Original Article

Role of New Chemotherapy Agents in Soft Tissue Sarcoma

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Key Words

Soft tissue sarcomas, Yondelis, mTOR, kinase inhibitors, VEGF, GIST, imatinib refractory

Abstract

Medical management of soft tissue sarcomas (STS) has been restricted by the limited availability of active drugs. A plethora of new oncologic agents are now available, many of which have specific therapeutic targets. Gemcitabine and docetaxel is a combination of drugs that have limited single-agent activity. Yondelis, a novel chemotherapeutic that binds DNA and functions partially by inhibiting transcription, is being tested alone and in combination with doxorubicin. Inhibitors of mTOR, a serine/threonine kinase that regulates cell cycle activation and cell growth, are also being tested. Growth factor receptor inhibitors are being evaluated in a variety of sarcomas that have been found to express the targets. In addition, a variety of agents are being assessed in gastrointestinal stromal tumors (GIST). Single agents and agents combined with imatinib are being tested in imatinib-refractory and in metastatic GIST. The increased use of targeted agents underscores the need for understanding sarcoma biology. (*JNCCN* 2005;3;198–205)

Soft tissue sarcomas (STS) are a rare set of diseases with an estimated incidence in the United States of 8,680 in 2004.[1] Although commonly referred to as sarcomas, they actually represent a set of diseases with heterogeneous histology and biology. Chemotherapy is primarily used for treatment of metastatic disease. Doxorubicin, ifosfamide, and dacarbazine alone or in combination have resulted in reported response rates up to 35%.[2] The majority of clinical trials in the past have enrolled patients with all types of histologies, not allowing for subtleties between tumor types to become evident. Current trials in soft tissue sarcoma are being designed to better assess the efficacy of an agent in specific histologies. This article reviews data on the role of gemcitabine in combination with other agents; new therapeutic agents under investigation in STS (including ectinascidin-743, mTOR inhibitors, and tyrosine kinase inhibitors); and novel approaches for gastrointestinal stromal tumors (GIST; including multitargeted tyrosine kinase inhibitors, src kinase inhibitors, and anti-angiogenic approaches).

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Gemcitabine in STS

Gemcitabine hydrochloride (Lilly Pharmaceuticals, Indianapolis, IN), a pyrimidine nucleoside analogue, inhibits DNA replication and synthesis. It requires intracellular phosphorylation for activation. Phase II clinical trials evaluated the efficacy of gemcitabine in STS in both first- and second-line settings for metastatic disease with overall response rates ranging from 3.3%[3] to 18%.[4] There did appear to be activity in leiomyosarcomas as well as in angiosarcomas, malignant fibrous histiocytoma, and spindle cell sarcomas. The Gynecologic Oncology Group (GOG) evaluated gemcitabine at 1000 mg/m^2 over 30 minutes in the second-line setting for uterine leiomyosarcoma. They determined a complete response (CR) rate of 2.3% and a partial response (PR) rate of 18.2%.[5]

The activity of gemcitabine in leiomyosarcoma was shown again in a phase II trial that combined gemcitabine with docetaxel in uterine or non-uterine leiomyosarcoma.[6] Patients received gemcitabine at 900 mg/m^2 on days 1 and 8 with docetaxel 100 mg/m^2 on day 8, except for patients who had received prior pelvic radiation, in whom there was a 25% dose reduction of

both agents. In addition, the rate of infusion of the gemcitabine was prolonged to 10 mg/m^2/min. In 52 patients, the RR was 40% in leiomyosarcomas and 10% in non-leiomyosarcomas.[7] Another single-institution retrospective analysis of patients treated with prolonged infusion of gemcitabine at 675 mg/m^2 on days 1 and 8 with docetaxel 100 mg/m^2 on day 8 showed activity in leiomyosarcoma, angiosarcoma, osteogenic sarcoma, peripheral neuroectodermal tumor (PNET), malignant peripheral nerve sheath tumor (MPNST), and malignant fibrous hystiocytoma (MFH), for an overall response rate of 43%.[8] The GOG is currently testing the combination in patients with recurrent or metastatic uterine leiomyosarcoma who have not received prior chemotherapy. In addition, the North American Sarcoma Study Group is conducting a randomized phase III trial comparing prolonged infusion gemcitabine alone or in combination with docetaxel.

Other gemcitabine combination trials have been reported. A phase I trial evaluated the combination of gemcitabine with dacarbazine.[9] Twenty-two patients were treated with gemcitabine at doses of 800 to 2,160 mg/m^2 at a rate of 10 mg/m^2/min with dacarbazine 500 mg/m^2. The dose limiting toxicity was transaminitis at the highest dose level; gemcitabine at 1,800 mg/m^2 with dacarbazine 500 mg/m^2 was recommended as the phase II dose. Five PR were seen in 19 evaluable patients, although two were inpatients treated above the maximum tolerated dose (MTD). Gemcitabine with doxorubicin resulted in 2 responses in 9 evaluable patients, one PR in a uterine leiomyosarcoma and another in MFH.[10] Gemcitabine with vinorelbine as first or second line chemotherapy for patients with metastatic soft tissue sarcoma was given at doses of vinorelbine 25 mg/m^2 followed by gemcitabine 800 mg/m^2 given on days 1 and 8 every 21 days.[11] Two responses have been reported to date, in a high grade leiomyosarcoma and in an MPNST.

Novel Agents in the Therapy of STS

A number of novel agents have been evaluated in the treatment of STS (Table 1).

Ecteinascidin-743

Ecteinascidin-743 (ET-743, Yondelis; Pharma Mar/Johnson & Johnson) is a tetrahydroisoquinolone alkaloid derived from a Caribbean tunicate *Ecteinascidia turbinate*. The drug binds the minor groove of DNA, alkylates guanine,[12] and blocks cell cycle progression and the organization and assembly of the microtubular cytoskeleton.[13,14] ET-743 also inhibits topoisomerase I.[15] In addition, tumor cell lines that are deficient in nucleotide excision repair are resistant to the effects of ET-743.[16,17] Preclinical data also suggest that ET-743 can function to inhibit binding of transcription factors to genes such as *MDR1*.[18] Preclinical studies show in vitro activity of ET-743 in multiple STS cell lines.[19] In particular, a fibrosarcoma and malignant fibrous histiocytoma cell line were shown to be very sensitive to ET-743.

ET-743 in conjunction with doxorubicin, trimetrexate, or paclitaxel on fibrosarcoma and liposarcoma cell lines showed synergy.[20] ET-743 followed by doxorubicin or paclitaxel was more effective than the opposite sequence. In particular, the ET-743 and

Table 1 Novel Agents with Their Targets and Phase of Testing

Target	Agent	Phase of Testing	Histology
Minor groove of DNA	ET-743	Phase II	Liposarcoma, leiomyosarcoma
mTOR inhibitor	AP23573	Phase II	Multiple
	RAD 001	Phase I	GIST
Growth factor inhibitors	Erlotinib	Phase II	MPNST
	Trastuzumab	Phase II	Synovial sarcoma
VEGF inhibitor	Bevacizumab	Phase II	Angiosarcoma, GIST
Multi-tyrosine kinase inhibitor	SU11248	Phase III	GIST
	AMG706	Phase I	GIST
	PKC412	Phase I	GIST
	BAY 439006	Phase II	Multiple

doxorubicin showed significant activity in a doxorubicin-resistant fibrosarcoma cell line.[21]

Phase I trials have tested many schedules of ET-743 (Table 2). In all schedules examined, the most common toxicities were neutropenia, thrombocytopenia, and transaminitis. Lengthening infusion times allows for a greater dose to be delivered and is associated with less hematologic toxicity,[22] but with an increase in liver toxicity.[23] A phase I trial and compassionate use program in patients with anthracycline-refractory disease used a 24-hour continuous infusion.[24] Two PRs were seen in 25 patients with STS, and 2 PRs were seen in 3 patients with osteosarcoma, with an additional 12 patients having stable disease (SD) for 2.8 to 15 months. Median duration of response was 10.5 months, with the median duration of disease stabilization being 5.2 months.

Another phase I trial used a daily times 5 schedule.[25] Five patients with STS were enrolled on this study with one minor response (greater than 25% decrease) in uterine leiomyosarcoma. A 72-hour continuous infusion repeated every 21 days tested 600, 900, 1,050, and 1,200 mcg/m^2.[22] The authors saw no responses in 4 patients with leiomyosarcoma and in 2 patients with GIST.

An analysis of 5 phase I trials evaluating parameters associated with toxicity revealed that patients with elevations of liver alkaline phosphatase after treatment with ET-743 were at an increased risk of severe side effects.[26] Patients with elevated alkaline phosphatase levels, but not elevated serum hepatic enzymes have had statistically higher area under the curve (AUC).[27] Protocols now incorporate frequent sampling of liver function tests between cycles of therapy. Preclinical studies in rats showed that pretreatment with dexamethasone decreased liver toxicity.[28] Dexamethasone is now a standard premedication for patients receiving ET-743.

Ongoing phase I clinical trials are testing the combination of ET-743 with pegylated doxorubicin (PLD), docetaxel, cisplatin, and gemcitabine. PLD with ET-743 has shown the ability to deliver significant doses of both agents, 30 mg/m^2 and 1,100 mcg/m^2 respectively.[29] Toxicities have been easily managed with PRs noted in patients with liposarcomas, PNET, and spindle cell sarcoma. Further testing of anthracyclines in combination with ET-743 in metastatic sarcoma is under development.

Phase II studies in STS used the 24-hour infusion schedule. Patients with metastatic, chemotherapy-naïve, and previously treated disease have been enrolled. For chemotherapy-naïve patients, objective RR were noted in 14%, with an additional 14% with SD.[30] Two phase II trials enrolled patients previously treated with doxorubicin and/or ifosfamide,[31,32] as well as patients with more extensive pretreatment in the latter study. In these 2 studies, OR were noted in 8% and 4% of patients respectively, with additional MR leading to an overall clinical benefit of 14% and 11%. Responses were primarily in leiomyosarcomas and liposarcomas. Patients have had significant progression-free survivals. Yovine et al.[32] found 24% of the patients free from progression at 6 months and 30% of patients alive at 2 years. Sixty percent of patients with responsive disease or disease stabilization showed resistance to anthracyclines and/or ifosfamide at study entry. Garcia-Carbonero et al.[31] estimated the one-year progression-free and overall survival rates to be 9% and 53%, respectively. This appears to be caused by a change in growth kinetics of sarcomas during ET-743 administration.[33] No clinical activity was seen in patients with GIST.[34]

Based on the activity seen in phase I and II trials, a phase II randomized trial is comparing the 24-hour infusion schedule to a 3-hour weekly infusion every 3 of 4 weeks in patients with metastatic leiomyosarcoma

Table 2 Phase I Trials of Yondelis

Reference	Schedule	Recommended Phase II Dose	Response
Taamma et al.[75]	24 hour CIV	1500 mcg/m^2	Liposarcoma Osteosarcoma
Villalona-Calero et al.[25]	Daily × 5	325 mcg/m^2	Leiomyosarcoma
Ryan et al.[22]	72 hour CIV	1050 mcg/m^2	No response in STS
van Kesteren et al.[23]	1 hour infusion every 21 days	1000 mcg/m^2	Not reported
van Kesteren et al.[23]	3 hour infusion	1650 mcg/m^2	Not reported

and liposarcoma that have progressed after doxorubicin and ifosfamide chemotherapy. An initial report suggested the weekly schedule was better tolerated; however, the 24-hour infusion schedule may be more effective.[35] The Children's Oncology Group will be testing ET-743 in recurrent or refractory soft tissue sarcomas and Ewing's family tumors for patients diagnosed before the age of 21.

mTOR Inhibitors

Growth factor receptors mediate signals that affect protein translation through mTOR and its upstream partners PKC, AKT, PI3-K, and PLCγ. mTOR, the mammalian target of rapamycin, is a member of the phosphatidylinositol kinase (PIK)-related kinase family and functions to regulate protein translation, cell cycle progression, and cellular proliferation.[36,37] CCI-779 (sirolimus; Wyeth Ayerst), RAD001 (everolimus; Novartis) and AP23573 (a non-prodrug of rapamycin; Ariad Pharmaceuticals) are derived from rapamycin, an immunosuppressive agent. These agents lead to G_1 cell cycle arrest and inactivation of protein synthesis. Loss of the tumor suppressor PTEN leads to enhanced sensitivity to the mTOR inhibitor CCI-779 in some tumor types.[38–40] In addition, in vitro activity exists for mTOR inhibitors in rhabdomyosarcoma cell lines.[41–44] Primary toxicities in phase I trials are dermatologic, myelosuppression, hepatic, and asymptomatic hypocalcemia.[45] Minor responses were noted in previously treated soft tissue sarcoma patients. Phase II trials of CCI-779 and AP23573 in STS sarcoma are under way. RAD 001 is being tested in GIST (see subsequent sections).

Growth Factor Receptor Inhibitors

Epidermal growth factor receptor (EGFR) expression has been shown on synovial cell sarcoma,[46] malignant peripheral nerve sheath tumors (MPNST),[47] and myxoid/round cell liposarcomas.[48] In addition, evidence exists for the expression on Her-2/neu on synovial sarcoma.[49] MPNST are derived from Schwann cells, which normally do not express EGFR. However, both clinical samples and animal models of MPNST have been shown to express EGFR.[50] Preclinical data support the efficacy of anti-EGFR approaches in MPNST cell line assays.[51] Two ongoing trials are evaluating erlotinib in MPNST and trastuzumab in synovial sarcoma.

VEGF/VEGFR Inhibitors

Sarcomas have been shown to express vascular endothelial growth factor receptor (VEGFR).[52] The data are mixed regarding the correlation of VEGF and VEGFR expression as a predictor of clinical outcome.[53–55] Human angiosarcomas,[56] hemangiosarcomas,[57] and hemangiopericytomas[58] all have been shown to express VEGFR and the mRNA for VEGF. This suggests that there may be autocrine or paracrine growth stimulation in these tumors. A phase II trial will assess bevacizumab, an antibody against VEGF, in angiosarcomas. BAY43-9006 also has activity against VEGFR as well as other kinases, and is discussed in a subsequent section.

Multi-Targeted Kinase inhibitors

Imatinib mesylate, with activity against the mutated tyrosine kinase named KIT, platelet-derived growth factor receptor (PDGFR), Abelson leukemia (ABL), and the *bcr-abl* translocation found in chronic myelogenous leukemia (CML) is being tested in a variety of sarcoma types. Initial reports suggest that the agent has no role outside of GIST.[59] A recent report of imatinib in multiple sarcoma types showed 4-month progression-free survivals of interest in liposarcoma, fibrosarcoma, leiomyosarcoma, and desmoid tumors.[60] Studies are planned to assess the presence of the targets of imatinib. Dermatofibrosarcoma protuberans (DFSP) has a translocation involving PDGFRβ and the *COL1A1* genes.[61] In vitro, imatinib causes apoptosis of DFSP cell lines through apoptosis.[62] Case reports have supported this activity.[63,64] Imatinib is being tested in patients with locally advanced and metastatic DFSP.

BAY 43-9006 is an inhibitor of wildtype and mutant B-raf and C-raf kinases, as well as VEGFR-2, Flt3, c-KIT, and p38α, a member of the mitogen actived protein (MAP) kinase family. Sarcomas are known to express PDGFR[65,66] and VEGFR[52] with mixed data on the correlation of VEGF expression as a predictor of clinical outcome.[53,54] In addition, KIT expression is seen in GIST.[67] This agent will be tested in a phase II trial. Three cohorts of patients will be enrolled: 1) angiosarcomas, malignant hemangiopericytomas, malignant hemangioendothelioma; 2) high-grade leiomyosarcomas; and 3) high-grade liposarcomas.

New Therapies in GIST

Following the initial success of imatinib mesylate for the treatment of GIST, clinicians are now challenged by patients who have experienced progression on imatinib or who cannot tolerate imatinib. The agent farthest in development is the multitargeted tyrosine kinase inhibitor SU11248 with activity against KIT,

PDGFR, VEGFR, and FLT-1/KDR. Phase I testing identified 50 mg orally for 28 days followed by 14 days rest as the best schedule. Toxicities included fatigue, nausea, vomiting, asymptomatic transient increases in lipase and amylase, neutropenia, hypertension, hand foot syndrome, anemia, and bleeding at site of tumor biopsies. Some patients with a history of coronary artery disease had asymptomatic cardiac enzyme elevations.

The phase I and II trials of SU11248 in patients with imatinib-refractory GIST or imatinib intolerance treated 97 patients, 96% of whom had progressive disease on imatinib. The PR was 8%, with SD rate of 58%.[68,69] Clinical benefit was seen in patients with *KIT* and *PDGFR*α mutations that are less sensitive to imatinib. SU11248 is now in phase III testing comparing placebo with SU11248 in a double-blind fashion in patients with imatinib refractory/intoleratnt GIST. The primary endpoint of the study is to determine if SU11248 treatment is associated with a prolongation in the time to tumor progression compared with placebo.

Another agent currently in clinical trials in patients who have progressed on imatinib is AMG706, a tyrosine kinase inhibitor with specificity against KIT and VEGFR. BMS 354825 is a SRC-family kinase inhibitor. Preclinical testing in a mouse model of CML refractory to imatinib mesylate revealed prolonged survival compared with untreated animals.[70,71] A phase I trial in imatinib refractory or imatinib intolerant CML has shown responses in 31 of 36 patients.[71] A phase I trial is currently evaluating this agent in GIST and other solid tumor patients.

Combination Therapy for GIST

GIST tumors are also being tested with anti-angiogenic therapies because they are very vascular tumors, and VEGF has been shown by immunohistochemistry in tumors and in serum from patients with metastatic tumors.[72] The efficacy of SU11248 raised the possibility that its anti-VEGFR inhibition was playing a role in tumor control. A phase II trial will be testing bevacizumab in combination with imatinib in patients with untreated metastatic and unresectable GIST.

KIT and PDGFR are cell surface receptors with complex signaling pathways intracellularly. RAD001, an inhibitor of mTOR, is being added to imatinib.[73] Phase I testing has shown pharmacokinetic interactions between the RAD001 and imatinib, with increases in the serum concentration of RAD001 when given concurrently with imatinib. No significant clinical activity has been noted to date. PKC 412 (an oral staurosporine derivative that has activity against multiple kinases including protein kinase C isotypes a, b, and g, KIT, WT and mutated PDGFRα and β, VEGFR2, FGFR, and FLT3) is also being combined with imatinib.[74] PKC412, when added to imatinib, results in up to a 70% decrease in serum concentrations of imatinib. In contrast, PKC412 serum levels increased when imatinib was added. To date, 3 of 17 evaluable patients show stable disease. The phase I trial of this combination is ongoing to define the phase II dose.

Conclusions

The management of patients with recurrent soft tissue sarcomas is challenging given the few effective drugs available and the many tumor histologies. Gemcitabine continues to show activity in leiomyosarcomas, particularly those originating in the uterus. The addition of other agents in combination with gemcitabine may or may not improve the response rate or, more importantly, the survival of patients with soft tissue sarcoma. Additional information will be gained from the ongoing phase III trial comparing gemcitabine alone versus gemcitabine with docetaxel. ET-743, although not associated with a large RR, seems promising in the second-line setting particularly because of the prolonged disease stabilizations observed. Combinations with ET-743 continue to be tested and may have a role in STS. The preclinical activity of the mTOR inhibitors requires further clinical testing to determine their efficacy in this disease setting. Kinase inhibitors are being tested in a variety of STS targeting specific receptors present in those tumor types. Ongoing trials are testing a variety of new agents in GIST, both in the metastatic disease setting and in patients refractory to imatinib. Future therapy for GIST will likely include additional agents beyond imatinib. The identification of biologic targets in STS combined with the availability of new targeted agents is leading to an increase in the number of clinical trials for this patient population, and hopefully improved outcomes as well.

References

1. Jemal A, Tiwari R, Murray T, et al. Cancer statistics, 2004. CA Cancer J Clin 2004;54:8–29.

2. Elias A, Ryan L, Aisner J, et al. Mesna, doxorubicin, ifosfamide, dacarbazine (MAID) regimen for adults with advanced sarcoma. Semin Oncol 1990;17:41–49.
3. Svancarova L, Blay J, Judson I, et al. Gemcitabine in advanced adult soft-tissue sarcomas. A phase II study of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 2002;38:556–559.
4. Patel SR, Gandhi V, Jenkins J, et al. Phase II clinical investigation of gemcitabine in advanced soft tissue sarcomas and window evaluation of dose rate on gemcitabine triphosphate accumulation. J Clin Oncol 2001;19:3483–3489.
5. Look K, Sandler A, Blessing J, et al. Phase II trial of gemcitabine as second-line chemotherapy of uterine leiomyosarcoma: a Gynecologic Oncology Group (GOG) study. Gynecol Oncol 2004;92:644–647.
6. Hensley M, Maki R, Venkatraman E, et al. Gemcitabine and docetaxel in patients with unresectable leiomyosarcoma: results of a phase II trial. J Clin Oncol 2002;20:2824–2831.
7. Hensley M, Anderson S, Soslow R, et al. Activity of gemcitabine plus docetaxel in leiomyosarcomas and other histologies: report of an expanded phase II trial. J Clin Oncol 2004;22:Abstract 9010.
8. Leu KM, Ostruszka LJ, Shewach D, et al. Laboratory and clinical evidence of synergistic cytotoxicity of sequential treatment with gemcitabine followed by docetaxel in the treatment of sarcoma. J Clin Oncol 2004;22:1706–1712.
9 Buesa J, Losa R, Fernandez A, et al. Phase I clinical trial of fixed-dose rate infusional gemcitabine and dacarbazine in the treatment of advanced soft tissue sarcoma, with assessment of gemcitabine triphosphate accumulation. Cancer 2004;101:2261–2269.
10. Lopez Pousa A, Buesa J, Maurel J, et al. Phase I/II trial of doxorubicin (DX) and dose escalation prolonged infusion gemcitabine (GMC) as first line treatment in advanced soft tissue sarcomas (STS). A study of the Spanish Group for Research in Sarcomas (GEIS) (Abstr), Chicago, IL: American Society of Clinical Oncology; 2003:A3317.
11. Morgan J, George S, Desai J, et al. Phase II study of gemcitabine/vinorelbine (GV) as first or second line chemotherapy in patients with metastatic soft tissue sarcoma (STS). J Clin Oncol 2004;22:Abstract 9009.
12. Pommier Y, Kohlagen G, Bailly C, et al. DNA sequence- and structure-selective alkylation of guanine N2 in the DNA minor groove by ecteinascidin 743, a potent antitumor compound from the Caribbean tunicate *Ecteinascidia turbinata*. Biochemistry 1996;35:13303–13309.
13. Garcia-Rocha M, Garcia-Gravalos MD, Avila J. Characterization of antimitotic products from marine organisms that disorganize the microtubule network: ecteinascidin 743, isohomohalichondrin-B and LL-15. Br J Cancer 1996;73:875–883.
14. Erba E, Bergamaschi D, Bassano L, et al. Ecteinascidin-743 (ET-743), a natural marine compound, with a unique mechanism of action. Eur J Cancer 2004;37:97–105.
15. Takebayashi Y, Pourquier P, Yoshida A, et al. Poisoning of human DNA topoisomerase I by ecteinascidin 743, an anticancer drug that selectively alkylates DNA in the minor groove. Proc Natl Acad Sci U S A. 1999;96:7196–7201.
16. Zewail-Foote M, Ven-Shun L, Kohn H, et al. The inefficiency of incisions of ecteinascidin 743-DNA adducts by the UvrABC nuclease and the unique structural feature of the DNA adducts can be used to explain the repair-dependent toxicities of this antitumor agent. Chem Biol 2004;8:1033–1049.
17. Damia G, Silvestri S, Carrassa L, et al. Unique pattern of ET-743 activity in different cellular systems with defined deficiencies in DNA-repair pathways. Int J Cancer 2001;92:583–588.
18. Bonfanti M, La Valle E, Fernandez Sousa Faro JM, et al. Effect of ecteinascidin-743 on the interaction between DNA binding proteins and DNA. Anticancer Drug Des 1999;14:179–186.
19. Li W, Takahashi N, Jhanwar S, et al. Sensitivity of soft tissue sarcoma cell lines to chemotherapeutic agents: indentification of ecteinascidin-743 as a potent cytotoxic agent. Clin Cancer Res 2001;7:2908–2911.
20. Takahashi N, Li W, Banerjee D, et al. Sequence-dependent enhancement of cytotoxicity produced by ecteinascidin 743 (ET-743) with doxorubicin or paclitaxel in soft tissue sarcoma cells. Clin Cancer Res 2001;7:3251–3257.
21. Meco D, Colombo T, Ubezio P, et al. Effective combination of Et-743 and doxorubicin in sarcoma: preclinical studies. Cancer Chemother Pharmacol 2003;52:131–138.
22. Ryan D, Supko J, Eder J, et al. Phase I and Pharmacokinetic study of ecteinascidin 743 administered as a 72-hour continuous infusion in patients with solid malignancies. Clin Cancer Res 2001;7:231–242.
23. van Kesteren C, Twelves C, Bowman A, et al. Clinical pharmacology of the novel marine-derived anticancer agent Ecteinascidin 743 administered as a 1- and 3-h infusion in a phase I study. Anticancer Drugs. 2002;13:381–393.
24. Delaloge S, Yovine A, Taamma A, et al. Ectinascidin-743: a marine-derived compound in advanced, pretreated sarcoma patients—preliminary evidence of activity. J Clin Oncol 2001;19:1248–1255.
25. Villalona-Calero M, Eckhardt S, Weiss G, et al. A phase I and pharmacokinetic study of ecteinascidin-743 on a daily x 5 schedule in patients with solid malignancies. Clin Cancer Res 2002;8:75–85.
26. Gomez J, Lopez Lazaro L, Guzman C, et al. Identification of biochemical parameters that predict the onset of severe toxicities in patients treated with ET-743 (Abstr). New Orleans, LA: American Society of Clinical Oncology; 2000:187a.
27. Puchalski T, Ryan D, Garcia-Carbonero R, et al. Pharmacokinetics of ecteinascidin 743 administered as a 24-h continuous infusion to adult patients with soft tissue sarcomas: associations with clinical characteristics, pathophysiological variables and toxicity. Cancer Chemother Pharmacol 2002;50:309–309.
28. Donald S, Verschoyle R, Greaves P, et al. Complete protection by high dose dexamethasone against the hepatotoxicity

of the novel antitumor drug ecteinascidin-743 (ET-743) in the rat. Cancer Res 2003;63:5902–5908.

29. von Mehren M, Schilder R, Cheng J, et al. Trabectedin in combination with pegylated doxorubicin (PLD) is a well tolerated regimen with good dose intensity in patients with advanced malignancies (Abstr). 29th ESMO Congress, Vienna, Austria: Elsevier; 2004:iii103.
30. Demetri GD: ET-743: the US experience in sarcomas of the soft tissues. Anti-Cancer Drugs 2002;13(suppl):S7–S9.
31. Garcia-Carbonero R, Supko J, Manola J, et al. Phase II and pharmacokinetic study of ecteinascidin 743 in patients with progressive sarcomas of soft tissues refractory to chemotherapy. J Clin Oncol 2004;22:1480–1490.
32. Yovine A, Riofrio M, Blay J, et al. Phase II study of Ecteinascidin-743 in advanced pretreated soft tissue sarcoma patients. J Clin Oncol 2004;22:890–900.
33. Lopez-Martin JA, Verweij J, Blay J, et al. An exploratory analysis of tumor growth rate (TGR) variations induced by trabectedin (ecteinascidin-743, ET-743) in patients (pts) with pretreated advanced soft tissue sarcoma (PASTS) (Abstr). Chicago, IL: American Society of Clinical Oncology; 2003:A3293.
34. Ryan D, Puchalski T, Supko J, et al. A phase II and pharmacokinetic study of ecteinascidin 743 in patients with gastrointestinal stromal tumors. Oncologist 2002;7:531–538.
35. Samuels B, Rushing D, Chawla S, et al. Randomized phase II study of trabectedin (ET-743) given by two different dosing schedules in patients (pts) with leiomyosarcomas (LMS) or liposarcomas (LPS) refractory to conventional doxorubicin and ifosfamide chemotherapy. J Clin Oncol 2004;22:A9000.
36. Schmelzle T, Hall MN. TOR, a central controller of cell growth. Cell 2000;103:253–262.
37. Brown EJ, Schreiber SL. A signaling pathway to translational control. Cell 1996;86:517–520.
38. Yu K, Toral-Barza L, Discafani C, et al. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. Endocr Relat Cancer 2001;8:249–258.
39. Neshat MS, Mellinghoff IK, Tran C, et al. Enhanced sensitivity of PTEN-deficient tumors to inhibition of FRAP/mTOR. Proc Natl Acad Sci U S A 2001;98:10314–10319.
40. Shi Y, Gera J, Hu L, et al. Enhanced sensitivity of multiple myeloma cells containing PTEN mutations to CCI-779. Cancer Res 2002;62:5027–5034.
41. Huang S, Houghton PJ. Mechanisms of resistance to rapamycins. Drug Resist Updat 2001;4:378–391.
42. Huang S, Houghton PJ. Resistance to rapamycin: a novel anticancer drug. Cancer Metastasis Rev 2001;20:69–78.
43. Dudkin L, Dilling M, Cheshire P, et al. Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. Clin Cancer Res 2001;7:1758–1764.
44. Gallicchio M, van Sinderen M, Bach L. Insulin-like growth factor binding protein-6 and CCI-779, an ester analogue of rapamycin, additively inhibit rhabdomyosarcoma growth. Horm Metab Res 2003;35:822–827.
45. Hidalgo M, Rowinsky E. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. Oncogene 2000;19:6680–6686.
46. Nielsen T, Hsu F, O'Connell J, et al. Tissue microarray validation of epidermal growth factor receptor and SALL2 in synovial sarcoma with comparison to tumors of similar histology. Am J Pathol 2003;163:1449–1456.
47. Perry A, Kunz S, Fuller C, et al. Differential NF1, p16, and EGFR patterns by interphase cytogenetics (FISH) in malignant peripheral nerve sheath tumor (MPNST) and morphologically similar spindle cell neoplasms. J Neuropathol Exp Neurol 2002;61:702–709.
48. Olofsson A, Willen H, Goransson M, et al. Abnormal expression of cell cycle regulators in FUS-CHOP carrying liposarcomas. Int J Oncol 2004;25:1349–1355.
49. Nuciforo P, Pellegrini C, Fasani R, et al. Molecular and immunohistochemical analysis of HER2/neu oncogene in synovial sarcoma. Hum Pathol 2003;34:639–645.
50. DeClue J, Heffelfinger S, Benvenuto G, et al. Epidermal growth factor receptor expression in neurofibromatosis type 1-related tumors and NF1 animal models. J Clin Invest 2000:105;1233–1241.
51. Li H, Velasco-Miguel S, Vass W, et al. Epidermal growth factor receptor signaling pathways are associated with tumorigenesis in the Nf1:p53 mouse tumor model. Cancer Res 2002;62:4507–4513.
52. Heymach J. Angiogenesis and antiangiogenic approaches to sarcomas. Curr Opin Oncol 2001;13:261–269.
53. Saenz NC, Heslin MJ, Adsay V, et al. Neovascularity and clinical outcome in high-grade extremity soft tissue sarcomas. Ann Surg Oncol 1998;5:48–53.
54. Chao C, Al-Saleem T, Brooks J, et al. Vascular endothelial growth factor and soft tissue sarcomas: tumor expression correlates with grade. Ann Surg Oncol 2001;8:260–267.
55. Yoon S, Segal N, Olshen A, et al. Circulating angiogenic factor levels correlate with extent of disease and risk of recurrence in patients with soft tissue sarcoma. Ann Oncol 2004;15:1261–1266.
56. Hashimoto M, Ohsawa M, Ohnishi A, et al. Expression of vascular endothelial growth factor and its receptor mRNA in angiosarcoma. Lab Invest 1995;73:859–863.
57. Amo Y, Masuzawa M, Hamada Y, et al. Expression of vascular endothelial growth factor in a human hemangiosarcoma cell line (ISO-HAS). Arch Dermatol Res 2001;293:296–301.
58. Hatva E, Bohling T, Jaaskelainen J, et al. Vascular growth factors and receptors in capillary hemangioblastomas and hemangiopericytomas. Am J Pathol 1996;148:763–775.
59. Verweij J, Von Oosterom A, Blay J, et al. Imatinib Mesylate is an active agent for GIST but does not yield responses in other soft tissue sarcomas that are unselected for a molecular target. Eur J Cancer 2003;39:2006–2011.
60. Chugh R, Thomas D, Wathen K, et al. Imatinib mesylate in soft tissue and bone sarcomas: Interim results of a Sarcoma

Alliance for Research thru Collaboration (SARC) phase II trial. J Clin Oncol 2004;22:Abstract 9001.

61. Simon M, Navarro M, Roux D, et al. Structural and functional analysis of a chimeric protein COL1A1-PDGFB generated by the translocation t(17;22)(q22;q13.1) in dermatofibrosarcoma protuberans (DP). Oncogene 2001;20:2965–2975.

62. Sjoblom T, Shimizu A, O'Brien K, et al. Growth inhibition of dermatofibrosarcoma protuberans tumors by the platelet-derived growth factor receptor antagonist STI571 through induction of apoptosis. Cancer Res 2001;61:5778–5783.

63. Maki R, Awan R, Dixon R, et al. Differential sensitivity to imatinib of 2 patients with metastatic sarcoma arising from dermatofibrosarcoma protuberans. Int J Cancer 2002;100:623–626.

64. Rubin BP, Schuetze SM, Eary JF, et al. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. J Clin Oncol 2002;20:3586–3591.

65. Weiner TM, Liu ET, Craven RJ, et al. Expression of growth factor receptors, the focal adhesion kinase, and other tyrosine kinases in human soft tissue tumors. Ann Surg Oncol 1994;1:18–27.

66. Franklin W, Christison W, Colley M, et al. In situ distribution of the beta-subunit of platelet-derived growth factor receptor in nonneoplastic tissue and in soft tissue tumors. Cancer Res 1990;50:6344–6348.

67. Fletcher CD, Berman JJ, Corless C, et al. Diagnosis of gastrointestinal stromal tumors: A consensus approach. Hum Pathol 2002;33:459–465.

68. Demetri G, George S, Heinrich MC, et al. Clinical activity and tolerability of the multi-targeted tyrosine kinase inhibitor SU11248 in patients (pts) with metastatic gastrointestinal stromal tumor (GIST) refractory to imatinib mesylate (Abstr). Chicago, IL: American Society of Clinical Oncology; 2003.

69. Demetri GD, Desai J, Fletcher JA, et al. SU11248, a multi-targeted tyrosine kinase inhibitor, can overcome imatinib (IM) resistance caused by diverse genomic mechanisms in patients (pts) with metastatic gastrointestinal stromal tumor (GIST) (Abstr). New Orleans, LA: American Society of Clinical Oncology; 2004:A 3001.

70. Shah N, Tran C, Lee F, et al. Overriding imatinib resistance with a novel ABL kinase inhibitor. Science 2004;305:399–401.

71. Sawyers C, Shah N, Kantarjian H, et al. Hematologic and cytogenetic responses in imatinib-resistant chronic phase chronic myelogenous leukemia patients treated with the dual SRC/ABL kinase inhibitor BMS-354835: results from a phase I dose escalation study (Abstr). San Diego, CA: American Society of Hematology; 2004:A1.

72. Takahashi R, Tanaka S, Kitadai Y, et al. Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. Oncology 2003;64:266–274.

73. Van Oosterom A, Dumez H, Desai J, et al. Combination signal transduction inhibition: a phase I/II trial of the oral mTOR-inhibitor everolimus (E, RAD001) and imatinib mesylate (IM) in patients (pts) with gastrointestinal stromal tumor (GIST) refractory to IM (Abstr). In: ASCO Annual Meeting Proceedings. New Orleans, LA: ASCO; 2004:3002.

74. Reichardt P, Pink P, Lindner T, et al. A phase I/II trial of the oral PKC inhibitor PKC412 and imatinib mesylate in patients with gastrointestinal stromal tumors (GIST) refractory to imatinib (IM) (Abstr). Vienna, Austria: Eur Soc Med Oncol; 2004.

75. Taamma A, Misset J, Riofrio M, et al. Phase I and pharmacokinetic study of Ecteinascidin-743, a new marine compound, administered as a 24-hour continuous infusion in patients with solid tumors. J Clin Oncol 2001;19:1256–1265.