

Ewing's sarcoma

Naomi J Balamuth, Richard B Womer

Lancet Oncol 2010; 11: 184–92

Division of Oncology and
Center for Childhood Cancer
Research, Children's Hospital of
Philadelphia; Department of
Pediatrics, University of
Pennsylvania School of
Medicine, Philadelphia, PA,
USA (N J Balamuth MD,
R B Womer MD)

Correspondence to:
Dr Richard B Womer, Division of
Oncology, 4303 Wood Building,
Children's Hospital of
Philadelphia, 3516 Civic Center
Boulevard, Philadelphia,
PA 19104, USA
rwomer@mail.med.upenn.edu

Progress in the treatment of Ewing's sarcoma, the second most common bone tumour in children and adolescents, has improved survival from about 10% in the period before chemotherapy was introduced to about 75% today for patients with localised tumours. However, patients with metastases still fare badly, and the therapy carries short-term and long-term toxicities. Multidisciplinary care is indispensable for these patients. Molecular techniques and new imaging modalities are affecting the diagnosis and classification of patients with Ewing's sarcoma. Cooperative group studies have led to chemotherapy regimens using the same drugs (vincristine, doxorubicin, cyclophosphamide, ifosfamide, and etoposide), although the exact regimens differ in Europe and North America. The EWS–ETS family of gene fusions and their downstream effects in Ewing's sarcomas provide opportunities for new approaches to treatment. These include the inhibition of the fusion gene or its protein product, and pathways related to IGF1 and mTOR. Inhibition of tyrosine kinases, exploitation of non-apoptotic cell death, and interference with angiogenesis are promising new approaches. With many new approaches and relatively few patients, it will be challenging to integrate new and established treatments through clinical trials.

Introduction

Some time in the future a teenager with a destructive bone lesion will be referred to a paediatric oncology centre, where he will have metabolic and anatomical imaging (perhaps a PET or CT and MRI, but perhaps more sophisticated) revealing the precise location and anatomical relation of the tumour and any metastases. A needle biopsy of the most metabolically active part of the tumour will provide tissue for histological and molecular diagnosis, and amplification techniques will provide abundant material for research. A multidisciplinary team will begin treatment using sophisticated algorithms and a combination of cytotoxic chemotherapy and agents directed against the biological features of the tumour. After one cycle, new imaging will reveal the tumour's response (figure), and the physicians will adjust therapy appropriately. After completing systemic and local therapy with only mild acute and late side-effects, the patient will enjoy almost assured survival.

Although such management is partly fantasy now, our deepening understanding of Ewing's sarcoma biology and increasingly sophisticated tools might herald rapid

changes. We review the current state of Ewing's sarcoma diagnosis and therapy, what new approaches are in development, and how we might get from the present to the future.

The Ewing's sarcoma family of tumours (ESFT) is an aggressive form of childhood cancer, which include classic Ewing's sarcoma, Askin tumour, and peripheral primitive neuroectodermal tumour. While significant progress has been made in the diagnosis and treatment of localised disease over the past 30 years, there is much room for improvement. Before chemotherapy was introduced, about 10% of patients with Ewing's sarcoma survived.¹ Progress since then has been dramatic, with 75% of patients with localised tumours now surviving. However, we calculate that only 55% of patients are receiving therapy that is appropriate (panel). The others are either receiving therapy that is ineffective or unnecessary. Current approaches also have many short-term toxicities, and leave survivors at risk for serious late effects.

Epidemiology

Primary bone tumours account for 5% of all child and adolescent cancers, and ESFT is the second most common primary bone tumour. Between 1973 and 2004, the incidence of Ewing's sarcoma in the US was 2·93 per 100000.² Ewing's sarcoma is much more common in white populations, and has a slight male predominance.³ About a quarter of Ewing's sarcomas arise in soft tissues rather than bone, and about a quarter of patients have detectable metastases at diagnosis. The lungs are the most common site for metastases (50%), followed by bone (25%) and bone marrow (20%).⁴

Diagnosis, imaging, and staging

Most patients with Ewing's sarcoma present with tumour-related symptoms, such as pain or a mass. The first step in the assessment phase should be imaging of the suspected tumour, preferably by MRI, encompassing the entire involved bone or compartment, and before the occurrence of bleeding and oedema from biopsy.

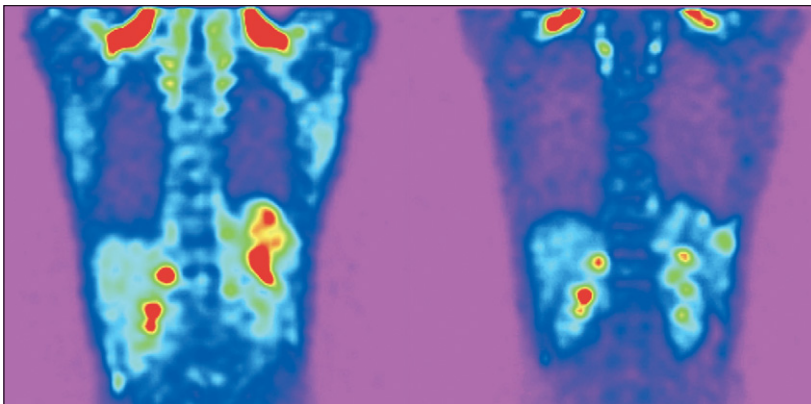


Figure: [¹⁸F]FDG-PET images of a patient with recurrent Ewing's sarcoma in the left lower pleural space, before (left) and after (right) treatment with two cycles of an anti-IGF-1R monoclonal antibody. Bilateral signal from the kidneys and brown fat (in the clavicular regions) is also apparent.

If possible, the surgeon who will do the local control surgery should do or participate in the biopsy. This enables expert placement of the biopsy tract and maintenance of tissue planes, to preserve subsequent reconstructive options. Closed, imaging-guided core biopsies are gradually supplanting the open biopsy, but require particular care and close cooperation between the surgeon and the pathologist. Ewing's sarcomas often have extensive necrosis, and there must be sufficient tissue for immunohistochemical and molecular diagnostic techniques. Thus, several cores are usually necessary, and frozen-section confirmation that a specimen contains adequate viable tumour is important. The biopsy site should be marked indelibly (eg, with a tattoo or incision) so that it can be included in the volume later excised or irradiated.

Following pathological confirmation of an ESFT, a complete staging assessment is needed. Traditionally, this is a CT scan of the chest to detect pulmonary metastases, bone scintigraphy to detect bony metastases, and bone marrow aspirate and biopsy. Each of these techniques has limited sensitivity and specificity and is constantly evolving, so clear criteria for clinical trials are needed, and comparisons of trials require careful comparisons of criteria. Interestingly, as the sensitivity of diagnostic imaging has improved over the past 30 years, the fraction of patients with detectable metastases at diagnosis has remained fairly stable at 20–25%.

Molecular diagnosis and staging

Identification of the characteristic t(11;22) chromosomal translocation and resulting *EWS-FLI1* gene fusion⁵ has had a considerable effect on diagnosis. The *EWS* gene on chromosome 22 encodes a widely-expressed and highly-conserved RNA-binding protein. In 85% of ESFTs, the amino terminus of *EWS* is fused with the DNA-binding domain of *FLI1*, a member of the ETS (erythroblastosis transforming-virus-1) family of transcription factors, which are involved in cellular proliferation, development, and tumorigenesis. ESFTs that lack an *EWS-FLI1* fusion usually have *EWS* joined to another member of the ETS family, such as *ERG*, *ETV1*, or *E1AF*.

Molecular diagnosis, using either fluorescent in-situ hybridisation (FISH) to detect the fusion gene, or reverse transcriptase PCR (RT-PCR) to detect its transcript, is now a routine part of pathological examination. Although retrospective studies have indicated that the specific exons involved in the *EWS-FLI1* fusion have a significant effect on prognosis, a recent large prospective European study showed no effect.^{6,7}

In addition to aiding in diagnosis, RT-PCR enables the detection of sub-microscopic metastatic disease in the blood (circulating tumour cells) or bone marrow. While detectable sub-microscopic tumour in the blood or marrow conferred a poor prognosis in a study of 172 patients with Ewing's sarcoma, the effect was not statistically separable from the effects of proximal site and large tumour size.⁸

Panel: Room for improvement in Ewing's sarcoma treatment

- 100 patients newly diagnosed with Ewing's sarcoma
 - 80 with localised tumours—60 survive (75%)
 - 20 with metastases
 - Five with lung metastases only—two survive (40%)
 - 15 with other metastases—one survives (6%)
- Total survivors: 63
 - Eight patients with localised tumours would have survived without chemotherapy
- Total patients treated appropriately: 55

Patterns of gene expression might contribute to future classification and treatment assignment. At least three groups have done retrospective microarray studies using tumour material from 20–30 patients, and identified gene-expression patterns associated with treatment response, metastasis, or prognosis.^{9–11} Although microarray analysis is not practical for routine clinical use, these results might lead to the identification of simpler genetic or even immunohistochemical markers.

Imaging

FDG-PET, whole body MRI, and spiral CT are widely used, and their effect on patient classification and prognosis will take time to assess. MRI is the current standard of care for primary tumour assessment. Spiral CT is more sensitive than traditional thin cut CT for the detection of metastases.¹² Whole body MRI is sensitive for the detection of metastases, although its use has been limited (particularly in paediatrics) by the time required. A small pilot study showed that rapid whole-body MRI with echoplanar and turbo short T1 inversion-recovery sequences was equivalent to conventional studies (ultrasound, CT, MRI, and bone scan) for the detection of metastases in children with sarcomas.¹³ Another group examined 46 paediatric sarcoma patients (23 with Ewing's sarcoma), and found that whole-body FDG-PET and conventional imaging were equivalent in the detection of the primary tumour, but PET was better at detecting lymph-node involvement and bony metastases. PET was not as good as CT for detecting pulmonary metastases.¹⁴

Risk groups

There is currently no internationally recognised risk classification scheme for patients with Ewing's sarcoma. The first clinical trials did not classify them, since the prognosis was uniformly poor. Early chemotherapy trials found that patients with pelvic primary sites or metastases at diagnosis had a higher risk of relapse and death than others. Cooperative group and single-institution studies have also identified patient sex and age, tumour size (with varied criteria), site, fever, serum lactate dehydrogenase concentration, anaemia, histological response to initial chemotherapy, and many treatment

variables as prognostic factors, but they vary widely between studies. This is because different studies use different criteria (eg, a large tumour might be defined as more than 100 mL, 200 mL, or 8 cm), and because the treatment used affects prognostic factors.

In the Children's Oncology Group studies in North America, there are three risk groups: patients with localised tumours, patients with lung metastases only, and patients with other or multiple metastases. The EuroEWING-99 study has a more complex scheme that uses metastatic status (none, lung, or other) and initial tumour size (with a 200 mL cutoff), and subsequently considers resectability and histological response to initial chemotherapy (for resected tumours) to assign patients to treatments or randomisations.

Cytotoxic chemotherapy

The use of chemotherapy has greatly improved survival rates for patients with localised ESFT, from about 10% to 70–80%. Unfortunately, it has had much less effect on the survival of patients with metastases at diagnosis.

Chemotherapy for Ewing's sarcoma began in the 1960s, with single-agent cyclophosphamide, dactinomycin, doxorubicin, vincristine, and carmustine, followed by single-arm multi-agent adjuvant chemotherapy trials using vincristine–actinomycin–cyclophosphamide (VAC) or VAC plus doxorubicin.^{1,15} The first North American randomised study (Intergroup Ewing's Sarcoma Study [IESS-I]; 1973–78) showed that VAC plus doxorubicin was better than VAC plus chest irradiation, which in turn was better than VAC alone for patients with localised, non-pelvic primary tumours.¹⁶ In the second IESS study (IESS-II, 1978–82) higher doses of doxorubicin earlier in therapy improved on the IESS-I regimen (overall survival 77% vs 56%).¹⁷

The subsequent Children's Cancer Group-Pediatric Oncology Group (CCG-POG) cooperative study (INT-0091, 1988–92) showed that ifosfamide and etoposide (IE), alternating with the standard regimen of vincristine, doxorubicin, cyclophosphamide (VDC), and dactinomycin markedly improved both overall and event-free survival (69% vs 54%, $p=0.005$, and 72% vs 61%, $p=0.01$, respectively) for patients with localised tumours.¹⁸ Curiously, it was a marked decrease in local (rather than metastatic) relapse that led to the improvement in outcome.

The most recent North American trials examined dose intensification. One can increase dose intensity either by increasing the doses, or decreasing the interval between doses. The first study (INT-0154, 1995–98) used the VDC–IE regimen from INT-0091 as the control; the experimental regimen increased alkylating agent dose intensity by increasing cyclophosphamide and ifosfamide doses, decreasing the length of treatment from 17 cycles in 48 weeks to 11 cycles in 30 weeks to maintain total doses. The experimental regimen was more toxic but no more effective.¹⁹ Most recently, a Children's Oncology Group (COG) study (AEWS0031, 2001–2005) compared

VDC–IE treatment every 2 weeks with VDC–IE treatment every 3 weeks for patients with localised disease, with 14 cycles and equal cumulative doses in each group. Interval compression provided a 25% increase in dose intensity of all agents without an increase in toxicity. Overall and event-free survival were both improved in the interval-compressed group (event-free survival 79% vs 70% at 4 years, $p=0.023$).²⁰ The regimen of alternating VDC–IE every 2 weeks has become standard for North American patients with Ewing's sarcoma.

A different approach evolved among the European cooperative groups, through independent single-group studies by the UK Children's Cancer Study Group (UKCCSG) and the German–Dutch–Swiss Cooperative Ewing's Sarcoma Studies (CESS). The CESS classified patients with localised tumours with radiographically determined volumes of 100 or 200 mL (depending on the study) as standard risk, and those with larger tumours or metastases as high risk. They also identified a poor histological response to initial chemotherapy as a poor prognostic factor.²¹ Both the CESS and UKCCSG adopted a chemotherapy design in which four drugs are given at once, and this evolved from VACA (vincristine–doxorubicin–cyclophosphamide–actinomycin), to VAIA (substituting ifosfamide for cyclophosphamide), to EVAIA (adding etoposide), to the current VIDE (omitting actinomycin). The only randomised controlled trial in this series, EICESS-92, found no difference between VACA and VAIA for standard risk patients with Ewing's sarcoma, and a slight advantage (although statistically insignificant) for EVAIA over VAIA in patients with high-risk localised or metastatic tumours.²²

The current Euro-EWING-99 study uses VIDE as initial chemotherapy for all patients. In a complex scheme, it compares VAC (vincristine–actinomycin–cyclophosphamide) with VAI as continuing chemotherapy for patients with good histological responses to VIDE, or small (<200 mL) tumours treated with radiation. For patients with poor histological responses, or large tumours treated with radiation, or lung metastases, it compares VAI with busulfan–melphalan megatherapy.²³ Outcome data are not yet available from this ongoing trial.

New chemotherapeutic approaches under study in the COG include adding topotecan–cyclophosphamide to VDC–IE, based on phase 1 and phase 2 data.^{24,25} Results of a COG phase 1 trial of the combination of vincristine, oral irinotecan, and temozolomide (VOIT) are pending.

Metastatic disease and megatherapy

There has not been similar progress in the treatment of patients with metastases. The addition of ifosfamide–etoposide to vincristine–doxorubicin–cyclophosphamide in the INT-0091 study did not improve the outcome for patients with metastases.¹⁸ Increasing the doses of doxorubicin, cyclophosphamide, and ifosfamide by 20%, 83%, and 56%, respectively, in regimen C of the same protocol also produced no improvement, and greatly

increased acute toxicity and the incidence of secondary leukaemia and myelodysplasia.²⁶

Patients with metastases outside the lungs at diagnosis seldom survive, and this has led to several studies using megatherapy (myeloablative high-dose chemotherapy with or without total-body irradiation, then rescue with marrow or peripheral haematopoietic stem cells). In a prospective Children's Cancer Group study of 36 patients with bone or marrow metastases at diagnosis, high-dose melphalan, etoposide and total-body irradiation did not improve outcomes over those obtained with conventional chemotherapy, with a 20% 2-year event-free survival.²⁷ A prospective French study of megatherapy with busulfan, melphalan, and autologous marrow or stem cells produced almost identical results: of 45 patients with bone or marrow metastases, there were 37 events, or 18% event-free survival.²⁸ EICESS enrolled 17 patients with bone, marrow, or other extra-pulmonary metastases, in a study of megatherapy with TBI, melphalan, and etoposide and either allogeneic (four patients) or autologous marrow or stem cells; there was only one event-free survivor.²⁹ A subsequent study used two sequential (tandem) transplants with high-dose melphalan and etoposide; there were four event-free survivors among 17 patients, which is a statistically insignificant improvement.³⁰

The ongoing European EuroEWING-99 trial has the first randomised test of megatherapy in patients with ESFT. Patients with localised tumours and a poor response to initial VIDE chemotherapy, or with lung metastases at diagnosis, are randomly assigned to either further chemotherapy (vincristine, dactinomycin and ifosfamide) or busulfan–melphalan megatherapy with autologous stem cells. In a few years we might have a first answer to the long-running controversy about the role of megatherapy in Ewing's sarcoma.

The current standard of care in North America remains VDC–IE, but cure rates are dismal. The COG recently completed a study adding metronomic anti-angiogenic therapy with vinblastine and celecoxib to this backbone; results are pending. New strategies for this difficult population are clearly needed.

Survival after relapse of Ewing's sarcoma is also poor, with only about 10% of patients event free at 5 years,^{31,32} sparking similar controversy over the use of megatherapy. In a single-institution series of 55 patients, eight of 13 patients who received high-dose therapy with autologous stem cell support were long-term survivors; however, those 13 patients were highly selected (a quarter of the 55 original relapsed patients, and only half of the patients who had a good response to retrieval chemotherapy).³³ In the EICESS series, 19 patients were treated with high-dose chemotherapy or chemotherapy with TBI, and either allogeneic marrow (six patients) or autologous marrow or stem cells. Seven patients remained in remission, while nine died of disease and three of complications.²⁹ Very few patients with relapsed

ESFT achieve a second remission and are eligible for megatherapy, and the benefit of megatherapy for them is uncertain.

Primary tumour treatment

Though Ewing's sarcomas are considered radiation-sensitive, the proportion of patients whose primary tumours are treated with radiation alone has steadily declined over the past 30 years. This is because of advances in orthopaedic surgery and a growing awareness of the late effects of radiation in children, particularly second malignancies and growth disturbances. Patients whose primary tumours are excised might survive more often, although the prognostic influences of site and size complicate the analyses.³⁴

Pelvic sites are particularly controversial, as excision can be very difficult. An analysis of 75 patients with pelvic tumours in INT-0091 showed that local relapse occurred in 25% of patients treated with surgery or radiation alone, and only 10·5% of patients treated with surgery and radiation, but the difference was not statistically significant ($p=0\cdot46$). Event-free survival was also not significantly affected.³⁵ However, among 56 patients treated at a single Italian institution over 25 years, there was a significant benefit of surgery in event-free survival (74% vs 30%; $p=0\cdot036$).³⁶ A notable difference between these two series is that larger tumours tended to be treated with surgery in INT-0091, and tended to be treated with radiation alone in the Italian series.

The INT-0091 study showed that systemic chemotherapy can have a large effect on local relapse,⁴ making comparisons between studies using different regimens difficult. The advent of proton radiation therapy will likely change the management of tumours adjacent to radiation-sensitive organs (such as the spinal cord). The issue of primary tumour treatment is not likely to be settled soon.

Biologically based approaches to treatment

Conventional cytotoxic chemotherapy is ineffective in a quarter of patients with localised tumours, and three-quarters of patients with metastases. The growing understanding of Ewing's sarcoma biology raises the hope that new more selective, effective, and less toxic agents will be developed. We will consider new and potential approaches beginning with the EWS-ETS fusion, then moving to the cell surface and working toward the nucleus, finishing with angiogenesis.

The EWS-ETS family fusion

Induced expression of EWS–FLI1 in human mesenchymal stem cells results in colonies with Ewing's sarcoma-like morphology in vitro, and with patterns of immuno-histochemical staining (including CD-99) and gene expression resembling those found in Ewing's sarcoma.³⁷ The Ewing's sarcoma gene signature includes genes associated with integrin, WNT, IGF, EGF, PDGF, and FGF

signalling; angiogenesis (including VEGF); and other mechanisms associated with metastasis and chemotherapy resistance.⁴¹ The EWS–FLI1 fusion protein seems to act both through DNA binding as a transcription factor, and by mechanisms not involving DNA binding. The unique fusion gene, its transcript and protein product, and the pathways it activates all provide opportunities for therapy.

Inhibition of the fusion product

Anti-sense cDNA against *EWS–FLI1* decreases endogenous EWS–FLI1 expression and inhibits growth in Ewing's sarcoma cell lines.³⁸ RNA interference with small interfering (si)RNA against *EWS–FLI1* decreases the invasiveness of EWS cells in culture, and causes an overall decrease in cell proliferation.³⁹ siRNA has been difficult to harness therapeutically, since no clinically useful delivery system yet exists.

Cytarabine inhibits the growth of Ewing's sarcoma cells in vitro and in xenografts by reducing the expression of the EWS–FLI1 fusion gene and its protein product. Unfortunately, in a phase 2 COG study, none of ten patients responded, and only one had transient stabilisation of tumour.⁴⁰

RNA helicase A

RNA helicase A (RHA) is a member of a protein complex with a crucial role in transcription, splicing, and mRNA translation. RHA is expressed in Ewing's cell lines as well as tumours, and cooperates with EWS–FLI1 in oncogenic transformation through a unique protein–protein interaction. A small molecule targeting the RHA-binding site on the EWS–FLI1 protein disrupted this interaction, slowed growth, and increased apoptosis in ESFT cells but not other malignant or normal cell lines in vitro. The molecule also inhibited xenograft growth and increased apoptosis in immunodeficient mice, without apparent toxicity, and with further development could become a treatment for Ewing's sarcoma.⁴¹

Unfortunately, the small number of patients with Ewing's sarcoma do not provide pharmaceutical companies with a market to balance the costs of developing and licensing specific inhibitors of EWS–FLI1. Improved understanding of the cellular mechanisms affected by the fusion proteins might be more attractive, as these pathways are probably shared with more common cancer types.

Insulin-like growth factors and the type 1 receptor

There is convincing evidence that the insulin-like growth factors (IGFs) have roles in human cancers, and that interfering with this system could be therapeutically useful.⁴² Ligand binding activates the type 1 IGF receptor (IGF-1R) through an intracellular tyrosine kinase domain, which leads to activation of the phosphatidylinositol 3 (PI3) kinase and mitogen-activated protein kinase (MAPK) pathways, and ultimately to cell proliferation, protection from apoptosis, invasion, and metastases.

The IGF system is a particularly attractive target in Ewing's sarcoma. Similar to some other solid tumours, Ewing's sarcoma cell lines and tumours express IGF-1R and produce IGF1, causing autocrine stimulation. Antibody blockade of the receptor inhibits growth, cell migration, and soft agar colony formation in vitro.⁴³

The interaction of IGF with its receptor is mediated in vivo by IGF binding proteins, particularly IGF binding protein 3 (IGFBP3). In Ewing's sarcoma, the EWS–FLI1 fusion protein binds to the IGFBP3 promoter and represses its activity.⁴⁴ Exogenous IGFBP3 inhibits Ewing's sarcoma cell growth and prevents metastasis in mouse xenografts, indicating that therapeutic use might be possible.⁴⁵

Monoclonal antibodies against the IGF-1R that block ligand binding are being examined as single agents and in combination with cytotoxic chemotherapy. In adult humans, the anti-IGF1R monoclonal antibody IMC-A12 was well tolerated in a phase 1 study, with hyperglycaemia the most significant adverse event.⁴⁶ These antibodies seem particularly promising in ESFT: in a recent phase 1 study of figitumumab, one of 16 patients had a complete response (ongoing at 21.5 months), one had a partial response (ongoing at 11.4 months), and six had stable disease lasting from 4 to 16 months.⁴⁷ Clinical trials of other, similar antibodies are in progress.

Combining an anti-IGF-1R antibody with doxorubicin or vincristine synergistically inhibits the growth of Ewing's sarcoma cell lines in vitro, and decreases their ability to form colonies in soft agar compared with chemotherapy alone.⁴⁸ Clinical trials combining antibodies and chemotherapy in patients with Ewing's sarcoma are in development.

In addition to antibodies targeting the receptor, small-molecule inhibitors of the IGF-1R tyrosine kinase are appealing because they seem to have favourable pharmacokinetics and oral bioavailability. Preclinical use in murine ESFT xenografts has produced tumour shrinkage and inhibition of vasculogenesis, invasion, and metastasis.⁴⁹

Small-molecule tyrosine kinase inhibitors

In addition to the IGFs, Ewing's sarcoma cell lines and primary tumours express the tyrosine kinase c-KIT;⁵⁰ its ligand, stem cell factor (SCF); and platelet-derived growth factor (PDGF) and its receptor. Imatinib, a small molecule designed to inhibit the BCR–ABL tyrosine kinase, also inhibits the c-KIT tyrosine kinase and the platelet-derived growth factor receptor (PDGFR). Although Ewing's tumours do not have transforming c-KIT mutations, work in vitro and in vivo has shown decreased proliferation following treatment with imatinib, albeit with much higher IC₅₀ values than are needed for chronic myeloid leukaemia or gastrointestinal stromal tumours, raising the possibility that imatinib is acting on other tyrosine kinases than c-KIT and PDGFR.⁵⁰ A subsequent COG phase 2 study of imatinib produced only one partial response among 24 evaluable patients with ESFT.⁵¹

VEGFR, PDGFR, and c-KIT are also inhibited by sunitinib, another orally available tyrosine kinase inhibitor that is approved in adults for the treatment of metastatic renal-cell carcinoma. The COG is running a phase 1 trial of this drug for patients with refractory solid tumours.

The RAS pathway is crucial to signal transduction and cell growth, and it is activated in many human cancers.⁵² RAF, a downstream target of RAS, is also mutated in various solid tumours.⁵³ The COG has a phase 1 trial of sorafenib, an inhibitor of RAF kinase (and other tyrosine kinases, including VEGFR2 and c-KIT) for children with refractory malignancies, including Ewing's sarcoma.

Src family kinases (SFKs) mediate signal transduction from a variety of different receptors, including epidermal growth factor receptor, PDGFR, HER2, and fibroblast growth factor receptor. Each of these receptors is important in cellular proliferation, adhesion, and invasion. LYN is a member of the SFK family, and EWS-FLI1 upregulates LYN expression.⁵⁴ Downregulation of LYN by siRNA or small-molecule kinase inhibition has been shown to inhibit ESFT growth in murine models and in vitro.

Rapamycin and analogues

Targeting downstream elements common to several signalling pathways, such as mammalian target of rapamycin (mTOR), might be a fruitful approach to therapy.⁵⁵ The macrolide rapamycin inhibits the kinase activity of mTOR by altering the activity of a ribosomal protein. The inhibition of mTOR inhibits pathways that lead to the translation of mRNAs required for progression from G1 to S phase. Rapamycin has been shown to inhibit the proliferation of Ewing's sarcoma cell lines by causing cell-cycle arrest and downregulating the EWS-FLI1 protein.⁵⁶ In xenograft models, combined therapy using rapamycin and EWS-FLI1 antisense oligonucleotides delayed tumour growth more than either agent alone.⁵⁷ Clinical trials using rapamycin alone, in combination with chemotherapy, or in combination with anti-IGF-1R antibodies, are both underway and planned.

Repair or bypass of apoptosis

Most cancer therapies (apart from excision or physical destruction) rely on apoptosis, and apoptotic defects probably underlie much chemotherapy and radiation resistance. In Ewing's sarcoma, mutations in both P53 and P16 are associated with a much worse prognosis.⁵⁸ Small-molecule approaches to restoring the function of mutant P53 are promising, but are still in very early stages of development.⁵⁹ Agents that cause non-apoptotic cell death also hold promise; for example, rapamycin (and analogues) and temozolomide might function by causing autophagy rather than apoptosis.⁶⁰

CD99 is a 32 kDa integral membrane protein that is highly expressed on haematopoietic cells and Ewing's sarcoma cells. In the haematopoietic system, CD99 seems involved in both cell adhesion and cell death. The binding of an activating monoclonal antibody to CD99 in

Ewing's sarcoma cells leads to caspase-independent apoptosis both in vitro and in vivo.^{61,62} Xenograft-bearing mice treated with the combination of anti-CD99 antibody and doxorubicin achieved significant tumour growth inhibition, including some complete responses. Additionally, the same group found a dose-dependent decrease in bony metastases in mice treated with the CD99 antibody as a single agent.⁶³

Fenretinide

Retinoic acid induces differentiation in several cancers, including neuroblastoma⁶⁴ and acute promyelocytic leukemia.⁶⁵ In ESFT, retinoic acid does not cause differentiation in vitro.⁶⁶ Fenretinide, a synthetic derivative of retinoic acid, causes apoptotic cell death. The exact mechanism of action is unclear, but it might involve the generation of reactive oxygen species. As in other solid tumours, fenretinide induces cell death in ESFT in vitro, and decreases tumour growth in murine xenograft models.^{67,68} In fact, cells are less sensitive to fenretinide following knockdown of EWS-FLI1, suggesting that the presence of the EWS-FLI1 translocation improves the response.⁶⁷ A major problem with fenretinide has been erratic oral bioavailability, but new formulations are being studied in paediatric neuroblastoma.⁶⁹

Angiogenesis

A large body of work has shown that solid tumour progression requires a newly formed vascular supply.⁷⁰ The vascular endothelial growth factors (VEGFs) are crucial mediators of angiogenesis, and interference with the VEGF pathway is a rational therapeutic target, particularly in ESFT.⁷¹ In a study of ESFT primary tumour samples taken from 40 patients, 10-year relapse-free and overall survival were higher when tumours had high VEGF-A expression (60% vs 29%, $p=0.02$ and 65% vs 25%, $p=0.01$, respectively). Multivariate analysis revealed that high VEGF-A expression was an independent predictor of survival, perhaps because VEGF-A-rich tumours have a denser and more permeable vasculature, enhancing chemotherapy delivery.⁷²

VEGF receptor tyrosine kinase inhibitors (eg, SU6668 and SU5416) and VEGF blockade agents (eg, bevacizumab and VEGF-Trap) have been shown to delay the growth of Ewing's sarcoma xenografts in mice.⁷¹ Unfortunately, combining bevacizumab with chemotherapy in humans might be problematic: a phase 2 study combining doxorubicin and bevacizumab in patients with metastatic soft-tissue sarcomas produced greater than expected cardiac toxicity.⁷³

Another anti-angiogenic strategy is metronomic chemotherapy, the chronic administration of anti-angiogenic therapy and low-dose chemotherapy. This approach was shown to be feasible in 20 paediatric patients with advanced cancer treated with thalidomide and celecoxib alternating with oral etoposide and cyclophosphamide every 21 days. 40% of the patients

completed the prescribed 6 months of therapy without toxicity or disease progression, and 16% had partial radiological responses.⁷⁴

From here to there

Bringing new diagnostic and therapeutic approaches to bear on Ewing's sarcoma will be neither easy nor neat. Progress in pathology and imaging have rarely waited for randomised controlled trials. Indeed, current techniques (radionuclide bone scans and chest CT scans) have never been re-assessed as their sensitivity has increased. That is unlikely to change, so we need to collect data and specimens carefully for retrospective assessment of new techniques.

Individual anti-cancer agents have been, and will continue to be, assessed in phase 1 and phase 2 clinical trials sponsored by governments (eg, the National Cancer Institute [NCI] in the US), charities (eg, Cancer Research UK), or pharmaceutical companies. The NCI-sponsored Pediatric Preclinical Testing Program (PPTP) has five Ewing's sarcoma xenografts in its panel, and might be useful for screening agents and combinations, though more experience correlating PPTP and phase 2 data are necessary. These mechanisms generally work well but have limited capacity, and it can be difficult to organise trials of combinations involving competing pharmaceutical companies.

Things get difficult in phase 3 trials. Ewing's sarcomas are rare, and improved survival decreases the events that provide power for randomised controlled trials. Most patients with metastases at diagnosis have events, but are even scarcer. One response is larger (ie, international) randomised controlled trials, such as the R2pulm component of the current EuroEWING-99 study. However, these are difficult to organise and maintain, with the myriad national and international research, funding, and regulatory agencies involved. This approach also greatly limits the number of ideas that are tested.

Changes in statistical approaches might make phase 3 randomised controlled trials in North America or Europe alone more practical. Two-tailed statistical tests are traditional, but usually one will add or intensify therapy only if it results in an improvement. About a third fewer patients are necessary using one-tailed tests. The traditional *p* value of less than 0.05 criterion for statistical significance has also outlived its usefulness. Two COG statisticians have modelled 25-year series of randomised controlled trials, varying the number of trials done, accrual, and the type 1 error. They showed that the probability of progress over 25 years increased as type 1 error increased and power decreased. For example, setting a type 1 error probability as high as 0.35 can cut accrual time in half, yet increase the expected gain in survival from 8% to 12% compared with the traditional approach over a 25-year series of trials, with less than a 10% chance of doing worse.⁷⁵ For some situations, Bayesian rather than traditional frequentist statistical designs might be more appropriate.

Search strategy and selection criteria

Data for this Review were identified by searches of Medline and PubMed, using the search terms "Ewing (and Ewing's) sarcoma", "PNET", and "pediatric sarcoma"; as well as references from relevant articles. We searched the same databases for articles written by leading investigators in the field. Initially we searched from 1997 onwards, but went back further to research specific areas. Abstracts and reports from meetings were included only when they related to previously published work. Only papers published in English were included.

The attitudes of clinical investigators might pose another challenge. Someday, improving therapy might involve substituting a new agent for doxorubicin; but risk aversion may make such a study difficult to launch. Courage to explore new therapies, and an acceptance of the attendant risk, will be important.

Conclusion

Our rapidly growing understanding of ESFT biology will lead to a growing arsenal of diagnostic and staging technologies and medicines; the challenge for clinical trials will be integrating them into our current protocols for assessment and treatment. Since the number of patients with Ewing's sarcoma is limited, such integration will require new statistical and study design strategies to be adopted, careful further use of international studies, further collaboration between industry and clinical cooperative groups, and a willingness to accept risk.

Contributors

NJB and RW contributed equally to searching and reviewing the literature and writing the manuscript. RW was responsible for revisions.

Conflicts of interest

The authors declared no conflicts of interest.

Acknowledgments

NJB was supported in part by the Hyundai Hope on Wheels Clinical Scholars Programme.

References

- 1 Rosen G, Wollner N, Tan C, et al. Disease-free survival in children with Ewing's sarcoma treated with radiation therapy and adjuvant four-drug sequential chemotherapy. *Cancer* 1974; **33**: 384–93.
- 2 Esiashvili N, Goodman M, Marcus R. Changes in incidence and survival of Ewing sarcoma patients over the past 3 decades: Surveillance Epidemiology and End Results data. *J Pediatr Hematol Oncol* 2008; **30**: 425–30.
- 3 Parkin D, Stiller C, Nectoux J. International variations in the incidence of childhood bone tumors. *Int J Cancer* 1993; **53**: 371–76.
- 4 Grier HE. The Ewing family of tumors: Ewing's sarcoma and primitive neuroectodermal tumors. *Pediatr Clin North Am* 1997; **44**: 991–1004.
- 5 Delattre O, Zucman J, Plougastel B, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumors. *Nature* 1992; **359**: 162–65.
- 6 de Alava E, Kawai A, Healey JH, et al. EWS-FLI1 fusion transcript structure is an independent determinant of prognosis in Ewing's sarcoma. *J Clin Oncol* 1998; **16**: 1248–55.
- 7 Kovar H, Le Deley M, Delattre O, et al. Impact of EWS-FLI1 fusion type on disease progression in Ewing's sarcoma: prospective results from the cooperative Euro-E.W.I.N.G.99 trial. *Proc Annu Meet Am Assoc Cancer Res* 2009; abstr 5680.

- 8 Schliermacher G, Peter M, Oberlin O, et al. Increased risk of systemic relapses associated with bone marrow micrometastasis and circulating tumor cells in localized Ewing tumor. *J Clin Oncol* 2003; 21: 85–91.
- 9 Scotlandi K, Remondini D, Castellani G, et al. Overcoming resistance to conventional drugs in Ewing sarcoma and identification of molecular predictors of outcome. *J Clin Oncol* 2009; 27: 2209–16.
- 10 Ohali A, Avigad S, Zaizov R, et al. Prediction of high risk Ewing's sarcoma by gene expression profiling. *Oncogene* 2004; 23: 8997–9006.
- 11 Schaefer K, Eisenacher M, Braun Y, et al. Microarray analysis of Ewing's sarcoma family of tumours reveals characteristic gene expression signatures associated with metastasis and resistance to chemotherapy. *Eur J Cancer* 2008; 44: 699–709.
- 12 Meyer J, Nadel H, Marina N, et al. Imaging guidelines for children with Ewing sarcoma and osteosarcoma: a report from the Children's Oncology Group Bone Tumor Committee. *Pediatr Blood Cancer* 2008; 51: 163–70.
- 13 Mazumdar A, Siegel M, Narra V, Luchtman-Jones L. Whole-body fast inversion recovery MR imaging of small cell neoplasms in pediatric patients: a pilot study. *Am J Roentgenology* 2002; 179: 1261–66.
- 14 Volker T, Kenecke T, Steffen I, et al. Positron emission tomography for staging of pediatric sarcoma patients: results of a prospective multicenter trial. *J Clin Oncol* 2007; 25: 5435–41.
- 15 Jaffe N, Paed D, Traggis D, Sallan S, Cassady R. Improved outlook for Ewing's sarcoma with combination chemotherapy (vincristine, actinomycin D and cyclophosphamide) and radiation therapy. *Cancer* 1976; 38: 1925–30.
- 16 Nesbit M, Gehan E, Burgert E, et al. Multimodal therapy for the management of primary, nonmetastatic Ewing's sarcoma of bone: a long-term follow-up of the First Intergroup Study. *J Clin Oncol* 1990; 8: 1664–74.
- 17 Burgert E, Nesbit M, Garmsey L, et al. Multimodal therapy for the management of nonpelvic, localized Ewing's sarcoma of bone: Intergroup Study IEES-II. *J Clin Oncol* 1990; 8: 1514–24.
- 18 Grier H, Krailo M, Tarbell N, et al. Addition of ifosfamide and etoposide to standard chemotherapy for Ewing's sarcoma and primitive neuroectodermal tumor of bone. *N Engl J Med* 2003; 348: 694–701.
- 19 Granowetter L, Womer R, Devidas M, et al. Dose-intensified compared with standard chemotherapy for nonmetastatic Ewing sarcoma family of tumors: a Children's Oncology Group study. *J Clin Oncol* 2009; 27: 2536–41.
- 20 Womer R, West D, Krailo M, Dickman P, Pawel B. Chemotherapy intensification by interval compression in localized Ewing sarcoma family tumors (ESFT). *Proc Am Soc Clin Oncol* 2008; 26: abstr 10504.
- 21 Paulussen M, Ahrens S, Dunst J, et al. Localized Ewing tumor of bone: final results of the cooperative Ewing's Sarcoma Study CESS 86. *J Clin Oncol* 2001; 19: 1818–29.
- 22 Paulussen M, Craft A, Lewis I, et al. Results of the EICESS-92 Study: two randomized trials of Ewing's sarcoma treatment—cyclophosphamide compared with ifosfamide in standard-risk patients and assessment of benefit of etoposide added to standard treatment in high-risk patients. *J Clin Oncol* 2008; 26: 4385–93.
- 23 Juergens C, Weston C, Lewis I, et al. Safety assessment of intensive induction with vincristine, ifosfamide, doxorubicin and etoposide (VIDE) in the treatment of Ewing tumors in the EURO-E.W.I.N.G. 99 clinical trial. *Pediatr Blood Cancer* 2006; 47: 22–29.
- 24 Saylor R, Stine S, Sullivan J, et al. Cyclophosphamide plus topotecan in children with recurrent or refractory solid tumors: a Pediatric Oncology Group phase II study. *J Clin Oncol* 2001; 19: 3463–69.
- 25 Hunold A, Weddeling N, Paulussen M, Ranft A, Liebscher C, Jurgens H. Topotecan and cyclophosphamide in patients with refractory or relapsed Ewing tumors. *Pediatr Blood Cancer* 2006; 6: 795–800.
- 26 Miser J, Goldsby R, Chen Z, et al. Treatment of metastatic Ewing sarcoma/primitive neuroectodermal tumor of bone: evaluation of increasing the dose intensity of chemotherapy—a report from the Children's Oncology Group. *Pediatr Blood Cancer* 2007; 49: 894–900.
- 27 Meyers P, Krailo M, Ladany M, et al. High-dose melphalan, etoposide, total-body irradiation, and autologous stem-cell reconstitution as consolidation therapy for high-risk Ewing's sarcoma does not improve prognosis. *J Clin Oncol* 2001; 19: 2812–20.
- 28 Oberlin O, Rey A, Desfachelles A, et al. Impact of high-dose busulfan plus melphalan as consolidation in metastatic Ewing tumors: a study by the Societe Francaise des Cancers de l'Enfant. *J Clin Oncol* 2006; 24: 3997–4002.
- 29 Burdach S, van Kaick B, Laws H, et al. Allogeneic and autologous stem-cell transplantation in advanced Ewing tumors: an update after long-term follow-up from two centers of the European Intergroup Study EICESS. *Ann Oncol* 2000; 11: 1451–62.
- 30 Burdach S, Meyer-Bahlburg A, Laws H, et al. High-dose therapy for patients with primary multifocal and early relapsed Ewing's tumors: results of two consecutive regimens assessing the role of total-body irradiation. *J Clin Oncol* 2003; 21: 3072–78.
- 31 Bacci G, Ferrari S, Longhi A, et al. Therapy and survival after recurrence of Ewing's tumors: the Rizzoli experience in 195 patients treated with adjuvant and neoadjuvant chemotherapy from 1979 to 1997. *Ann Oncol* 2003; 14: 1654–59.
- 32 Leavey PJ, Mascarenhas L, Marina N, et al. Prognostic factors for patients with Ewing sarcoma (EWS) at first recurrence following multi-modality therapy: a report from the Children's Oncology Group. *Pediatr Blood Cancer* 2008; 51: 334–38.
- 33 Barker L, Pendergrass TW, Sanders J, Hawkins D. Survival after recurrence of Ewing's sarcoma family of tumors. *J Clin Oncol* 2005; 23: 4354–62.
- 34 Schuck A, Ahrens S, Paulussen M, et al. Local therapy in localized Ewing tumors: results of 1058 patients treated in the CESS 81, CESS 86, and EICESS 92 trials. *Int J Rad Oncol Biol Phys* 2003; 55: 168–77.
- 35 Yock T, Krailo M, Fryer CJ, et al. Local control in pelvic Ewing sarcoma: analysis from INT-0091—a report from the Children's Oncology Group. *J Clin Oncol* 2006; 24: 3838–43.
- 36 Donati D, Yin J, Di Bella C, et al. Local and distant control in non-metastatic pelvic Ewing's sarcoma patients. *J Surg Oncol* 2007; 96: 19–25.
- 37 Riggi N, Suva M, Suva D, et al. EWS-FLI-1 expression triggers a Ewing's sarcoma initiation program in primary human mesenchymal stem cells. *Cancer Res* 2008; 68: 2176–85.
- 38 Kovar H, Aryee D, Jug G, et al. EWS/FLI-1 antagonists induce growth inhibition of Ewing tumor cells in vitro. *Cell Growth Differ* 1996; 7: 429–37.
- 39 Chansky H, Barahmand-Pour F, Mei Q, et al. Targeting of EWS/FLI-1 by RNA interference attenuates the tumor phenotype of Ewing's sarcoma cells in vitro. *J Orthoped Res* 2004; 22: 910–17.
- 40 DuBois SG, Krailo MD, Lessnick SL, et al. Phase II study of intermediate-dose cytarabine in patients with relapsed or refractory Ewing sarcoma: a report from the Children's Oncology Group. *Pediatr Blood Cancer* 2009; 52: 324–27.
- 41 Erkizan H, Kong Y, Merchant M, et al. A small molecule blocking oncogenic protein EWS-FLI1 interaction with RNA helicase A inhibits growth of Ewing's sarcoma. *Nat Med* 2009; 15: 750–56.
- 42 Larsson O, Girmata A, Girmata L. Role of insulin-like growth factor 1 receptor signalling in cancer. *Br J Cancer* 2005; 92: 2097–101.
- 43 Scotlandi K, Benini S, Sarti M, et al. Insulin-like growth factor I receptor-mediated circuit in Ewing's sarcoma/peripheral neuroectodermal tumor: a possible therapeutic target. *Cancer Res* 1996; 56: 4570–74.
- 44 Prieur A, Tirode F, Cohen P, Delattre O. EWS/FLI1 silencing and gene profiling of Ewing cells reveal downstream oncogenic pathways and a crucial role for repression on insulin-like growth factor binding protein 3. *Mol Cell Biol* 2004; 24: 7275–83.
- 45 Benini S, Zuntini M, Manara M, et al. Insulin-like growth factor binding protein 3 as an anticancer molecule in Ewing's sarcoma. *Int J Cancer* 2006; 119: 1039–46.
- 46 Higano C, Yu E, Whiting S. A phase I, first in man study of weekly IMC-A12, a fully human insulin like growth factor-I receptor IgG1 monoclonal antibody, in patients with advanced solid tumors. *Proc Am Soc Clin Oncol* 2007; 25: Abstr 269.
- 47 Olmos D, Postel-Vinay P, Molife LR, et al. Safety, pharmacokinetics, and preliminary activity of the anti-IGF-1R antibody figitumumab (CP-751,871) in patients with sarcoma and Ewing's sarcoma: a phase 1 expansion cohort study. *Lancet Oncol* 2010; 11: 129–35.
- 48 Benini S, Manara M, Baldini N, et al. Inhibition of insulin-like growth factor I receptor increases the antitumor activity of doxorubicin and vincristine against Ewing's sarcoma cells. *Clin Cancer Res* 2001; 7: 1790–97.

- 49 Manara M, Landuzzi L, Nanni P, et al. Preclinical *in vivo* study of new insulin-like growth factor-1 receptor-specific inhibitor in Ewing's sarcoma. *Clin Cancer Res* 2007; **13**: 1322–30.
- 50 Ricotti E, Fagioli F, Garelli E, et al. c-kit is expressed in soft tissue sarcoma of neuroectodermic origin and its ligand prevents apoptosis of neoplastic cells. *Blood* 1998; **91**: 2397–405.
- 51 Bond M, Bernstein M, Pappo A, et al. A phase II study of imatinib mesylate in children with refractory or relapsed solid tumors: a Children's Oncology Group study. *Pediatr Blood Cancer* 2008; **50**: 254–58.
- 52 Rodenhuis S. Ras and human tumors. *Semin Cancer Biol* 1992; **3**: 241–47.
- 53 Mercer K, Pritchard C. Raf proteins and cancer: B-Raf is identified as a mutational target. *Biochim Biophys Acta* 2003; **1653**: 25–40.
- 54 Guan H, Zhou Z, Gallick G, et al. Targeting Lyn inhibits tumor growth and metastasis in Ewing's sarcoma. *Mol Cancer Ther* 2008; **7**: 1807–16.
- 55 Hidalgo M, Rowinsky E. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene* 2000; **19**: 6680–86.
- 56 Mateo-Lonzano S, Tirado O, Notario V. Rapamycin induces the fusion-type independent downregulation of the EWS/FLI-1 proteins and inhibits Ewing's sarcoma cell proliferation. *Oncogene* 2003; **22**: 9282–87.
- 57 Mateo-Lonzano S, Gokhale P, Soldatenkov V, Dritschilo A, Tirado O, Notario V. Combined transcriptional and translational targeting of EWS/FLI-1 in Ewing's sarcoma. *Clin Cancer Res* 2006; **12**: 6781–90.
- 58 Huang H, Illei P, Zhao Z, et al. Ewing sarcomas with p53 mutation or p16/ARF homozygous deletion: a highly lethal subset associated with poor chemoresponse. *J Clin Oncol* 2005; **23**: 548–58.
- 59 Dey A, Verma C, Lane D. Updates on p53: modulation of p53 degradation as a therapeutic approach. *Br J Cancer* 2008; **98**: 4–8.
- 60 LeFranc F, Facchini V, Kiss R. Proautophagic drugs: a novel means to combat apoptosis-resistant cancers, with a special emphasis on glioblastomas. *The Oncologist* 2007; **12**: 1395–403.
- 61 Sohn H, Choi E, Kim S, et al. Engagement of CD99 induces apoptosis through a calcineurin-independent pathway in Ewing's sarcoma cells. *Am J Pathol* 1998; **153**: 1937–45.
- 62 Scotlandi K, Baldini N, Cerisano V, et al. CD99 engagement: an effective therapeutic strategy for Ewing tumors. *Cancer Res* 2000; **60**: 5134–42.
- 63 Scotlandi K, Perdichizzi S, Bernard G, et al. Targeting CD99 in association with doxorubicin: an effective combined treatment for Ewing's sarcoma. *Eur J Cancer* 2006; **42**: 91–96.
- 64 Matthay K, Villablanca J, Seeger R, et al. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation and 13-cis-retinoic acid. *N Engl J Med* 1999; **341**: 1165–73.
- 65 Fang J, Chen S, Tong J, Wang Z, Chen G, Chen Z. Treatment of acute promyelocytic leukemia with ATRA as As2O3: a model of molecular target-based cancer therapy. *Cancer Biol Ther* 2002; **1**: 614–620.
- 66 Burchill S, Berry P, Bradbury F, Lewis I. Contrasting levels of p21ras activation and expression of neurofibromin in peripheral primitive neuroectodermal tumor and neuroblastoma cells, and their response to retinoic acid. *J Neurol Sci* 1998; **157**: 129–37.
- 67 Myatt S, Burchill S. The sensitivity of the Ewing's sarcoma family of tumours to fenretinide-induced cell death is increased by EWS-FLI1-dependent modulation of p38(MAPK) activity. *Oncogene* 2008; **27**: 985–96.
- 68 Myatt S, Redfern C, Burchill S. p38MAPK-Dependent sensitivity of Ewing's sarcoma family of tumors to fenretinide-induced cell death. *Clin Cancer Res* 2005; **11**: 3136–48.
- 69 Maurer B, Kalous O, Yesair D, et al. Improved oral delivery of N-(4-hydroxyphenyl)retinamide with a novel LYM-X-SORB organized lipid complex. *Clin Cancer Res* 2007; **13**: 3079–86.
- 70 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. *Nat Med* 1995; **1**: 27–31.
- 71 Dalal S, Berry A, Cullinane C, et al. Vascular endothelial growth factor: a therapeutic target for tumors of the Ewing's sarcoma family. *Clin Cancer Res* 2005; **11**: 2364–78.
- 72 Kreuter M, Paulussen M, Boeckeler J, et al. Clinical significance of vascular endothelial growth factor-A expression in Ewing's sarcoma. *Eur J Cancer* 2006; **42**: 1904–11.
- 73 D'Adamo D, Anderson S, Albritton K, et al. Phase II study of doxorubicin and bevacizumab for patients with metastatic soft-tissue sarcomas. *J Clin Oncol* 2005; **23**: 7135–42.
- 74 Kieran M, Turner C, Rubin J, et al. A feasibility trial of antiangiogenic (metronomic) chemotherapy in pediatric patients with recurrent or progressive cancer. *J Pediatr Hematol Oncol* 2005; **27**: 573–81.
- 75 Spoto R, Stram DO. A strategic view of randomized trial design in low-incidence pediatric cancer. *Stat Med* 1999; **18**: 1183–97.