this is due to inhibition of the mTOR/HIF-1 α /VEGF pathway via S6 kinase.⁵⁸

Sapi et al. reported a differential activation of mTOR-1 among 108 cases with GIST with c-KIT, PDGFR- α and WT mutations (38.4% versus 83.3% versus 73.9%, respectively).⁵⁹ In imatinib-resistant GIST cell lines, despite treatment with imatinib, there is continual hyperactivation of c-KIT with no increase in total c-KIT expression. Either PI3 kinase, MEK or mTOR inhibition leads to decrease in growth, but only PI3 kinase inhibition led to apoptosis.⁶⁰ Similarly, prolonged treatment with sunitinib leads to silencing of pTEN expression through methylation, which in turn leads to activation of the PI3K/AKT pathway.⁶¹ Ikezoe et al. demonstrated synergism of sunitinib with PI3K or mTOR in imatinib-resistant GIST.⁶²

There are currently 3 rapamycin-analogues targeting mTOR-1, temsirolimus, everolimus and ridaforolimus, being developed in the clinics. Only temsirolimus (CCI-779) and ridaforolimus (AP 23573) have been tested in soft tissue sarcomas. Okuno et al.⁶³ reported a phase II study of temsirolimus in metastatic soft tissue sarcoma patients and 22% had prior chemotherapy. Two out of 40 evaluable patients attained a partial response for 3 and 17 months, respectively. But disappointingly, the median time-to-progression (TTP) was only 2 months or 6-month progression-free rate (PFR) was only 14%, which is deemed to be inactive according to the EORTC criteria.⁶⁴ The median survival was 7.6 months.

A phase II study of ridaforolimus in osteosarcoma, leiomyosarcoma, liposarcoma and other soft tissue sarcomas was reported in abstract form. 93% of patients had prior chemotherapy. The primary endpoint was clinical benefit rate at 16 weeks, which were 30% (4 partial response), 33%, 30% and 23%, respectively and were deemed active by the authors. The corresponding 6-month PFR were 23%, 22%, 25% and 23%. The median overall survival of the entire population was 40.1 weeks (range 37.9-44.1 weeks).⁶⁵

In 2011, the phase III study of ridaforolimus versus placebo as a maintenance therapy after first to third-line therapy in the metastatic settings for soft tissue and bone sarcomas was reported with progression free survival (PFS) as the primary endpoint and overall survival (OS) as the main secondary endpoint. In the overall population, the PFS and OS was improved from 14.6 weeks to 17.7 weeks in the ridaforolimus treated patients (HR=0.72, $p<0.0001$) and from 19.2 months to 21.4 months (HR=0.88, $p=0.22$) after first analysis. Subgroup analysis including histology, number of prior lines of therapy, etc is pending.⁶⁶

A combined phase I/II study combining everolimus and imatinib in pretreated metastatic GIST patients was reported. The recommended phase II dose of the combination was 2.5 mg daily of everolimus in combination with 600 mg daily of imatinib, denoting pharmacokinetic interaction. Two strata were enrolled: stratum 1 failed imatinib alone and stratum 2 failed imatinib and other therapies. A 37% progression free rate was observed in stratum 2 with a corresponding PFS and OS at 3.5 and 10.7 months, respectively.⁶⁷ This combination was suggested by the authors to take forward for further development. The biological activity seen may be related to the silencing of pTEN as reported by Yang and colleagues after treatment with sunitinib.⁶¹ Unfortunately, no biopsy samples were available in this trial to further elucidate the biology underlying this combination in this heavily-pretreated GIST population.

Toxicities of rapalogues are considered as tolerable with <10% patients experienced grade 3 or 4 toxicities. Common toxicities include stomatitis, hyperglycemia, hyperlipidemia, fatigue and thrombocytopenia.

In summary, the clinical efficacy of rapalogues in sarcomas has been modest, which may be explained by the following resistance mechanisms. Wan et al. reported inhibition of mTOR by rapalogues led to the loss of the negative feedback of S6 kinase on IRS-1, leading to activation of pAKT.⁶⁸ Cao et al.⁶⁹ found that after initial inhibition of IGF-1R and Akt phosphorylation by IGF-1R antibody, at 72 hours such inhibition was lost through mechanisms independent to pTEN loss or activation of epidermal growth factor. The activation of the Ras/Raf/MEK/Erk pathway led to inhibition of mTOR leading to loss of addiction to the IGF pathway.^{70,71}

To circumvent these resistance mechanisms, phase I/II studies of combined PI3K/mTOR inhibitors such as BEZ235, IGF-1R-mTOR and Akt-MEK (MK-2206 and AZD 6244) combinations in solid tumours are currently being performed. The improvements in clinical efficacy in soft tissue sarcomas remain to be reported.

Complicating the development of rapalogues is the lack of biomarkers. Despite pTEN loss leads to constitutional activation of mTOR, pTEN status did not predict clinical benefit.⁷² Similarly, inhibition of S6 kinase and p70 activation did not predict clinical benefits.

Targeting Akt or PI3K upstream from mTOR may offer an alternative strategy in the treatment of soft tissue sarcomas. Barrentina et al.⁷³ reported 18% of myxoid/round cell liposarcoma had PIK3CA mutation leading to Akt activation, which is associated with poor survival. Zhu et al.⁷⁴ found activation of PI3K and Akt in all the soft tissue sarcoma cell lines. Inhibition of PI3K or Akt led to inhibition of downstream pathway, leading to cycle cell arrest at G2 phase through upregulation of GADD45 α . Activation of Akt and mTOR was observed in 61% and 66% of the 140 sarcoma samples, particularly in malignant nerve sheath tumours, rhabdomyosarcoma and synovial sarcoma, independent from EGFR activation. Phosphorylation of Akt was associated with metastatic disease.⁷⁵

Inhibition of both PI3K and mTOR by BEZ235 leads to G1 phase cell cycle arrest without apoptosis in both *in vitro* and *in vivo* sarcoma models including Ewing's and rhabdomyosarcoma. As a single agent, no apoptosis was observed. But synergistic anti-tumour effect was observed when BEZ235 is combined with classical active chemotherapeutic agents in sarcoma, including doxorubicin, when chemotherapy is given first. Simultaneous targeting with IGF-1R showed synergistic activity probably due to decrease in IC50 for PI3K inhibition by BEZ235.⁷⁶ Therefore, clinical studies of PI3K inhibitor in myxoid/round cell liposarcoma and of PI3K or Akt inhibitors in malignant nerve sheath tumour, rhabdomyosarcoma and synovial sarcoma may be of value.

As mentioned previously, PI3K inhibition with concurrent imatinib not only leads to growth inhibition but also apoptosis in imatinib-resistant GIST cell lines as compared to mTOR inhibition. Therefore, clinical investigation of PI3K inhibitor in combination with imatinib in imatinib failure GIST patients may be of value. Similarly, the activation of PI3K/AKT/mTOR due to methylation of pTEN from prolonged sunitinib treatment can be exploited as a novel therapeutic option for sunitinib failure.

3. Vascular endothelial growth factors, fibroblast growth factors and receptors

Angiogenesis is a complex biological process involved in the formation of new blood vessels in normal and pathological tissues, including cancer.^{77,78} A battery of proangiogenic and antiangiogenic factors is involved in angiogenesis, which is tightly controlled in normal tissues, but not in pathological processes including cancer.^{77,78} In cancer, angiogenesis is a result from the interaction of various proangiogenic and antiangiogenic factors and their receptor produced or present in tumour cells, endothelial cells, extracellular matrix and inflammatory cells.⁷⁹

A number of proangiogenic and antiangiogenic factors have been identified (Table 3). The most important proangiogenic factor is vascular endothelial factor (VEGF) which is present in six isoforms, VEGF A to E and placental growth factor (PlGF).⁸⁰ Furthermore, VEGFA exists as 6 proangiogenic isoforms (VEGF 121, 145, 148, 165, 183, 189 and 206) and 6 corresponding antiangiogenic isoforms, generated through alternative splicing of exons 6, 7 and 9 of VEGFA gene.^{79,81}

There are a total of 4 VEGFR, namely VEGFR-1, VEGFR-2, VEGFR-3 and VEGFR-4. Interaction of VEGFR-2 and its ligands, VEGFA and PlGF, represents the most important angiogenic pathway. Whereas, VEGFA, VEGF-B and PlGF bind to VEGFR-1 for angiogenesis and VEGF-C and D bind to VEGFR-3 for lymphogenesis.⁸⁰

Increasing level of angiogenesis or elevation of VEGF expression in soft tissue sarcoma samples has been reported to be associated with high grade, increase risk of metastases and poor prognosis.⁸²⁻⁸⁴ Serum VEGF expression is elevated in all soft tissue sarcomas⁸⁵⁻⁸⁷ especially epithelial subtypes including epithelioid sarcoma and alveolar soft part sarcoma (ASPS),⁸⁸ and high grade fibrosarcoma of no specific subtype (previously known as malignant fibrous histiocytoma) and leiomyosarcoma.⁸⁹ In addition, VEGF A, B and C and their corresponding VEGFR-1, -2 and -3 are overexpressed in angiosarcoma, demonstrating an autocrine or paracrine growth pathway, with corresponding activation of the PI3K/Akt/mTOR pathway.⁹⁰⁻⁹² Antonescu et al. also demonstrated overexpression of TIE-2, VEGFR-1 and -2 in angiosarcoma, and 10% of the samples demonstrated mutations in either exon 15 in the extracellular domain and exon 16 in the transmembrane domain.⁹³

Thus, targeting VEGFs and their receptors is an attractive strategy for the treatment of soft tissue sarcomas, especially angiosarcoma, and ASPS.

Targeting VEGF can be achieved through

1. Monoclonal antibody to VEGF-A by Bevacizumab: A phase II study combining 7.5 mg/kg of bevacizumab with standard dose doxorubicin at 75 mg/m² every 3 weeks in patients with metastatic soft tissue sarcomas was reported D'Adamo et al. 17 patients were treated and only 2 PRs were observed with a median time-to-progression and median overall survival of 8 and 16 months, respectively. Despite dexrazoxane, 6 patients experienced at least grade 2 congestive heart failure, making it too toxic for further development of the combination.⁹⁴ Bevacizumab at 15 mg/kg every 3 weeks was tested in a phase II study of patients with metastatic angiosarcoma and epithelioid hemangioendothelioma. 29 patients were enrolled and out of 26 evaluable patients, 3 PRs were observed. Currently, the development of bevacizumab is unclear despite this level of activity in angiosarcoma.⁹⁵ Common toxicities include hypertension, macular or papular erythematous rash, diarrhea, mucosal bleeding (most common as epistaxis) and proteinuria. Other rare toxicities are hemorrhage, hypertensive emergency and reversible posterior leukoencephalopathy syndrome, arterial thromboembolism (especially in patients over 65 years), bowel perforation and fistulae.
2. Aflibercept is a decoy recombinant protein of the second Ig domain of VEGFR-1 and third Ig domain of VEGFR-2 fused to the Tc domain of human IgG1, with pM affinity to VEGF-A, -B and PlGF. In vivo rhabdomyosarcoma model treated with aflibercept at 2.5 mg/kg twice weekly demonstrated significant anti-tumour activity.^{96,97} Late stage clinical development in metastatic non-small cell lung cancer, colorectal cancer and other solid tumours are either ongoing or reported. But no development plan in soft tissue sarcoma has been made. Toxicities are consistent with other anti-VEGF/VEGFR agents, such as hypertension, headache, proteinuria, fatigue, dysphonia, bleeding (epistaxis and hemoptysis), anorexia, abdominal pain, nausea, diarrhea or constipation, and arthralgia.
3. Small molecules ATP-mimetic receptor tyrosine kinase inhibitors: VEGFR is a member of the split kinase class III receptor which shares structural homology in the ATP binding domain with platelet derived growth factor (PDGFR), FLT-3, c-kit, and Tie-2. Although in preclinical models, co-inhibition of PDGFR and VEGFR may yield synergistic anti-tumour activity through targeting of the endothelial cells and pericytes in the tumour vasculature, respectively and delay in tumour resistance,⁹⁸⁻¹⁰² additional toxicities are expected. Additional receptor tyrosine kinases are inhibited by some of these small molecules at clinically relevant plasma concentration, such as RET in motesanib, vandatinib, FGFR-1, 2 or 3 in BIBF 1120 and brivanib^{103,104} and c-met in foretinib, cabozantinib and MGCD265,¹⁰⁵ leading to possible synergistic anti-tumour activity.

The early phase development of sunitinib,^{106,107} sorafenib¹⁰⁸⁻¹¹¹ and pazopanib¹¹² in various types of soft tissue sarcomas is summarized in Table 4. With the observed progression-free survival benefit in the phase II trial of pazopanib across all soft tissue sarcoma subtypes, a phase III trial comparing pazopanib and placebo in metastatic soft tissue sarcoma was reported in the 2011 annual meeting of the American Society of Clinical Oncology with progression-free survival as the primary endpoint. Patients had prior anthracyclin but had no more than 4 prior lines of therapy, and performance status of 0-1. With a median follow-up of 15 months, and 19% of patients still on study, the median progression-free survival in

the pazopanib treated patients was improved from 1.5 to 4.6 months ($p < 0.0001$). The interim median overall survival was 11.9 versus 10.4 months ($p > 0.05$), favouring the pazopanib arm.¹¹³

Based on the success of sunitinib as second-line therapy for metastatic GIST, other antiangiogenic compounds have been in clinical development in pretreated GIST. Campbell et al. reported a phase II study of sorafenib in imatinib- and sunitinib-pretreated metastatic GIST patients. An impressive 13% response rate was observed with median PFS at 5.2 months and OS at 11.6 months which were compared to that of sunitinib.¹¹⁴ In the annual meeting of the American Society of Clinical Oncology Meeting in 2011, the final result of a phase II study of regorafenib in pretreated GIST was reported. Regorafenib is a multi-targeted tyrosine kinase inhibitor targeting VEGFR 1-3, TIE2, PDGFR- β , FGFR-1, as well as B-RAF, C-RAF and p38 MAPK at nanomolar concentration. Three PRs and a median PFS of 10 months were observed for the entire population. Due to the small patient numbers, median PFS were 10, 6 and 8 months for those harboured exon 11, 9 and WT c-KIT mutation respectively. Interesting, one patient was found to have B-RAF mutation in exon 15.¹¹⁵ Agaram et al. reported up to 7% of WT GIST had V600E mutation in B-RAF.¹¹⁶ In preclinical models, sorafenib has superior inhibitory activity in secondary mutations in the activation loop, such as D816H, D820A/G, V822K, and Y823D, of c-KIT as that of sunitinib, and similar activity towards ATP pocket secondary mutations, such as V654A and T670I, and exon 9 c-kit mutations.¹¹⁷ Sorafenib, most likely regorafenib, inhibits cell proliferation, induces apoptosis and decreases angiogenesis through the inhibition of Ras/Raf/MEK/ERK pathway as well as induction of p15 and p27 and decrease in p21, cyclin A and B1 as well as cdc-2.¹¹⁸ Based on these biological rationales, sorafenib and regorafenib will expect to have substantial clinical activity in GIST. A phase III study of regorafenib in the imatinib- and sunitinib-pretreated metastatic GIST patients has just finished enrollment.

The VEGF and FGF pathways are associated with growth and poor prognosis in soft tissue sarcomas.¹¹⁹ Upregulation of other proangiogenic pathways, such as the FGF pathway, has been conferred as resistance mechanism for anti-VEGF strategies.¹²⁰ Tumour growth suppression has been observed in preclinical soft tissue sarcoma xenografts by FGF inhibition.¹²¹ Therefore, combined FGFR and VEGFR tyrosine kinase inhibitors may have improved anti-tumour activity and duration of response in soft tissue sarcomas. In a retrospective analysis of 43 solid tumour samples through a phase I study of brivanib, expression of FGF-2 was associated with longer progression-free survival and higher disease control rate.¹²² Schwartz et al. reported the preliminary result of a randomized discontinuation study of brivanib in metastatic soft tissue sarcomas to detect an HR of 0.5 in progression free survival in the FGF-2 positive patients. 251 patients were enrolled with a median age of 54, and all patients were ECOG 0-1 and 80% had prior systemic therapy with a median of 2 regimens. During the first 12 weeks of open label brivanib, 130 patients had PD and 35 patients were taken off study due to reasons other than PD. Seven patients had a PR, and 3 were either angiosarcoma or hemangiopericytoma. Out of the 78 patients who had SD and underwent randomization to brivanib or placebo, 53 patients had FGF-2 positive tumours, and the median progression-free survival was 2.8 months in the brivanib arm as compared to 1.4 months in the placebo arm ($HR = 0.58$, $p = 0.08$). The progression-free rate at 12 weeks in the overall population was 31% and no difference was observed across all histological subtypes. In the FGF-2 negative tumours, the corresponding progression-free survival was 2.6 and 1.4 months ($HR = 0.8$). The median progression-free survival was 4.1

months in those in the placebo arm who crossed over to brivanib at progression. Brivanib was considered as well tolerated. All grade 3 or higher toxicities occurred in less than 5% except for fatigue (10%) and hypertension (15%).¹²³

Clinical benefit with tyrosine kinase inhibitors observed in angiosarcoma is particularly of interests as clinical response with classical chemotherapy, such as doxorubicin, liposomal doxorubicin and paclitaxel, is usually short-lived.¹⁰⁸⁻¹¹⁰ Various reports have demonstrated overexpression of VEGFR-2 and VEGFR-3 and their corresponding ligands, VEGF-A, VEGF-C, in pulmonary and cutaneous angiosarcomas, denoting the paracrine and/or autocrine role in their development and proliferation.^{90,124,125}

Alveolar soft part sarcoma (ASPS) is known to be chemotherapy resistant and therefore development of novel agents is essential. Stacchiotti et al.¹²⁶ reported a series of 10 patients. Five patients had prolonged PR and 3 other had prolonged stable disease, with a median duration of response of more than 9 months. Similar impressive clinical benefit of 4 out of 7 patients had a PR and 3 patients with SD by RECISTS were reported for cediranib.¹²⁷ The investigators in the National Cancer Institute reported the preliminary results of a phase II study of cediranib with 14 PRs in 33 untreated or second-line metastatic ASPS patients. Biopsies for gene expression profile alterations were performed before and after treatment and VEGFR-1, VEGFR-2 and angiopoietin-2 expression were downregulated whereas CXCR7, CCL-2 and transgelin were upregulated.¹²⁸ Currently, a cross-over phase II trials comparing cediranib and sunitinib is being planned. The transcription factor activity of the ASPACK1-TFE3 chromosomal translocation leads to overexpression of growth factor receptors, including VEGFR-2, PDGFR, c-Met, RET and EGFR, and transcription factor, HIF-1 α . The activation of these pathways leads to activation of both the PI3K/Akt/mTOR and ERK pathway.^{126,129-131}

4. Monoclonal antibody to the extracellular domain of VEGFRs or PDGFR: IMC1121B (ramucirumab) and IMC18F-1, targeting the VEGFR-2 and VEGFR-1 extracellular domain respectively, are currently under clinical development as a single agent or in combination with chemotherapy in other solid tumours. IMC-3G3, a monoclonal antibody against the extracellular domain of PDGFR, is currently in clinical development including in PDGFR mutated metastatic GIST.

The preliminary phase II and III trial result in antiangiogenic compounds look promising in all soft tissue sarcomas, especially for angiosarcoma, hemangiopericytoma, ASPS, which devoid of effective systemic therapy. Further development of this class of agent in these uncommon subtypes is definitely worthwhile. Given the activation of multiple growth factor pathways, like VEGFR, PDGFR, Tie-2, the use of tyrosine kinase inhibitor may be more likely to provide clinical benefit, unlike that in other solid tumours. With the activation of downstream pathways, including the PI3K/Akt/mTOR or MEK/ERK pathway, combination with antiangiogenic strategy, especially for tyrosine kinase inhibitors, may be at least additive. Furthermore, combinations with doxorubicin and other cytotoxic chemotherapy will be reported in the next couple of years, which have the potential of improving their anti-tumour activity. Like the identification of FGF-2 expression as a potential biomarker for efficacy for brivanib, further exploration for biomarker for antiangiogenic therapeutics is important which allows us to identify the corresponding patient subgroups and to improve the understand of the biology of angiogenesis and thus the mechanisms of resistance and corresponding therapeutic strategies.

4. Epigenetic modification and histone deacetylase inhibitors

Histones and histone deacetylase

There are 5 members of the histone family, H1, H2A, H2B, H3 and H4 in eukaryotic cells. Tetramer of H3 and H4 and dimmers of H2A and H2B make up nucleosomes, around which DNA winds. H1 makes this complex more compact. The amino terminal of histones undergoes post-translational modification by acetylation, phosphorylation and methylation, leading to change in the structure and function of the histones. Acetylation and deacetylation are the most studied processes. The lysine residues in histones, particularly H3 and H4, undergo acetylation by histone acetyltransferase (HAT) and deacetylation by histone deacetylase (HDAC) which determine the fate of gene expression.¹³² Acetylation leads to neutralization of the positive charges in the lysine residues of H3, decrease in the affinity to DNA, and thus generating an open or euchromatin structure which allows the binding of transcription factors. Conversely, deacetylation leads to tight interaction between the histones and DNA, generating a closed or heterochromatin structure which prevents gene transcription.^{132,133}

There are 4 classes of HDAC: Class I includes HDAC 1, 2, 3, and 8 and is ubiquitously present in the nucleus; Class II includes Class IIa (HDAC 4, 5, 6 and 7) and Class IIb (9 and 10), which shuttles between the cytoplasm and nucleus and is tissue specific, Class III includes SIRT 1-7 and is NAD⁺ dependent for its activity and Class IV includes HDAC 11 and properties of Classes I and II.¹³⁴⁻¹³⁶ Classes I, II and IV are homologous in their structure with a zinc-containing catalytic domain.

Recently, HDAC Class II is found to modulate acetylation status of proteins, such as heat shock protein 90 (Hsp 90). Acetylation of Hsp 90 leads to release of oncoproteins, such as Her-2, Akt, c-kit, bcr-Abl, and subsequently subject to proteasome-mediated degradation.¹³⁵ This process is believed to account for at least a good portion of the anti-tumour activity of HDAC inhibitors. Specifically, inhibition of HDAC 6 and 10 leads to downregulation of VEGFR1 and 2 expression through acetylation of Hsp90.¹³⁷ Other members of Class II HDAC are involved in either cell proliferation and survival or cell migration, which is crucial for angiogenesis.^{138,139}

Histone deacetylase and sarcoma

Overexpression of one or more members of Class I and II HDAC has been well reported in breast, prostate and colorectal cancer,¹³⁵ which has not been reported in soft tissue sarcoma.

Preclinical studies of HDAC inhibitors demonstrated not only tumour growth suppression but also tumour regression and differentiation in human synovial sarcoma xenografts.^{140,141} Synovial sarcoma is characterized by the expression of oncoprotein, SYT-SSX, which co-localizes with the Polycomb repressor complex leading to HDAC-mediated chromatin condensation.¹⁴²⁻¹⁴⁴ In particular, the interaction among the Polycomb protein, the SS18 component of the oncoprotein and a transcriptional corepressor protein called TLE was observed.¹⁴⁵ One of the repression targets of this complex is the tumor suppressor gene EGR1.¹⁴⁶ HDAC inhibitor will reverse the repression by the Polycomb protein, leading to de-repression of EGR1 and followed by apoptosis.

Similar preclinical anti-tumour effect has been observed in Ewing sarcoma,¹⁴⁷⁻¹⁴⁹ clear cell sarcoma,¹⁵⁰ endometrial stromal sarcoma,¹⁵¹ myxoid chondrosarcoma¹⁵² and alveolar rhabdomyosarcoma.¹⁴⁹ Treatment of HDAC inhibitor leads to increase in expression of p21

and G2/S arrest and decrease in mTOR expression and activity, leading to apoptosis and autophagy.¹⁵¹

In addition, Lopez et al. reported G2/M arrest and S phase depletion of fibrosarcoma, leiomyosarcoma and rhabdomyosarcoma cell lines with PCI-24781, a novel HDAC inhibitor, followed by decrease in RAD51 and apoptosis.¹⁵³ Synergistic anti-tumour activity has been observed when combining doxorubicin with HDAC inhibitor, when modest anti-tumour activity was observed after treatment with doxorubicin or HDAC alone.^{153,154}

Based on these promising preclinical data, clinical development of HDAC inhibitor in translocation associated sarcoma is definitive of interest. Combination with doxorubicin will be of interests in both translocation associated sarcomas and in soft tissue sarcomas with complex karyotypes. Inhibition of HDACs can lead to at least partial decrease of VEGFR-1 and -2 and significant clinical efficacy has been observed with VEGFR tyrosine kinase inhibitors in patients with ASPS, angiosarcoma, etc, so clinical development of these combination will likely provide improved clinical efficacy, with caution about the possibility of increase toxicity. Other combinations that may be of interests include HDAC inhibitor with therapeutics targeting the PI3K/Akt/mTOR pathway and PARP inhibition.

Double strand DNA damage due to radiation leads to activation of ATM which subsequently activates other downstream DNA repair proteins, including p53, CHK-2, BRCA-1, leading to cell cycle delay or apoptosis.¹⁵⁵ HDAC1 interacts with ATM which leads to its activation and DNA repair.¹⁵⁶ HDAC inhibitor is a radiosensitizer through suppression of DNA repair by decreasing the expression of DNA repair proteins such as DNA protein kinase.¹⁵⁷ Therefore, combination of HDAC inhibitor and radiation in the management of localized limb or retroperitoneal sarcomas will be worth studying.

HDAC inhibitors in clinical development

There are four classes of HDAC inhibitor, short fatty acid, hydroxamic acid, cyclic tetrapeptide, and benzamide. Except for the first class, all the others are in clinical development. Focus will be placed on the hydroxamic acid and benzamide class of HDAC inhibitors.

Hydroxamic acid analogues

Vorinostat or suberoylanilide hydroxamic acid (SAHA) is the first orally administered HDAC inhibitor in clinical development, which targets Class I, IIa and IIb HDAC. A dose of 300 mg twice daily 3 days every week or 400 mg daily or 200 mg twice daily.¹⁵⁸ It is currently licensed for the treatment of cutaneous T-cell lymphoma. The result of vorinostat as a second-line therapy in malignant pleural mesothelioma is anticipated in later part of 2011. Clinical development in combination with chemotherapy in various solid tumours has been slow due to toxicity compared to chemotherapy alone. Marked production of reactive oxygen species was observed in preclinical model was treated with the combination of bortezomib and HDAC inhibitor through suppression by bortezomib on NF- κ B production induced by HDAC inhibitor, which in turn leads to reactive oxygen species production.^{159,160} A phase II study of vorinostat in combination with bortezomib in second-line soft tissue sarcoma reported with no response in 16 evaluable patients.¹⁶¹

Panobinostat (LBH-589) is another Class I, IIa, and IIb HDAC inhibitor in clinical development with both oral and intravenous formulation. Dose-limiting toxicity is prolonged QTc, thrombocytopenia, neutropenia and hypophosphatemia and diarrhea, fatigue and thrombocytopenia for intravenous¹⁶² and oral panobinostat,^{163,164} respectively.

Early phase studies of either as a single agent or in combination with chemotherapy and novel agents to Hsp 90, Her-2, EGFR, VEGFR, etc are ongoing.

Belinostat (PDX-101) is again a Class I, IIa, and IIb HDAC inhibitor. An intravenous formulation entered clinical development as a daily for 5 day every 3 week schedule. 1000 mg/m²/day was determined to be the phase II dose. Histone H4 hyperacetylation lasted for 4-24 hours after each infusion. Best response was SD, and amongst which 2 soft tissue sarcoma patients lasted for 7 and 14 months, respectively.¹⁶⁵ A preliminary report of 14 patients using various schedules, daily, twice or three times daily, at doses ranging from 900-1000 mg/m²/dose and for one day to for 5 days every 21 days.¹⁶⁶ Clinical development plan of both single agent in various solid tumours and haematological malignancies and combination with chemotherapy and demethylating compounds are ongoing including a phase I/II trial in combination with doxorubicin in soft tissue sarcoma.

Other hydroxamic acid analogues are in clinical development including PCI-24781, a Class I, IIa and IIb HDAC inhibitor with high potency against HDAC 1 and 3, which is being developed in haematological malignancies and solid tumours.¹⁶⁷ Based on the synergistic anti-tumour effect in combination with doxorubicin, possibly through hyperacetylation of topo-II, a phase I/II study is currently recruiting patients.

Benzamide analogues

MS-275 was initially developed at the US NCI using various schedules, daily for 4 out of 6 weeks, once every 2 weeks and weekly for 4 out 6 weeks.^{168,169} Due to excess toxicity and prolonged half-life, only the latter 2 schedules are being developed mostly in combination with other protein targets of HDAC class IV.

MGCD0103 is HDAC 1, 2, 3 and 11 specific HDAC inhibitor. Siu et al.¹⁷⁰ reported the phase I study using a three times a week schedule with 45 mg/m²/day as the phase II dose. Acetylation at H4 increased in a dose-dependent fashion and significantly acetylation in white cells was observed at doses above 45 mg/m²/day. No objective response was observed except SD in 5 patients for 4 or more months. Currently, development of MGCD0103 is concentrated in lymphoma and other haematological malignancies.¹⁷¹

SB939, a HDAC Class I, II, and IV, has been tested in weekly 3 out 4 weeks and daily for 5 day every 2 weeks schedules with improved intra-tumour accumulation and transit time, HDAC inhibition as well as half-life.¹⁷² 60 mg a day was determined to be the dose for either schedule.^{173,174} Only SDs for a median of 5 months, as best response, were observed. Proof of concept phase II study in translocation associated soft tissue sarcomas led by the NCIC-CTG is accruing patients.

Fatigue, nausea, vomiting, diarrhea and anorexia are common to all classes of HDAC inhibitors in oral or intravenous formulations. In the absence of comparative trials, cardiac toxicity, such as arrhythmia and prolongation of QTc, seems to be occur more frequently in the intravenous formulation, which is probably related to the peak concentration of HDAC inhibitor is approached the inhibitory concentration of cardiac ion channels. This observation may favour the development of oral formulation but careful cardiac monitoring will be essential when combined with therapeutics that have been associated with cardiac toxicities including HER-2, VEGF/VEGFR. Thrombocytopenia is the only observed haematological toxicities, though mostly grade 1 and 2. The mechanism is still being investigated.

HDAC inhibition in peripheral blood mononuclear cells as a pharmacodynamic marker for anti-tumour activity and drug exposure has not been consistent. Identification of such a marker is still essential.

The mechanisms of action of HDAC inhibitor are likely multi-fold and inhibition of different classes of HDAC may have different anti-tumour effects.¹³⁵ Thus clinical activity may be different and whether cross-resistance will occur. Fantin et al. reviewed the potential mechanisms of resistance for HDAC inhibitors such as increase in DNA hypermethylation, upregulation of protection against oxidative stress, and overexpression of anti-apoptotic proteins, such as Bcl-2 and Bcl-XL.¹⁷⁵

5. DNA repair by poly (ADP-ribose) polymerases (PARP)

Poly (ADP-ribose) polymerases (PARP)

DNA damage leads to the activation of PARP, which consists of 17 members^{176,177} that have a conserved catalytic domain.¹⁷⁸ PARP1 is the most well characterized nuclear protein with three functional domains: the zinc finger containing DNA binding domain, which is responsible for the detection of DNA breaks and localization to the nuclei; the auto-modification domain which includes the breast cancer gene-1 (BRCA-1) C-terminal domain; and the C-terminal catalytic domain.^{179,180} Any single strand DNA break detected by the DNA binding domain of PARP1 leads to ADP-ribose polymerization with base excision repair proteins, such as XRCC-1, DNA polymerase- β and DNA ligase III, histones and PARP1, by the catalytic domain, which is NAD-dependent.^{181,182} In turn, this complex process affect DNA replication, transcription, differentiation, gene regulation, protein degradation and mitotic spindle maintenance. Only in the absence of PARP-1, which accounts for 90% of PARP activity, PARP2 will be involved in DNA repair.¹⁸³

Relevance of PARP and soft tissue sarcomas

Farmers et al.¹⁸⁴ demonstrated homozygous deletion of BRCA-1 or BRCA-2 led to sensitivity to PARP1 inhibition, resulting in apoptosis. Apoptosis is postulated to be the loss of homologous recombination repair after single strand DNA breaks as a result of both PARP1 inhibition and loss of BRCA-1 or -2. To date no BRCA-1 or -2 germline loss has been documented in soft tissue sarcomas. But Xing et al. reported 29% of uterine leiomyosarcoma had decrease or absent BRCA-1 protein expression, which is postulated to be due to methylation of BRCA-1 gene promoter.¹⁸⁵ Schoffski et al.¹⁸⁶ reported decrease in BRCA-1 in 50% of the soft tissue sarcoma samples.

In addition, members of the Fanconi family proteins are involved in double strand DNA repair through activation of ATM and ATR and formation of a nuclear complex of 5 Fanconi family proteins. This complex subsequently co-localizes with BRCA1 and -2 for DNA repair.¹⁸⁷ Loss of function or expression of any of these protein or "BRCA-ness" confers sensitivity to PARP1 inhibition.¹⁸⁸ ATM is a serine/threonine kinase that activates checkpoint kinase -2 (Chk-2) in the presence of DNA damage and sequentially, leads to increase in RAD51 and BRCA1 and -2 activity.¹⁵⁵ A study of leiomyosarcoma and gastrointestinal stromal sarcoma samples showed frequent 13q21-q32 amplification, which corresponds to BRCA-2 and RB1 gene location, and 11q22-q24 loss, which contains the ATM gene, in high grade and >5 cm leiomyosarcoma. 83% of the samples had either absence or weak ATM expression by immunohistochemistry and out of which 50% had 11q loss.¹⁹⁰

Similarly, loss of ATM was reported in 41% of alveolar rhabdomyosarcoma.¹⁹¹ Concurrent ATR loss and K-ras G12D mutation in p53 heterozygous mice developed lung adenocarcinoma and sarcomas.¹⁹² Germline Chk-2 loss has not been reported in sarcoma except in osteosarcoma¹⁹³, and a variant of Li-Fraumeni syndrome-related malignancies RAD51 is transcriptionally controlled by pTEN. Loss of pTEN⁴³ and rarely pTEN mutation^{194,195} has been documented in leiomyosarcoma and uterine leiomyosarcoma, respectively and 12% of various soft tissue sarcomas had hypermethylation of the pTEN gene.¹⁹⁶ Interestingly, only truncated pTEN but not point mutation confers sensitivity to PARP1 inhibition.¹⁹⁷ Whereas increase in RAD51 expression in soft tissue sarcoma leads to resistance to doxorubicin due to S/G2 phase arrest. In the presence of wild type p53 through activator protein 2, RAD51 expression was downregulated through decrease in promotor activity.¹⁹⁸

PARP inhibitor potentiates chemotherapy and radiation

Potential of preclinical anti-tumour efficacy was observed when combining PARP inhibitor with methylating agents (DTIC, temozolamide), alkylating agent (cyclophosphamide, ifosfamide), doxorubicin, topoisomerase I inhibitors and platinum agents, which are of relevance in soft tissue sarcomas.¹⁹⁹⁻²⁰² Soft tissue sarcomas that have low BRCA1 expression had statistically significantly higher response rate, PFR at 6 months, and OS to trabectedin, thus, one may suspect that the combination of PARP inhibition with trabectedin may confer additive or synergistic anti-tumour activity.

Radiation is commonly being given pre-operatively or post-operative for localized soft tissue sarcomas of the limbs and retroperitoneum. Single strand and sometimes double strand DNA breaks occur after radiation lead to PARP1 activation followed by DNA repair. Similar potentiation of radiation induced anti-tumour efficacy has been observed when given with PARP1 inhibitor.¹⁹⁹⁻²⁰²

Thus PARP1 inhibitors either as a single agent or in combination with DNA damaging agents and radiation will be worthwhile to explore in soft tissue sarcomas. One may predict that single agent activity will be observed only in those soft tissue sarcomas that have a low BRCA1 protein expression resulting from promoter methylation or other post-translational mechanisms. To select this selected population, immunohistochemistry of BRCA1 and BRCA2 will suffice. When use in combination with DNA damaging agents and radiation, PARP1 inhibitor will act as a potentiating agent. But one may still expect those tumours with a low BRCA1 protein level will have a better response.

PARP inhibitor in clinical development

Olaparib (AZD2281; KU-0059436) is a phthalazone class PARP1 and 2 inhibitor. A phase I study of 60 solid tumour patients were treated at doses from 100 mg to 600 mg twice daily on either a 2 weeks on 1 week off or continuous schedules. Grade 1 or 2 drug-related anemia, lymphopenia, fatigue, vomiting, dysphagia and anorexia were reported. Dose-limiting toxicity included grade 3 mood alteration, grade 3 fatigue, grade 3 somnolence and grade 4 thrombocytopenia were observed in 1 and 2 patients, respectively in the continuous schedule at 400 and 600 mg twice daily. As predicted by preclinical data, responses were observed in BRCA 2 mutation positive ovarian and breast cancer. One BRCA2 mutation positive prostate cancer had 50% reduction of PSA and resolution of bone metastases.²⁰³ Forty percent of BRCA1/2 mutation positive BRCA1/2 mutation positive ovarian cancer had a PR or reduction of CA125 for a median of 28 weeks.²⁰⁴

AG014699 (PF-01367338) is a PARP1 and 2 inhibitor administered intravenously. Based on synergistic preclinical anti-tumour activity with temozolomide, a phase I study was reported. The addition of PF-01367338 did not increase temozolomide related toxicity, allowing administration of full dose temozolomide at 200 mg/m²/day for 5 days. Increase in single strand DNA break was observed only when PF-01367338 was administered concurrently with temozolomide. A PR was observed in one melanoma, one pretreated desmoids tumour. Stable disease for more than 6 months was observed in 4 patients, including one leiomyosarcoma.²⁰⁵

Adoption of the novel phase 0 design, Kummar et al. found a good correlation of PARP inhibition in tumour and peripheral blood mononuclear cells by ABT 888. A minimum of 50% reduction of PARP activity was observed with 25-50 mg of ABT 888 daily. Due to an average 70% of ABT 888 is excreted in urine unchanged during the 24 hours at 50 mg or higher daily, continuous dosing will be necessary.²⁰⁶

BSI-201 was initially thought to be an intravenously administered irreversible PARP1 inhibitor, but due to recent preclinical and clinical data showed that PARP1 is not inhibited at clinically relevant concentration. Thus no further discussion will be presented in this book chapter.

MK-4827 is an orally administered PARP1 and 2 inhibitor. Sandhu et al. reported a phase I study of MK-4827 given daily at doses 30-210 mg. Dose-limiting toxicity included grade 3 fatigue, pneumonitis and anorexia. Common toxicities were all grade 1-2 such as nausea and myelosuppression. At doses of 110 mg daily, sustained PARP inhibition was observed in peripheral blood mononuclear cells. Responses and SD for more than 16 weeks were observed in sporadic and BRCA-related breast or ovarian cancer. One heavily-pretreated non-small cell lung cancer patient had SD for more than 42 weeks.²⁰⁷

A number of other PARP inhibitors are in phase I clinical development, including E7016 (PARP1 and 2 inhibitor) and CEP-8983 and its prodrug, CEP-9722 (PARP1 and 2 inhibitor).

Based on the preclinical and translational research information, PARP inhibitor in combination with DNA damaging agents (anthracyclin, ifosfamide, DTIC and trabectedin) and radiation will be of interest. As reported by Plummer et al., enhanced DNA breaks is observed only when PARP inhibitor is administered concurrently with chemotherapy. Thus careful preclinical and clinical study of the schedule of these combination should be performed not only to enhance anti-tumour activity, but also to reduce unnecessary toxicities.

Development as a single agent may only be applicable to those with low BRCA1 protein expression, low ATM expression such as gastrointestinal stromal tumour, leiomyosarcoma and alveolar rhabdomyosarcoma and low RAD51 expressing sarcomas.

Development of resistance is the rule rather than exception for PARP inhibitor. Reversal of loss of nonsense mutation in BRCA2 due to a second mutation has been reported.²⁰⁸ Pre- and post-treatment biopsies in the setting of localized disease or metastatic disease may proven to be valuable in further our understanding of the predictive biomarker for response, the biological effect of PARP inhibitors alone and/or in combination with other agents and more importantly, the mechanisms of resistance. For example, the activation of PARP 1 by Ras/Raf/Erk pathway has been observed.²⁰⁹ Thus, such combination may be of clinical relevance. PARP activity is at least in part responsible for HIF-1 α expression.²⁰⁸ Building on the clinical benefit of anti-VEGFR TKI in soft tissue sarcoma, the combinations with PARP inhibitor have been initiated.

Protective effects of PARP inhibition on doxorubicin related cardiac toxicities²¹⁰, platinum- and taxane related neurotoxicities²¹¹ and cisplatin-related nephrotoxicities²¹² without compromising anti-tumour activities have been reported in preclinical models. Careful study of the incidence of toxicity will be fruitful during clinical development such as randomized phase II studies with toxicities as one of the main endpoints will be worthwhile.

Apoptosis pathways

Apoptosis, otherwise known as programmed cell death, is a critical process required for maintaining tissue homeostasis. Furthermore, it prevents tumorigenesis by eliminating damaged cells. Apoptosis occurs through two major pathways. The extrinsic or death receptor pathway is turned on in response to extracellular signals resulting in activation of plasma membrane receptors. This ultimately leads to processes that trigger initiator caspases which in turn activate executioner caspases.²¹³ The intrinsic or mitochondrial pathway is turned on in response to a diverse set of apoptotic signals which include DNA damage, growth factor withdrawal and viral infection. Mitochondria release co-factors from their intermembrane space which promote and amplify the apoptotic cascade including the formation and activation of apoptosomes.²¹⁴ Although these pathways have their own distinct regulatory processes, there is also significant cross-talk in many situations.²¹⁵ Defects in apoptosis can lead to oncogenic transformation and contribute to therapeutic resistance.²¹⁶

Bcl-2 family

The members of the Bcl-2 family of proteins are the key regulators of mitochondrial response to apoptotic signals and are divided into three subfamilies based on function and structure. The anti-apoptotic members include Bcl-2, Bcl-X_L, Mcl-1, Bcl-w, and A1/Bfl1. The pro-apoptotic members include Bax and Bak. The third subfamily is the BH3-only group which is largely responsible for sensing apoptotic signals and transmitting them to the other Bcl-2 family members.²¹⁷ Members of this subfamily include Bid, Bad, PUMA, and Noxa.²¹⁴ A network of interactions between these subfamilies decides the apoptotic fate of the cell and an imbalance within this network leads to a variety of disease states.

The Bcl-2 family of proteins is a common target for deregulation in cancers. Anti-apoptotic members may be overexpressed or pro-apoptotic members may be mutated or silenced. These abnormalities have been demonstrated in soft tissue sarcomas. Bcl-2 overexpression has been demonstrated in synovial sarcoma, Kaposi's sarcoma, solitary fibrous tumor and gastrointestinal stromal tumor (GIST).^{218,219} Focal positivity has been observed in both benign and malignant peripheral nerve sheath tumors. Sarcomas of fibroblastic type, including low-grade myxofibrosarcoma, malignant fibrous histiocytoma and fibrosarcoma showed variable expression of Bcl-2.²¹⁸ The bcl-2 overexpression in synovial sarcoma include immunohistochemical expression of bcl-2, bax, bcl-x, and bac. Furthermore, this has been correlated with poor prognosis.^{220,221}

Oblimersen sodium (G3139, Genasense), the first agent targeting Bcl-2 to enter clinical trials, is a phosphorothioate Bcl-2 antisense oligodeoxynucleotide that targets Bcl-2 mRNA. The cell death mechanisms of Bcl-2 antisense can be classified in two categories exerting either an apoptotic or a nonapoptotic effect based on their involvement in cell death pathways. Pre-clinical data in combination with doxorubicin in the FU-SY-1 synovial sarcoma cell line enhanced doxorubicin-induced cell kill.²²² Clinical development of oblimersen is unknown.

Similarly ABT 737 (A-779024, Abbott Laboratories), a small molecule that targets anti-apoptotic Bcl-2 family proteins (Bcl-2, Bcl-X_L, and Bcl-w), in combination with imatinib in GIST cell lines inhibits the proliferation and induces apoptosis in all GIST cell lines. Strong synergistic drug interactions at clinically relevant in vitro combinations were reported.²²³ Clinical development of ABT-263 is ongoing in various solid tumours and chronic lymphocytic leukemia and in combination with other chemotherapeutic and targeted agents. ABT-263 is administered orally and thrombocytopenia is the dose-limiting and most common toxicity, which occurs on day 15 followed by a rebound by days 21-28 and due to mechanistic inhibition of Bcl-XL in mature platelets.²²⁴ Impressive anti-tumour activity has been observed in commonly Bcl-2 amplified chronic lymphocytic leukemia.²²⁵ SDs and a few PRs have been observed in solid tumours, including small-cell lung cancer which has overexpression of Bcl-2.^{224,226}

Many additional agents have been identified or designed to target the Bcl-2 family at the mRNA or protein level and pre-clinical data suggests these agents should be developed further in the subtypes of soft tissue sarcomas demonstrating Bcl-2 overexpression.

Stem cell pathways

The developmental pathways important to normal stem cells are also important to cancer stem cells (CSC). Like normal stem cells, CSC are thought to possess the capacity for unlimited self renewal through symmetric division, the ability to give rise to progeny cells through assymetric division, and an innate resistance to cytotoxic therapies.^{227,228} However where the process of differentiation initiated by the normal stem cell results in a specialized progeny that has no proliferative potential, the CSC gives rise to progeny that do not undergo terminal differentiation but instead exhibit uncontrolled proliferation.²²⁹

Due to similarities to normal stem cells, CSCs are predicted to rely on the same pathways that govern development, self-renewal and cell fate. In embryonic stem cells, the Notch, Wnt, and Hedgehog (HH) pathways largely regulate these processes.²³⁰ These pathways are frequently dysregulated in many types of cancers, and specifically within subpopulations of these cancers that possess stem cell like properties (Li et al, 2010; Barker et al, 2006; Haegbarth et al, 2009; Wang et al, 2010).²³¹⁻²³³

Hedgehog (HH) pathway²³⁴

Under normal conditions, HH signaling plays important roles in embryonic development tissue regeneration in adults (Ingham PW et al, 2001; Vajosalo M et al, 2008). HH signaling is comprised of multiple ligands that regulate receptor activity. HH signaling is initiated when one of three HH ligands (Sonic, Indian and Desert) bind the receptor Patched (Ptch1). The ligand/receptor interactions may be autocrine or paracrine. Receptor engagement results in activation of the transmembrane Smoothened (Smo), which is inactive state in the absence of a ligand. Subsequently, Smo activation regulates transcription of genes involved in HH signaling such as Gli1, Gli2 and Gli3. Gli1/2/3 regulate the transcription of genes involved in HH signaling such as Gli1 and Ptch1 as well as genes involved in epithelial-mesenchymal transition (EMT), such as SNAIL1.²³⁵

Dysregulation of nearly every step of the HH signaling pathway has been linked to cancer development and progression. In rhabdomyosarcoma (RMS), the most common soft tissue sarcoma in children, HH signaling is preferentially activated in specific subgroups of RMS.²³⁶⁻²³⁹ Ptch1, Gli1, and Gli3 are expressed at significant higher levels in embryonal RMS

than in fusion-gene negative alveolar RMS than in fusion-gene positive alveolar RMS. High expression of *Ptch1* significantly correlated with reduced cumulative survival.²³⁸

Betullinic acid possesses anti-tumoral activity and overcomes resistance by inducing apoptosis in a variety of human cancers.²⁴⁰ In preclinical study using RMS-13 cells, which is known to display an activate HH pathway caused by DNA amplification of the *Gli1* locus, betullinic acid effectively suppressed HH target gene expression.²³⁸

Early phase clinical trials are underway which include patients with soft tissue sarcomas with agents such as GDC 0449 (Genetech),²⁴¹⁻²⁴⁴ LDE225 (Novartis Pharmaceuticals)²⁴⁵ and BMS-833923²⁴⁶ which both selectively inhibit Smo. Preliminary anti-tumour activity in consistently in basal cell carcinoma and medulloblastoma and in sporadic cases of non-small cell lung cancer. All are orally administered on a daily schedule. Only IPI-926 is a cyclopamine analogue that is administered intravenously.²⁴⁷ Toxicity includes gastrointestinal (nausea, vomiting), anorexia, muscle spasm/cramps, fatigue and dysguesia. Due to the long half-life of days, various alternative dosing schedules are being tested to optimize toxicity and anti-tumour activity. Combination with various chemotherapy and targeted agents are ongoing, such as PI3K inhibitor.

Notch signaling pathway

The Notch pathway is a highly conserved regulatory signaling network with the basic molecular players in the pathway including 5 ligands (*Dll* 1, 3, 4 and *Jagged* 1, 2), Notch receptors (Notch 1 to Notch 4) and the transcription factors. A complex signaling pathway is initiated when a ligand expressed on one cell engages a receptor expressed on another cell. Upon ligand/receptor interaction a cleavage event removes the Notch/ligand complex from the membrane bound portion of Notch. The cytoplasmic region of Notch then undergoes a proteolytic cleavage mediated by γ -secretase, releasing an intracellular domain peptide which translocates to the nucleus and drives transcription of Notch target genes.²³⁰ The physiologic functions of Notch signaling are multifaceted and include maintenance of stem cells, specification of cell fate, and regulation of differentiation in development as well as oncogenesis.^{248,249}

γ -secretase inhibitors are a promising therapeutic approach as they act downstream of the ligand/receptor interactions and therefore should not be affected by the diversity of ligands, receptors and combinations thereof. Currently several γ -secretase inhibitors are being evaluated in early Phase human studies.

Aberrant Notch signaling has been reported in rhabdomyosarcoma and Kaposi sarcoma cell lines.^{250,251} A striking increase in Notch 2 expression was observed. In addition a slight upregulation of Notch 3 was also noted. However, Notch 1 and 4 were not significantly increased. Increased expression of two downstream effectors, *Hes1* and *Hey1*, correlated with invasiveness of the cell lines. The addition of γ -secretase inhibitors significantly decreased the *Hes1* and *Hey1* expression. Furthermore, with addition of γ -secretase inhibitors, a significant reduction in cell mobility was observed suggesting the Notch pathway may play a role in the invasiveness of the cell lines.²⁵⁰ In pre-clinical studies, a γ -secretase inhibitor also blocked Notch activation and induces apoptosis in Kaposi's sarcoma tumor cells.²⁵¹ These findings show that the Notch pathway is important in regulating these subtypes of soft tissue sarcoma and may γ -secretase inhibitors be useful therapeutic agents.

Wnt pathway

Wnts are secreted glycoproteins that bind to cell surface receptors initiating signaling cascades which are important in many physiologic settings including embryogenesis,

development, cell polarization, differentiation and proliferation.²⁵²⁻²⁵⁶ The Wnt signaling pathways fall into two categories: canonical and non-canonical, characterized by their dependence on β -catenin. Canonical Wnt will be the focus of this section as it is better characterized in mammalian systems. Canonical Wnt signaling is initiated when a Wnt ligand engages co-receptors of the Frizzled (Fzd) and low-density lipoprotein receptor-related protein (LRP) families, leading to β -catenin stabilization, nuclear translocation and activation of target genes.^{230,257}

The relevance of Wnt signaling in human cancers is highlighted by the frequency of which this pathway is aberrantly activated across a vast range of malignancies. There are numerous mechanisms that drive aberrant Wnt/ β -catenin signaling than nearly always occur in a mutually exclusive manner. In one study, 50% of human sarcomas and 65% of sarcoma cell lines of diverse histological subtypes exhibit upregulated autocrine canonical Wnt signaling. Furthermore, in Wnt autocrine cell lines, alterations included overexpression or gene amplification of Wnt ligands and/or LRP5/6 coreceptors and epigenetic silencing of different cell surface Wnt antagonists.²⁵⁸ High level of nuclear expression of β -catenin has reported in solitary fibrous tumor, endometrial stromal sarcoma, synovial sarcoma, dedifferentiated liposarcoma, and malignant fibrous histiocytoma.²⁵⁹⁻²⁶²

There are three major areas of targeting the Wnt pathway which include receptor/ligand interactions, cytosolic signaling components, and nuclear signaling components. In pre-clinical studies, monoclonal antibodies against Wnt1 induced apoptosis in a number of cell lines including sarcoma.^{263,264} *In vitro* results are encouraging and warrant further development. An alternate approach to inhibiting ligand/receptor interactions would be to target the Wnt co-receptors. The LRP family is comprised of 2 highly homologous members, LRP5 and LRP6 which are long single-pass transmembrane receptors which could be targeted through antibodies.²⁶⁵ The Fzd family of transmembrane receptors also shares a high degree of homology in their cysteine rich domain which could be targeted through an antibody to block Wnt signaling.²⁶⁶

Targeting the cytosolic signaling components of the Wnt pathway are in preclinical or phase I early development. No clinical information is currently available. The small molecules in development include those targeting Dvl, Tankyrase1 and 2, Axin, Porcupine, and β -catenin.²³⁰ Similarly nuclear signaling small molecules which would interrupt the β -catenin interaction with TCF/LEF are also in early development.

6. Conclusions

With decades of research into the biology of each target, its interaction with other pathways or targets, and the relevance in sarcoma, it is an exciting time in drug development in soft tissue sarcomas. But the academia and industry must remember that soft tissue sarcoma is a collective term for a florid of many different diseases, though some share similar characteristics, such as the translocation associated sarcomas and their fusion proteins with transcription factor activity, each target may only be relevant to a small number of subtypes. A lot of investment and time will be needed in order to bring these agents to the market with a small return.

During the development of these agents, it is important to understand the biology of these targets and their effects on the sarcoma cells, cytostatic or cytotoxic, as it will influence the clinical endpoints, progression-free survival or progression-free rate versus response rate,

respectively, in proof of concept phase II studies. This approach will lessen our chance of failure in the phase III setting.

Furthermore, how can a target, expression/overexpression, amplification, mutation, or translocation, be considered to be of clinical relevance for clinical therapeutics? There seems to be a different answer for different cancer. Therefore, detail preclinical modeling and translational research using archival tumour samples will be important. It is important to collect tumour (fresh biopsies before and after therapy and archival samples), blood or even urine samples during proof of concept studies in order to validate the target identify and to evaluate novel predictive markers for clinical benefit.

Resistance is universal for any cancer therapeutics. Preclinical, translational and clinical research should be done to elucidate these escape mechanisms. Such information can help us not only to develop combinatorial therapeutics to delay or prevent resistance and thus more prolonged clinical benefits, but also to understand the biology of sarcomas and thus new therapeutic options when resistance emerges.

With the constant quest of the sarcoma community to understand and to develop new agents for this vastly diverse cancer, it is the hope that more effective and less toxic agents will be available for the treatment of this disease.

7. References

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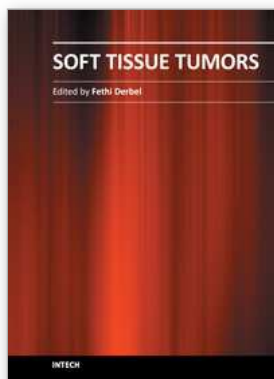
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Soft tissue tumors include a heterogeneous group of diagnostic entities, most of them benign in nature and behavior. Malignant entities, soft tissue sarcomas, are rare tumors that account for 1% of all malignancies. These are predominantly tumors of adults, but 15% arise in children and adolescents. The wide biological diversity of soft tissue tumors, combined with their high incidence and potential morbidity and mortality represent challenges to contemporary researches, both at the level of basic and clinical science. Determining whether a soft tissue mass is benign or malignant is vital for appropriate management. This book is the result of collaboration between several authors, experts in their fields; they succeeded in translating the complexity of soft tissue tumors and the diversity in the diagnosis and management of these tumors.

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