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Clinical features of Ewing and chondrosarcoma

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Annemiek van Maldegem

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Clinical features of Ewing and chondrosarcoma

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Voor mam

Hoe je omgaat met tegenslagen toont je ware karakter

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Chapter 1

General introduction

Sarcoma

For the last couple of years cancer has become the number one cause of death in Europe [1]. The most common form of cancer is carcinoma, which means that the cancer arises in epithelial tissues. A rarer form of cancer is sarcoma, this cancer arises in tissues of mesenchymal origin. Sarcoma can be divided in 2 subtypes, namely bone sarcoma such as Ewing sarcoma, chondrosarcoma, and osteosarcoma, and soft-tissue sarcoma for example rhabdomyosarcoma and liposarcoma. Soft tissue sarcoma is the most common form accounting for 76% of sarcomas. Of all new diagnosed cancers 1% is a sarcoma which calculates to 15,000 new cases a year in the United States [2] and almost 28,000 in Europe [3]. In the Netherlands a total of 973 new cases were reported in 2015 (www.cijfersoverkanker.nl).

Ewing sarcoma

Ewing sarcoma (ES) is the third most common primary malignant bone tumor after osteosarcoma and chondrosarcoma, however it is still rare with approximately 500 new diagnoses in Europe each year. It affects mostly children and adolescents with a peak

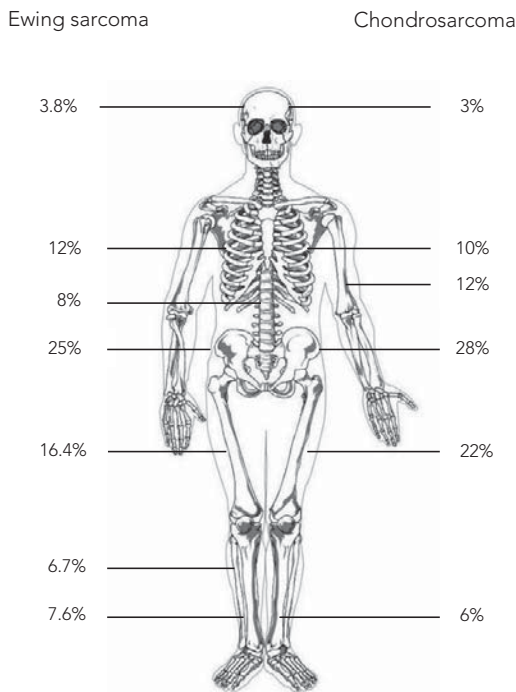


Figure 1.1 Most commonly diagnosed locations for Ewing sarcoma and chondrosarcoma.

incidence at age 15. ES is diagnostically defined by a reciprocal translocation, causing a fusion of the EWSR1-gene on chromosome 22 with a member of the ETS family of transcription factors. The most common translocation (85%) is the $t(11;22)(q24;q12)$, fusing EWSR1 to FLI1. An example of the histology ES cells is shown in Figure 1.2. ES is most commonly diagnosed in the pelvis, femur, humerus, ribs and clavicle (Figure 1.1), an example of a typical radiological presentation is shown in Figure 1.2. With current treatment options including surgery, conventional chemotherapy, radiotherapy and high dose chemotherapy and peripheral blood stem cell transplant (PBSCT), the 5 year survival for local disease is 60%. However, for patients that present with metastatic disease the 5-year survival decreases to only 20% and the outcome for patients with relapsed or refractory Ewing sarcoma is even worse with a 5-year survival of no more than 10% [4]. During the last four decades, patient outcome has been improved significantly in patients with localized disease, by dose intensification of conventional therapeutics and the use of multidisciplinary treatments, such as chemotherapy (including Vincristine, Doxorubicin, Cyclophosphamide, Ifosfamide and Etoposide), surgery and radiation therapy. However, advances in the treatment of ES patients with metastatic or relapsed disease have not been made and their outcome is still poor.

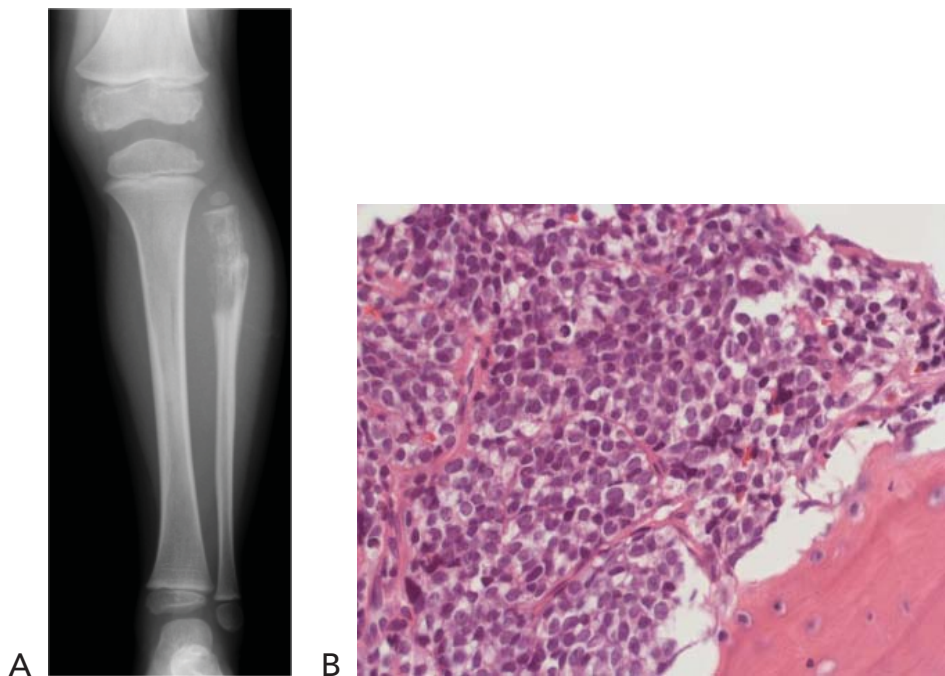


Figure 1.2 X-ray of a typical radiological presentation for ES (A) with a lytic lesion in the proximal fibula of the right leg and an example of Haematoxylin and eosin staining on ES (B) showing small round blue cells without any differentiation.

Chondrosarcoma

Chondrosarcoma is the second most common primary bone cancer. It is an orphan disease and accounts for 20% of all the new primary bone malignancies [5]. It is a heterogeneous group that have in common the production of chondroid matrix. Chondrosarcoma affects adults mostly over 40 years of age with an incidence that is gradually increasing and no difference of occurrence between sex and race. It mostly affects the pelvis, femur and humerus (Figure 1.1), a typical radiological presentation is shown in Figure 1.3. Chondrosarcoma consist of different subtypes with conventional chondrosarcoma being the most common (75%), more rare subtypes include dedifferentiated, clear cell and mesenchymal chondrosarcoma. Histologically, conventional chondrosarcoma can be subdivided in grade 1 (now called “atypical cartilaginous tumour”), grade 2 and grade 3 according to the nuclear size, matrix composition, mitotic activity and degree of cellularity. An example of histology of Haematoxylin and eosin staining of chondrosarcoma cells is shown in Figure 1.3. The grade of the tumour is one of the most important predictors of outcome [6, 7]. From two benign cartilaginous lesions it is known that they can progress to chondrosarcoma. The first is osteochondroma which is a cartilage-capped bony projection arising on the external surface of a bone. It can arise as a single lesion or as an inherited condition within the genetic disorder multiple osteochondromas (previously called

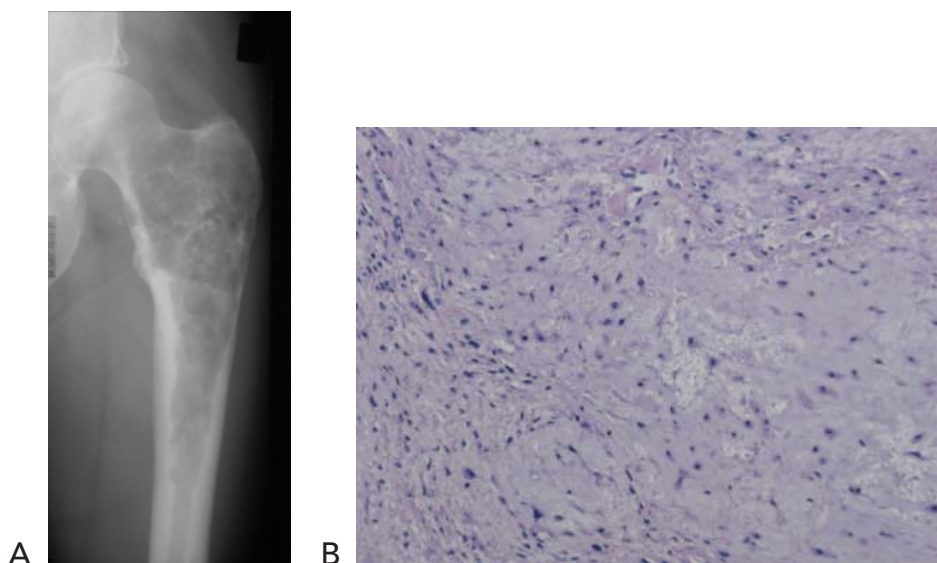


Figure 1.3 X-ray of a typical radiological presentation for chondrosarcoma (A) with ill-defined lytic bone lesion with calcifications of the right proximal femur and an example of Haematoxylin and eosin staining on chondrosarcoma cells (B) showing tumour cells displaying chondrogenic differentiation by depositing cartilaginous of extracellular matrix.

“hereditary multiple exostoses”). Malignant transformation occurs in 1 percent of patients with a solitary and 2 till 6 percent of patients with multiple osteochondromas [8-15]. The average time between initial diagnosis and malignant transformation was 9.8 years [8]. All chondrosarcoma arising in the setting of an osteochondroma are secondary peripheral tumors. The other precursor lesion for chondrosarcoma is enchondroma which is a benign cartilaginous tumor that develops in the medulla (marrow cavity) of bone. Enchondromas can occur in a non-hereditary syndrome of which the most common forms are Ollier disease and Maffucci syndrome. The risk of malignant transformation in these forms is up to 50 percent, depending on the location of the tumors [16-21].

Grade 1 chondrosarcoma / atypical cartilaginous tumours are slowly growing and metastasize rarely with a 10 years survival rate of ~80% [22]. In contrast, grade 3 chondrosarcomas have a high metastatic rate and a 10 year survival of ~38% [7]. For the grade 2 and 3 chondrosarcomas the most important treatment option is surgery and it is considered to be the only chance for cure. Most subtypes of chondrosarcoma have always been considered chemotherapy resistant and it was assumed that patients would not benefit from non-surgical treatment options.

Several studies were conducted to investigate new treatment options for patients diagnosed with chondrosarcoma. Recent preclinical and retrospective studies show that patients may benefit from non-cytotoxic, chemo- and radiotherapy. These studies enrolled only small numbers of patients because of the orphan state of the disease. Because of the rarity of chondrosarcoma and especially the grade 2 and 3 tumours this is difficult to assess with few reported series consisting mostly of small numbers of patients [23, 24].

In the last decades pre-clinical and clinical studies have been conducted including patients diagnosed with local or advanced ES or chondrosarcoma. Because both ES and chondrosarcoma are orphan diseases with few new patients diagnosed each year most studies can enrol only small numbers of patients and the conclusions from these studies are limited. There are no uniform treatment regimens for patients with advanced disease and the treatment options in different countries vary greatly. Systemic analysis of the available pre-clinical and clinical data is needed to identify the most optimal treatment regimen and collaborations between countries need to be formed to conduct clinical studies based on pre-clinical work.

Aim and outline of this thesis

The aim of this thesis was to categorise the clinical studies executed for Ewing sarcoma and chondrosarcoma patients in the past, to determine the outcome for unresectable Ewing and chondrosarcoma patients and to find new treatment options based on pre-clinical studies on molecular pathways for the future.

In the last twenty years the outcome for patients with metastasized bone sarcoma shows no major improvement. To identify which clinical studies have been conducted **chapter 2** is a literature search of published phase I/II clinical trials which included Ewing sarcoma patients between 1990 and 2010.

Several new treatment options are being tested in a preclinical or clinical setting for patients diagnosed with Ewing sarcoma. Two interesting pathways for potential targeted therapy are the IGF-1R and PARP pathway. **Chapter 3** summarises the preclinical data available of these pathways and the results of the clinical studies conducted so far.

There are limited treatment options for patients diagnosed with unresectable Ewing sarcoma. Several sarcoma centres in Europe treat these patients with the combination of etoposide and either cisplatin or carboplatin. **Chapter 4** shows the outcome for patients treated with this combination and several possible prognostic factors were tested.

Gene transcription is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) which modulates chromatin and thereby influences the wrapping of eukaryotic DNA. HDACs prevent gene transcription and upregulation is associated with silencing of tumour suppressor genes and oncogenesis. Panobinostat is a HDAC inhibitor, **chapter 5** describes one of the first Ewing sarcoma patients treated with panobinostat monotherapy.

Chapter 6 gives an overview of the published prospective and retrospective studies with systemic therapies for chondrosarcoma patients between 2000 and 2013. The results on outcome are compared and promising therapies are emphasized.

Overall survival for patients who are diagnosed with unresectable chondrosarcoma remains poor. To gain insight, **chapter 7** gives the retrospective overall survival data from all patients diagnosed with unresectable conventional chondrosarcoma between 1980 and 2011 in two major European bone sarcoma centres.

The effect of systemic therapy of patients diagnosed with unresectable chondrosarcoma, per subtype, is shown in **chapter 8**.

Chapter 9 gives a general discussion of this thesis with future perspectives.

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Comprehensive analysis of published phase I/II clinical trials between 1990–2010 in osteosarcoma and Ewing sarcoma confirms limited outcomes and need for translational investment

Annemiek M. van Maldegem, Aparna Bhosale, Hans Gelderblom,
Pancras C.W. Hogendoorn, Andrew B. Hassan

Background

High grade primary bone sarcomas are rare cancers that affect mostly children and young adults. Osteosarcoma and Ewing sarcoma are the most common histological subtypes in this age group, with current multimodality treatment strategies achieving 55–70% overall survival. As there remains an urgent need to develop new therapeutic interventions, we have reviewed published phase I/II trials that have been reported for osteosarcoma and Ewing sarcoma in the last twenty years.

Results

We conducted a literature search for clinical trials between 1990 and 2010, either for trials enrolling bone sarcoma patients as part of a general sarcoma indication or trials specifically in osteosarcoma and Ewing sarcoma. We identified 42 clinical trials that fulfilled our search criteria for general sarcoma that enrolled these patient groups, and eight and twenty specific trials for Ewing and osteosarcoma patients, respectively. For the phase I trials which enrolled different tumour types our results were incomplete, because the sarcoma patients were not mentioned in the PubMed abstract. A total of 3,736 sarcoma patients were included in these trials over this period, 1,114 for osteosarcoma and 1,263 for Ewing sarcoma. As a proportion of the worldwide disease burden over this period, these numbers reflect a very small percentage of the potential patient recruitment, approximately 0.6% for Ewing sarcoma and 0.2% for osteosarcoma. However, these data show an increase in recent activity overall and suggest there is still much room for improvement in the current trial development structures.

Conclusion

Lack of resources and commercial investment will inevitably limit opportunity to develop sufficiently rapid improvements in clinical outcomes. International collaboration exists in many well founded co-operative groups for phase III trials, but progress may be more effective if there were also more investment of molecular and translational research into disease focused phase I/II clinical trials. Examples of new models for early translational and early phase trial collaboration include the European based EuroBoNeT network, the Sarcoma Alliance for Research through Collaboration network (SARC) and the new European collaborative translational trial network, EuroSarc.

Background

Primary bone sarcomas are rare, and account for approximately 6% of all childhood malignancies, with Ewing sarcoma and osteosarcoma accounting for approximately 3% of tumours arising in teenagers. Here we focus on osteosarcoma and Ewing sarcoma, the two most common bone sarcomas, whose treatments are similar as they involve multimodality treatment with dose intensive and toxic chemotherapy, combined with potentially mutilating surgery.

Osteosarcoma is the most common primary malignant tumour arising from bone. It is a pleiomorphic tumour of bone, and based on animal model systems, thought to arise in mesenchymal stem cells in which the mutant proliferating spindle cells produce osteoid or immature bones [1]. The EMEA published data in 2009 estimating approximately five people in 1,000,000 in the European Union (EU) are affected by osteosarcoma. Recently, data from the RARECARE surveillance network revealed an incidence of 0.23 per 100,000, amounting to approximately 1,135 new cases per year in EU27 [2]. Osteosarcoma mostly affects children and young adults with the median age of diagnosis being fifteen. Seventy-five percent of patients are between eight and twenty-five years old. Osteosarcoma is often located in the extremities of long bones near metaphyseal growth plates. Current treatments for osteosarcoma achieve 60–70% event-free survival (EFS) for patients who present with localized disease and approximately 20% EFS for patients with clinically detectable metastatic disease [3]. Of all patients, 20% have clinically detectable metastatic disease at first presentation. Surgical resection of all clinically detectable sites of disease and systemic therapy to control microscopic metastatic disease is currently the therapy of choice for early stage osteosarcoma. Since the introduction of neo-adjuvant and adjuvant chemotherapy to surgery in the early 1980s, the long term survival of patients with osteosarcoma has remained stable at about 60–65%. In terms of chemotherapy, several agents have demonstrated activity in osteosarcoma including cisplatin, doxorubicin, high-dose methotrexate with leucovorin rescue (HDMTX) and ifosfamide. For current treatment options these agents are combined. Since the early 1980s, trials have been conducted in which the variations in doses and scheduling between these four agents were tested, but these have not result in improvement of EFS [4]. Since the introduction of ifosfamide, more than two decades ago, the only new agent that has been approved is muramyl tripeptide, a drug that activates the innate immune system [5]. Thus, despite surgical resection of the primary tumour and aggressive adjuvant chemotherapy, 30–40% still die of metastases that are resistant to conventional therapies [6–8]. For osteosarcoma patients with resectable pulmonary metastases it has become more standard to treat these patients with metastectomy. This has been shown to improve relapse free survival and a subgroup of patients may even be cured [9].

Ewing Sarcoma/Primitive Neuroectodermal (PNET) are the second most common bone malignancy after osteosarcoma in children and young adults with a peak incidence at age fifteen. Ewing sarcoma are diagnostically defined by a Ewing sarcoma EWS (Chromosome 22) translocation resulting in fusion with an ETS transcription factor, the commonest abnormality (85%) being EWS-FLI1 (Chromosome 11) or rarely with a non ETS family partner [10, 11]. Although claimed in the past that the transcript type reflected a difference in prognoses [12] this proved not to be the case in prospective randomised trials [13]. RARECARE estimates an EU27 incidence rate for bone and soft tissue Ewing sarcoma of 0.13 per 100,000 and 0.05 per 100,000, respectively [2]. This translates to approximately 647 bone and 263 soft tissue new Ewing sarcoma diagnoses per year (EU27), with a predicted 65–75% 5 year survival for non-metastatic disease using conventional chemotherapy, radiotherapy, high dose chemotherapy and peripheral blood stem cell transplant (PBSCT). However, survival for the 25% of patients that present with metastatic disease is approximately 20% [14], and for those who develop relapsed and/or refractory disease, the survival is no more than 10%. To date, studies in the patient population requiring salvage treatment have been confined to chemotherapy combinations and high dose chemotherapy, with variable response rates and little or no impact on survival. During the last two decades, the outcome has improved in patients with localized disease. This has been achieved by dose intensification and standardisation of conventional therapeutics and radiotherapy. With the use of multidisciplinary treatments, such as chemotherapy (including vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide), surgery and radiation therapy, the five year overall survival probability exceeds 75% for patients with non-metastatic low volume Ewing sarcoma. However, advances in the treatment of Ewing sarcoma have not impacted on the outcome of patients with large volume and metastatic disease [15].

In the last two decades the treatment outcome for these bone sarcomas has not improved greatly, even though some new treatment interventions have been successfully tested. Large scale phase III trials with long durations of recruitment have established material for prognostic and treatment related correlative studies with survival and toxicity outcomes, and importantly, have formed the basis of international collaboration. However, the smaller phase I/II studies are also important in order to develop proof of principle single agent and combination treatments, particularly with newer molecular and biological based interventions that directly test disease specific molecular mechanisms.

We wished to establish the effective level of phase I/II activity that has been reported in peer reviewed publications in the last twenty years. We report an overview of the phase I/II trials that have been conducted for osteosarcoma and Ewing sarcoma, where we detail the

kind of drugs that have been tested, what the published study outcomes were and what interventions have progressed to testing in phase III trials. The results show an improvement in overall activity, but that the number of studies and International collaborations in early phase trials remain at a low and limited level. We discuss potential routes to improve the number and quality of early phase trials in Ewing and osteosarcoma.

Methods

Search strategy

We report data available in the public domain only. Publications were identified from searches of PubMed, the Cochrane Controlled Trials Register, American Society of Clinical Oncology (ASCO) abstracts and ClinicalTrials.gov. databases for the period 1990–2010. The search strategies used terms for osteosarcoma; (osteosarcoma) AND (phase I OR phase II) AND (clinical trial), and was supplemented with a text word search. For Ewing sarcoma the search algorithm was; (Ewing sarcoma) AND (phase I OR phase II) AND (clinical trial). To validate the search we broadened the search algorithm to; (sarcoma) AND (phase I OR phase II) AND (clinical trial), and compared the results from the narrow search with the ones from the broad search. The latest search was performed in April 2010.

Whenever multiple reports from the same trial were published, we used only the report with the longest follow-up to avoid any duplication of information. Publications were eligible if they: (1) described (or cited a paper that described) osteosarcoma or Ewing sarcoma study of early phase clinical trials; (2) were published in English; and (3) came from industrialized countries. All types of evaluation were accepted (full papers, conference abstracts, reports) as long as results (including data) were presented.

Data extraction

Data extraction was conducted independently by two authors (A. B. and A.M. van M.). We used a systematic method for the search normally used for meta-analysis [16]. Differences in data extraction were resolved by consensus with a third author (A.B. H.). From each eligible trial we recorded authors' names, journal and year of publication, number of patients enrolled, number of osteosarcoma or Ewing sarcoma patients, study phase and the outcome of the trial.

Results and discussion

Eligible trials

A flow-chart indicating the identification of clinical trials for inclusion in the analysis is reported for Ewing sarcoma (Figure 2.1A) and osteosarcoma (Figure 2.1B). During the search many reports had to be excluded because no results were published. When we searched the reports using full text, we had to exclude some papers because neither osteosarcoma nor Ewing sarcoma patients were included in these studies. When we combined the results, we identified 42 trials enrolling patients with any histological diagnosis of sarcoma that were eligible for our study, in that they included osteosarcoma and Ewing sarcoma. Of the 42 clinical trials twenty-one were phase I, two were phase I/II and nineteen were phase II trials. We found eight clinical trials which included only Ewing sarcoma patients; of this group two were phase I and six were phase II trials. We identified twenty trials that included only osteosarcoma patients. There were two phase I, sixteen were phase II and two were phase I/II trials. A total of 3,736 patients were included in all the clinical trials, of which 1,263 were Ewing sarcoma and 1,114 were osteosarcoma patients.

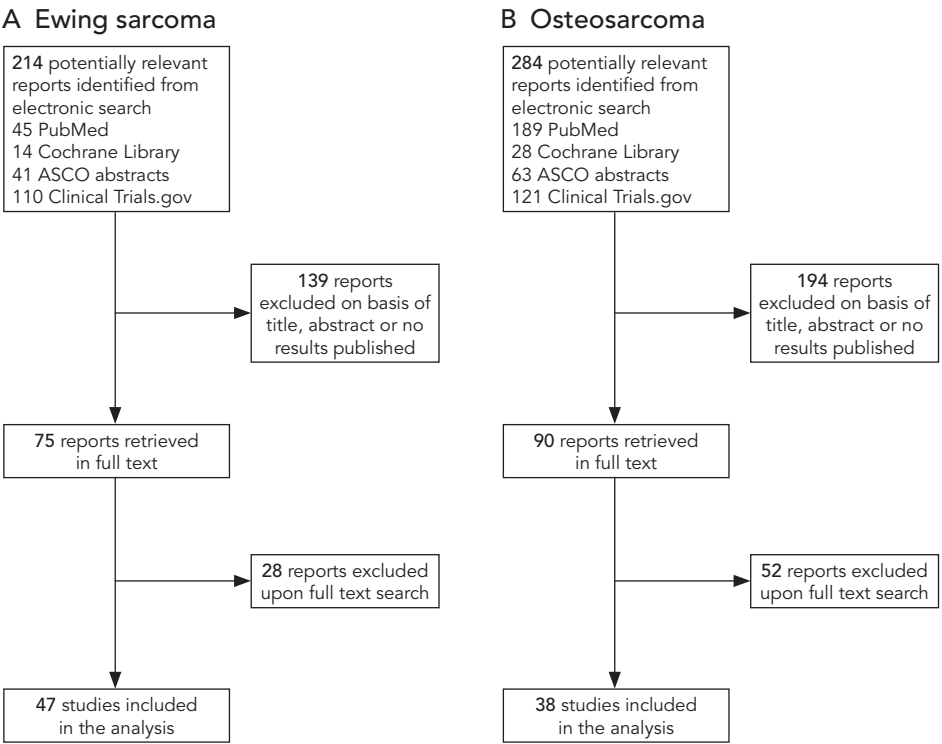


Figure 2.1 Flowchart diagrams of the clinical trial selection criteria outcomes for Ewing sarcoma (A) and osteosarcoma (B).

Primary outcome

Tables 2.1 and 2.2 show the search results for clinical trials conducted in patients with the wider diagnosis of general sarcoma, and specifically the trials that included either osteosarcoma (Table 2.3) or Ewing sarcoma patients only (Table 2.4). Table 2.1 and 2.2 are trials testing either chemotherapy or biological treatments, respectively. From the analysis of the number of trials conducted which included only osteosarcoma or Ewing sarcoma patients from 1990 to the present, (Figure 2.2A) it was clear that the number of trials reported for osteosarcoma has been stable since 1995, with approximately five trials in five years. For Ewing sarcoma there has been an increase in the number of trials published, with the no early phase trials reported between 1990 and 1999, an increasing number of trials in the period 2000–2005 and even more between 2006–2010.

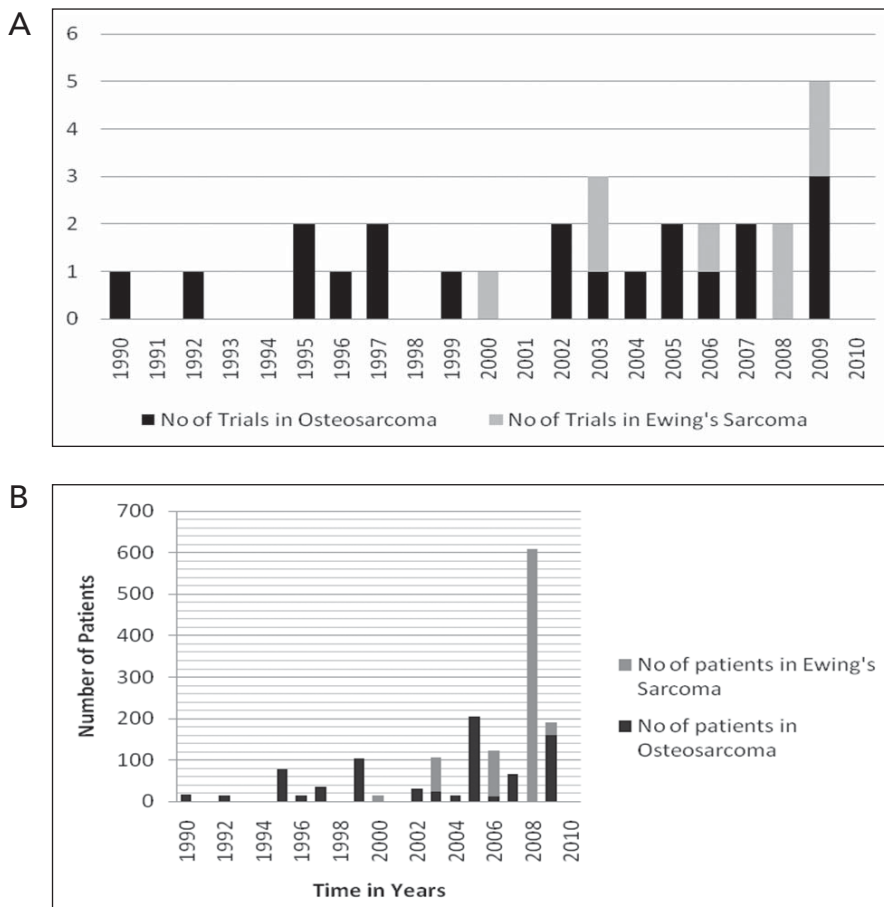


Figure 2.2 Distribution of the number of trials published that have recruited either osteosarcoma or Ewing sarcoma patients between 1990–2010 (A). The total number of either osteosarcoma or Ewing Sarcoma patients entered in phase I/II trials published between 1990–2010 (B).

Table 2.1 Characteristics of eligible trials for general sarcoma with chemotherapy based treatment for phase I and phase II

Author	Year	Intervention	No Osteo pts	No Ewing pts	Phase	Outcome
Pratt et al, Cancer, 74: 2593-8 [22]	1994	Leucovorin, 5-Fluorouracil	22	12	II	Ewing: 3 (25%) SD, Osteo: 1 (5%) SD
Kushner et al, J Clin Oncol, 13 (11): 2796-804 [23]	1995	Cyclo, Doxo, Etop, Ifos, Vin	NA	NA	II	6 (17%) CR, 2 (6%) PR, 34 (95%) CIR
Antman et al, Cancer, 82 (7): 1288-95 [24]	1998	Doxo, Dacarbazine, Ifos	31	13	II	10 (12%) CR, 34 (42%) PR
Blaney et al, Clin Cancer Res, 4 (2): 357-60 [25]	1998	Topotecan	18	25	II	1 (1%) CR, 1 (1%) PR, 8 (9%) SD
Lucidarme et al, Bone Marrow Transplant, 22 (6): 535-40 [26]	1998	Thiotepa combined with SCR	7	3	II	11 (50%) PR
Berg et al, J Pediatric Hematol Oncol, 22 (6): 506-9 [27]	2000	Pyrazoloacridine	8	10	II	0% response
Delaloge et al, J Clin Oncol, 19 (5): 1248-55 [28]	2001	Trabectedin	3	1	I	4 (14%) CR, 10 (35 %) SD
Saylors et al, J Clin Oncol, 19 (15): 3463-9 [29]	2001	Cyclo and Topotecan	18	17	II	6 (35%) OR
Wagner et al, Clin Cancer Res, 10 (3): 840-8 [30]	2004	Temozolomide, Irinotecan	0	7	I	1 (8%) CR, 2 (17%) PR
Lau et al, Clin Cancer Res, 11: 672-7 [31]	2005	Trabectedin	4	3	I	1 (8%) CR
van Winkle et al, Pediatr Blood Cancer, 44 (4): 338-47 [32]	2005	Ifos, Carboplatin and Etop	35	22	I/II	Not mentioned

Table 2.1 Continued

Author	Year	Intervention	No Osteo pts	No Ewing pts	Phase	Outcome
Hawkins et al, Pediatr Blood Cancer, 47 (6): 328-37 [33]	2006	Topotecan	11	20	II	2 (4%) PR
Navid et al, Cancer, 106 (8): 1846-56 [34]	2006	Vin, Doxo, Cyclo, Ifos and Etop	0	11	II	47 (66%) CR
Wagner-Bohn et al, Cancer, 46 (2): 262 [35]	2006	Gemcitabine	2	4	II	2 (10%) SD
Zauch et al, Int J Rad Oncol Biol Phys, 64 (1): 227-34 [36]	2006	TBR after HD-chemotherapy	0	10	II	13 (25%) CR, 10 (19%) PR
Zwerdling et al, Cancer, 106 (8): 1821-8 [37]	2006	Docetaxel	23	21	II	2 (1%) CR, 6 (3%) PR, 17 (10%) SD
Georger et al, J Clin Oncol, 26: 4394-400 [38]	2008	Oxaliplatin	6	2	I	2 (4%) PR, 17 (6%) SD
Geler et al, Pediatr Blood Cancer, 52	2009	Paclitaxel, Ifos	1	3	I	3 (20%) PD, 5 (33%) SD
Wagner et al, ASCO abstract	2009	Vin, Irinotecan, Temozolomide	3	5	I	1 (3%) PR, 2 (6%) OR
McGregor et al, Cancer, 115 (8): 1765-75 [39]	2009	Oxaliplatin, Irinotecan	6	1	I	1 (8%) CR, 1 (8%) SD

CR, clinical response; CR, complete response; Cyclo, cyclophosphamide; Doxo, doxorubicine; EFS, event free survival; Etop, etoposide; Ewing, Ewing sarcoma; HD, high dose; Ifos, ifosfamide; NA, not available; No, number; OR, objective response; Osteo, osteosarcoma; PD, progressive disease; PR, partial response; pts, patients; RR, response rate; SCR, stem cell rescue; SD, stable disease; TBR, total body radiation; TMR, total marrow radiation; Vin, vincristine.

Table 2.2 Characteristics of eligible trials for general sarcoma with biologically based treatment for phase I and phase II

Author	Year	Intervention	No Osteo pts	No Ewing pts	Phase	Outcome
Daw et al, J Clin Oncol, 23 (25): 6172-80 [40]	2005	Gefitinib	6	3	I	1 (4%) PR, 4 (16%) SD
Maki et al, Cancer, 103 (7): 1431-8 [41]	2005	Bortezomib	1	2	II	1 (4%) OR
Villablanca et al, J Clin Oncol, 24 (21): 3423-30 [42]	2006	Fenretinide	2	5	I	1 (2%) CR, 13 (24%) SD
Biron et al, ASCO abstract	2007	Gimatecan	0	NA	II	3 (8%) SD
Bagatell et al, Clin Cancer Res, 13 (6): 1783-8 [43]	2007	Tanespimycin	6	2	I	0% OR
Jimeno et al, Pediatr Blood Cancer, 49 (3): 352-7 [44]	2007	Gefitinib	3	1	I	1 (7%) PR, 3 (20%) SD
Kramer et al, J Clin Oncol, 25 (34): 5465-70 [45]	2007	Monoclonal antibody(131-I-3F8)	0	2	I	3 (23%) CIR
Bond et al, Pediatr Blood Cancer, 50 (2): 254-8 [46]	2008	Imatinib mesylate	10	24	II	1 (1%) PR
Chao et al, ASCO abstract	2008	Imatinib mesylate	0	7	II	1 (14%) PR
Fox et al, Clin Cancer Res, 14 (4): 1111-5 [47]	2008	Tubulin Inhibitor (ABT-751)	3	3	I	0% OR
Jakacki et al, J Clin Oncol, 26 (30): 4921-7 [48]	2008	Erlotinib, Temozolomide	1	0	I	1 (2%) SD
Langevin et al, Pediatr Blood Cancer, 50 (3): 577-80 [49]	2008	Rebeccamycin	16	14	II	% OR

Table 2.2 Continued

Author	Year	Intervention	No Osteo pts	No Ewing pts	Phase	Outcome
Mita et al, J Clin Oncol, 26 (3): 361-7 [50]	2008	Deforolimus	1	1	I	4 (13%) PR, 18 (56%) SD
Olmos et al, ASCO abstract	2008	Figitumumab	0	9	I	1 (5%) PR, 6 (27%) SD
Chawla et al, Mol Ther, 17 (9): 1651-7 [51]	2009	Rexin-G	3	1	I/II	13 (65%) SD
Chugh et al, J Clin Oncol, 27 (19): 3148-53 [52]	2009	Imatinib	27	13	II	1 (0.5%) CR, 3 (2%) PR
Malempati et al, ASCO abstract	2009	Cixutumumab	NA	10	I	1 (4%) PR
Patel et al, ASCO abstract	2009	IGF-1R antibody (R1507)	43	71	II	NA
Widemann et al, J Clin Oncol, 27 (4): 550-6 [53]	2009	Ixabepilone	3	2	I	4 (21%) SD
Jacobs et al, Clin Cancer Res, 16 (2): 750-4 [54]	2010	Ixabepilone	11	9	II	0% OR
Kurzrock et al, Clin Cancer Res, 16 (8): 2458-65 [55]	2010	IGF-1R antibody (R1507)	0	9	I	2 (5%) PR, 13 (35%) SD
Olmos et al, Lancet Oncol, 11: 129-35 [56]	2010	Figitumumab	0	16	I	1 (3%) CR, 1 (3%) PR, 8 (28%) SD

CR, clinical response; CR, complete response; Cyclo, cyclophosphamide; Doxo, doxorubicin; EFS, event free survival; Etop, etoposide; Ewing, Ewing sarcoma; Ifos, ifosfamide; NA, not available; No, number; OR, objective response; Osteo, osteosarcoma; PR, partial response; pts, patients; RR, response rate; SCR, stem cell rescue; SD, stable disease; TMR, total marrow radiation; Vin, vincristine.

Table 2.3 Characteristics of eligible trials for osteosarcoma only

Author	Year	Intervention	No of pts	Phase	Outcome
Salesh et al., Cancer, 65: 861-5	1990	Etoposide 72-h i.v. 600 mg/m ² . Cyclophosphamide 300 mg/m ² every 12 hours for a total dose of 1800 mg/m ² .	17	II	15 (88%) CR or PR
Kleinerman et al, J Clin Oncol, 10: 1310-6 [57]	1992	L-MTP-PE 2 mg/m ² infused during a 1-h period twice a wk for 12 wks, then once a wk for 12 wks	16	II	NA
Kleinerman et al, Am J Clin Oncol, 18 (2): 93-9 [58]	1995	L-MTP-PE, 2 mg/m ² , i.v. over a 1-h twice a wk for 12 wks in 12 pts (Group 1). 16 pts (Group 2) had 2 mg/m ² LTP-PE twice a wk for 12 wks, then once a wk for 12 wks, for a total of 24 wks.	36	II	2 (6%) SD
Kleinerman et al, J Immunother Emphasis Tumor Immunol, 17: 181-93 [59]	1995	IFO 1.8 g/m ² for 5 days every 21 days for up to 8 cycles. L-MTP-PE twice weekly for 12 weeks, then once weekly for 12 weeks.	9	IIb	NA
Harris et al, Med Ped Oncol, 24: 87-92 [60]	1995	Two courses of ifosfamide (2,400 mg/m ² ×5 days) administered 3 wks apart	33	II	Stratum 1: 1 (11%) CR, 8 (24%) PR Stratum 2: 1 (3%) CR, 2 (7%) PR
Patel et al, Cancer, 78: 741-4 [61]	1996	Paclitaxel 175 mg/m ² 24-h i.v.	15	II	0% OR
Gentet et al, Eur J Cancer, 33 (2): 232-7 [62]	1997	Two courses of IFO 3g/m ² /day and etoposide 75 mg/m ² /day for 4 days	27	II	6 (23%) CR, 7 (25%) PR, 5 (23%) SD
Worth et al, Clin Cancer Res, 3 (10): 1721-9 [63]	1997	IL-1alpha followed by ICE daily for 5 days/3 wks	9	II	3 (34%) PR, 1 (11%) SD

Table 2.3 Continued

Author	Year	Intervention	No of pts	Phase	Outcome
Voûte et al, Annals of Oncology, 10: 1211-8 [64]	1999	IFO 3 g/m ² /dl-2, DOX 25 mg/m ² /dl-3 i.v. bolus and CDDP 100 mg/m ² /dl	103	II	5-year survival was 62% in limb-non-metastatic, 41% in axial skeletal and 16% in limb metastatic patients
Fagiol et al, Journal of Clinical Oncology, 20 (8): 2150-6 [65]	2002	High-dose chemotherapy consisted of carboplatin and etoposide followed by stem-cell rescue	32	I	25 (78%) CR, 6 (19%) PD 3-year OS 20% 3-year DFS 12%
Laverdiere et al, Cancer, 98: 832-40 [66]	2003	Trabectedin 1,500 micro g/m ² as a 24-h i.v. every 3 wks	25	II	0% OR
McTiernan et al, Sarcoma, 8 (2/3): 71-6 [67]	2004	Docetaxel 100 mg/m ² 1-h i.v. every 3 wks	14	II	1 (7%) PR, 2 (14%) SD
Ferrari et al, J Clin Oncol, 23: 8845-52 [68]	2005	Two blocks of high-dose IFO (15 g/m ²), MTX (12 g/m ²), CDDP (120 mg/m ²), and DOX (75 mg/m ²)	182	II	5-year EFS 64% and OS 77%
Arpaci et al, Cancer, 104: 1058-65 [69]	2005	2 cycles of CDDP, DOX, and IFO followed by HDC and APBSCT	22	II	3-year OS was 83% and 70% DFS
McTiernan et al, Pediatr Blood Cancer, 46 (3): 345-50 [70]	2006	IFO 2.5 g/m ² etoposide 150 mg/m ² and DOX 20 mg/m ² on days 1-3, every 21 days, with interval MTX 12 g/m ² given on day 14, for a maximum of 8 cycles	13	I/II	0% OR

Table 2.3 continues on next page.

Table 2.3 Continued

Author	Year	Intervention	No of pts	Phase	Outcome
Seibel et al, Cancer, 109: 1646-53 [71]	2007	Topotecan daily x5 followed by chemotherapy (IFO, carboplatin, ICE, alternating with CDDP and CD	28	I	1 (6.6%) PR, 1 (6.6%) CLR 2- and 5-year EFS rates 7% and 4%, resp., 2- and 5-year OS rates 44% and 22%, resp.
Basaran et al, Oncology, 72: 255-60 [72]	2007	Epirubicin 90 mg/m ² cisplatin 100 mg/m ² on day 1 and IFO 2.0 g/m ² day with an equivalent dose of mesna on days 2–4, repeated every 21 days	38	II	10 (26%) CR, 5-year DFS 41.9%, OS 48.2%
Iwamoto et al, J Orthop Sci, 14: 397-404 [73]	2009	Preoperative chemotherapy: HD-MTX, CDDP, and ADR	113	II	5-year OS 77.9% 5-year EFS 65.5%
Berger et al, Cancer, 115: 2980-7 [74]	2009	Cyclophosphamide 4 g/m ² on Day 1 followed by etoposide at 200 mg/m ² on Days 2, 3, and 4.	26	II	9 (35%) SD, OS at 1 yr 50%, PFS 42%
Chawla et al, Mol Ther, 17: 1651-7 [51]	2009	Escalating doses of Rexin-G i.v. from 8×10 ¹¹ to 24×10 ¹¹ colony forming units (cfu)/cycle	Osteo=22 Sar=20 Osteo=0	II I/II	10 (58.8%) SD 3 (50%) SD (lowest dose) 10 (71%) SD (higher dose)

ADR, adriamycin; APBSCT, autologous peripheral blood stem cell support transplantation; CDDP, cisplatin; Cfu, colony forming units; CR, complete response; DOX, doxorubicin; EFS, event free survival; GCSF, granulocyte colony-stimulating factor; HD-MTX, high-dose methotrexate; ICE, etoposide; IFO, ifosfamide; IL-6, interleukin -6; IL-8, interleukin-8; L-MTP-PE, liposomal muramyl tripeptide phosphatidyl ethanolamine; MTX, methotrexate; NA, not available; OR, objective response; PD, progressive disease; PFS, progression free survival; pts, patients; OS, overall survival; RDI, relative dose intensity; resp, respectively; Sar, sarcoma; TNF, tumour necrosis factor.

For the number of patients enrolled in the eligible trials the results were disappointing (Figure 2.2B), with an almost stable number of patients in osteosarcoma trials, except for 1999, 2005 and 2009 when the results from larger phase II trials were published (Table 2.3). For Ewing sarcoma there were increasing numbers of patients in early phase trials, especially in 2008 when the result of the largest trial in Ewing sarcoma patients was published (Table 2.4). Out of all the 780 osteosarcoma patients enrolled in phase I/II trials including only osteosarcoma patients, 762 were evaluable and 58 patients (8%) achieved complete response (CR), 21 (2.8%) showed partial response (PR) and 30 (4%) developed stable disease (SD). For the trials enrolling only Ewing sarcoma patients, 869 were recruited and 840 were evaluable, 80 had CR (9.5%), 63 (7.5%) showed PR and 23 (3%) developed SD.

Looking at the trials subdivided for chemotherapy or biological treatment (Tables 2.1 and 2.2) in the past twenty years, there seems to have been a shift towards biologically based treatments instead of chemotherapy. Even so most of the phase III trials are still chemotherapy based treatments.

For current ongoing phase I and phase II studies we found 156 trials that are open and recruiting sarcoma patients. These trials recruit sarcoma patients in general or patients with solid tumours or soft tissue sarcoma. Studies enrolling only osteosarcoma or Ewing sarcoma patients are rare. For osteosarcoma we found four trials that are recruiting patients and two that are already active but not yet recruiting patients (Table 2.5). Of the trials two were testing chemotherapy treatment, three biologically agents and one a combination of both. For Ewing sarcoma we found no trials that enrol only Ewing patients and two that are still active but not currently enrolling patients.

When searching for studies that are enrolling sarcoma patients we found a number of trials that were preliminary stopped. For example SARC011, this is a phase II trial of R1507, a recombinant human monoclonal antibody to the Insulin-like growth factor-1 receptor for the treatment of patients with recurrent or refractory Ewing sarcoma, osteosarcoma, synovial sarcoma, rhabdomyosarcoma and other sarcomas. In December 2009, Roche/ Genentech decided to discontinue the development of their IGF-1R antibody although the drug had shown important clinical benefit <http://www.cancer.gov/ncicancerbulletin/111610/page5>. Roche said the decision was due to the available clinical data, the large number of molecules targeting the same pathway that are presently in development and the prioritization of the Roche portfolio. The decision was not as a result of safety concerns. Both this study, and a Phase I/II with figitumumab (Pfizer) have been published since completing this survey, and show low but durable response rates in a subset of relapsed Ewing patients (10–20%) [17, 18].

Table 2.4 Characteristics of eligible trials for Ewing sarcoma only

Author	Year	Intervention	No of pts	Phase	Outcome
Hawkins et al, Med Pediatric Oncol, 34 (5): 328-37 [33]	2000	Myeloablative therapy followed by HSCT	16	I	3-year EFS 36%
Kolb et al, J Clin Oncol, 21 (18): 3423-30 [75]	2003	7 cycles of chemotherapy consisted of Cyclo, Doxo, Vin, Ifos and Etop	68	II	29 (43%) CR, 13 (19%) PD or SD
Meyer et al, Sarcoma, 7 (1): 13-7 [76]	2003	Doc thrice weekly for a maximum of six cycles	14	II	1 (14%) PR, 2 (28%) SD
Bernstein et al, J Clin Oncol, 24 (1): 152-9 [77]	2006	High-dose induction therapy followed by window period. Randomization between topo, topo + cyclo or no treatment. No window treatment patients received Amifostine.	117	II	45 (43%) CR, 41 (39%) PR, 14 (14%) SD
Womer et al, ASCO abstract	2008	Vin, Doxo and Cyclo alternating with Ifos and Etop, for 14 cycles. Regimen A: 3 weeks cycle, regimen B: 2 week cycle. Primary tumor treatment was scheduled to begin week 13.	587	II	EFS at a median of 3 years was 65% in Regimen A and 76% in Regimen B
Rosenthal et al, Bone Marrow Transplantation, 42: 311-8 [78]	2008	HDT followed by HSCT	22	I	3-year EFS 47%, 3-year OS 45%
DuBois et al, Pediatric Blood Cancer, 52 (3): 324-7 [79]	2009	Cytarabine	10	II	1 (10%) SD
Casey et al, Pediatric Blood Cancer, 53 (6): 1029-34 [80]	2009	Irinotecan and Temozolomide	20	II	5 (25%) CR, 7 (36%) PR
Olmos et al, Lancet Oncol, 11: 129-35 [56]	2010	A subgroup of Ewing Sarcoma patients were treated with Figitumumab	15	I	1 (6%) CR, 1 (6%) PR, 6 (37%) SD

CR, complete response; Cyclo, cyclophosphamide; Doc, docetaxel; Doxo, doxorubicine; EFS, event free survival; Etop, etoposide; G-CSF, granulocyte colony stimulating factor; HDT, high-dose chemotherapy; HSCT, hematopoietic stem cell transplantation; Ifos, ifosfamide; OR, overall response; OS, overall survival; PD, progressive disease; PR, partial response; pts, patients; SD, stable disease; Topo, topotecan; Vin, vincristine.

Table 2.5 Ongoing phase I or II trials in osteosarcoma (Assessed from www.ClinicalTrials.gov)

ClinicalTrials.gov Identifier	Sponsors	Title	Status
NCT00102531	Transave	Phase Ib/Ila non-randomised study of SLIT Cisplatin by inhalation in the treatment of patients with relapsed/ progressive osteosarcoma metastatic to the lung	Recruiting
NCT01002092	Shandong Simcere-Medgenn Bio-pharmaceutical Co., Ltd	A randomized, controlled multicenter trial of Endostar combined with chemotherapy for treatment of osteosarcoma (phase II)	Recruiting
NCT00889057	Italian Sarcoma Group	Phase II, open label, non-randomized study of second or third line treatment with Sorafenib (BAY 43-9006) in patients affected by relapsed high-grade osteosarcoma	Recruiting
NCT00752206 NCT00923286	Sarcoma Alliance for Research through Collaboration (SARC)	A randomized, double-blinded, placebo-controlled, multi-institutional, phase II study of AZD0530, a selective Src kinase inhibitor, in patients with recurrent osteosarcoma localized to the lung	Recruiting
NCT01005043	University of Heidelberg	Phase I/II therapy non-randomised trial to determine the safety and efficacy of heavy ion radiotherapy in patients with osteosarcoma	Not yet recruiting
NCT00902044	Baylor College of Medicine	Administration of Her2 Chimeric antigen receptor expressing T Cells for subjects with advanced osteosarcoma (HEROS) (phase I)	Not yet recruiting

Bone sarcoma is fatal in approximately one third of the children and young adults in whom it is diagnosed. This mortality rate has not changed greatly in the two decades since the initial introduction of surgery and chemotherapy. The primary cause of death in treated patients is recurrent metastatic disease often to the lung. In the group of patients who present either with metastatic or relapsed disease, it is especially important to conduct biologically based trials with small groups of patients from which quantitative and quick answers of whether a treatment intervention is either working or not can be obtained. Importantly, tumour material obtained may provide informative clues for future studies especially if analysed with genomic technologies.

It is difficult to do trials in an orphan disease, mainly because of the problems of recruiting enough eligible patients for the trial. In our literature search, we identified 42 phase I/II trials for sarcoma patients which were published between 1990 and 2010. However the phase I trials for general sarcoma (Table 2.1) are not complete, this is because of our search criteria. With a PubMed search you only search the abstract of the article, so if sarcoma is not mentioned there we didn't find the article. In phase I trials quite a few different tumour types are sometimes enrolled and only the one who show results are mentioned in the abstract. Some of these articles we found in the Cochrane Controlled Trial Register or the ClinicalTrials.gov database, but not all trials are registered in these databases. Except with a very wide PubMed search using (phase 1) as only search criteria we don't think that it is possible to find all phase I trials enrolling sarcoma patients.

Of the trials we did find with our search criteria twenty-one were phase I, two phase I/II and nineteen phase II trials. Only eight clinical trials included only Ewing sarcoma patients; of this group two were phase I and six were phase II trials. We found only twenty trials which included only osteosarcoma patients. There were two phase I, sixteen phase II, and two phase I/II trials. A total of 3,736 patients were included in the clinical trials, of which 1,263 were Ewing sarcoma and 1,114 were osteosarcoma patients. From the identified trials, the results are not convincing for benefit and most of the time even disappointing, from osteosarcoma trials we found 8% CR, 2.8% PR and 4% SD. For Ewing sarcoma the results are 9.5% CR, 7.5% PR and 3% SD. And if the drugs seem to be effective, a lot of toxicity is reported (Tables 2.1, 2.2, 2.3, 2.4). Looking at the number of trials we found for sarcoma patients, the results seem encouraging as a high number of patients were included. However, if we make a calculation of the number of new Ewing sarcoma patients expected in the last twenty years, assuming a world population of five billion and an incidence of 2 in 1,000,000, approximately 200,000 new cases would be expected in twenty years worldwide. Of these patients, we could only find 1,263 reported and included in published clinical trials. For osteosarcoma the number is even worse, with 500,000 new cases expected in twenty years and only 1,114 reported in trials.

To improve these results changes in conducting trials for orphan state diseases have to be made. It is important to collaborate between nations; the Sarcoma Alliance for Research through Collaboration (SARC) is a good example based principally in North America. SARC is a non-profit organization started in 2003. Funding is provided by generous donations, sponsors and grants. SARC works with healthcare professionals as part of a collaborative multidisciplinary team from over 35 institutions in the USA. It works with a number of international institutions and provides the infrastructure for collaboration between physicians, researchers, and medical institutions from across the worlds who are engaged in clinical research for development of new standards of sarcoma

patient treatment, education, and prevention. This organization facilitates dialogue and collaboration among sarcoma researchers and clinicians, assists in the development and dissemination of protocols for clinical trials and information relating to sarcoma research and the results of clinical trials, administers research grants and funding for clinical trials, and acts as a primary resource for those treating patients with sarcoma. The EORTC Soft Tissue and Bone Sarcoma Group is one of SARC's European counterpart, conducting multicenter academic studies in sarcoma, including some early stage translational studies, although it's main contribution in the past has been in the field of larger phase III trials soft tissue sarcoma. The European Osteosarcoma Intergroup, EURAMOS and EuroEwing consortium are examples of groups acting primarily as platforms for phase III studies in bone sarcomas.

The EC-granted European network, EuroBoNeT <http://www.eurobonet.eu>, was the first central organized network of excellence for integrated bone sarcoma research and staff exchange, in order to increase and disseminate knowledge of primary bone tumours at the molecular level for development of new tools for patient care. This integration, exchange of material (virtual Bio-Bank), standard operating protocols and the use of technology platforms enabled researchers to obtain statistically significant datasets, otherwise not achievable due to the rareness of the condition and the large number of sub-entities. Without these kinds of collaborations, the development of new treatment strategies in osteosarcoma and Ewing sarcoma is going to be very difficult. But perhaps most importantly is that phase I/II clinical trials have to be conducted. To achieve this funding needs to be established from industry and governments to feed through these collaborative structures over time frames that might result in enduring progress. Recent progress has also been made with the FP7 funding of EuroSarc, European trials in rare sarcomas within an integrated translational trial network (2011–2016). In this new translational trial network, the challenges of combining high quality disease research into clinical trials in being addressed, and progresses the field beyond conventional trials and the existing clinical trial networks.

In addition to the challenge of identifying the most promising agents for clinical trials in bone sarcoma, obstacles inherent to this disease further complicate the successful design and completion of trials of novel agents. In evaluating the efficacy of novel agents, the standard approach is to use objective response criteria, such as Response Evaluation Criteria in Solid Tumours (RECIST) [19], to compare the size and/or volume of lesions pre-treatment and at regular intervals during and post-treatment. New methods for tumour response which may be more precise are radiological response based on new criteria, e.g. 18FDG-PET-CT and contrast enhanced MRI. For a patient to be eligible for a trial

using this approach, he or she must have measurable disease. The traditional approach for patients with relapsed bone sarcoma, in particular pulmonary relapse, has been surgical resection, which at least temporarily renders a patient free of radiographic measurable disease and thus, ineligible for most trials of novel agents. Unfortunately novel targeted therapies may be most effective in the setting of minimal residual disease. The use of creative surrogate end points to estimate response and perhaps render the combination of surgical resection and treatment on trials of new agents not mutually exclusive would allow for better evaluation of new therapies in patients with bone sarcoma [20].

When conducting the literature search we found that the results of many trials are not published. We can only guess what the reason for this is, but it seems logical that the trial results were disappointing. From the phase I/II trials only a very limited number of treatments proceeded to phase III trial. For Ewing sarcoma we found three clinical trials conducted in the last twenty years and two trials which are being recruiting at this moment and for osteosarcoma we found twenty-two phase III clinical trials and four active trials currently recruiting patients and one is ongoing trial but not recruiting.

Most of the phase I/II trials that have been conducted recruited patients with general sarcoma or solid tumours. In these trials it is very difficult to say anything about disease specific response and it is therefore almost impossible to translate the results to clinical practise and patients with a specific sort of cancer. Of course it is understandable why researchers test a drug in different patient populations in the same trial, especially in rare cancers like bone sarcoma, but it makes it more difficult to proceed to a disease specific phase II or even phase III trial.

While the current standard of care continues to be multi-agent chemotherapy and surgical resection, it is clear that further intensification of traditional chemotherapy regimens is limited by toxicity, and new approaches are needed. Some promising novel agents are currently in or are entering into phase II trials in children with relapsed bone sarcoma. For example, Muramyl Tripeptide Phosphatidylethanolamine (MTP-PE) is the first drug to be approved for osteosarcoma in Europe for ten years [21]. In Ewing sarcoma the identification of the Insulin-like Growth Factor 1 Receptor (IGF1R) pathway deregulation, as a consequence of the EWS-FLI1 translocation seems to be a new treatment strategy to be explored. The incorporation of these new treatments into the up-front therapy for bone sarcoma in the near future holds promise for improving outcomes.

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Ewing sarcoma: the clinical relevance of the insulin-like growth factor 1 and the poly-ADP-ribose-polymerase pathway

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Background

In the last three decades the outcome for patients with localized Ewing sarcoma has improved significantly since the introduction of multimodality primary treatment. However, for patients with (extra-) pulmonary metastatic and/or non-resectable relapsed disease the outcome remains poor and new treatment options are urgently needed. Currently the insulin-like growth factor 1 receptor (IGF-1R) pathway and the poly ADP ribose polymerase (PARP) pathway are being investigated for potential targeted therapies.

IGF-1R

The IGF-1R pathway is known to be deregulated by the EWSR1-FLI1 translocation which makes it a potential target for therapy. Clinical trials have been reported in which only Ewing sarcoma patients were treated with an IGF-1R inhibitor, either as single agent or in combination. In total 291 Ewing sarcoma patients were included in these trials, in which two (0.7%) complete responses, 32 (11%) partial responses of which some durable, and 61 (21%) stable diseases were observed.

PARP

In the presence of a PARP-inhibitor DNA strand breaks cannot be efficiently repaired, leading to cell death. The first phase II trial with Ewing sarcoma patients was recently published and showed no clinical responses, which may have been due to the drug being non-effective as single agent.

Discussion

The IGF-1R pathway is an interesting target for Ewing sarcoma and should be explored further, as biomarkers to select patients that might benefit from treatment are lacking. PARP inhibitors as single agent have so far failed to show improvement in outcome. Future directions include dual insulin receptor/IGF-1R blockade with linsitinib as well as chemotherapy-PARP combinations. Both therapeutic strategies are currently being explored.

Background

Ewing sarcoma is the third most common primary bone sarcoma after osteosarcoma and chondrosarcoma. However it is still rare with an overall incidence of 2.9 new cases/1,000,000 every year in the United States [1]. In children and adolescents Ewing sarcoma is mainly localized in bone, with a peak incidence at fifteen years of age [2]. In adults, Ewing sarcoma localizes more frequently primarily in soft tissue or organs. The tumour often harbors a reciprocal translocation, causing a fusion of the EWSR1-gene on chromosome 22 with a member of the ETS family of transcription factors [3]. The most common translocation (85%) is the t(11;22)(q24;q12), fusing EWSR1 to FLI1. Other rare ETS and non-ETS fusion partners have been described, and so far it is unclear whether the latter should be considered a separate entity [3]. The EWSR1-ETS translocation type does not influence the outcome or reaction on chemotherapy [4, 5]. Proven genetic prognostic factors are TP53 mutations [6], CDKN2A deletions [7], 1q gain [8] and Stag2 mutations [9-12].

With current multimodal treatment options, including surgery, conventional chemotherapy and radiotherapy, the 5 year survival for localized disease is 60%. However, for patients that present with metastatic disease other than lung involvement only, the 5-year survival is below 20%. The outcome for patients with relapsed or refractory Ewing sarcoma is even worse with a 5-year survival as low as 10% [13]. Therefore, new treatment strategies are urgently needed to advance the treatment of Ewing sarcoma patients with metastatic or relapsed disease. Currently the insulin-like growth factor 1 receptor (IGF-1R) pathway and the poly ADP ribose polymerase (PARP) pathway are being investigated for potential targeted therapies. In our opinion these two pathways represent the main area of early clinical studies in Ewing sarcoma in the recent years deserving an in-depth review. Therefore, here we summarize current knowledge in an attempt to stimulate further treatment development for Ewing sarcoma.

IGF-1R pathway in Ewing sarcoma

IGF-1R pathway

IGF-1R is a tyrosine kinase receptor which is 84% homologous to the insulin receptor (IR) and is widely expressed in human tissues [14]. Binding of the ligands (IGF-1 and IGF-2) to the IGF-1R or the IR induces receptor dimerization, resulting in trans-autophosphorylation of the receptors (Figure 3.1). This receptor phosphorylation recruits the downstream signaling proteins insulin receptor substrate (IRS) 1, 2 and 4 and the Src homology 2 domain containing transforming proteins (Shc) to the cell membrane [14]. The subsequent

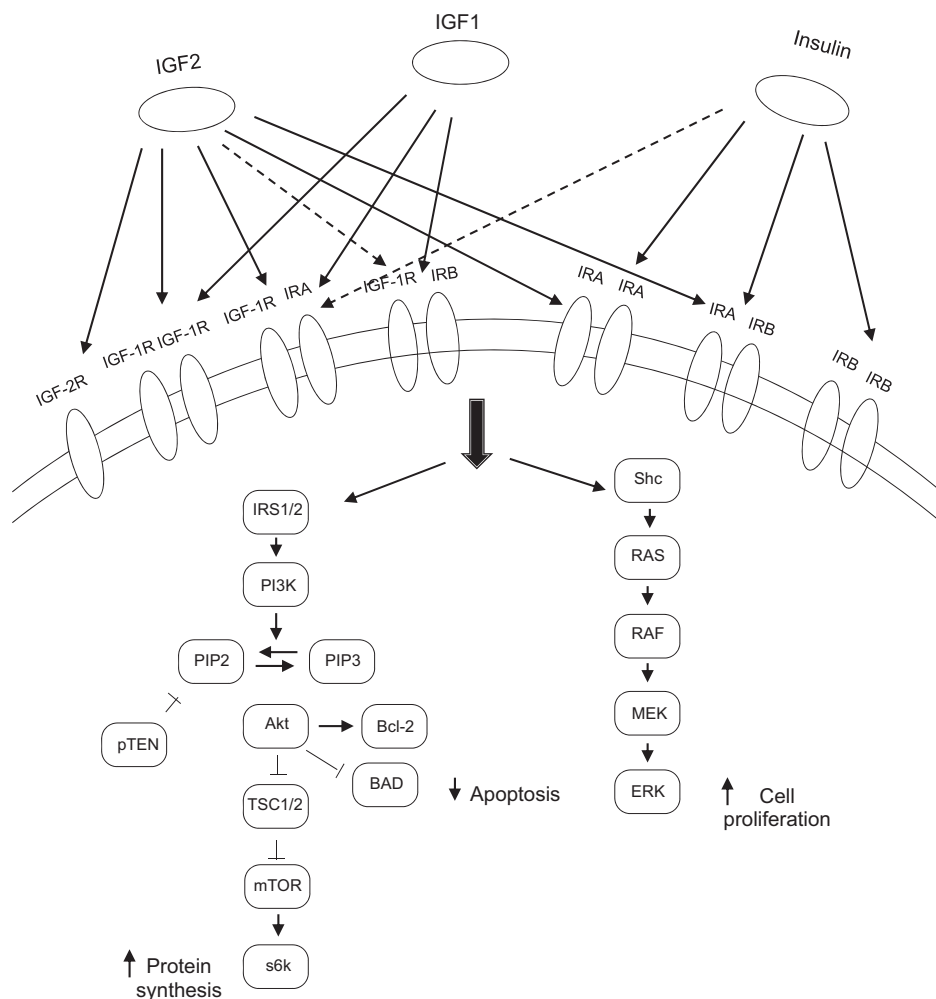


Figure 3.1 The IGF pathway showing the stream molecules as well as the parallel activation route of the insulin pathway.

Activation of the IGF or IR receptor by IGF1, IGF2 or insulin activates either the P13K-Akt pathway which can upregulate Bcl-2 and downregulate BAD resulting in apoptosis and it can down regulate the mTOR pathway resulting in increased protein synthesis. It can also activate the RAS-RAF-MEK-ERK pathway resulting in increased cell proliferation.

phosphorylation of these proteins induces the activation of the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinases (MAPK) pathways resulting in stimulation of cellular proliferation, cell motility and inhibition of apoptosis [14]. IGF-1 and IGF-2 are mainly produced by the liver in response to the presence of growth hormone and are found in the circulation bound to a family of proteins called IGF binding proteins

(IGFBP1-6), which regulate their bioavailability in peripheral tissues. The bioavailability of IGF-2 is also regulated by the IGF-2R, which does not confer intracellular signaling [15]. IGFBP3 is the most abundant IGF binding protein, which forms a ternary complex with insulin-like growth factor acid-labile subunit and accounts for 80% of all IGF binding. In addition to its role in normal cellular development (fetal growth and linear growth of the skeleton and other organs), the IGF signaling pathway has been implicated in malignant transformation and disease progression [16-18]. Interestingly, patients with congenital deficiency of IGF-1 seem protected from the development of malignancies [19] and cells with a dominant negative mutation in IGF-1R fail to undergo malignant transformation and *in vivo* tumourigenesis [20]. Furthermore, many tumours and cell lines have increased expression of IGF1 or IGF-1R [21]. Also, numerous studies have shown that higher plasma concentrations of IGF1 are associated with increased cancer risk, in particular for breast, prostate and colon cancer [22-31].

IGF-1R pathway activity and its inhibition in Ewing sarcoma; preclinical data

Interestingly, the peak incidence of primary bone Ewing sarcoma correlates with the increased levels of the IGF ligands in puberty. In 1990 it was already shown that IGF1 is expressed in Ewing sarcoma carrying a t(11;22) translocation and that blocking the IGF-1 loop inhibits cell growth [32]. Subsequent studies using Ewing sarcoma cell lines confirmed these findings [33]. In 1997 a study using fibroblast cell lines from a IGF-1R knock-out mouse and a wild type mouse was described in which the altered expression of IGF-1R was studied in the presence of the EWSR1-FLI1 fusion [34]. It was shown that the wild type cells that contained the fusion protein had a greater degree of ligand-stimulated insulin receptor substrate-1 phosphorylation and thus giving evidence that altered IGF-1R signaling by expression of the EWSR1-FLI1 fusion protein is required to transform fibroblasts. Despite activity of the pathway, IGF-1R expression is relatively low in Ewing sarcoma cell lines and in patient tumour tissues [33]. Higher expression levels of IGF-1R, IR and IGF-1 were significantly correlated with better clinical outcome in Ewing sarcoma, in which high levels of IGF-1 were associated with lower risk of disease progression and death [35]. Interestingly, it has been described that the EWSR1-FLI1 fusion protein can directly bind the IGFBP3 promoter, thereby repressing its activity [36].

However other fusions in addition to EWSR1-FLI1 have been described in Ewing sarcoma including EWSR1-ERG and FUS-ERG. When the three different fusions were introduced into mouse progenitor cells the expression profiles differed, but all three fusions were able to activate the IGF-1 promotor and induced IGF-1 expression, while FLI-1 or ERG alone were not able to do this [37]. In Ewing sarcoma mouse xenografts, a selective IGF-

1R kinase inhibitor (NVP-AEW541) decreased migration, metastases, vasculogenesis and angiogenesis [38]. Side effects included significant weight loss at the start of treatment and high blood glucose levels.

Combined inhibition of IGF-1R and other pathways

IGF-1R inhibitors have been combined with other anti-tumour drugs in Ewing sarcoma. For example, an inhibitor of endocytosis was studied in combination with an IGF-1R tyrosine kinase inhibitor. It was shown that blockade of receptor internalization inhibited the phosphorylation of AKT and MAPK, reduced proliferation rate and increased apoptosis. Strikingly, this effect was greatly enhanced by combining the endocytosis inhibitor with a tyrosine kinase inhibitor, thereby identifying a new therapeutic approach for IGF-1R dependent neoplasms [39].

Very recently, promising results were achieved *in vitro* and *in vivo* by combining IGF-1R inhibition with the DNA minor groove binding agent trabectedin [40]. Trabectedin was shown to increase IGF-1R expression by improving the binding of ESWR1-FLI1 to the IGF-1R promotor. Inhibition of IGF1R, either by the specific AVE1642 human antibody or by the dual IGF1R/insulin receptor inhibitor OSI-906 (linsitinib) greatly potentiated the efficacy of trabectedin in Ewing sarcoma cell lines as well as in Ewing sarcoma xenografts providing rationale for combination treatment.

The mammalian target of rapamycin (mTOR) is a serine/threonine protein kinase which is critical in many cellular processes such as cell proliferation and survival [41]. Aberrant mTOR signaling is known to conduct tumour cell survival and is therefore an interesting target for therapy [42-44]. Because mTOR plays an important role in the PI3K/Akt pathway downstream of the IGF-1R receptor, inducing cell proliferation by phosphorylation of the s6k protein [45], inhibition of mTOR could be a promising therapeutic option in tumours with upregulated IGF-1R signalling [46]. However, tumour cells *in vitro* and *in vivo* treated with mTOR inhibitor as monotherapy can develop resistance, probably due to the activation of a feedback loop after blocking of the mTOR pathway, resulting in upregulated Akt phosphorylation via an IGF-1R dependent mechanism [47-49]. This provides rationale for the combination of an mTOR inhibitor with an IGF-1R inhibitor to overcome resistance to either treatment as monotherapy. Another reason for combination treatment is that using immunohistochemistry and immunofluorescence it was found that approximately 25% of Ewing sarcoma harbour a PTEN deficiency which leads to enhanced AKT activation with decreased apoptosis and increased growth, rendering cells less sensitive to IGF-1R inhibition [50]. The combination of an IGF-1R and an mTOR inhibitor may give a therapeutic

benefit. *In vivo* xenograft models it has been shown that the combination gives an enhanced antitumour activity compared to monotherapy [51, 52].

Another important player in activation of the PI3K/Akt/mTOR pathway is ErbB3, a tyrosine kinase receptor [53] and combined IGF-1R and ErbB3 inhibition may result in complete blockage of the IGF pathway thereby inducing apoptosis. Recently, MM-141, an IGF-1R and ErbB3 bispecific antibody was developed and the first preclinical results are published [54]. ErbB3 activation was proven to be an escape mechanism for different solid tumour cells, including Ewing sarcoma cells, treated with IGF-1R inhibitors. MM-141 has demonstrated to decrease the levels of IGF-1R and ErbB3 *in vivo*, thereby providing a strong clinical rationale for further development of IGF-1R and ErbB3 antibodies. Currently, a phase I study is enrolling patients with solid tumours who are being treated with MM-141 as monotherapy and a phase II is recruiting patients with metastatic pancreatic cancer who are being treated with MM-141 combined with paclitaxel and gemcitabine (NCT01733004 and NCT02399137).

Clinical trials with IGF-1R inhibition in Ewing sarcoma

Recently a phase II trial was published treating patient with recurrent or refractory bone or soft tissue sarcomas with the IGF-1R monoclonal antibody R1507 [55]. This study concluded that the IGF-1R antibody R1507 has limited activity in bone and soft tissue sarcomas but a favorable toxicity profile. A subgroup of the enrolled patients did have a durable response and further studies are needed.

Several clinical trials have been conducted to evaluate the efficacy of IGF-1R inhibition specific in Ewing sarcoma patients (Table 3.1). In six trials different IGF-1R antibodies were tested as monotherapy, including two trials with figitumumab® [56, 57], two trials with cixutumumab® [58, 59], one trial with R1507 [60] and one with ganitumumab® [61]. A total of 311 patients were included in these trials with two (0.6%) complete responses (CR), 31 (9.9%) partial responses (PR) and 66 (21%) patients with stable disease (SD). Some of these responses were durable up to more than two years. These results and the results from other studies show that these antibodies are well tolerated and require almost no dose reductions, with the exception of ganitumumab® in which all patients had an adverse event reported and 45% of the patients required a dose reduction.

Sarcoma patients were treated with cixutumumab®, an IGF-1R monoclonal antibody, combined with the mTOR inhibitor temsirolimus® [62]. The study was divided into two cohorts. The first cohort received cixutumumab® 6 mg/kg weekly intravenously and temsirolimus® 25 mg intravenously weekly. In the second cohort patients received

Table 3.1 Trials conducted with an IGF-1R inhibitor enrolling exclusively Ewing sarcoma patients showing the name of the author, the phase of the study, the drug tested, the dosage of the tested drugs, number of patients included, the response rate and the biomarkers tested

Author	Phase	Drug	Dose	Nb of ES pts	Response for ES pts	Biomarkers tested
Juergens (56)	II	Figitumumab®	30 mg/kg/4w	107	15 PR, 25 SD	Free IGF-I levels, Total IGF-I level
Olmos (57)	Expansion cohort	Figitumumab®	20 mg/kg/4w	16	1 CR, 1 PR, 6 SD	None
Malempati (58)	II	Cixutumumab®	6 mg/kg/w and 9 mg/kg/w	36	3 PR, 5 SD	IGF-I level, IGF-II level, IGF-IR level
Schöffski (59)	II	Cixutumumab®	10 mg/kg/2w	18	1 PR, 5 SD	None
Pappo (60)	II II	R1507 R1507	9 mg/kg/w 27 mg/kg/3w	109 6	1 CR, 10 PR, 18 SD	Total IGF-I level
Tap (61)	II	Ganitumumab®	12 mg/kg/3w	19	1 PR, 7 SD	IGF-I level
Naing (62)	II	Cixutumumab® and Temsirolimus®	6 mg/kg/w and 25 mg/w	17	2 CR, 5 MR	None

CR = complete response ES = Ewing sarcoma; MR = minor response; Nb = number; PR = partial response; pts = patients; OR = overall response; sar = sarcoma; SD = stable disease.

cixutumumab® 6 mg/kg intravenously weekly and temsirolimus® 37.5 mg intravenously weekly. All 17 Ewing sarcoma patients were enrolled in the first cohort. In total, 7 Ewing sarcoma patients (35%) had an objective response, being 5 minor responses and 2 CR. In one of these patients the response lasted more than 27 months although the patient had previously developed resistance to another IGF-1R inhibitor. The treatment was well tolerated and most common adverse events were thrombocytopenia, mucositis, hypercholesterolemia, hypertriglyceridemia and hyperglycemia. Three patients required dose reductions but they were re-escalated without recurrence of toxicity.

PARP pathway in Ewing sarcoma

PARP pathway

Poly-ADP-ribose-polymerases (PARPs) are a family of proteins which are activated upon DNA damage [63]. They detect single strand breaks and recruit the enzymatic DNA repair machinery (Figure 3.2). Mostly PARP-1, and to a lesser extent PARP-2, are responsible

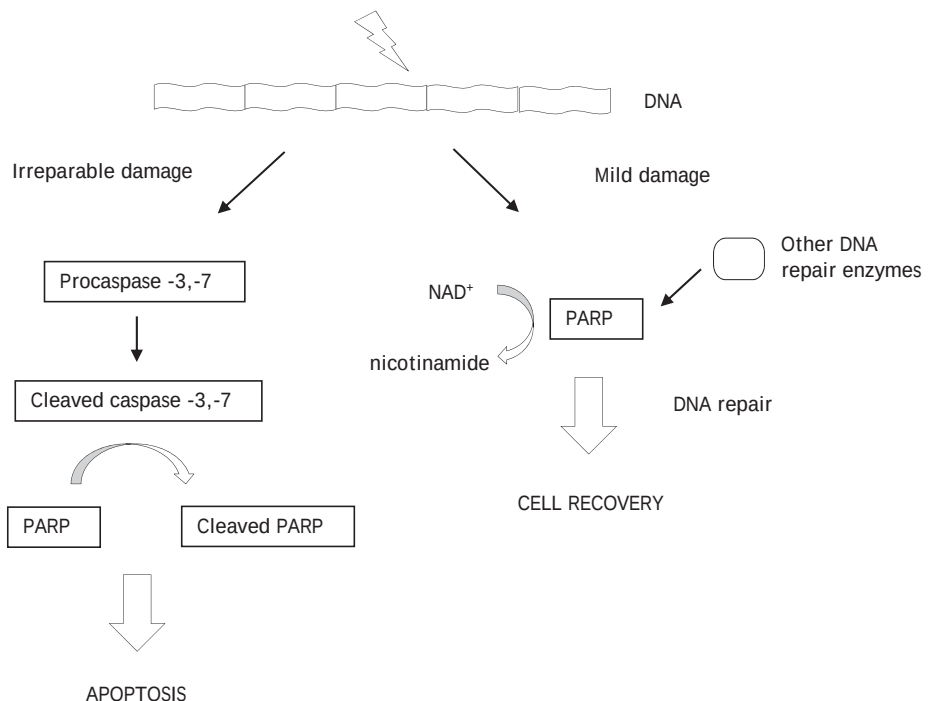


Figure 3.2 The PARP pathway showing activation of the apoptosis route.

By mild DNA damage PARP is activated and with the aid of other DNA repair enzymes NAD⁺ is transformed in nicotinamide and the cell recovers. If there is irreparable DNA damage, the procaspase -3,-7 are cleaved which subsequently cleaves PARP, resulting in apoptosis.

for this mechanism [64]. If PARP is inactivated by caspase cleavage, cells are more prone to go into apoptosis upon DNA damage. PARP inhibitors seem a promising therapeutic approach for tumours harboring a germline BRCA1/2 mutation, including ovarian and breast cancer [65, 66].

PARP inhibition in Ewing sarcoma

The sensitivity of ES cells to PARP inhibitors was discovered when several hundred cancer cell-lines were screened for drug-sensitivity [67]. Moreover, EWSR1-FLI1 positive Ewing sarcoma cell lines and xenografts were shown to be highly sensitive to the PARP-1 inhibitor olaparib, especially in comparison to osteosarcoma and rhabdomyosarcoma cell lines without the ETS rearrangement [68]. More specifically, the EWSR1-FLI1 and the EWSR1-ERG fusion genes were shown to interact with PARP-1. Strikingly, even cell lines from heavily pretreated and relapsed patients were extremely sensitive to olaparib. Although treatment with olaparib accentuated DNA damage, this did not have an effect on short-term cell viability. In 2012, an additional mode of action of PARP-inhibitors has been found, in which trapped PARP-DNA complexes are highly toxic to cells because of DNA replication blockade [69]. It is shown that the trapped PARP-DNA complexes are even more toxic to cells than the accumulating DNA damage in the presence of PARP-inhibitors. The combination of PARP inhibitors and cytotoxic drugs was tested in *in vitro* and *in vivo* assays [70]. Interestingly, synergy was most notable in medulloblastoma and Ewing sarcoma cell lines. In Ewing sarcoma xenograft models the maximum tolerated dose of olaparib, cyclophosphamide and topotecan was determined and used in a combination treatment schedule [70]. With single treatment of olaparib 88% of PARP-1 inhibition was detected, which increased to 100% when combined with cyclophosphamide and topotecan. A synergistic effect was found between olaparib and irinotecan with 2–3 times more growth inhibition than with single treatment. Talazoparib, another PARP inhibitor, *in vitro* showed a synergistic effect when combined with temozolomide with a 30-fold increase in toxicity in Ewing sarcoma cell lines [71]. The PARP inhibitor olaparib was combined with trabectedin and showed a synergistic effect in several Ewing sarcoma cell lines and an increase in apoptotic rate [72]. When the combination was tested in a xenograft model a reduce in tumour growth was seen. The combination of PARP-1 inhibition with radiotherapy was also investigated [73]. It was found that after low levels of radiation, PARP-1 activation was significantly increased in Ewing sarcoma cell lines and only mild in non-Ewing sarcoma cells. The combination treatment of olaparib and radiotherapy in Ewing sarcoma cells lead to a synergistic decrease in proliferation and colony formation. Moreover, in xenograft models the combination treatment stopped tumour growth, emphasizing the potential as new therapeutic approach for Ewing sarcoma [73].

Clinical trials with PARP-inhibitors in Ewing sarcoma

Since the discovery of PARP-inhibitors as a therapeutic option for Ewing sarcoma patients in 2012, only one clinical trial including Ewing sarcoma patients has been published [74].

This phase II trial included Ewing sarcoma patients with recurrent/metastatic disease who were not responding to previous therapy. A total of 12 patients were treated with olaparib 400 mg twice a day, administered orally. All patients were evaluated for response, and no CR or PR was seen. However, in four patients SD was achieved ranging from 10.9–18.4 weeks. The median progression free survival was only 5.7 weeks. The drug was well tolerated with two grade 3 toxicities (anemia and thrombocytopenia).

Discussion

Preclinical studies have revealed the IGF-1R and PARP pathway as promising new targets for Ewing sarcoma and these observations have led to several clinical studies.

Regarding the IGF-1R pathway, five pharmaceutical companies went in parallel to the niche indication Ewing sarcoma for rapid approval in an orphan disease. The results of these studies were disappointing and as a result all companies stopped further development of IGF-1R pathway inhibitors for Ewing sarcoma [75]. We think that this decision was made too fast and that IGF-1R antibodies can work in Ewing sarcoma, but they should be tested in the right patient population. To find the right patient population it is very important to understand the biological rationale behind this treatment so that specific patients, schedules and doses can be selected to reach positive results for the individual patients. Good non heterogeneous biomarkers are needed to stratify patients to assess the right treatment schedule and to determine response during treatment [76, 77]. In preclinical studies the resistance mechanism for Ewing sarcoma to IGF-1R has been studied. Resistant cells switch from IGF-1/IGF-1R signaling to IGF-2/IR-A signaling [39, 78, 79]. Therefore, one could postulate that Ewing sarcoma patients should be treated with a dual IGF-1R and IR-A inhibitor, such as linsitinib (OSI-906). The EuroSARC consortium (www.eurosarc.eu) initiated the LINES study (ISRCTN 94236001), which is currently the only remaining open study for Ewing sarcoma patients with a drug targeting the IGF-1R receptor. This is a phase II study in which refractory and/or relapsed Ewing sarcoma patients are being treated with the dual IGF-1R and IR inhibitor linsitinib in a single arm Bayesian design. Patients will undergo mandatory tumour biopsies during treatment for translational research and this will be compared to pretreatment material to monitor response at the molecular level and to look for possible biomarkers. Pharmacokinetic assays will be conducted testing for IGF1, IGF2, IGFBP3 and Insulin levels on blood samples. The tumour core biopsies

will be used to test for phosphoproteome/kinase assays of pre-treatment kinome IGF-1R pathway activation, qPCR will be conducted for expression of trabectedin related DNA repair genes, quantitative immunoassays for IGF-1R, IRS1, pS6, FOXO and EGFR1 will be performed using multi-spectral confocal image segmentation and random forest analysis. This study will hopefully answer the question why inhibition of the IGF-pathway may work in some patients and not in others.

Small molecule inhibitors targeting proteins in pathways involved in the immune system may theoretically have a dual working mechanism. On one hand they block pathways having an anti-tumour working mechanism, on the other hand they may also induce an immunological response inducing a reaction of the patients own immune system against the tumour cells [80]. Natural killer (NK) cells were found to play an important role in this antibody-dependent cell-mediated cytotoxicity (ADCC) as an immunological form of anti-tumour action [81]. NK cells can be activated by Fc-receptors (FcR) bound to the target cells by antibodies thereby being able to lyse the target cells and shred cytokines to activate the adaptive immune system. Several studies show that patient have a different FcR genotype and those with a higher FcR affinity respond better to monoclonal antibodies, this finding can be used as a biomarker and to select patients for treatment with specific antibodies [82-86]. This dual working mechanism provide a theoretical advantage of antibody treatment compared to tyrosine kinase inhibitors which lack the ADCC anti-tumour mechanism.

Regarding the PARP inhibitors, they have been developed more recently and have been tested with various successes in other tumour types such as breast, lung, prostate, fallopian tube and pancreatic cancer, glioblastoma and hematologic malignancies. So far, only the results of one trial with Ewing sarcoma patients was presented and results of monotherapy treatment in this study were unsatisfactory. Different theories about the lack of treatment effect with PARP monotherapy were suggested by the authors of this study, for example secondary epigenomic alterations that may render the PARP pathway insignificant [74]. However, the exact mechanism still has to be found. Preclinical models suggest that PARP inhibition may show better results in combination with chemotherapy or radiotherapy [70, 73, 87]. A phase I combination study of the PARP inhibitor olaparib with temozolomide in Ewing sarcoma is open for recruitment (NCT01858168) and a phase I study combining olaparib with niraparib, another PARP inhibitor, and temozolomide, an alkylating agent, is also enrolling patients with incurable Ewing sarcoma (NCT02044120). To evaluate a possible effect of PARP inhibition as monotherapy versus combined therapy, more clinical studies must be conducted. Measuring the PARP pathway down regulation by recording levels of the PARP pathway caspases before and after drug exposure in tumour biopsies in different treatment conditions will help to find the optimal dosage and combination schedule.

Biomarkers need to be tested in preclinical and clinical studies to identify patient populations with a higher likelihood to respond to certain treatment schedules. Hereby more personalized medicine can be conducted with hopefully better outcome results. IGF-1 was found to be a negative prognostic marker in different types of carcinoma [88, 89]. In Ewing sarcoma patients with elevated levels of free or total IGF-1 before treatment had a better overall survival [56]. Another protein which has been found to be overexpressed in various tumour types, like breast, head and neck, colon, pancreas and non-small cell lung cancer, and can be used as a biomarker to predict response is Rad51 [90-97]. Rad51 functions as a DNA double strand break repair protein thereby inducing stabilisation of the genome. Overexpression of Rad51 has been found to result in reduced proliferation and a more instable genome [98, 99]. Preclinical testing has to be conducted to determine if this biomarker is also usable in Ewing sarcoma. Hopefully a lesson was learned from the problems which rose with the development of IGF-1R antibodies in clinical trials, so far the development of PARP inhibitors seems to go more structured.

In conclusion, both pathways are interesting potential targets for Ewing sarcoma as they either hypothetically interact with the EWSR1-FLI1 or EWSR1-ERG fusion product and/or show promising (pre)clinical data. Therapeutic strategies should therefore definitely be explored further, and we will need to better understand which patients are responding and as a result of which underlying molecular mechanism. Other ways forward may include dual blockade such as with linsitinib and conventional chemotherapy or radiotherapy combined with PARP inhibitors. Both strategies are currently being explored clinically.

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Etoposide and carbo-or cisplatin combination therapy in refractory or relapsed Ewing sarcoma: a large retrospective study

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Introduction

Patients with Ewing sarcoma who develop refractory or relapsed disease have limited treatment options. In some sarcoma centres in Europe the combination of etoposide with carbo- or cisplatin is being used for these patients, however there are no published data available yet. Here we investigated the outcome of the combination treatment for patients with advanced Ewing sarcoma in progression after standard treatment.

Materials and methods

All patients diagnosed with Ewing sarcoma between 1980 and 2012 in one of 6 major sarcoma centres in Europe and treated with either carboplatin and etoposide or cisplatin and etoposide were included and data were retrospectively collected for analysis.

Results

A total of 107 patients enrolled in this study of which 61 received the combination of etoposide and carboplatin and 46 received etoposide and cisplatin. The median overall survival (OS) was 23 months for both patient groups and the 5-year OS was 24.5% for the patients who received carboplatin and etoposide and 20% for those who received cisplatin and etoposide. The progression free survival was better in patients treated with the combination of carboplatin and etoposide (14.5 versus 6.3 months $p=0.023$).

Conclusion

This is a retrospective study on the combination treatment of etoposide and carbo- or cisplatin in refractory Ewing sarcoma. The results justify exploring the combination in a prospective study with relapsed patients.

Introduction

Ewing sarcoma (EWS) is a rare disease with an annual incidence of approximately 3 per million new cases each year [1]. EWS affects mostly children and adolescents with a peak incidence at age fifteen [2]. It arises in bone and to a lesser extent in soft tissue with the most common primary sites being the pelvis and the long tubular bones. EWS is associated with chromosomal translocations involving the *EWS* gene on chromosome 22 and an ETS transcription factor gene, the commonest translocation, t(11;22)(q24;12), resulting in the EWS-FLI1 fusion protein [3]. For patients with localised disease 5 year survival rates have improved to 70% in the last decades [4, 5]. However, 25% of all patients present with metastatic disease, mostly to the lungs or bones. The survival rates for these patients are much lower, around 50% for patients with only lung metastases and around 20% for patients with bone metastases [6, 7]. Following relapse, the prognosis is poor with 5 year overall survival of 13% [8].

When patients develop relapsed or refractory disease with no local treatment options several palliative chemotherapy regimens are used. In some sarcoma centres in Europe these patients are treated with either the combination of carboplatin and etoposide (carbo-eto) or cisplatin and etoposide (cis-eto). Carboplatin and cisplatin belong to the group of platinum-based antineoplastic agents and generating several types of DNA damage that are difficult to repair and lead to cell death. Carboplatin has fewer side effects than cisplatin; however, in some tumour types, namely testis, the drug is considered to be less effective. Etoposide belongs to the topoisomerase inhibitor family and forms a complex with DNA and topoisomerase II enzyme thereby preventing re-ligation of DNA strands and causing them to break [9].

Because there is no published literature on the effect of the two agent combination of etoposide with a platinum-based antineoplastic agent for patients with relapsed or refractory Ewing sarcoma, we retrospectively collected the overall survival data of these patients who received the combination and also looked at possible prognostic factors for survival.

Materials and methods

Patient data were collected retrospectively from the archives of 5 sarcoma centres in Europe (Leiden University Medical Center, Leiden, the Netherlands; Royal Marsden Hospital, London, United Kingdom; Memorial Cancer Center, Warsaw, Poland; Emma Children's Hospital, Amsterdam, the Netherlands; Centre Leon Berard, Lyon, France;). The data for

the patients treated in Germany were collected from the sarcoma relapse registry (SAREZ) and the Ewing relapse registry of the cooperative Ewing sarcoma study group (CESS). All successive patients diagnosed with Ewing sarcoma between 1980 and 2012 were identified. From these a subpopulation was made for patients treated with either carbo-eto or cis-eto. These patients were included in the analysis and the following data were collected: patient identification number, gender, date of birth, date of first diagnosis, date of last contact or death, tumour location at onset, location of metastases, outcome of previous treatment lines, date of start carbo-eto or cis-eto treatment, number of treatment cycles, date of progression and major toxicity. Overall survival (OS) and progression free survival (PFS) was calculated using the Kaplan and Meier method and compared with the log-rank test.

Results

A total of 107 patients were treated with the carbo- or cisplatin and etoposide combination from 1980 until 2012 and all were enrolled in this study, 61 received carbo-eto, 46 received cis-eto. The characteristics of these patients are shown in Table 4.1. The treatment was given either weekly carboplatin or cisplatin on day 1 and etoposide on day 1–3 or 3-weekly carboplatin or cisplatin on day 1 and etoposide on day 1–2 or 3. The most common schedule for the carbo-eto patients was for the weekly treatment 120 mg/m² carboplatin and 100 mg/m² etoposide both given intravenously and for the 3-weekly regimen 200 mg/m² carboplatin and 100 mg/m² etoposide also given intravenously. For the cis-eto patients the most frequently used treatment regimen was for the weekly schedule 60 mg/m² cisplatin intravenously and 50 mg etoposide orally and for the 3-weekly regimen 50 mg/m² cisplatin intravenously and 150 mg/m² etoposide intravenously. Within the limitations of evaluating toxicity data in a retrospective study no cases of hearing loss or secondary cancers were reported, but there were 2 cases of renal insufficiency. However this data may be incomplete and toxicity was not always reported according to common toxicity data. The median OS calculated from start of treatment until death or last patient contact was 23 months for both patient groups (Figure 4.1). The PFS after start of the treatment is better in the patients treated with the combination of carbo-eto (mean = 14.5 months) than patients treated with cis-eto (mean = 6.3 months) (Figure 4.2). Of the patients in the carbo-eto group 68% were pretreated with etoposide, for the cis-eto group this was 75%. The response data are shown in Table 4.2, with 10 CR (31%), 7 PR (20%), 3 SD (8%) and 14 PD (40%) in the carbo-eto group and 4 CR (10%), 7 PR (19%), 10 SD (27%) and 16 PD (43%) for the cis-eto group. In the cis-eto group one patient received CR after pulmonary metastasectomy, the other patients in both groups received CR after only the combination treatment. This data was available for 90% of all patients. The patients

Table 4.1 Characteristics of the 61 patients with Ewing sarcoma who received etoposide and carboplatin treatment and 46 patients with Ewing sarcoma who received etoposide and cisplatin treatment between 1980 and 2012

Carboplatin + etoposide		Cisplatin + etoposide	
Male	41	Male	31
Female	20	Female	15
Age		Age	
Mean	20	Mean	25
Range	2–48	Range	5–46
Primary site		Primary site	
Pelvis	19	Pelvis	15
Vertebra	6	Rib	4
Scapula	2	Femur	3
Rib	15	Mediastinum	3
Femur	5	Retroperitoneal	2
Fibula	3	Bone	9
Skull	3	Foot	4
Chest wall	2	Scapula	1
Soft tissue	1	Humerus	1
Humerus	1	Mesenterium	1
Toe	1	Neck	1
Clavicle	2	Soft tissue	2
Axilla	1		
Site metastases		Site metastases	
Bone	3	Bone	5
Lung	13	Lung	17
Lung + bone	15	Lung + bone	2
Lung + bone + other	9	Lung + bone + other	2
Lung + other	4	Lung + other	2
Bone + other	6	Bone + other	1
Other	4	Other	7
Treatment line		Treatment line	
First	0	First*	5
Second	48	Second	21
Third	9	Third	9
Fourth	2	Fourth	2
Fifth	1	Fifth	4
Sixth	1	Unknown	5

Other = lymph nodes, bone marrow, mediastinum, liver, CNS, soft tissue, skin, peritoneal, pleura.

* Primary systemic treatment after surgery in patients with primary extensively metastatic disease.

in the carbo-eto group received an average of 3.1 treatment cycles (range 1–11 cycles), the patients in the cis-eto group received this treatment longer with a mean of 3.8 cycles (range 1–15 cycles). Concerning possible prognostic factors, we found that there was a better prognosis for patients with tumours of the extremities than in the axial skeleton in the carbo-eto group ($p=0.012$) (Figure 4.3). For the patients in the cis-eto group tumour

Table 4.2 Overview of studies for patients with refractory or relapsed Ewing sarcoma published in the last decade. For comparison we added the current study.

Author	Year published	Study type	Number of patients	Treatment	Outcome*	Main toxicity
El Weshi [21]	2004	Prospective	27	Etoposide 75 mg/m ² /day for 5 days Ifosfamide 1.2 mg/m ² /day for 5 days Cisplatin 20 mg/m ² /day for 5 days Q 3wks	1 CR (4%) 8 PR (30%) 9 SD (33%) PFS 6.6 months OS 8.1 months	19 grade IV granulocytopenia 15 grades III/IV thrombocytopenia
Whelan [22]	2004	Retro-spective	39	Carboplatin AUC 6 for 1 day Etoposide 120 mg/m ² for 3 days Cyclophosphamide 500–750 mg/m ² for 2 days Q 3 wks	1 CR (2.5%) 9 PR (23%) PFS 10 weeks OS not reported	Haematological, leading to 56% dose reductions 16% grade III/IV infection 7% grade III/ IV mucositis
Hunold [23]	2006	Retro-spective	54	Topotecan 0.75 mg/m ² /day, days 1–5 Cyclophosphamide 250 mg/m ² /day, days 1–5 Q 3 wks	16 PR (32.6%) 13 SD (26.5%) PFS not reported OS 61% at 1 year	4.4% grade III/IV infection 1 toxic death
Wagner [24]	2007	Retro-spective	16	Temozolomide 100 mg/m ² /day on days 1–5 Irinotecan 10–20 mg/m ² /day on days 1–5 and 8–12 Q 3–4 wks	1 CR (6%) 3 PR (18.7%) PFS 30 weeks OS not reported	11% grade III/IV diarrhea
Ferrari [25]	2009	Prospective	37	Ifosfamide 15 g/m ² over 5 days Q 3wks	2 CR (5.5%) 10 PR (27%) 11 SD (30%) PFS not reported OS 29% at 2 year	97% grade IV neutropenia 54% grade IV thrombocytopenia

Table 4.2 Continued

Author	Year published	Study type	Number of patients	Treatment	Outcome*	Main toxicity
Fox [10]	2012	Prospective	14	Gemcitabine 675 mg/m ² days 1 and 8 Docetaxel 75 mg/m ² day 8 Q 3wks	0 CR 2 PR (14%) PFS not reported OS not reported	Mostly haematological
Kebudi [26]	2013	Retro-spective	13	Vincristine 1.5 mg/m ² Cyclophosphamide 600 mg/m ² /day Topotecan 1 mg/m ² Q 3 wks	2 CR (15%) 5 PR (38.5%) 2 SD (15%)	55% grade III/IV neutropenia 44% grade III/IV thrombocytopenia
Raciborska [27]	2013	Retro-spective	22	Vincristine 1.5 mg/m ² day 1 Irinotecan 50 mg/m ² /day days 1–5 Temozolomide 125 mg/m ² /day days 1–5 Q 3 wks	5 CR (22.7%) 7 PR (32%) 3 SD (13.6%)	3 grade III/IV diarrhea 1 grade III/IV neutropenia 1 grade III/IV thrombocytopenia
Maldegem (this article)	2014	Retro-spective	61	Carboplatin day 1 average 480 mg Etoposide day 1–3 average 300mg	11 CR (31%) 7 PR (20%) 3 SD (8.5%)	42% haematological 6% alopecia 6% mucositis#
			46	Cisplatin day 1 average 60 mg Etoposide dag 1–3 average 90 mg	4 CR (10%) 7 PR (19%) 10 SD (27%)	

OS, overall survival; PFS, progression free survival; CR, complete response; PR, partial response; SD, stable disease.

* Response, PFS and OS when documented.

Data was not available for all patients, therefore these percentages are probably an underestimation of the real toxicity encountered.

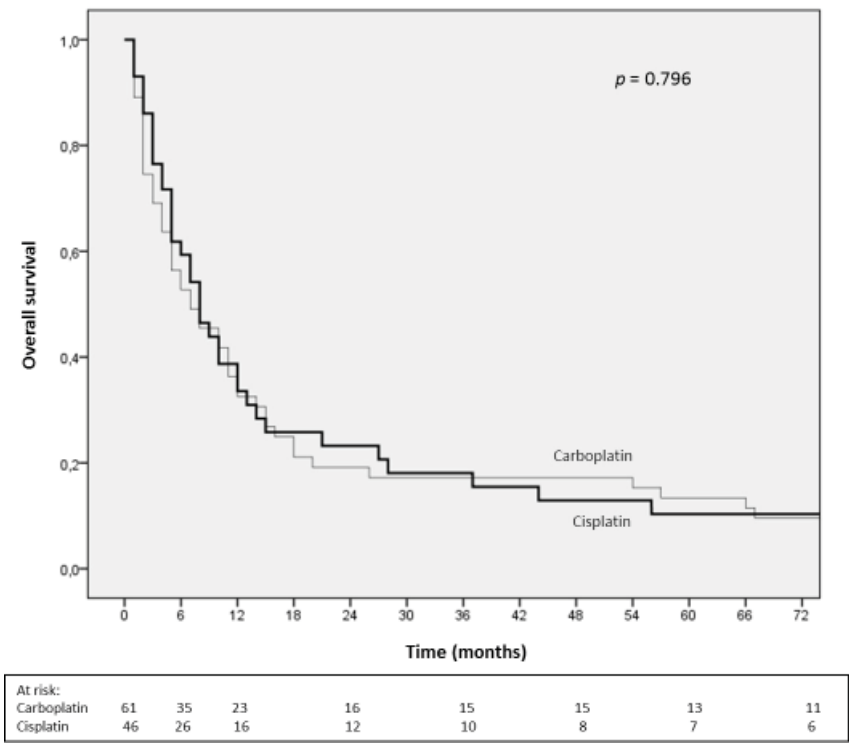


Figure 4.1 Overall survival calculated from start of carboplatin and etoposide or cisplatin and etoposide treatment till death or last patients contact.
The thin line is the carboplatin and etoposide group, the thick line is the cisplatin and etoposide group.

localisation was not significant in relation to OS ($p=0.939$). In the different age groups of children (1–15 years), adolescence and young adults (15–30 years) and adults (30 years and older) we found no difference in OS. In both groups response on previous treatment lines, gender, the localisation of metastases and metastases located in one versus multiple organs has no effect on OS. Toxicity data were only available for 33 patients (30%) and were mainly haematological (42%), alopecia (6%) and mucositis (6%), 36% of the patients had no grade II, III or IV toxicity. At the time of data collection a total of 12 patients were still alive, 6 in the carbo-eto group and 6 in the cis-eto group.

Discussion

For patients with Ewing sarcoma the standard treatment is surgery with or without radiotherapy combined with pre- and post-operative chemotherapy. However for patients who develop refractory or relapsed disease the treatment options are limited. In the last

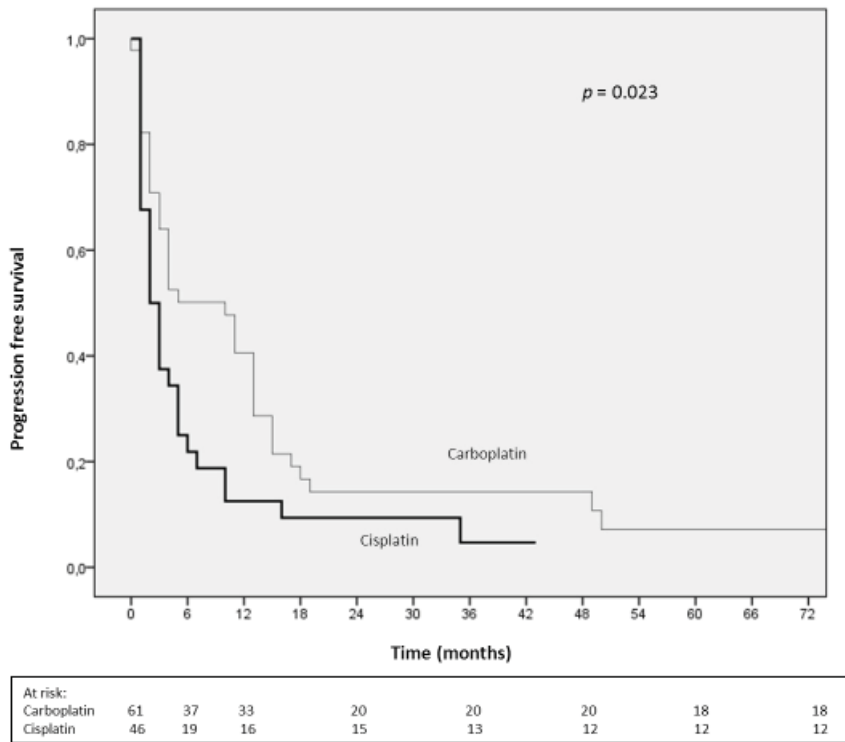


Figure 4.2 Progression free survival calculated from start of carboplatin and etoposide or cisplatin and etoposide treatment till disease progression.

The thin line is the carboplatin and etoposide group, the thick line is the cisplatin and etoposide group.

decade several studies have been published in which second line treatment options for advanced Ewing sarcoma were tested (Table 4.2). Generally there are 3 different commonly used schedules: the combination of irinotecan and temozolomide with diarrhea as the main toxicity, the combination of topotecan and cyclophosphamide with bone marrow toxicity, and high dose ifosfamide with known bone marrow and renal toxicity. The combination of docetaxel and gemcitabine is also used in some countries despite the fact that the published literature on this combination is scarce [10-13]. The response rate in these studies (CR and PR) ranges from 10 to 50% and PFS from 2 to 10 months. Another regimen that is sometimes used is ifosfamide, carboplatin and etoposide (ICE). A study was published in which recurrent or refractory sarcoma patients were treated with this combination [14]. The overall response rate was 51% with a median survival of 11.8 months. Due to the different ways of reporting the outcome it is difficult to compare the outcomes between these studies. Given our results with a response rate of 51%, a PFS of 14.5 months and OS of 23 months for the carbo-eto combination and a response

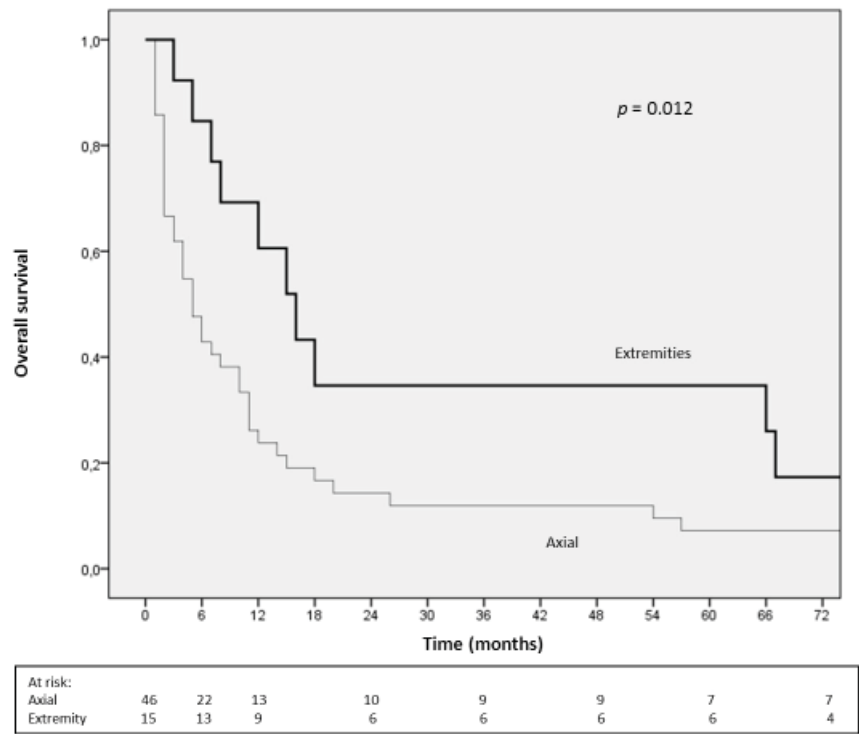


Figure 4.3 Overall survival for the patients who received carboplatin and etoposide treatment subdivided in the primary tumour location being either in the extremity or axial. The thick line is the extremity group, the thin line is the axial group.

rate of 29%, a PFS of 6.3 months and a OS of 23 months for the cis-eto combination we conclude that the combination of a platinum with etoposide is a potential additional treatment option in this patient population. This is especially the case for the carboplatin combination for patients with impaired renal function, which is quite often the case in this heavily pretreated population. A prospective randomised multi-arm study for recurrent disease will be conducted within FP7 IEC project for Ewing sarcoma (rEECur-study).

The PFS for carbo-eto with a mean of 14.5 months is significantly better than the 6.3 months for the patients treated with cis-eto. But because of the retrospective nature of this study it is not possible to draw conclusions out of the comparison of the efficacy of these treatments. Patients that received carboplatin were treated on the weekly schedule with an average of 190 mg carboplatin and on the 3-weekly schedule with 630 mg, using the calculation of drug exposure relative to renal function giving an area under the curve (AUC) of 2.2 for carboplatin in the weekly schedule, and 7.5 on the 3-weekly schedule.

Comparing this to published reports, the dosages used are similar to other combination treatment regimens [15-19]. To make a comparison between the two treatment groups we also looked at the differences in side effects, but because this is a retrospective study the side effects of the patients were at the time not collected or scored in detail, as for instance by the common toxicity criteria and therefore comparison was not possible. These two platinum-based drugs have a lot of similar side effects, with two important differences namely the ototoxicity of cisplatin which occurs frequently ($>10\%$ [20]), and is experienced by the patients as a very severe side effect, and the nephrotoxicity of cisplatin, which is much greater than that of carboplatin. Another major difference between the two drugs is that carboplatin can be given on an outpatient basis while for high dosage cisplatin given in the 3-weekly schedule the patient has to stay overnight in the hospital, which together with the difference in toxicity may be a reason to prefer carboplatin instead of cisplatin. We found that in the carbo-eto group the OS for patients with a tumour located in the extremities is significantly better than for the patients with an axial located tumour, however this could be biased because of a different spread in tumour localisation as we see especially a lot of rib localization in the carbo-eto group, and could be by chance, again due to the retrospective nature of this study.

In conclusion, our results show, within the limitations of a retrospective study, that carboplatin and etoposide maybe is a good additional option for patients with recurrent metastatic Ewing sarcoma and worthy of investigation in a prospective trial with relapsed patients.

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Panobinostat, a potential treatment for metastasized Ewing sarcoma? A case report

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Abstract

Ewing sarcoma (ES) is a form of primary bone cancer, with few treatment options for patients who develop relapse with an overall 5 year survival of 13%. New treatment options are needed and histone deacetylase (HDAC) inhibitors show encouraging results in pre-clinical studies.

Our patient developed inoperable progressive lung metastases and was treated with the HDAC inhibitor panobinostat. During eighteen months of treatment no new lesions appeared, treatment was stopped due to progression.

This clinical observation warrants further evaluation of HDAC inhibitors in ES. Combination with chemotherapy and biomarker studies could improve the therapeutic index of these class of compounds.

Background

Ewing sarcoma (ES) is the second most common form of bone sarcoma in children and adolescents, characterised by a chromosomal translocation involving the EWSR1 gene on chromosome 22 and an ETS transcription factor gene with EWSR1-FLI1 being the most common fusion protein [1].

In the last decade the effect of epigenetic changes on the onset and development of cancer is found to play an increasingly important role [2]. Epigenetic changes influence gene expression, without altering the nucleotide sequence of the DNA, and include changes in histone modification and chromatin remodelling, DNA methylation, loss of imprinting and microRNAs interference [3]. To study the influence of the EWSR1-FLI1 translocation on epigenetics changes, the epigenomes of an ES cell line with and without knockdown of EWSR1-FLI1 were compared [4]. High EWSR1-FLI1 levels were associated with gene expression, open chromatin formation, nucleosome depletion and DNA methylation reduction [5]. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate gene transcription by modification of chromatin, which is important for wrapping eukaryotic DNA. HDACs prevent transcription of genes that regulate cell cycle, cell differentiation and apoptosis and up-regulation of HDACs are associated with silencing of tumour suppressor genes and oncogenesis [3]. Preclinical data showed that in ES cells the expression of the EWSR1-FLI1 results in a repression of HATs and an upregulation of HDACs [6]. EWSR1-FLI1 interacts with histone deacetylase 1 (HDAC1) and overexpression results in a significant inhibition of TP53 promotor activity and inactivation of the p53 pathway, while knockdown of HDAC1 restores the p53 function [7]. In vitro, HDAC inhibitors block the expression of EWSR1-FLI1 at protein and mRNA level and in vivo with a nude mice model apoptosis was seen after intraperitoneal administration of the HDAC inhibitor FK228 [6]. EWSR1-FLI1 mainly acts as a repressor of transcription rather than an upregulator [8]. EZH2 also plays an important role in transcriptional repression. It is a methyltransferase enhancer and thereby can function as a gene silencer. It regulates the trimethylation of H3K27 hereby regulating cell cycle, cell development and differentiation. Overexpression of EZH2 gives cells the ability to grow independent of cell contact and stimulates tumour growth. In microarray analysis EZH2 was found to be strongly up-regulated in ES [9]. Down-regulation of EWSR1-FLI1 by siRNA leads to lower expression levels of EZH2, while up-regulation of EWSR1-FLI1 showed a dose dependent up-regulation of EZH2 thereby suggesting that the EZH2 gene is a direct target of EWSR1-FLI1 [10]. When EZH2 is suppressed in ES cells they lose the ability to grow independent of contact and a significant delay in tumour growth can be seen. EZH2 needs HDAC activity for gene silencing and the effects of EZH2 in vitro can be down-regulated by HDAC inhibitors [11]. These

preclinical results suggest that HDAC inhibitors may be an interesting treatment option for ES.

Here we describe a case of a patient with metastasized ES treated with the HDAC inhibitor panobinostat as mono treatment.

Case report

A 48 year old woman was diagnosed with an ES of the right first rib growing into the lung top and brachial plexus. The presence of a translocation involving the EWSR1 gene was confirmed by FISH analysis. The primary treatment consisted of 6 courses of doxorubicin, ifosfamide, mesna and etoposide (DIME) followed by 2 courses of vincristine and ifosfamide (VI) combined with radiotherapy up to 54Gy and 1 course of vincristine, adriamycin and ifosfamide (VAI). A CT-scan 14 months after diagnosis showed a tumour reduction from 3.5 cm to 2.5 cm. Fifteen months after the first diagnosis she underwent surgery and histologically a good response to prior treatment was seen with a R0 removal of the tumour. After the operation the patient received adjuvant treatment consisting of 5 additional VAI courses. She was closely monitored and showed no signs of recurrence of the tumour. Forty-four months after primary diagnosis, multiple bilateral lung metastases were found. The patient was first operated on the left lung revealing multiple metastases (Figure 5.1) and extensive lymphangitis sarcomatosa which thus reflects non-resectable disease and

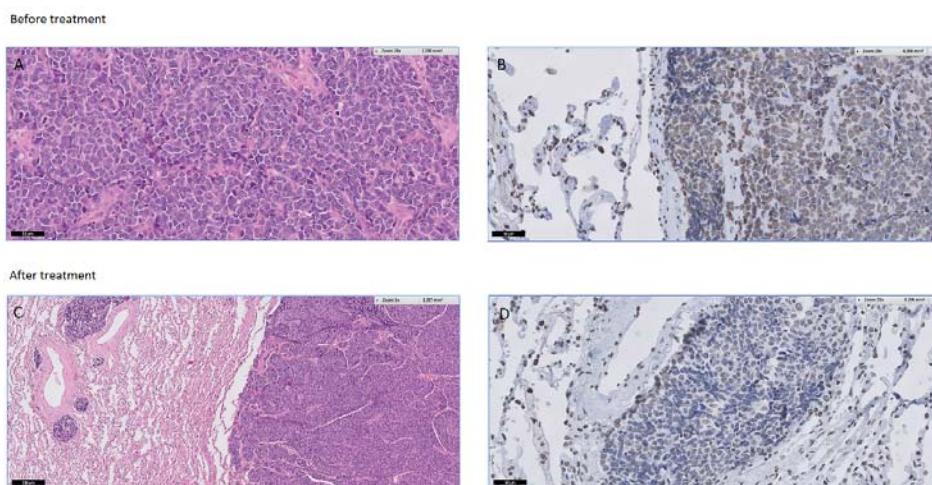


Figure 5.1 Histology of a Ewing sarcoma metastasis in the left lung of our patient showing before treatment with panobinostat small blue round cells (haematoxylin and eosin) (A) and H3K27me3 staining (B) and after treatment with panobinostat small blue round cells (C) and H3K27me3 (D) (scale bars: 50 µm). For Figure B, C and D normal lung tissue can be seen on the left part of the picture.

it was decided not to operate on the right lung. Because of impaired renal function with an estimated creatinine-clearance of 27–30 mL/min no conventional chemotherapy could be given. Five months after her presentation with metastatic disease, while having disease progression (Figure 5.2), the patient entered a phase I study exploring panobinostat safety and pharmacokinetics in patients with renal impairment and she started panobinostat in a dose of 30mg three times a week [12]. Because of low thrombocyte count a switch was made to three times a week every other week after 20 days of treatment. The 2 monthly CT-scans during treatment showed a progression arrest, indicating stable disease for a duration of eighteen months after treatment with panobinostat in a patient that had progressive disease prior to start of treatment (Figure 5.2).

Discussion

Non-resectable metastasized ES has a dismal prognosis with a progression-free and overall survival generally not exceeding 3 and 6 months, respectively. Our patient was not able to receive standard chemotherapy because of a reduced kidney function. Based on preclinical data supporting HDAC inhibition in Ewing sarcoma the opportunity was given to enter a renal impairment study with panobinostat. In general, HDAC inhibitors have proven efficacy in patients with different forms of hematologic malignancies but not yet in solid tumours [13]. The phase I study in which she participated showed that panobinostat can be given to patients with reduced renal function without increasing the drug exposure by altered renal clearance [12], despite the fact that 50% of the drug is being eliminated by the urinary route [14]. Our patient had metastasectomy of a metastasis of her left lung before and after treatment with panobinostat. Staining for H3K27me3 before and after treatment showed a slight decrease in H3K27me3 activation after treatment which may suggest that HDAC inhibition suppresses the activation of EZH2 (Figure 5.1). Our patient was treated with panobinostat as monotherapy most clinical studies in solid tumours were conducted with a combination of panobinostat and chemotherapy. For instance one phase I trial studied the effect of panobinostat compared to the combination of panobinostat with docetaxel in patients with prostate cancer [16]. In the group treated with the combination, two out of 16 patients had a partial response while in the single treatment group only one stable disease was seen and no disease reduction, thus suggesting that combination treatment is better. This could imply that combination therapy in ES would be an interesting avenue to explore and further studies should be conducted to search for synergistic combinations of panobinostat and chemotherapy. To predict which patients are going to respond to the treatment with HDAC inhibition and which do not, biomarkers need to be identified, such as E-cadherin expression which is currently being tested in non-small cell lung carcinomas [17].

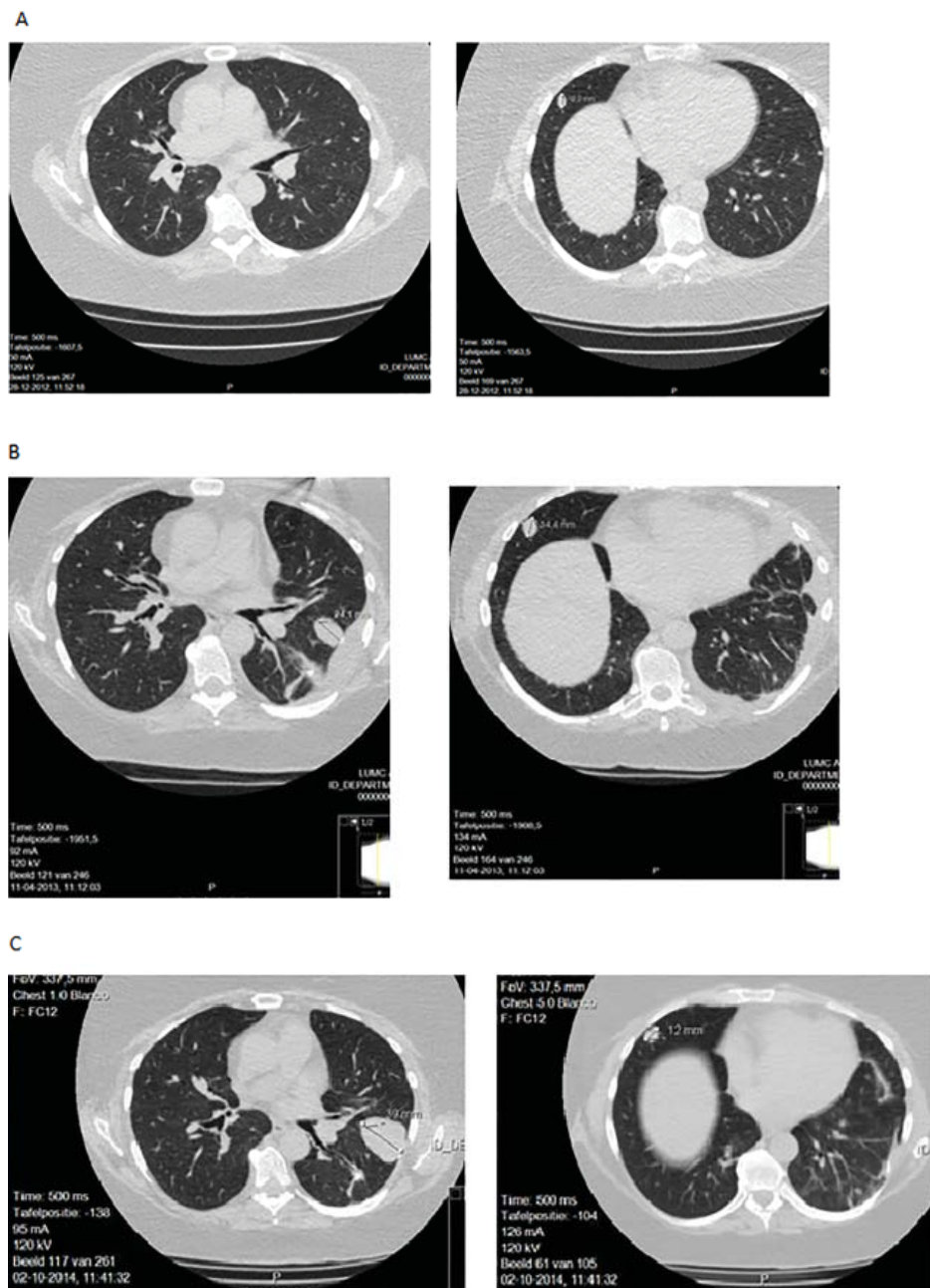


Figure 5.2 CT-scan of the lungs of our patients 4 months before start of treatment with panobinostat with only 9.9 mm lesion in right lung (A), at start of treatment with increase of lesion right lung to 14.4 mm and new lesion left lung 24.1 mm plus pleural lesion left (B) and when treatment was stopped after 18 months due to progressive disease, no new lesions are visible and still decrease in size lesion right lung to 12 mm, resolution of pleural lesion, but significant increase in target lesion left lung to 39 mm (C).

In summary, we explained the preclinical rationale for HDAC inhibition in ES and showed a prolonged clinical response in a patient with metastasized ES. We expect that HDAC inhibition could act synergistically with chemotherapy in this setting and strongly support these combination studies in ES. Biomarkers studies should accompany these clinical studies to select the right patient population and the right combination of HDAC inhibitor and chemotherapy.

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Comprehensive analysis of published studies involving systemic treatment for chondrosarcoma of bone between 2000 and 2013

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Background

The majority of patients with chondrosarcoma of bone have an excellent overall survival after local therapy. However, in case of unresectable locally advanced or metastatic disease the outcome is poor and limited treatment options exist. Therefore we conducted a survey of clinical phase I or II trials and retrospective studies that described systemic therapy for chondrosarcoma patients.

Materials and methods

Using PubMed, clinicaltrials.gov, the Cochrane controlled trial register and American Society of Clinical Oncology (ASCO) abstracts a literature survey was conducted. From the identified items, data were collected by a systematic analysis. We limited our search to semi-recent studies published between 2000 and 2013 to include modern drugs, imaging techniques and disease evaluations.

Results

A total of 31 studies were found which met the criteria: 9 phase I trials, 11 phase II and 8 retrospective studies. In these studies 855 chondrosarcoma patients were reported. The tested drugs were mostly non-cytotoxic, either alone or in combination with another non-cytotoxic agent or chemotherapy. Currently two phase I trials, one phase IB/II trial and three phase II trials are enrolling chondrosarcoma patients.

Conclusion

Because chondrosarcoma of bone is an orphan disease it is difficult to conduct clinical trials. The meagre outcome data for locally advanced or metastatic patients indicate that new treatment options are needed. For the phase I trials it is difficult to draw conclusions because of the low numbers of chondrosarcoma patients enrolled, and at different dose levels. Some phase II trials show promising results which support further research. Retrospective studies are encouraged as they could add to the limited data available. Efforts to increase the number of studies for this orphan disease are urgently needed.

Background

Chondrosarcoma (CS) is the second most common primary bone sarcoma in humans, but with an estimated incidence of 0.2 in 200,000 patients per year it is still a very rare disease [1]. CS mostly affects adults between the age of 20 and 60 [2]. CS belong to a very diverse group of tumors having in common the production of cartilaginous matrix. Almost 90% of the CS are of the conventional subtype, but there are also the more rare subtypes with their own distinct histological and clinical features including clear cell, mesenchymal and dedifferentiated CS [3]. The prognosis for patients with CS is very diverse with a very good prognosis for atypical cartilaginous tumour/CS grade I which are slow growing and do not metastasize and a poor prognosis for grade III CS which have a high risk, up to 70%, for local recurrence and metastasis [4, 5]. Currently the most commonly used treatment option for atypical cartilaginous tumour/CS grade I is curettage with local adjuvants which is usually enough to cure the patient. However, for grade II and grade III CS en bloc resection is required. If a patient has unresectable or metastasized disease the current treatment options are very limited. CS has always been considered to be chemotherapy and radiotherapy resistant and it was assumed that patients would not benefit from non-surgical treatment. However, new preclinical work and retrospective studies show that there may be a place for non-cytotoxic, chemo- and radiotherapy in the treatment of CS [6, 7]. In the last decades more knowledge has become available about the molecular background of the different CS subtypes (for review see [8-10]). Investigators have been trying to find new systemic treatment options for these patients through phase I and II clinical trials (no phase III studies were ever conducted). Because CS is such a rare disease, and high grade metastatic or unresectable disease is even more uncommon, the number of patients in these trials is however low and thereby it is difficult to give a clear answer to the question whether a new drug improves outcome or not. Here we report an overview of a survey we conducted on published and presented phase I and II clinical trials and retrospective studies on systemic therapy enrolling CS patients, published from 2000 until 2013. We also include the studies that are enrolling patients at this moment.

Materials and methods

Search strategy

To collect phase I and II and retrospective studies which included CS patients we used the search machines PubMed, clinicaltrials.gov, the Cochrane controlled trial register and American Society of Clinical Oncology (ASCO) abstracts. For the search criteria we used the terms [chondrosarcoma] AND [phase I OR phase II OR retrospective] AND [clinical trial].

To check for missed articles we widened the search to [sarcoma] AND [phase I OR phase II] AND [clinical trial] and compared the results. When multiple reports from the same trial were published we only used the article with the longest follow up time. Publications were used if they 1) described results from an early phase clinical trial in which CS patients were included, either prospective or retrospective, 2) were written in English. The latest search was performed in December 2013. Studies on extraskeletal myxoid CS were excluded.

Data were collected from trials published between 2000 and 2013 to include modern drugs, imaging techniques and disease evaluations. Data extraction was done by one of the authors (A.v.M) and a systemic analysis was applied that is normally used for meta-analysis [11]. From all reports author name, year of publication, number of patients, intervention and outcome data were noted.

Results

From 2000 until 2013 a total of 31 phase I, II or retrospective clinical trials were reported that enrolled 1 or more CS patients: 11 phase I, 11 phase II and 8 retrospective studies. Figure 6.1 shows a flow-chart indicating the search method for clinical trials included in this analysis. Figure 6.2 shows a timetable with the number and type of clinical trials that met our criteria and their time of publication showing an increasing number of publications from 2004 onwards. The data from the trials that were included in this study are shown in Table 6.1 for the phase I trials, Table 6.2 for phase II trials and Table 6.3 for the results of the retrospective studies. In the clinical trials that were identified a total of 1,927 patients were included of which 855 are patients with CS. Histological subtypes included were conventional, dedifferentiated and mesenchymal. The actual number of patients with CS may be higher because in some of the phase I trials only the CS patients who had an objective response or stable disease were reported but more may have been enrolled. The drugs that were being tested were mostly non-cytotoxic in the phase I trials, either alone or in combination with another non-cytotoxic agent or conventional chemotherapy in the phase II trials. For the retrospective studies all treatments were conventional chemotherapy based. In the phase I trials of the 13 included CS patients there were no complete response (CR), 2 (15%) partial response (PR) and 7 (54%) stable disease (SD). For the phase II trials 156 CS patients were enrolled with 2 (1.3%) CR, 2 (1.3%) PR and 21 (13.4%) SD. The results on the clinicaltrials.gov website for current trials are shown in Table 6.4. Two phase I trials, one phase IB/II trial and three phase II trials were found. There are no phase III trials that are currently or were ever recruiting CS patients.

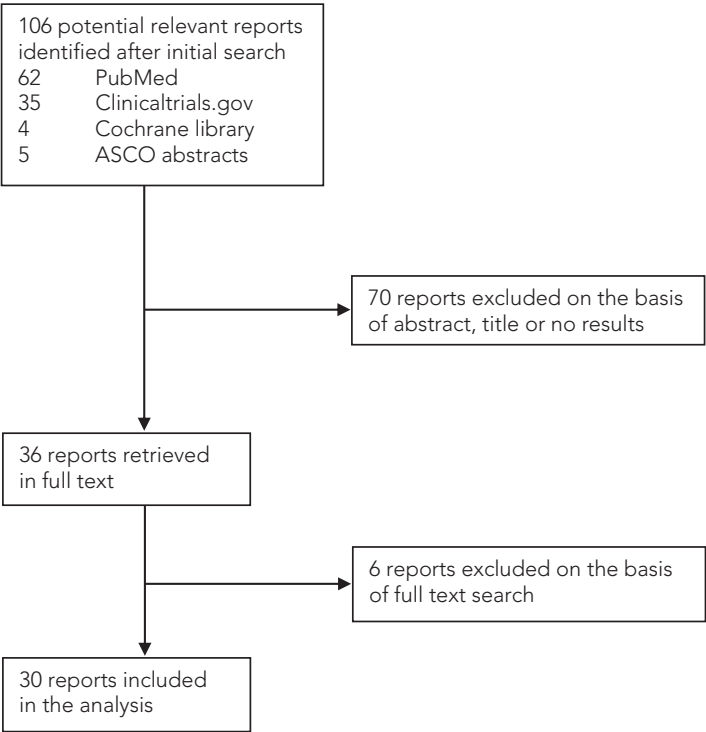


Figure 6.1 Flow-chart showing the search method to identify the relevant clinical reports for our analysis.

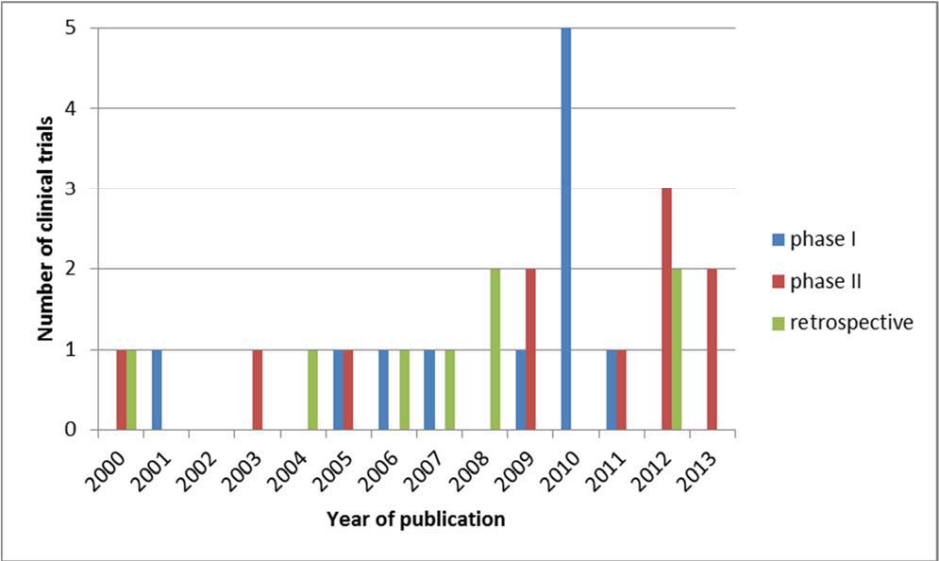


Figure 6.2 Overview of the number of clinical trials included in the analysis according to the year of publication with phase I trials in blue, phase II trials in red and the retrospective trials in green.

Table 6.1 Phase I trials included in the analysis showing the name of the author, year of publication, number of patients enrolled in the trial, the intervention that was tested and the outcome

Author	Year	No of patients	Intervention	Outcome for CS patients
Serrone et al [26]	2001	44 advanced sarcoma (2 CS)	Group A: IFOS short infusion + EPI Group B: IFOS continuous infusion + EPI	Not mentioned
Schwartz et al [27]	2005	38 advanced solid tumors with 6 sarcoma with at least 1 CS	LY293111 (Leucotriene-B4 receptor antagonist)	1 SD
Levine et al [28]	2006	51 advanced malignancies with 7 sarcoma with at least 1 CS	Veglin (VEGF antisense oligodeoxynucleotide)	1 SD
Lockhart et al [29]	2007	18 advanced malignancies (1 CS)	MAC-321 (analogue of docetaxel)	1 SD
Chawla et al [30]	2009	20 advanced sarcoma (1 CS)	Rexin-G (tumor-targeted retrovector) Group A: 2 times a week Group B: 3 times a week	Not mentioned
Camidge et al [31]	2010	50 advanced malignancies with 11 sarcoma with at least 1 CS	PRO95780 (death receptor 5 antibody)	1 SD
Herbst et al [32]	2010	71 advanced malignancies with 9 sarcoma with at least 2 CS	rhApo2L/TRAIL (death receptor activator) Cohort 1: dose escalation Cohort 2: treated at MTD	2 PR
Olmos et al [33]	2010	29 advanced sarcoma (1 CS)	Figitumumab (IGF1R antibody)	1 SD
Qeuk et al [34]	2010	21 advanced solid tumors (1 CS)	Everolimus (mTOR inhibitor) and figitumumab (IGF1R antibody)	1 SD
Stroppa et al [35]	2010	8 advanced sarcoma (1 CS)	Doxorubicin + IFOS	Not mentioned
Pacey et al [36]	2011	25 advanced solid tumors with at least 1 CS	Alvespimycin (heat shock protein 90 inhibitor)	1 SD

CS, chondrosarcoma; EPI, epirubicin; IFOS, ifosfamide; PR, partial response; SD, stable disease.

Table 6.2 Phase II trials included in the analysis with the name of the author, year of publication, number of patients enrolled in the trial, the intervention that was tested and the outcome

Author	Year	No of patients	Intervention	Outcome for CS patients
Merimsky et al [37]	2000	18 advanced sarcoma (3 CS)	Gemcitabine	2 SD
Skubitz et al [38]	2003	47 advanced sarcoma (1 CS)	Pegylated-liposomal doxorubicin	Not mentioned
Nooij et al [23]	2005	37 bone sarcoma (16 CS) Group A: operable, non-metastatic (4 CS) Group B: inoperable, metastatic (12 CS)	Doxorubicin + cisplatin	2 CR 3 SD
Maki et al [39]	2009	145 advanced sarcoma (2 CS)	Sorafenib (multi-tyrosine kinase inhibitor)	Not mentioned
Pacey et al [40]	2009	26 advanced sarcoma (2 CS)	Sorafenib(multi-tyrosine kinase inhibitor)	1 SD
Grignani et al [20]	2011	26 advanced chondrosarcoma	Imatinib mesylate (c-kit/PDGFR inhibitor)	8 SD OS 11 months
Fox et al [24]	2012	53 advanced sarcoma (25 CS)	Gemcitabine + docetaxel	2 PR
Italiano et al [17]	2012	40 advanced CS	GDC-0449/vismodegib (hedgehog inhibitor)	4 SD (of first 17 patients)
Schuetze et al [41]	2012	49 advanced sarcoma (2 CS)	Sirolimus (mTOR inhibitor) + cyclophosphamide	1 SD
Ha et al [42]	2013	36 advanced sarcoma Group A: EGFR+ Group B: EGFR- (1 CS)	Cetuximab (EGFR antibody)	PD
Schwartz et al [43]	2013	388 advanced sarcoma Group A: IGF-1R+ soft tissue sarcoma Group B: IGF-1R+ bone sarcoma (20 CS) Group C: IGF-1R- sarcoma (18 CS)	Cixutumumab (IGF1R antibody) + temsirolimus (mTOR-inhibitor)	Not mentioned

CR, complete response; CS, chondrosarcoma; OS, overall survival; PD, progressive disease; PFS, progression free survival; PR, partial response; SD, stable disease.

Table 6.3 Retrospective trials included in the analysis showing the name of the author, year of publication, number of patients enrolled in the trial, the intervention that was tested and the outcome

First author	Year of publication	No of patients	Intervention and number of patients	Outcome of systemic therapy
Mitchell [44]	2000	22 resectable dediff CS	Doxorubicin based chemotherapy + surgery 5 preoperative chemotherapy and 6 postoperative, 11 no chemotherapy	1/5 >90% necrosis after preoperative chemo 5-year OS with chemotherapy 36%, without 0%
Dickey [45]	2004	42 local or advanced dediff CS	15 surgery 22 surgery and chemotherapy 5 other	5-year OS and median OS: with surgery 11.8 % and 6.4 months With surgery and chemotherapy 4% and 8.4 months
Staals [46]	2006	102 local or advanced dediff central CS	68 surgery 25 surgery + chemotherapy 9 palliative care	Median survival 18 vs 23 months (not significant)
Cesari [7]	2007	24 local or advanced mesenchymal CS	24 surgery of which: 5 + RT 12 + chemotherapy	10-year OS: Surgical remission 27% non-surgical remission 0% Complete surgical remission 10 year DFS: With chemotherapy 76%, without 17%

Table 6.3 Continued

First author	Year of publication	No of patients	Intervention and number of patients	Outcome of systemic therapy
Grimer [47]	2008	266 local and 71 advanced dediff CS	207 surgery 90 chemotherapy 40 surgery + chemotherapy	Local disease 5-year OS: With chemotherapy 33%, without 25% (p=0.11) Advanced disease Median OS: With chemotherapy 7 months, without 3 months (not significant)
Dantonello [48]	2008	14 local mesenchymal CS	12 surgery + chemotherapy 2 surgery + RT	10-year OS 64%
Bernstein-Molho [49]	2012	9 advanced CS	Sirolimus + cyclophosphamide	OR 11% SD 56% PFS 15 months
Italiano [5]	2012	98 advanced CS	Doxorubicin based chemotherapy	OR 14% SD 31% PFS 5.3 months OS 19 months

CS, chondrosarcoma; Dediff, dedifferentiated; DFS, disease free survival; OR, objective response; OS, overall survival; PFS, progression free survival; RT, radiotherapy; SD, stable disease.

Table 6.4 Overview of the trials currently recruiting chondrosarcoma patients, showing the clinicaltrials.gov number, title of the study and the phase

Clinicaltrials.gov number	Title	Phase
NCT01522820	Vaccine therapy with or without sirolimus in treating patients with NY-ESO-1 expressing solid tumors	I
NCT01643278	Dasatinib and ipilimumab in treating patients with gastrointestinal stromal tumors or other sarcomas that cannot be removed by surgery or are metastatic	I
NCT01154452	Vismodegib and gamma-secretase/notch signalling pathway inhibitor RO4929097 in treating patients with advanced or metastatic sarcoma	IB/II
NCT01330966	Study of pazopanib in the treatment of surgically unresectable or metastatic chondrosarcoma	II
NCT00928525	Imatinib in patients with desmoid tumor and chondrosarcoma	II
NCT01653028	Alisertib in treating patients with advanced or metastatic sarcoma	II
Not yet assigned	A phase 2, single arm, multi center trial evaluating the efficacy of the combination of sirolimus and cyclophosphamide in metastatic or unresectable myxoid liposarcoma and chondrosarcoma	II

Discussion

CS is a primary bone cancer that in most patients can be cured by local treatment alone. When tumors are unresectable, either because of locally advanced or metastatic disease, systemic treatment options are very limited due to the current view that non-surgical treatment options have no benefit. Currently for advanced CS patients the outcome is poor with an overall survival of less than two years [5, 12, 13].

The general insensitivity to chemotherapy in CS may be due to activation of anti-apoptotic and pro-survival pathways and therefore future treatment of advanced CS patients could benefit from targeted agents that specifically interfere with these pathways, rendering the tumours more sensitive to the conventional chemotherapeutic agents [8, 9, 12]. For instance, the anti-apoptotic proteins Bcl-2 and Bcl-XL are highly expressed in all CS subtypes and the BH-3 mimetic ABT-737 renders CS cell lines sensitive to the conventional chemotherapeutic agents doxorubicin and cisplatin [10, 12]. Survivin, a member of the inhibitor of apoptosis protein family, is expressed in CS samples and RNA interference targeted on survivin results in cell cycle arrest and increased apoptotic rates in CS cell lines [19]. However, despite promising results in vitro, some novel approaches never make

it to a clinical trial. An example of this is the combination treatment of the Bcl-2 inhibitor ABT-737 and doxorubicin. Drug companies were not interested to supply drug for a clinical trial so it remains unclear if this combination is beneficial to patient outcome.

Two recent retrospective studies as well as animal studies suggested that a subgroup of the patients may benefit from non-cytotoxic agents, chemotherapy, radiotherapy or a combination [6, 7, 13]. In systemic treatment, being either a doxorubicin-containing chemotherapy regimen or non-cytotoxic drugs as imatinib and sirolimus, significantly improved survival compared to no treatment in central CS patients with unresectable disease was shown [13]. For patients with only locally advanced disease radiotherapy may be a good therapeutic option with a significant survival benefit compared to no treatment. Patients with mesenchymal or dedifferentiated CS may also benefit from systemic treatment [6], and further clinical studies are warranted looking at specific CS subtypes.

Recently also new treatment options were tested in clinical trials such as the hedgehog (Hh) inhibitor IPI-926. Hedgehog signaling was previously shown to be important in CS genesis [14-16]. CS xenograft models were treated with IPI-926 and showed a downregulation of the Hh pathway in the tumors and a significant growth inhibition in both newly planted as well as established CS tumours with a mean of 43% [17]. Because of the strong preclinical results a randomized phase II trial studying the effect of IPI-926 compared to placebo in metastatic or locally advanced CS patients was conducted (NCT01310816). The study showed that IPI-926 is well tolerated but there was no difference in PFS or OS compared to placebo. However a small subset of patients had minor reductions in tumour size. Another Hh inhibitor that was tested in a phase II trial including advanced CS patients was GDC-0449, also called vismodegib (NCT01267955). This study did not meet its primary endpoint, but the results suggested activity of the drug in a subset of patients with progressive grade 1 or 2 conventional CS [18]. Despite the fact that in both studies only a small subset showed benefit, it is important to do these trials even if the results do not seem promising for the whole patient group. By studying the tumor tissue of the small subset of responders, we may learn to better understand the mode of action. Moreover, this will enable the identification of biomarkers to predict which patients do respond to the treatment to improve patient selection in future trials.

According to the clinicaltrials.gov website currently 6 clinical phase I or II trials are enrolling CS patients, in all of these trials non-cytotoxic agents are being tested either alone or in combination. These treatments are based on preclinical work that has been conducted over the last years and which shows promising results. An example of this is the mTOR pathway. Dysregulation of mTOR signaling can be found in many tumor types, however in clinical trials inhibitors of mTOR so far show only modest results, which may be due

to activation of the PI3K/Akt pathway. In CS, both mTOR and AKT were shown to be activated [19, 20]. Dual inhibition using BEZ235 dramatically decreased growth of CS cell lines and xenografts [20]. The euroSARC consortium, www.eurosarc.eu, is starting a phase II study in unresectable conventional, dedifferentiated and mesenchymal CS with the combination of sirolimus and cyclofosfamide. An exploratory analysis of the mTOR pathway is foreseen in this study with pharmacokinetic assays on tumour biopsies taken before and after treatment (www.eurosarc.eu).

We conducted a search for all clinical phase I or II trials and retrospective studies that included CS patients. Because we intended to include modern imaging and study designs the survey was limited to the period from 2000 until 2013. The list of the phase I trials that met our search criteria is probably not completely reflecting the actual number of CS patients enrolled in phase I clinical trials, which is caused by the search strategy by which trials that included CS patients without mentioning them in the abstracts are very difficult to find. Some of the phase I trials could be found by using the Cochrane Controlled Trial Register or the clinicaltrials.gov database, although not all trials are registered in these databases. In the phase I trials, with an average of 34 recruited patients, only a small number of CS patients were enrolled at various dose levels which make it difficult to conclude on the effect of these new treatment options specifically for CS patients. For the phase II trials sarcoma patients were enrolled in different strata. Some strata included CS patients while the studies by Grignani and Italiano were restricted to CS patients only [18, 21].

For the retrospective studies it was difficult to make definite conclusions on the effect of systemic therapy because many different chemotherapy treatment regimens were used in patients with different stages of disease and different CS subtypes. As the biological behavior of the subtypes differs much with therefore expected different outcomes it is hard to conclude from studies where the specific subtype is not reported. The study by Cesari [22] is very interesting because it shows that mesenchymal CS patients who had a complete surgical remission may benefit from adjuvant chemotherapy with a 10 year disease free survival that improves from 10% without chemotherapy to 76% with chemotherapy. This is in line with previous studies and the current opinion is that mesenchymal CS is a chemotherapy sensitive tumor and that patients therefore benefit from chemotherapy treatment [23, 24]. For the other CS subtypes it has generally been thought that they are insensitive to conventional chemotherapy, although for dedifferentiated CS activity was shown in individual cases and it is still undefined if chemotherapy treatment is effective [3, 25, 26]. A retrospective study with 337 dedifferentiated CS patients shows that the prognosis remains dismal, however an improvement of survival, not significant, in patients receiving chemotherapy who are under 60 years of age and had limb salvage treatment

was found [27]. Also for conventional CS more evidence is currently found that systemic treatment does improve survival [13].

In conclusion, CS is a difficult patient population to study in clinical trials. It is a very rare disease and most patients can be cured by surgery alone, which makes the group of patients that might need adjuvant or palliative treatment even smaller. This is also one of the reasons why it is very difficult to receive funding for these studies. However, from the current poor prognosis of non-resectable locally advanced or metastatic CS patients it is very clear that there is an unmet medical need and new treatment options are warranted. To improve the number of new treatment options for these patients it is essential to collaborate and share data on research so that future clinical trials have a sound biological rationale and can be conducted with as few patients as possible in a short timeframe.

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Outcome of advanced unresectable conventional central chondrosarcoma

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Background

For chondrosarcoma patients with unresectable disease, due to tumor location, tumor size or extensive metastatic disease, treatment options are very limited because of relative resistance to radio- and chemotherapy. The overall survival of this patient population is poor, however specific studies are lacking and large series have not been published. Therefore we conducted this retrospective two-center study in order to gain insight in the outcome of patients with advanced unresectable conventional central chondrosarcoma.

Patients and methods

All unresectable conventional central chondrosarcoma patients diagnosed between 1-1-1980 and 31-12-2011 in two major European bone sarcoma centres (Rizzoli Institute Bologna, Leiden University Medical Centre) were selected. Relevant information was collected from the medical records at both centers.

Results

A total of 171 patients met our selection criteria, overall survival for all patients was 48% at 1 year, 24% at 2 years, 12% at 3 years, 6% at 4 years and 2% at 5 years. Patients with unresectable locally advanced disease without distant metastases had a significant better survival than patients with metastatic disease ($p=0.0014$). Systemic treatment, either doxorubicin based chemotherapy or the non-cytotoxic drugs imatinib and sirolimus, improved survival significantly compared to no treatment ($p=0.0487$). For patients with locally advanced disease without metastases, radiotherapy was associated with a survival benefit ($p=0.0032$).

Conclusion

This study provides a standard for overall survival rates after unresectable conventional central chondrosarcoma. Systemic treatment and radiotherapy may improve survival although selection bias due to the retrospective nature of this study may have influenced the outcome. The poor survival underlines the need for new therapeutic options for this patient population.

Introduction

Chondrosarcomas are a heterogeneous diverse group of tumors that share at least the characteristic of chondroid matrix production. After osteosarcoma it is the second most common primary bone tumor in adults and accounts for 20 percent of the new primary bone cancer cases. Conventional central chondrosarcoma is the most common subtype, and the majority is of low histological grade. Histological grade is so far the best prognostic indicator [1-3]. Low grade chondrosarcoma tumors rarely metastasize, grow slowly and have a favorable prognosis after surgery, and therefore in the new WHO classification grade I chondrosarcomas are reclassified as atypical cartilaginous tumours [www.WHO.it]. Approximately 10% of conventional central chondrosarcomas are histologically high grade (grade II or III) with a high risk for distant metastases and/or local recurrence and therefore a poor prognosis after resection alone.

In the last decades there has been no significant improvement in survival for patients with chondrosarcoma. The only treatment option with curative intent is surgical resection [4]. However for some locations in the body, such as the pelvis or the skull, resection with a wide margin is difficult to achieve and so local recurrence and metastatic disease are more common. The currently available systemic therapy options are not believed to improve outcome although randomized studies and large series have not been published. Therefore we conducted this study in order to gain insight in the outcome and the effectiveness of systemic treatment of patients with advanced unresectable conventional central chondrosarcoma.

Materials and methods

For our study we selected all unresectable central chondrosarcoma patients diagnosed between 1-1-1980 and 31-12-2011 in two major European bone sarcoma centres (Rizzoli Institute in Bologna, and Leiden University Medical Centre) in January 2012. Reasons for unresectable disease were: complete resection of primary tumor and/or metastases was not deemed feasible because of technical inability due to the size or location of the primary tumor or extensive metastatic sites, or because complete resection would lead to unacceptable morbidity as judged by the multidisciplinary teams at the referral sites in Bologna and Leiden. Relevant information was retrospectively collected from the archives at both centers. Baseline information consisted of gender, age at first presentation of disease, date of first disease presentation, disease location, type, grade and TNM stage of chondrosarcoma at first diagnosis and at recurrence, treatment received, date of development of local recurrence or metastatic disease, pattern of metastases, overall survival from disease onset and post-relapse survival.

The overall survival (OS) was calculated from the day of non-resectability until death or the last follow-up examination. The day that the decision was taken not to perform surgery in the multidisciplinary sarcoma board meeting was taken as the day of non-resectability. The survival curves were calculated according to the Kaplan and Meier method and compared using the log-rank test.

Results

A total of 171 patients were diagnosed with unresectable central conventional chondrosarcoma in the given timeframe at the two centers: 49 cases at first presentation, 122 cases after one or more relapses. The patient characteristics are shown in Table 7.1. From the 171 unresectable patients 72 (42%) had unresectable metastatic disease in the lungs

Table 7.1 Patient characteristics from the 171 unresectable central conventional chondrosarcoma patients

Male : Female	107 : 64
Age at diagnosis (years)	
Mean	53
Range	17–90
Primary site	
Limb proximal	51
Limb distal	19
Pelvis	63
Rib	14
Scapula	7
Vertebra	9
Other ^a	8
Grade at diagnosis	
1	9
2	118
3	44
Margins of primary surgery	
Wide	87
Marginal	24
Intralesional	28
Inoperable	18
Refused surgery	10
Unknown	4
Unresectable disease localization	
Local	45
Lung	72
Local and lung	39
Multi-organ	15

^a Other sites = foot, neck, sternum and hand.

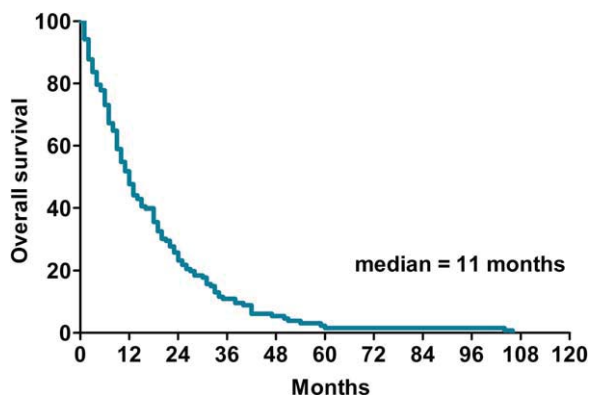


Figure 7.1 Median overall survival for all included patients calculated from the time point of non-resectability till death or last patient contact.

only, 45 (26%) had only local unresectable disease, 39 (23%) had local and unresectable lung disease and 15 (9%) had multi-organ involvement. The median OS was 11 months with a range of 1 to 106 months (Figure 7.1).

OS for all patients was 48% at 1 year, 24% at 2 years, 12% at 3 years, 6% at 4 years and 2% at 5 years. The OS was additionally analysed according to location of the disease. For patients with only local unresectable disease OS was 26% after 3 years compared to 7% in patients with only lung involvement and 8% for patients with lung and local disease involvement. Patients with multi-organ involvement had an OS of 0% after 2 years with a mean survival time of 7 months (Figure 7.2). The difference between OS of patients with only local unresectable disease and patients with metastatic disease is significant with a p-value of 0.0014.

The OS after 3 years was the same for the patients who received only cytotoxic drugs and the patients who received systemic treatment, this is probably due to the small group of patients who were treated with these drugs. It was not possible to calculate the progression free survival because patients were often not followed by scans as this is a palliative setting, and if scans were made they were not done with standard time intervals.

Next we investigated which variables may influence survival after unresectable disease. Both age below 40 years ($p=0.001$) and grade II ($p=0.022$) were correlated with a better OS, no correlation with OS was found for gender, site and resectable versus non-resectable at primary diagnosis (data not shown).

Of the 171 patients 37 received systemic treatment, most patients received doxorubicin-based chemotherapy, but also the non-chemotherapy based agents imatinib and sirolimus

were adopted. This group had an OS of 26% after 3 years compared to 8% for the patients without systemic treatment ($p \leq 0.05$) (Figure 7.3) with a median OS of 20 months for the patients with systemic treatment versus 15 months for the patients without treatment.

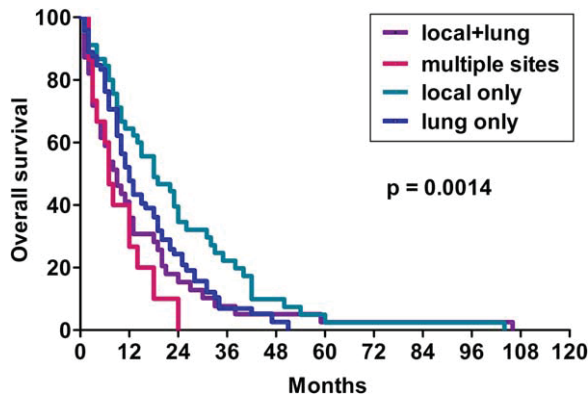


Figure 7.2 Median overall survival calculated from the time point of non-resectability till death or last patient contact for the patients subdivided in the groups local disease involvement only, local and lung, lung only and multiple sites.

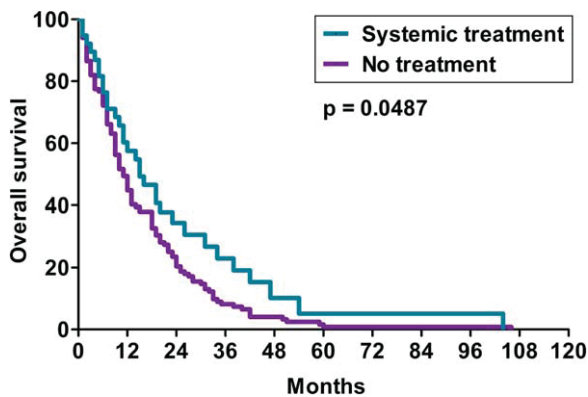


Figure 7.3 For the included patients median overall survival is correlated to systemic treatment. Patients were subdivided in those who received systemic treatment versus patients who received no treatment.

Patients with only metastatic disease, thus excluding patients with local disease or local disease with metastases, had a better OS if they received any systemic or radiotherapy treatment compared to the patients who received no treatment ($p=0.0082$) (Figure 7.4). The patient characteristics of these subpopulations are shown in Table 7.2.

Thirty-three patients received radiotherapy mainly with palliative intent. Patients were treated with radiotherapy according to the standard protocol in the centers. A broad range of dose fractionation schedules was used ranging from 66 Gy in 2 Gy fractions in an attempt to maximize local control to 24 Gy in 8 Gy fractions when the intent was palliative in patients with a poor prognosis. These patients had an OS of 27% after 3 years versus 8% for the patients without radiotherapy treatment. After separating the 33 patients receiving radiotherapy in groups depending on the disease location a survival benefit for patients with only local disease was shown with a p-value of 0.0032 (Figure 7.5). The patient characteristics of these subpopulations are shown in Table 7.3.

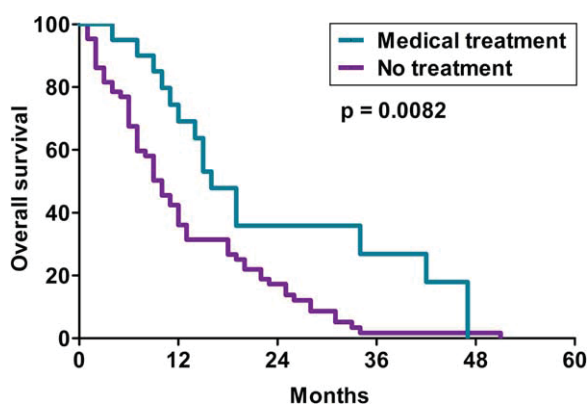


Figure 7.4 A subpopulation was defined with patients with only metastatic disease, patients with local or local and metastatic disease were excluded. Patients were subdivided in those who received treatment (systemic and/or radiotherapy) and those who received no treatment.

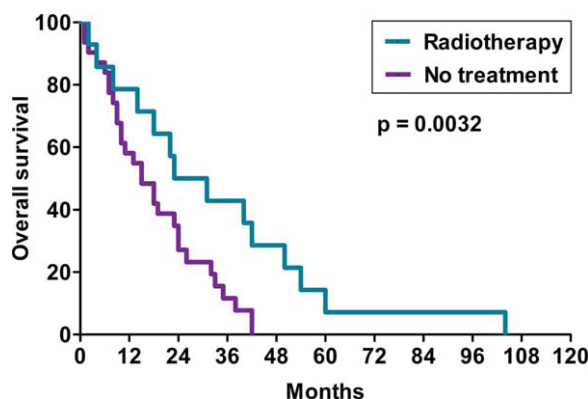


Figure 7.5 A subpopulation was defined with patients with only locally advanced unresectable disease. The median overall survival for these patients is better after radiotherapy versus no radiotherapy.

Table 7.2 Patients characteristics comparing the subpopulation of metastatic only patients who receive treatment (systemic and/or radiotherapy) versus those who received no treatment

	Metastatic only patients	
	Systemic treatment	No systemic treatment
Male : Female	15 : 24	49 : 25
Age at diagnosis (years)		
Mean	49	49
Range	27–68	17–90
Primary site		
Limb proximal	12	25
Limb distal	1	15
Pelvis	6	9
Rib	2	4
Scapula	1	4
Vertebra	2	0
Other ^a	1	5
Grade at diagnosis		
1	2	1
2	18	32
3	6	28
Outcome first surgery		
Wide	14	48
Marginal	6	8
Intralesional	4	6
Inoperable	0	0
Refused surgery	0	0
Unknown	1	0

Patients are compared for gender, age primary site, histological grade at diagnosis and outcome of first surgery.

^a Other sites = sternum, foot, hand, calcaneus.

Discussion

Conventional central chondrosarcoma is a primary bone tumor which, if the grade is low, can be treated with surgery with curative intent. However the patients who present or develop unresectable disease have a poor prognosis. In the present study the outcome of all patients diagnosed with unresectable conventional central chondrosarcoma at two major bone sarcoma centres was evaluated, in order to explore the OS in this condition and to set the standard for future studies.

The overall survival for all patients is poor, with a 3 year OS of 12%. The patients with only local unresectable disease had a better prognosis as compared to patients with metastatic disease, with an OS of 26% after 3 years. Comparing these figures with the published overall survival data for resectable chondrosarcoma the difference with our

Table 7.3 Patients characteristics comparing the subpopulation of locally advanced only patients who receive radiotherapy versus those who received no radiotherapy

	Locally advanced only patients	
	RT	No RT
Male : Female	8 : 6	19 : 12
Age at diagnosis (years)		
Mean	49	59
Range	26–81	26–89
Primary site		
Limb proximal	1	5
Limb distal	0	2
Pelvis	8	20
Rib	1	0
Scapula	0	0
Vertebra	3	4
Other ^a	1	0
Grade at diagnosis		
1	0	4
2	14	27
3	0	0
Outcome first surgery		
Wide	3	10
Marginal	0	2
Intralesional	6	4
Inoperable	2	8
Refused surgery	3	6
Unknown	0	1

Patients are compared for gender, age primary site, histological grade at diagnosis and outcome of first surgery.

^a Other sites = neck.

patient population is striking: 5-years OS of 99% for grade I tumours and 77% for grade III tumours [5]. The results of our study show how urgent the need for new therapeutic options is in this patient population. Currently trials with targeted single agent or combinations are being conducted to address this unmet medical need. A phase II study for unresectable or metastatic conventional chondrosarcoma patients treated with saridegib had to be terminated because the interim analyses showed no treatment benefit compared to placebo. A phase II study testing the hedgehog inhibitor GDC-0449 in patients with advanced chondrosarcoma showed some activity and was well tolerated [6]. A phase II study investigating pazopanib for unresectable or metastatic conventional chondrosarcoma patients (NCT01330966) and a phase II study with imatinib in advanced conventional chondrosarcoma patients (NCT00928525) are still recruiting.

Because of the dismal survival for patients with unresectable disease it is important to determine prognostic factors able to identify the subpopulation of chondrosarcoma patients at high risk of developing unresectable disease. Several studies have already been conducted to search for prognostic factors [1, 7, 8]. So far, no molecular marker has been shown that is independent from and superior to histological grading in combination with clear margins in predicting outcome.

In contrast to what is generally stated in literature we found that for metastatic patients there is a survival benefit when receiving chemotherapy: various regimes of alkylating drugs were being used. Due to heterogeneity of the chemotherapy treatments employed it was not possible to do sub-analysis on type of treatment. We did compare the patients who received cytotoxic drugs to those who got non-cytotoxic drugs, but the difference was not significant ($p=0.436$). This outcome may not be representative because of the small number of patients and the wide time range in which the patients were treated. The general result of improved survival after chemotherapy is rather unexpected because the common opinion is that chondrosarcoma patients do not benefit from non-surgical treatment. However some preclinical studies have already shown promising data that contradicts this assumption [9]. Moreover, patients with only locally advanced unresectable disease have a survival benefit from radiotherapy, although this benefit disappears when patients have metastatic disease. We were also interested in the progression free survival after radiotherapy. However, no routine scans were made after radiotherapy, most likely due to the palliative intent of radiotherapy in many patients. This hampers correct interpretation of time to progression and thus the progression free survival endpoint in this retrospective study. The results of these non-surgical treatment could however be affected by selection bias which may have played a role in this retrospective study of two bone tumor referral centers.

The patients with only locally advanced disease eventually die because of local problems due to increasing tumor load, local tumor pressure on important structures and side effects of the tumor like increasing need of pain medication like morphine and anemia.

Italiano et al very recently published an article in which they retrospectively describe the survival data of advanced chondrosarcoma patients [10]. One hundred and eighteen advanced chondrosarcoma patients were analyzed and the median PFS was 4.7 months with a median OS 18 months. Performance status, number of metastatic sites and palliative surgery were all associated with OS. Their population is heterogeneous due to the different subtypes of chondrosarcoma included and thereby different expected outcome. The authors also mention this as they find a significant difference in objective response rate for the different histological subtypes. This is why we decided to study outcomes in a larger cohort of a homogenous population of central conventional chondrosarcoma.

Also our study has some limitations. Our retrospective study, despite using data from two experienced bone cancer centers in Europe may have encountered some difference in the definition of unresectability between the centers which may have had limited impact on the outcome of this study. Differences in risk-benefit ratio may be perceived differently between sites and surgeons, and patients. Also due to the retrospective nature of this study not all possible prognostic items could be reliably retrieved from the records, such as performance status which had been found in earlier studies to be related to OS [10].

In conclusion, our study adds significantly to the limited data available on overall survival for both locally and metastatic unresectable conventional central chondrosarcoma. Our data show that chemotherapy in unresectable chondrosarcoma patients may increase survival, but further studies are warranted. Radiotherapy shows a survival advantage and is common practice for locally advanced conventional central chondrosarcomas in both reference centers.

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Outcome of first-line systemic treatment for unresectable conventional, dedifferentiated, mesenchymal and clear cell chondrosarcoma

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Background

Chondrosarcoma is a heterogeneous group of primary bone sarcoma with an excellent overall survival after local therapy. However the small percentage of patients who have no surgical treatment options, have a very poor prognosis. We retrospectively collected data from these patients in four sarcoma centers and compared the progression free survival (PFS) for the different treatment regimens used for the four chondrosarcoma subtypes.

Material and method

Patients diagnosed with unresectable chondrosarcoma in one of four major sarcoma centres were included and data on first line systemic therapy were retrospectively collected for analysis.

Results

A total of 112 patients were enrolled in this retrospective analysis, 50 conventional, 25 mesenchymal, 34 dedifferentiated and 3 clear cell chondrosarcoma patients. In conventional chondrosarcoma patients the longest mean PFS (6.7 months) was found in the group treated with anti-hormonal therapy. Patients diagnosed with mesenchymal chondrosarcoma were all treated with multidrug chemotherapy and the mean PFS was 6.7 months. Doxorubicin monotherapy seems to have an unexplained better PFS than doxorubicin based combination therapy in patients with dedifferentiated chondrosarcoma (5.5 versus 2.8 months respectively, $p=0.275$).

Conclusion

Prospective studies need to be conducted based on preclinical work to develop a uniform regimen to treat advanced chondrosarcoma patients according to the diagnosed subtype and improve survival.

Background

Chondrosarcoma consists of a heterogeneous group of tumours that share the common feature of cartilage matrix production [1]. Conventional chondrosarcoma is the most common (90%) subtype, the remaining 10% are mesenchymal, dedifferentiated and clear cell chondrosarcoma. For conventional chondrosarcoma metastatic disease is rare and most patients can be cured with surgical interventions. Overall survival (OS) depends on the subtype with dedifferentiated and mesenchymal chondrosarcoma being the more aggressive with a worse overall survival [2-5]. Retrospective studies have shown that these patients may benefit from (neo-) adjuvant chemotherapy or radiotherapy, but large prospective studies still need to be conducted [2, 5-7]. The small percentage of patients who present or develop metastatic disease for which no surgical options are available, still have a very poor prognosis and limited treatment options [8]. First line of treatment for any chondrosarcoma is surgery, as this is currently the only treatment option to cure a patient. For patients with metastatic disease it remains unclear what the best chemotherapy regimen is and if chemotherapy has any benefit on overall survival. Recent data on mesenchymal chondrosarcoma patients with localised disease show a significant reduction in local disease recurrence with (neo) adjuvant chemotherapy [9]. For patients with metastatic disease the numbers were too small for the authors to make any recommendations. The data from another retrospective study including advanced chondrosarcoma patients suggest that chemotherapy results in a better progression free survival for patients with mesenchymal and dedifferentiated chondrosarcoma. No effect on overall survival was seen [10].

Here we retrospectively collected the data from patients diagnosed with unresectable chondrosarcoma in four major sarcoma treatment centers. The data was shown separately for the four chondrosarcoma subtypes, conventional, mesenchymal, dedifferentiated and clear cell. The progression free survival after different treatment regimens was calculated and compared.

Materials and method

Patients diagnosed with unresectable chondrosarcoma in one of four centers (Leiden University Medical Centre, the Netherlands; University of Texas MD Anderson Cancer Center, USA; Maria Skłodowska-Curie Institute – Oncology Center Warsaw, Poland; Radboud University Medical Centre Nijmegen, the Netherlands) receiving palliative systemic treatment between 1980 and 2016 were selected. Information was collected retrospectively regarding date of birth, gender, date of last contact or death, histological subtype, grade, tumour location at onset, tumour location of unresectable disease,

received treatments, overall survival and progression free survival. Patients were regarded unresectable if complete resection of the primary tumour and/or metastatic sites was viewed as technically not possible due to the size or (multiple) location(s) of the tumour or if complete resection would lead to unacceptable morbidity for the patient as assessed in multidisciplinary team meetings in centers of expertise.

The progression free survival (PFS) after the first treatment line is compared between the different treatment regimens for each chondrosarcoma subtype.

The PFS was calculated from start of first treatment line until disease progression, death or the last follow-up examination. The survival curves were calculated according to the Kaplan and Meier method and compared using the log-rank test.

Results

Patients

A total of 112 patients were enrolled in this study of which 50 (45%) had conventional, 25 (22%) mesenchymal, 34 (30%) dedifferentiated and 3 (3%) clear cell chondrosarcoma. The patient's characteristics are described in Table 8.1. All patient data is shown in Supplementary Table S8.1.

Table 8.1 Patient characteristics

	Conventional*	Mesenchymal	Dedifferentiated	Clear cell
Total patients number	50 (45%)	25 (22%)	34 (30%)	3 (3%)
Gender				
Male	30 (60%)	9 (36%)	17 (50%)	3 (100%)
Female	20 (40%)	16 (64%)	17 (50%)	0 (0%)
Primary tumour location				
Axial	35 (70%)	16 (64%)	18 (53%)	1 (33%)
Extremity	15 (30%)	9 (36%)	16 (47%)	2 (67%)
Unresectable at diagnosis				
Yes	10 (20%)	17 (68%)	11 (32%)	0 (0%)
No	40 (80%)	8 (32%)	23 (68%)	3 (100%)
Alive				
Yes	15 (30%)	11 (44%)	7 (21%)	2 (67%)
No	35 (70%)	14 (56%)	27 (79%)	1 (33%)

* Six of these patients were also included in the study published by Meijer et al [11].

Of the conventional chondrosarcoma patients four had atypical cartilaginous tumours/ chondrosarcoma grade 1 at time of diagnosis. Three had biopsy proven progression to grade 2 at disease recurrence/progression. Of the fourth patient no new biopsy was performed to establish progression to a higher grade tumour. Thus, except this one patient, all patients in the analysis had a high grade chondrosarcoma at time of unresectability.

Treatment

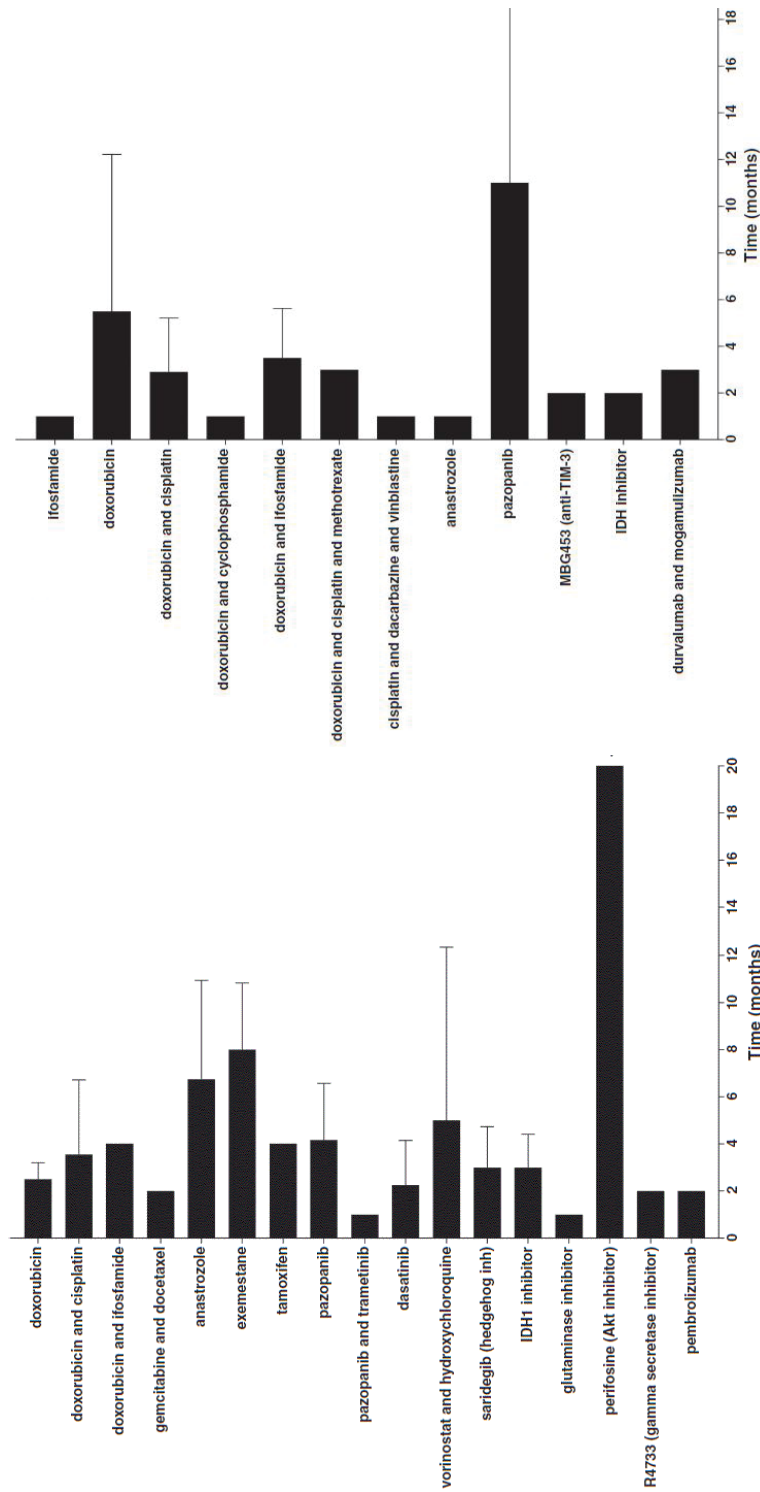
The specific treatment regimens that patients received were compared using a swimmer plot for each chondrosarcoma subtype (Figure 8.1). The numbers of different treatment regimens were too small for a statistical analysis but trends can be observed.

For the conventional chondrosarcoma the patients treated with doxorubicin monotherapy (n=2) had a mean PFS on first treatment line of 2.5 months (Figure 8.1A). Patients treated with a combination of doxorubicin with either cisplatin or ifosfamide (n=10) had a mean PFS of 3.6 months. The combination of gemcitabine and docetaxel has a mean PFS of 2 months (n=3). Seven patients, six males and one female patient, treated with anti-hormonal therapy, aromatase-inhibitors and anti-oestrogen drugs, had a mean PFS of 6.7 months. Six of these were also included in the study published by Meijer et al [11]. Pazopanib and the combination of pazopanib with trametinib had a mean PFS of 3.7 months (n=7) and dasatinib treatment 2.2 months (n=4), these patients were treated in clinical trials. Patients treated with the combination of vorinostat and hydroxychloroquine (n=4) had a mean PFS of 5 months. Hedgehog inhibitors (saridegib) and IDH1 inhibitors had a mean PFS of 3 months (n=5).

For dedifferentiated chondrosarcoma, six patients treated with doxorubicin monotherapy had a mean PFS of 5.5 months (Figure 8.1B). The combination of doxorubicin with cisplatin and doxorubicin with cisplatin and methotrexate had a mean PFS of 2.9 months (n=13).

The 25 patients diagnosed with mesenchymal chondrosarcoma were all treated with chemotherapy based combination regimens (Figure 8.1C). The mean PFS for all chemotherapy regimens is 6.7 months. Patients treated with the combination of ifosfamide and doxorubicin (n=4) had a PFS of 3.7 months, the combination of cisplatin and doxorubicin (n=4) had a PFS of 7.7 months (p=0.04).

The three patients diagnosed with clear cell chondrosarcoma were either treated with sunitinib (n=2) or the combination of pazopanib and denosumab (n=1) and on average their PFS is the same (Figure 8.1D).



A

B

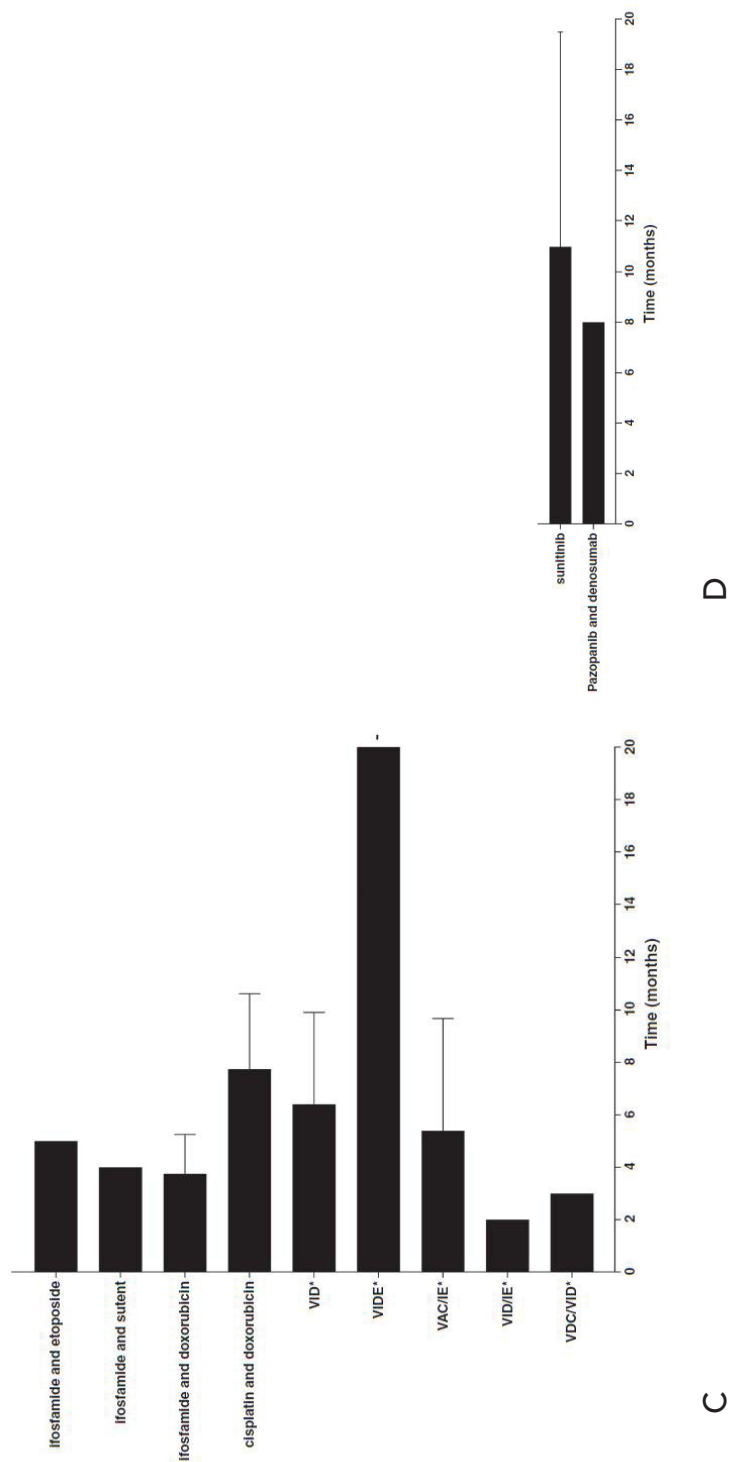


Figure 8.1 Swimmer plot of the different treatment regimens used after unresectability comparing the PFS for conventional (A), dedifferentiated (B), mesenchymal (C) and clear cell (D) subtype.
VID, vincristine, ifosfamide, doxorubicin; VIDE, vincristine, ifosfamide, doxorubicin, etoposide; VAC / IE, vincristine, doxorubicin, cyclophosphamide / ifosfamide, etoposide; VID / IE, vincristine, ifosfamide, doxorubicin / ifosfamide, etoposide; VDC / VID, vincristine, doxorubicin, cyclophosphamide / vincristine, doxorubicin, ifosfamide.

Response

No complete responses according to RECIST criteria were seen after the first treatment line. Seven patients had a partial response, one patient diagnosed with conventional CS, four with mesenchymal CS and two dedifferentiated CS patients. The patient diagnosed with conventional CS was treated with the combination of doxorubicin and cisplatin. Of the four mesenchymal CS patients three were treated with VAC/IE (vincristine, doxorubicin, cyclophosphamide/ifosfamide, etoposide) and one with VID (vincristine, doxorubicin, ifosfamide). The two dedifferentiated CS patients were treated with doxorubicin monotherapy and doxorubicin in combination with cisplatin, respectively.

Survival

The mean progression free survival (PFS) as calculated using the Kaplan and Meier method for the first systemic treatment line after patients became unresectable was 11 months for patients diagnosed with conventional chondrosarcoma, 16 months for mesenchymal, 15 months for dedifferentiated and 10 months for clear cell chondrosarcoma. The differences in outcome for PFS of first systemic treatment line between the histological subtypes were not significant (Figure 8.2).

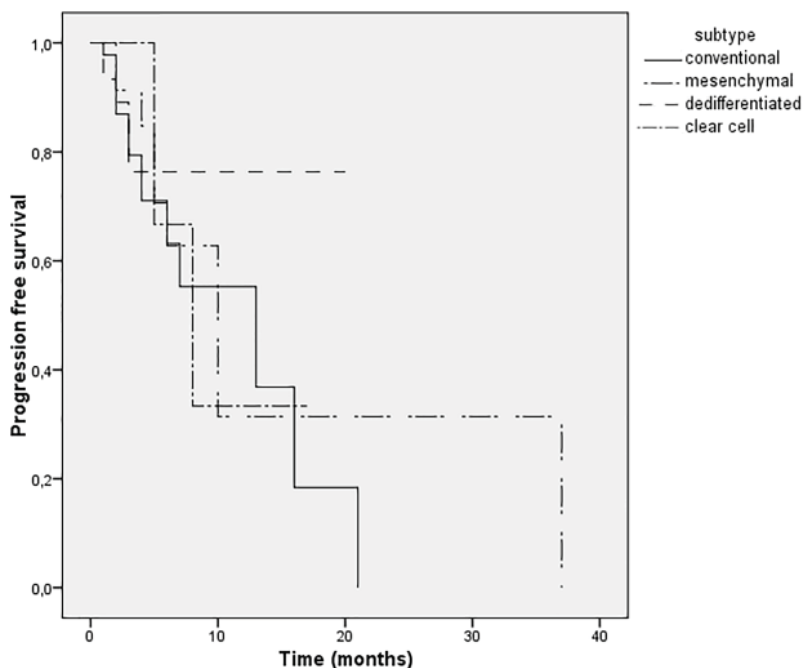


Figure 8.2 Progression free survival for first treatment regimen after unresectability for patients divided in the different chondrosarcoma subtypes.

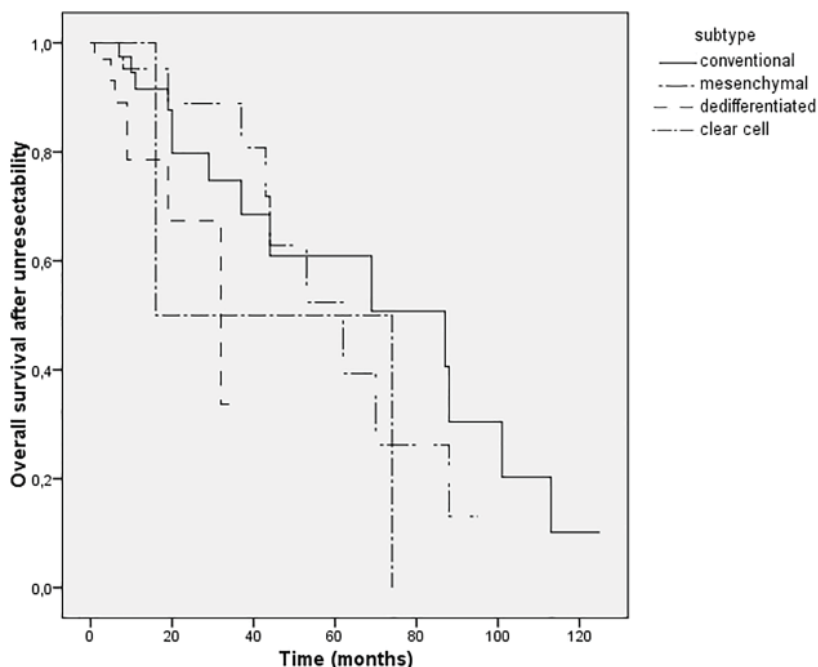


Figure 8.3 Overall survival after unresectability for all patients subdivided in chondrosarcoma subtypes.

At the time of data collection 35 (31%) of the 112 patients were still alive. The OS after unresectability was 87 months for conventional, 62 for mesenchymal, 32 for dedifferentiated and 16 for clear cell chondrosarcoma patients (Figure 8.3). The patients diagnosed with conventional chondrosarcoma or mesenchymal chondrosarcoma show a trend towards having a significant better OS than patients diagnosed with dedifferentiated chondrosarcoma ($p=0.53$ and $p=0.26$ respectively).

Prognostic markers

Different prognostic markers were tested for significance for PFS after unresectability. For dedifferentiated chondrosarcoma male patients had a significant better PFS ($p=0.042$). For the other subtypes no difference in PFS between genders was found. Age (≤ 50 versus >50 years) and tumour localization showed no significant difference (data not shown). There is no difference in PFS between the different treatment centers ($p=0.443$).

Discussion

Patients with unresectable chondrosarcoma still have a very poor prognosis with an overall 2-year survival around 24 to 37% [8, 10]. The conventional, mesenchymal, dedifferentiated and clear cell subtypes should not be considered the same disease and should not be treated equally. Even within the different subtypes there is a wide range of clinical and biological heterogeneity. But to make clinical research feasible for this rare disease, patients with the same subtype are considered to have comparable disease.

Conventional chondrosarcoma is the most common form and the low-grade form (now called atypical cartilaginous tumor) infrequently develops metastases. Dedifferentiated CS is a high grade chondrosarcoma with a worse prognosis. From previous studies we know that the overall survival depends on the histological subtype of chondrosarcoma [10, 12]. In line with these previous studies, the patients in our study diagnosed with conventional or mesenchymal chondrosarcoma also seem to have a better, non-significant, OS than patients diagnosed with dedifferentiated chondrosarcoma (Figure 8.3).

In our cohort several conventional chondrosarcoma patients (n=7) were treated with hormonal based therapies and had a mean PFS of 6.7 months compared to 3.1 months for the patients treated with chemotherapy. Six of these patients were included in a previously published study [11]. In previous preclinical work the presence of the estrogen receptor alpha and activity of aromatase in chondrosarcoma cell lines was confirmed providing rationale for inhibition of estrogen and aromatase as a treatment strategy for chondrosarcoma [13, 14]. However, a beneficial effect could not be convincingly and reproducibly shown *in vitro*, and an *in vivo* pilot study in 6 patients showed no clear therapeutic benefit for advanced conventional or dedifferentiated chondrosarcoma patients treated with an aromatase inhibitor [11]. It is interesting though, that in the current study that includes six of these patients, anti-hormonal therapy gave the longest PFS with a mean of 6.7 months among the different treatment regimens. So far we cannot explain the benefit in PFS for the conventional CS patients treated with these treatment regimens. Further preclinical and clinical studies need to be conducted to clarify if anti-hormonal therapy is a possible therapeutic strategy for CS patients.

In our cohort three patients diagnosed with conventional CS were treated with saridegib. The mean PFS for these three patients was 3.6 months. Preclinical studies suggest that hedgehog signaling plays an important role in the pathogenesis of chondrosarcoma. The hedgehog pathway regulates chondrocyte proliferation and differentiation during endochondral bone development. In chondrosarcoma hedgehog signaling is dysregulated. In previous preclinical work only one out of six chondrosarcoma cell cultures was responsive

to the Hh inhibitor cyclopamine [15]. However, in primary human chondrosarcoma tissue xenotransplanted in mice, treatment with the hedgehog inhibitor IPI-926 results in down-regulation of the hedgehog pathway and inhibition of tumour growth [16]. In addition, chondrosarcoma xenografts responded to the hedgehog inhibitor triparanol with a 60% decrease in tumor volume [17]. Preclinical evidence suggests that maybe a small subset of chondrosarcoma patients may benefit from hedgehog inhibition, currently there are no biomarkers to predict which patients will respond. In a phase 2, randomized, placebo controlled trial inoperable chondrosarcoma patients were treated with the hedgehog inhibitor saridegib. The results of the study showed no improvement in PFS when compared to placebo and the study was stopped [18]. Future studies should be performed to investigate whether the subgroup of patients with mutations in hedgehog pathway genes may benefit from hedgehog inhibitors.

In our study, the median PFS for patients with conventional chondrosarcoma treated with dasatinib was 2.2 months. Using kinome profiling the Src pathway was identified as a possible therapeutic target for chondrosarcoma [19]. Inhibition of the Src pathway with dasatinib, a tyrosine kinase inhibitor, resulted in a decrease of cell growth. Combination treatment of dasatinib with the chemotherapeutic agent doxorubicin results in a synergistic effect on inhibition of cell viability and inducing apoptosis in chondrosarcoma cell lines [20]. This may suggest that blocking the Src pathway may overcome chemo resistance in chondrosarcoma. A recent phase 2 study with the tyrosine kinase inhibitor dasatinib included patients diagnosed with chondrosarcoma, who were incurable with conventional therapy. The median PFS for the patients in the study, enrolling all different chondrosarcoma subtypes, was 5.5 months [21].

Three conventional CS patients in the current study were treated with inhibitors of mutant isocitrate dehydrogenase (IDH). In ~50% of the conventional and the dedifferentiated chondrosarcomas driver mutations are found in the IDH1 or the IDH2 gene [22, 23] providing a strong rationale to treat chondrosarcomas with inhibitors of the mutant enzyme. Promising results have been obtained in other IDH mutant tumours including glioma and leukemia [24]. Preclinical data have shown that while IDH mutations are important for the development of the benign precursor lesion enchondroma [25, 26], however after progression to malignant chondrosarcoma the cells do not depend on the IDH mutation anymore for survival [27, 28]. This would imply that chondrosarcoma patients will not benefit from these inhibitors as single agent therapy, which is in line with the poor PFS in the three patients in the current study. Several clinical trials are currently investigating the effect of IDH inhibition for patients with solid tumors with an IDH1 or IDH2 mutation (ClinicalTrials.gov Identifier: NCT02746081, NCT02481154, NCT02073994, NCT02273739).

All patients diagnosed with mesenchymal chondrosarcoma were treated with chemotherapy. The combination of doxorubicin with cisplatin (n=4) seems to have a better PFS than the regimens that contain 3 or more different chemotherapy agents (n=13). Although numbers are small, one may – with reservation - conclude that the need for regimens with multiple different chemotherapy agents are doubtful.

The fusion gene HEY1-NCOA2 is considered a diagnostic molecular marker [29]. Data on HEY1-NCOA2 translocations, described in 73% of mesenchymal chondrosarcomas, and for IDH1 or -2 mutations, described in ~50% of conventional and dedifferentiated chondrosarcoma were not available for our patients. However, the IDH1 or -2 molecular alterations have no known prognostic value [30], for HEY-NCOA2 translocation it is still unclear.

Patients diagnosed with dedifferentiated chondrosarcoma had a wide range of PFS when comparing the outcome with the same treatment regimens. Doxorubicin monotherapy seems to have a better outcome than treatment with the different combination regimens of doxorubicin. This difference in PFS is notable, however non-significant ($p=0.275$) and thus far unexplained. No difference in toxicity as a reason for discontinuing the treatment was seen, 16.6% in the monotherapy group versus 12.5% for the combination regimens.

From this database we observed that there was a wide variation in effect of the same treatment between patients with the same subtype of CS. This may be related to the low number of patients per subgroup. This makes it hard to draw firm conclusions and propagate one treatment over the other. On the other hand this is still one of the largest series of systemic treatment outcome in chondrosarcoma and the only study taking into account both: line of systemic therapy and CS subtype.

Currently there are no uniform treatment guidelines for advanced chondrosarcoma patients, which results in very diverse treatment regimens being used. In this study we collected the data of 112 patients, but the numbers were still too small to do a statistical analysis on the different systemic treatments. Within the limitations of this study, with low numbers and a retrospective nature causing a selection bias, we can conclude that some treatment regimens seem to have a better PFS as compared to others, and that these results differ between the chondrosarcoma subtypes. Because of the retrospective nature of this study we were not able to do a central pathological review of all the tumours, this may have affected the results. However, all diagnoses were established in a multidisciplinary setting in a center of expertise. Prospective studies need to be conducted based on preclinical work to develop a uniform regimen to treat advanced chondrosarcoma patients according to the histological subtype in order to improve their survival.

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Supplementary Table S8.1 The patient data used for the analysis

Center	Subtype	Gender	Age	Localization	Treatment	PFS first systemic treatment line after unresectability (months)
Netherlands	Conventional	Male	48	Axial	Doxorubicin	2
Netherlands	Conventional	Male	53	Axial	Doxorubicin + cisplatin	5
Netherlands	Conventional	Male	57	Extremity	Doxorubicin + cisplatin	1
Netherlands	Conventional	Female	36	Axial	Doxorubicin + cisplatin	3
Netherlands	Conventional	Male	32	Axial	Doxorubicin + cisplatin	1
Netherlands	Conventional	Female	38	Axial	Ifosfamide	3
Netherlands	Conventional	Male	47	Axial	Arimidex	5
Netherlands	Conventional	Male	31	Extremity	Arimidex	4
Netherlands	Conventional	Male	53	Extremity	Arimidex	5
Netherlands	Conventional	Male	43	Axial	Aromasin	10
Netherlands	Conventional	Male	36	Axial	Aromasin	6
Netherlands	Conventional	Female	58	Axial	Pazopanib	7
Netherlands	Conventional	Female	49	Axial	Dasatinib	5
Netherlands	Conventional	Male	54	Extremity	Dasatinib	1
Netherlands	Conventional	Female	69	Axial	Dasatinib	2
Netherlands	Conventional	Male	47	Axial	Dasatinib	1
Netherlands	Conventional	Female	43	Axial	Dasatinib	2
Netherlands	Conventional	Male	51	Axial	Saridegib	2
Netherlands	Conventional	Male	72	Axial	Saridegib	2
Netherlands	Conventional	Male	55	Axial	Saridegib	5
Netherlands	Mesenchymal	Male	24	Extremity	Doxorubicin + cisplatin	4
Netherlands	Mesenchymal	Female	46	Axial	Doxorubicin + cisplatin	10
Netherlands	Mesenchymal	Male	21	Axial	Doxorubicin + ifosfamide	3
Netherlands	Mesenchymal	Male	45	Extremity	Sutent + ifosfamide	4

Supplementary Table S8.1 continues on next page.

Supplementary Table S8.1 Continued

Center	Subtype	Gender	Age	Localization	Treatment	PFS first systemic treatment line after unresectability (months)
Netherlands	Mesenchymal	Female	38	Extremity	Vincristine + ifosfamide + doxorubicin + etoposide	37
Netherlands	Dedifferentiated	Female	29	Axial	Doxorubicin	2
Netherlands	Dedifferentiated	Male	49	Extremity	Doxorubicin	4
Netherlands	Dedifferentiated	Female	45	Axial	Doxorubicin	3
Netherlands	Dedifferentiated	Male	45	Extremity	Doxorubicin	19
Netherlands	Dedifferentiated	Male	51	Axial	Doxorubicin + cisplatin	3
Netherlands	Dedifferentiated	Female	53	Axial	Doxorubicin + cisplatin	1
Netherlands	Dedifferentiated	Female	50	Extremity	Doxorubicin + cisplatin	4
Netherlands	Dedifferentiated	Male	62	Extremity	Doxorubicin + cisplatin	4
Netherlands	Dedifferentiated	Female	41	Axial	Doxorubicin + cisplatin	9
Netherlands	Dedifferentiated	Male	68	Extremity	Arimidex	1
Netherlands	Dedifferentiated	Male	77	Axial	Dasatinib	3
Netherlands	Dedifferentiated	Male	50	Extremity	MBG453	2
Poland	Conventional	Female	32	Axial	Doxorubicin + cisplatin	1
Poland	Conventional	Male	69	Extremity	Doxorubicin + cisplatin	10
Poland	Conventional	Male	56	Extremity	Doxorubicin + cisplatin	2
Poland	Mesenchymal	Male	27	Extremity	Doxorubicin + cisplatin	10
Poland	Mesenchymal	Female	45	Axial	Doxorubicin + cisplatin	7
Poland	Mesenchymal	Female	19	Axial	Vincristine + doxorubicin + cyclofosfamide/ ifosfamide + etoposide	2
Poland	Mesenchymal	Male	21	Axial	Vincristine + doxorubicin + cyclofosfamide/ ifosfamide + etoposide	11
Poland	Mesenchymal	Female	39	Axial	Vincristine + doxorubicin + cyclofosfamide/ ifosfamide + etoposide	2

Supplementary Table S8.1 Continued

Center	Subtype	Gender	Age	Localization	Treatment	PFS first systemic treatment line after unresectability (months)
Poland	Mesenchymal	Female	28	Extremity	Vincristine + doxorubicin + cyclofosfamide/ ifosfamide + etoposide	9
Poland	Dedifferentiated	Female	73	Extremity	Cisplatin + dacarbazine + vinblastine	1
USA	Conventional	Male	67	Extremity	Doxorubicin	2
USA	Conventional	Female	41	Axial	Doxorubicin	2
USA	Conventional	Female	62	Axial	Doxorubicin + cisplatin	7
USA	Conventional	Male	25	Extremity	Doxorubicin + cisplatin	2
USA	Conventional	Male	48	Extremity	Gemcitabine + docetaxel	4
USA	Conventional	Male	52	Axial	Gemcitabine + docetaxel	13
USA	Conventional	Female	64	Axial	Gemcitabine + docetaxel	2
USA	Conventional	Male	38	Axial	Aldoxorubicin	2
USA	Conventional	Female	64	Axial	Vincristine + doxorubicin + cyclophosphamide / ifosfamide + etoposide	1
USA	Conventional	Female	53	Extremity	Arimidex	7
USA	Conventional	Female	65	Axial	Tamoxifen	4
USA	Conventional	Male	55	Axial	Pazopanib	3
USA	Conventional	Male	56	Extremity	Pazopanib	1
USA	Conventional	Female	51	Axial	Pazopanib	4
USA	Conventional	Female	32	Extremity	Pazopanib	3
USA	Conventional	Female	32	Axial	Pazopanib + trametinib	1
USA	Conventional	Male	64	Axial	Vorinostat + hydroxychloroquine	2
USA	Conventional	Female	44	Axial	Vorinostat + hydroxychloroquine	16
USA	Conventional	Male	43	Axial	Vorinostat + hydroxychloroquine	3
USA	Conventional	Male	47	Extremity	Perifosine	21
USA	Conventional	Female	44	Axial	R4733	2

Supplementary Table S8.1 continues on next page.

Supplementary Table S8.1 Continued

Center	Subtype	Gender	Age	Localization	Treatment	PFS first systemic treatment line after unresectability (months)
USA	Conventional	Female	28	Extremity	Vismodegib	2
USA	Conventional	Male	28	Axial	Pembrolizumab	2
USA	Conventional	Female	47	Axial	CB-839	1
USA	Conventional	Male	40	Extremity	IDH305	2
USA	Conventional	Male	61	Extremity	AG-881	1
USA	Mesenchymal	Female	45	Extremity	Doxorubicin + ifosfamide	3
USA	Mesenchymal	Male	37	Axial	Doxorubicin + ifosfamide	3
USA	Mesenchymal	Female	38	Axial	Doxorubicin + cisplatin	3
USA	Mesenchymal	Female	21	Axial	Ifosfamide + etoposide	5
USA	Mesenchymal	Male	30	Extremity	Gemcitabine + docetaxel	1
USA	Mesenchymal	Female	18	Axial	Vincristine + doxorubicin + ifosfamide	10
USA	Mesenchymal	Female	48	Extremity	Vincristine + doxorubicin + ifosfamide	5
USA	Mesenchymal	Female	45	Axial	Vincristine + doxorubicin + ifosfamide	2
USA	Mesenchymal	Female	18	Axial	Vincristine + doxorubicin + ifosfamide	10
USA	Mesenchymal	Female	48	Axial	Vincristine + doxorubicin + ifosfamide	5
USA	Mesenchymal	Female	24	Extremity	Vincristine + doxorubicin + cyclofosfamide / vincristine + doxorubicin + ifsofamide	3
USA	Mesenchymal	Male	17	Axial	Vincristine + doxorubicin + cyclofosfamide / ifosfamide + etoposide	3
USA	Mesenchymal	Male	16	Axial	Vincristine + ifosfamide + doxorubicin / ifosfamide + etoposide	6
USA	Mesenchymal	Female	7	Extremity	Pazopanib	2
USA	Dedifferentiated	Male	49	Axial	Pazopanib	2
USA	Dedifferentiated	Male	54	Axial	Ddoxorubicin + cisplatin	2

Supplementary Table S8.1 Continued

Center	Subtype	Gender	Age	Localization	Treatment	PFS first systemic treatment line after unresectability (months)
USA	Dedifferentiated	Female	78	Extremity	Durvalumab + mogamulizumab	3
USA	Dedifferentiated	Male	63	Axial	Doxorubicin + cisplatin	2
USA	Dedifferentiated	Female	47	Axial	Doxorubicin + cyclofosfamide	1
USA	Dedifferentiated	Female	36	Axial	Doxorubicin + cisplatin + methotrexate	3
USA	Dedifferentiated	Male	44	Axial	Pazopanib	20
USA	Dedifferentiated	Female	56	Axial	Doxorubicin + cisplatin	2
USA	Dedifferentiated	Male	67	Axial	Doxorubicin + ifosfamide	5
USA	Dedifferentiated	Female	58	Axial	Doxorubicin + cisplatin	4
USA	Dedifferentiated	Male	66	Extremity	Doxorubicin + cisplatin	2
USA	Dedifferentiated	Female	44	Axial	Doxorubicin + cisplatin	1
USA	Dedifferentiated	Female	46	Extremity	Doxorubicin + cisplatin	1
USA	Dedifferentiated	Female	43	Extremity	Doxorubicin + cisplatin + methotrexate	3
USA	Dedifferentiated	Male	66	Axial	AG-120	1
USA	Dedifferentiated	Female	76	Extremity	Doxorubicin + cisplatin	2
USA	Dedifferentiated	Female	59	Axial	Vorinostat + hydroxychloroquine	3
USA	Dedifferentiated	Male	61	Extremity	Doxorubicin + ifosfamide	2
USA	Dedifferentiated	Female	57	Axial	Doxorubicin + ifosfamide	4
USA	Dedifferentiated	Male	42	Axial	Doxorubicin + ifosfamide	1
USA	Dedifferentiated	Male	53	Extremity	Sunitinib	1
USA	Clear cell	Male	42	Extremity	Ifosfamide	17
USA	Clear cell	Male	43	Axial	Sunitinib	8
USA	Clear cell	Male	42	Extremity	Pazopanib + denosumab	5

PFS, progression free survival; USA, United States of America.



Chapter 9

General discussion and
future perspectives

Sarcoma

Sarcomas are tumours that arise in tissue of mesenchymal origin. Of all new cancers diagnosed each year only 1% is a sarcoma. The incidence of sarcoma is low with less than 1,000 new cases in the Netherlands each year. Sarcoma can be further subdivided into bone and soft tissue sarcoma, including gastrointestinal stromal tumours. Ewing- and chondrosarcoma are two of the 3 most common subsets of bone sarcoma (osteosarcoma being the third).

Ewing sarcoma

Ewing sarcoma is characterized by a chromosomal translocation involving the *EWSR1* gene on chromosome 22 and an *ETS* transcription factor gene, with *EWSR1-FLI1* being the most common fusion protein. With a median age of 15 years, most patients diagnosed with Ewing sarcoma are adolescents. The average 5-year overall survival (OS) for localised disease is 60–65%, for metastatic disease the outcome is poor with a 5 year OS of only 20% [1-2]. New treatment options to improve the outcome for these patients are needed. An overview of clinical studies enrolling Ewing sarcoma patients published between 1990 and 2010 is given in **chapter 2**. With a total of 42 phase I/II clinical trials conducted in a time period of 20 years the results are disappointing. Of these only nine trials were designed specifically for Ewing sarcoma patients. It is however encouraging that during the study period (till 2010), the number of trials started per year gradually increased. A general PubMed search shows that from 2010 till 2017 the results of at least 12 phase I, two phase I/II and 17 phase II studies enrolling Ewing sarcoma patients have been published. There is an increase in publications from 2012 till 2014 with an average of 6 new articles each year. From 2015 the number decreases with only 3 articles published in 2017. Treatments tested were mostly non-cytotoxic drugs like mTOR inhibitors, histone deacetylase inhibitors or insulin growth factor receptor antibodies. For most studies the results are non-superior to the standard treatment regimens and the outcome in metastatic or primary chemotherapy refractory disease remains poor.

Preclinical data

A clinical trial is ideally based on treatment models which are tested in *in vitro* and *in vivo* studies. One pathway that has been extensively studied in cancers is the insulin growth factor (IGF) signalling pathway. IGF plays an important role in normal cell development, while overexpression of the pathway components have been implicated in malignancy [3-5].

Chapter 3 gives an overview of the *in vitro* and *in vivo* work conducted in Ewing sarcoma for understanding the role of the IGF pathway. Several drugs are available for inhibition of the insulin growth factor 1 receptor (IGF-1R) and these have been tested in Ewing sarcoma patients either as monotherapy or in combination with other types of drugs. The results of these trials were not as promising as expected and the pharmaceutical companies decided to stop further development. Subgroups of patients did have a good and long response to treatment with IGF-1R inhibition and there is an urgent need for better biomarkers to select patients. Of the conventional biomarkers, patient age, male gender, tumour size and tumour location were proven to be clinically relevant for predicting prognosis [6-8]. Many other biological biomarkers for outcome prediction were tested and interesting markers, like genes and proteins of the 9p21 locus, have to be further tested for validation [9]. For clinical practice no good biological biomarkers are available. Cancer related inflammation and smouldering inflammation in the tumour microenvironment is being recognised as an important factor in genetic instability of cancer cells [10]. It contributes to proliferation, angiogenesis and development of metastases [11]. The CRP/Albumin ratio can be used as an inflammatory indicator in patients' blood and a high ratio is significantly correlated with a poor prognosis in Ewing sarcoma, but needs validation before it can be used clinically [12]. New biomarkers for predicting outcome at time of diagnosis of patient populations with comparable clinical disease stage are being studied, but predicting individual patient response to therapy is still very difficult. Obtaining tumour cells with biopsy before and after treatment of patient and comparing responders to no responders to the treatment can be used to identify novel biomarkers. These materials could be obtained by adding tumour biopsies as a part of a phase I or II trial design for new treatment strategies. Our aim would be to find biomarkers for which patients can be tested before start of treatment to predict response to therapy and hereby compile a patient specific treatment regimen.

Another interesting treatment target also described in **chapter 3** is the Poly-ADP-ribose-polymerase (PARP) pathway. PARPs are proteins involved in DNA repair; they detect single strand breaks and activate the repair process by recruitment of the enzymatic DNA repair mechanism [13]. Ewing sarcoma cell lines were found to be very sensitive to PARP inhibition [14], and even tumour cells from heavily pre-treated and relapsed patients were extremely sensitive *in vitro*. Only one phase II clinical trial enrolling Ewing sarcoma patients has been published until now. With no complete or partial responses the results of this study were disappointing. Preclinical models suggest that better results may be found when combining PARP inhibition with chemotherapy or radiotherapy [15-17]. *In vitro* a synergistic effect was proven when combining PARP inhibition with cytotoxic drugs. Several phase I studies treating Ewing sarcoma patients with combination regimens are currently recruiting or recently finished but do not yet have published results.

Of all new developed drugs or treatment regimens with promising results in preclinical testing approximately 90% when tested in a phase II or III trials fail [18-19]. Most of the negative results are due to inadequate preclinical testing. *In vitro* screening for new drugs is done on cell lines or xenografts of immune-deficient mice and the response criteria are cell death or tumour regression. However, these techniques cannot replicate the complex alterations in tumours on cellular and tissue functions [20]. Animal models play an important role in testing pharmacokinetics of new anticancer drug. However, these animal models are not perfect with uncertainties in interpretation of the results and high costs. For testing on human cells, cell cultures are used but with limitations regarding representatively after prolonged culture. Moreover, the blood flow which delivers oxygen and nutrition cannot be mimicked. The chemical and physical stimulation from the surrounding environment is usually also absent *in vitro*. This gap between *in vitro* and *in vivo* testing needs to be solved. Organ-on-a-chip or body-on-a-chip is a new technology which may be able to fill this gap. With this technique the behaviour of different organs and their physical and chemical (micro-)environment is being reproduced in *in vitro* models by microfluidic devices.

Clinical experience

For patients diagnosed with inoperable Ewing sarcoma there are limited standard treatment regimens. When these patients fail to respond to standard treatment they are usually enrolled in a clinical trial or are treated with a drug regimen based on expert opinion. In several major European sarcoma centers oncologists treat unresectable Ewing sarcoma patients with the combination of etoposide with either cisplatin or carboplatin. The data of 107 of these patients were collected and the results are summarized in **chapter 4**. With a 5-year OS of 24.5% for the patients who received carboplatin and etoposide and 20% for those who received cisplatin and etoposide this regimen is a potential additional treatment option in this patient population. The combination of etoposide and carboplatin had a better progression free survival and fewer side effects, especially with regards to nephrotoxicity, than expected.

Epigenetic changes are increasingly thought to play an important role in the development of cancer [21]. Epigenetic changes regulate gene expression, without altering the nucleotide sequence of the DNA. It alters gene expression by histone modification and chromatin remodelling, DNA methylation, loss of imprinting, and microRNAs interference [22]. Epigenetic changes play an important role especially in Ewing sarcoma. The molecular basis of Ewing sarcoma is very homogeneous with the EWSR1-FLI1 fusion gene. But the disease behaviour is very heterogeneous between different patients. This heterogeneity might be caused by epigenetic changes. Analysing DNA methylation in a Ewing sarcoma

patient cohort showed epigenetic tumour heterogeneity between patients but also within a tumour [23]. A correlation was found between high intra-tumour heterogeneity and metastatic disease at diagnosis. The histone demethylase KDM3A has recently been found to be upregulated by the EWSR1-FLI1 fusion [24]. Activation of KDM3A *in vitro* upregulates metastatic promotor pathways and is an important factor in aggressive behaviour of Ewing sarcoma. MCAM was found to be a target of KDM3A. In non-cancer cells MCAM is involved in regulating cell adhesion, angiogenesis and extravasation [25-27]. In different cancer types expression of MCAM has been correlated with a poor prognosis and metastatic disease [28-29].

Preclinical data prove that high expression of EWSR1-FLI1 is associated with open chromatin formation [30]. Chromatin modification is regulated by histone deacetylases (HDACs) and upregulation of HDACs are associated with silencing of tumour suppressor genes and oncogenesis [22]. In Ewing sarcoma cells the expression of EWSR1-FLI1 is associated with upregulation of HDAC and HDAC is thereby an interesting therapeutic target [31]. In **chapter 5** a patient treated with the HDAC inhibitor panobinostat is reported. This patient had a relapse after primary treatment with chemotherapy and surgery. On panobinostat treatment the patient had stable disease for a total of 18 months. In other solid tumours panobinostat is given in a combination with conventional chemotherapy and these results suggest that combination treatment is better. Combination with checkpoint-inhibitors in phase I studies is currently also being explored.

Chondrosarcoma

With an average age of 50 years chondrosarcoma affects mostly adult patients. It consists of five subtypes, the most common form being the conventional (75%) and more rare subtypes include mesenchymal, dedifferentiated, clear cell and periosteal chondrosarcoma. Conventional chondrosarcoma can be subdivided in atypical cartilaginous tumours (previously called chondrosarcoma grade 1), grade 2 or 3 depending on nuclear size, matrix composition, mitotic activity and degree of cellularity. The most important predictor of outcome is grade, with grade 3 having the worst outcome with a ten year survival of approximately 38% [32]. The only cure for patients diagnosed with chondrosarcoma is surgery as most subtypes of chondrosarcoma have always been considered chemotherapy resistant. Lately new preclinical and retrospective studies show that individual patients may benefit from non-cytotoxics, chemo- and radiotherapy depending on subtype. From 2000 till 2013 31 phase I/II or retrospective studies enrolling chondrosarcoma patients have been published, an overview of these studies can be found in **chapter 6**. In the phase I

trials mostly non-cytotoxic drugs were tested and in the retrospective studies all treatments were conventional chemotherapy based. The outcome for most enrolled patients was still poor but a small subset did seem to respond to treatment. New biomarkers need to be found to predict response before start of treatment.

Clinical data

For patients with advanced, unresectable conventional chondrosarcoma the outcome is regarded to be poor but clinical studies were so far almost lacking. In **chapter 7** the outcome of advanced chondrosarcoma patients in two major European bone sarcoma centers is being investigated. A total of 171 patients were identified. The OS data show that chemotherapy in unresectable chondrosarcoma patients may increase survival, but further studies are warranted. Radiotherapy shows a survival advantage and is common practice for locally advanced conventional central chondrosarcomas in both reference centers. There are currently no standard treatment regimens for unresectable chondrosarcoma patients. Most patients are treated according to expert opinion treatment schedules and it is unclear which schedule improves outcome significantly. To answer this question, in **chapter 8** the treatment and response data from 112 patients diagnosed with unresectable chondrosarcoma in four major sarcoma treatment centers worldwide was collected. No patient had a complete response, seven patients had a partial response and they were all treated with chemotherapy based regimens. Patients with conventional or mesenchymal chondrosarcoma had a better OS than dedifferentiated chondrosarcoma patients. Patients were treated with a diverse range of treatment regimens and the numbers were too small to do statistical analysis. Many non-cytotoxic drugs were based on preclinical data with promising results: in the clinical setting these results were not yet seen. Better patient selection before start of treatment based on biomarkers needs to be conducted.

Future perspectives

A recurring theme in this thesis on systemic therapy in Ewing and chondrosarcoma is the need to select the right patient population before start of treatment. Currently used biomarkers as age, gender, grade and subtype are not capable to distinguish between possible responders and non-responders. Several molecular markers have been identified and are being tested in the (pre-)clinical setting but no distinctive marker has been found yet [33].

Ewing- and chondrosarcoma are rare forms of cancer which makes it difficult to perform good (pre-)clinical studies. Most treatment decisions for patients with recurrent or

unresectable disease are based on expert opinion. Centers of expertise need to be established where preclinical research can be performed and patients need to be referred to these centers to increase the number of patients enrolled in clinical trials. Until now many studies, including the ones in this thesis, are retrospective which can be used to generate hypotheses but they cannot fully answer the question if a treatment regimen improves outcome and what the side-effects are. The rEECur trial is an example of an international study enrolling patients with recurrent or primary refractory Ewing sarcoma. In this trial four different chemotherapy regimens are compared and the goal is to recruit 525 patients. During the trial biospecimens are collected for preclinical research. From the start in 2015 311 patients are enrolled and the first preliminary results were presented at ASCO 2019 [34]. This trial will be used as a platform to study new prospective arms and has the potential to change clinical practice in the future. A similar trial for advanced or metastatic chondrosarcoma would be interesting, but even harder to start because of the even rarer occurrence.

Several new therapeutic options with non-chemotherapy regimens are also being explored. In different cancer types mutations in *IDH1* or *-2* genes lead to tumorigenesis by altered DNA methylation and subsequently gene expression [35]. More than 50% of conventional chondrosarcomas harbour an IDH mutation [36]. These mutations are also found in solitary enchondromas as well as in enchondromas in Ollier disease and Maffucci syndrome, which are precursor lesions for chondrosarcoma, suggesting that the *IDH* gene mutation is an early event. Moreover, inhibition of mutant *IDH1* in chondrosarcoma cell lines does not affect tumorigenic properties: no effect is seen on viability, colony formation or migration [39]. This raises the question whether inhibition of mutant IDH is a therapeutic option for chondrosarcoma, or that it is non drug able as it is an early event and not important anymore after malignant progression. In other forms of cancer, especially acute myeloid leukaemia (AML), inhibition of mutant IDH seems more promising. In the last years two inhibitors, Enasidenib and Ivosidenib, are approved by the FDA for relapsed or refractory AML [38]. The mutant IDH inhibitors Ivosidenib and Olutasidenib are currently being tested in solid tumours, including chondrosarcoma. The results from these and future trials will answer the question if mutant IDH inhibition is a possible treatment regimen for chondrosarcoma.

A way to find new therapeutic options is to combine registered drugs with newly developed therapeutic options. The pharmaceutical companies could be more interested in these kind of trials if they see new treatment options for their already registered drugs or when it can lead to a registration for orphan disease treatment. An example is the COSYMO study which enrolls patients with metastatic or unresectable chondrosarcoma or myxoid liposarcoma. The treatment regimen is a combination of cyclophosphamide with the mTOR

inhibitor sirolimus. This study, the retrospective studies in this thesis and the previously mentioned rEECur study were financially supported by the European Commission granted FP7 clinical trials network (278742).

In the last years immune modulating therapies are being explored as a possibility for cancer treatment. The concept of the working mechanism is that an antitumor immune response is activated, which was suppressed by the tumour adaptive mechanisms [39-40]. In several malignancies, like melanoma and lung carcinoma, impressive results were found after treatment with check-point inhibitors. The most frequently used checkpoint inhibitors are antibodies against programmed cell death receptor-1 (PD-1) and its ligand (PD-L1). In various subtypes of bone sarcomas high expression of PD-1 and PD-L1 was observed [41-42]. The level of expression is found to be correlated with recurrence in chondrosarcoma and metastatic disease in Ewing sarcoma [43-44]. A phase II trial including 13 Ewing sarcoma patients treated with the PD-1 inhibitor pembrolizumab showed no objective response and only 2 patients had stable disease [45]. Thoughtful patient selection is needed and biomarkers to make this selection have to be found. A possible biomarker for immune modulating therapies is mutational load. This is a measure of the number of mutations in a tumor genome [46]. In melanoma and non-small cell lung cancer treated with PD-1 inhibitors, patients with higher mutational loads showed better response rates [47-48]. However, in sarcoma mutational load is often low [49-50].

Altogether, finding new systemic therapies for Ewing and chondrosarcoma remains a challenging field, but international collaborations between centers of excellence, pharmaceutical industry and public grants may lead to much needed progress in the future.

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Chapter 10

Summary
Nederlandse samenvatting
List of publications
Curriculum vitae
Nawoord

Summary

Ewing sarcoma and chondrosarcoma are two of the most common forms of primary malignant bone sarcoma, osteosarcoma being the third. They are rare forms of cancer with an incidence of less than a thousand new patients diagnosed with sarcoma in the Netherlands each year. For patients with surgical treatment options the diagnosis is good. However if they presents themselves with or develop metastatic disease the outcome is poor.

Ewing sarcoma affects mostly children and adolescents with a peak incidence at age 15. It is characterized by a chromosomal translocation involving the EWSR1 gene on chromosome 22 and an ETS transcription factor gene, with EWSR1- FLI1 being the most common fusion protein.

In the last decades the outcome for patients with Ewing sarcoma has not improved greatly. Conducting phase I/II studies is import in order to develop proof of principle for new treatment strategies. **Chapter 2** gives an overview of the peer reviewed studies enrolling Ewing sarcoma patients between 1990 and 2010. In this time period two phase I and six phase II were published which enrolled only Ewing sarcoma patients. No trials for Ewing sarcoma patient were published between 1990 and 1999 but we found an increasing number of early phase trials in the following years. The results of the trials were disappointing, 9.5% of all Ewing sarcoma patients enrolled achieved a complete response, 7.5% had a partial response and 3% stabile disease. More trials were performed which enrolled patients with all forms of sarcoma or solid tumours. The results of these trials are difficult to translate to patients with a specific form of cancer.

For patients with metastatic or relapsed Ewing sarcoma new treatment strategies are urgently needed. Two interesting targets for potential therapies are the insulin-like growth factor 1 receptor (IGF-1R) pathway and the poly ADP ribose polymerase (PARP). Current knowledge of these pathways are summarized in **chapter 3**. Activation of the IGF-1R pathway results in stimulation of cellular proliferation, cell motility and inhibition of apoptosis. The IGF signalling pathway has been implicated in malignant transformation and disease progression. Higher plasma concentrations of IGF1 are associated with increased cancer risk. It is interesting that the peak incidence of Ewing sarcoma correlates with the increased levels of the IGF ligands in puberty. Several clinical trials have been conducted to evaluate the efficacy of IGF-1R inhibition in Ewing sarcoma patients. The results of these trials were disappointing and further development was stopped. Possibly the wrong patient population was treated. Another option is to treat the patients with a dual IGF-1R and IR inhibitor, preventing possible of the cells in which they switch from IGF-1/IGF-1R

to IGF-2/IR-A signalling. A phase II trial with a dual inhibition antibody is currently being conducted and we eagerly await the results.

PARPs are a family of proteins which are activated upon DNA damage. If PARP is inactivated cells are more prone to go into apoptosis upon DNA damage. EWSR1-FLI1 positive Ewing sarcoma cell lines and xenografts were shown to be highly sensitive to PARP-1 inhibition. Since this discovery in 2012, the results of one clinical trial were published. Unfortunately no complete or partial responses were seen. Preclinical work suggests that better results can be expected with combination treatment but this needs to be tested in clinical trials.

For patients who develop relapsed or refractory Ewing sarcoma several palliative chemotherapy treatment regimens are being used. In several sarcoma centers in Europe these patients are treated with the combination of etoposide and carboplatin or etoposide and cisplatin. There is no published literature on the effect of this combination treatment. A retrospective analysis of the survival data for these patients is reported in **chapter 4**. The data of 107 patients were collected, 61 were treated with carboplatin-etoposide and 46 with cisplatin-etoposide. The median overall survival is 23 months for both groups. Response rate in the carboplatin-etoposide treatment group was 51%, with a progression free survival of 14.5 months. In the cisplatin-etoposide group the response rate was 29% and a progression free survival of 6.3 months. Comparing the outcome data of this analysis to published data of other palliative treatment regimens, we conclude that it is a good additional treatment option in this patient population.

Epigenetic changes were found to play an important role on the onset and development of cancer. Gene expression is being influenced by these changes, without altering the nucleotide sequence of the DNA. Gene transcription is being regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) by modification of chromatin, which plays an important role in wrapping of DNA. Up-regulation of HDAC's are associated with silencing of tumour suppressor genes and oncogenesis. Several preclinical studies suggest that HDAC inhibition may be a new treatment option for Ewing sarcoma. For example, it was found that expression of EWSR1-FLI1 results in an upregulation of HDAC and hereby inactivation of the p53 pathway. In **chapter 5** a case of a patient with metastatic Ewing sarcoma who was treated with a HDAC inhibitor is described. The patient had progressive disease prior to start of the treatment and was treated with panobinostat monotherapy three times a week. After 20 days of treatment a low thrombocyte count was found and a switch was made to three times a week every other week. Two monthly CT-scans showed stabile disease for a duration of eighteen months. Clinical trials conducted for other solid tumours showed that combination treatment with chemotherapy may improve the results further. New clinical trials should be conducted to search for synergistic combinations of panobinostat and chemotherapy.

Chondrosarcoma is a more heterogeneous group of tumours that have in common the production of chondroid matrix. It affects mostly adults over 40 years of age. Chondrosarcoma consist of different subtypes, conventional being the most common and mesenchymal, dedifferentiated a clear cell the more rare forms.

Chondrosarcoma is a very rare disease and metastatic or unresectable disease is even more uncommon. Because of this conducting early phase clinical trials for patients diagnosed with chondrosarcoma is very difficult. **Chapter 6** gives an overview of clinical trials and retrospective studies published between 2000 and 2013. A total of 31 studies enrolling 1 or more chondrosarcoma patients were identified, 11 phase I, 11 phase II and 8 retrospective studies. No phase III trials enrolling chondrosarcoma patients were published. For a long time chondrosarcoma was thought to be insensitive to chemotherapy and radiotherapy. However, several retrospective studies show that this is not correct and these patients may have a survival benefit. New trials need to be conducted in which drugs are tested with a sound biological rational.

For patients with advanced unresectable chondrosarcoma the predicted survival and benefit of treatment for this survival remains unclear. In **chapter 7** data from two large sarcoma centers in Europe treating these patients was collected retrospectively. All patients diagnosed with unresectable central chondrosarcoma in either the Rizzoli Institute in Bologna Italy or Leiden University Medical Centre in Leiden the Netherlands between 1-1-1980 and 31-12-2011 were selected. A total of 171 patients were enrolled in this study. Overall survival for all patients was 48% at 1 year, 24% at 2 years, 12% at 3 years, 6% at 4 years and 2% at 5 years. Patients with only local unresectable disease had a significant better overall survival compared to patients with metastatic disease. The overall survival was significant better for patients who received systemic treatment versus the patients who received no treatment. This is in line with the changing opinion and published preclinical and clinical trials that chondrosarcoma patients due benefit from systemic treatment.

From previous studies it became clear that for patients diagnosed with chondrosarcoma histological subtype is a prognostic marker for overall survival. However, all patients are treated with the same regimens. **Chapter 8** gives an overview of patients with unresectable chondrosarcoma, the different treatment regimens used and the outcome data. Retrospectively, the data was collected from patients treated in four sarcoma centers between 1980 and 2016. The data of 112 patients was collected, 50 (45%) with conventional, 25 (22%) mesenchymal, 34 (30%) dedifferentiated and 3 (3%) clear cell chondrosarcoma. No complete responses according to RECIST criteria were seen after the first treatment line. Seven patients had a partial response, one patient diagnosed with conventional, four with mesenchymal and two dedifferentiated chondrosarcoma patients. All these patients

were treated with a chemotherapy based regimen. The mean progression free survival and overall survival were respectively 11 and 87 months for patients diagnosed with conventional chondrosarcoma, 16 and 62 months for mesenchymal, 15 and 32 months for dedifferentiated and 10 and 16 months for clear cell chondrosarcoma. In line with previous studies the patients from our cohort with conventional or mesenchymal chondrosarcoma subtype had a better outcome than patients with dedifferentiated chondrosarcoma. The same treatment regimens gave different outcomes for the four subtypes, indicating that they should not be considered the same disease and should not be treated equally.

Nederlandse samenvatting

Ewing-sarcoom en chondrosarcoom zijn twee van de meest voorkomende vormen van primaire kwaadaardige botsarcomen, met osteosarcoom als derde vorm. Het zijn zeldzame vormen van kanker met een incidentie van minder dan duizend nieuw gediagnosticeerde patiënten met een sarcoom in Nederland per jaar. Voor patiënten met chirurgische behandelopties is de diagnose goed. Als ze zich echter presenteren met een gemetastaseerde ziekte of dit ontwikkelen dan is de uitkomst slecht.

Ewing-sarcoom komt vooral voor bij kinderen en jongvolwassenen, met een gemiddelde incidentieleeftijd van 15 jaar. Het wordt gekarakteriseerd door een chromosomale translocatie van het EWSR1-gen op chromosoom 22 en een ETS transcriptie factorgen, waarbij EWSR1-FLI1 het meest voorkomende fusie-eiwit is.

In de laatste decennia is de uitkomst voor patiënten met een Ewing-sarcoom niet significant verbeterd. Het uitvoeren van fase I/II studies is belangrijk voor het uitbreiden van bewijs van principes voor nieuwe behandelopties. Hoofdstuk 2 geeft een overzicht van alle peer-reviewed studies die Ewing-sarcoom patiënten includeerden tussen 1990 en 2010. In deze periode zijn er twee fase I en zes fase II studies gepubliceerd die alleen Ewing-sarcoom patiënten includeerden. Tussen 1990 en 1999 zijn geen studies voor Ewing-sarcoom patiënten gepubliceerd, maar in de daarop volgende jaren wordt een toename van publicaties van eerste fase studies gezien. De resultaten van de studies zijn teleurstellend: van de geïnccludeerde Ewing-sarcoom patiënten had 9,5% een complete remissie, 7,5% een partiële remissie en 3% een stabiele ziekte. In studies waarbij patiënten met alle vormen van sarcomen of solide tumoren zijn geïnccludeerd zijn de resultaten moeilijk te vertalen naar patiënten met een specifieke vorm van kanker.

Voor patiënten met een gemetastaseerd of recidief Ewing-sarcoom zijn dringend nieuwe behandelopties nodig. Twee interessante behandelopties voor mogelijke therapieën zijn de insulin-like groeifactor 1 receptor (IGF-1R) route en de poly ADP ribose polymerase (PARP). De huidige kennis van deze routes is samengevat in hoofdstuk 3. Activatie van de IGF-1R-route zorgt voor stimulatie van celproliferatie, celbeweeglijkheid en remming van apoptose. De IGF-signaleringsroute is betrokken bij kwaadaardige transformatie en ziekteprogressie. Hogere plasmaconcentraties van IGF1 zijn geassocieerd met een verhoogd risico op kanker. Het is interessant dat de piekincidentie van het Ewing-sarcoom is gecorreleerd met een verhoogd niveau van IGF-liganden in de puberteit. Meerdere klinische studies zijn uitgevoerd om de werkzaamheid van IGF-1R-remming in Ewing-sarcoom patiënten te evalueren. De resultaten van deze studies zijn teleurstellend en toekomstige ontwikkeling is gestopt. Mogelijk is de verkeerde patiëntenpopulatie getest.

Een andere optie is om patiënten te behandelen met tweevoudige blokkade van IGF-1R en IR, waarmee mogelijk voorkomen kan worden dat cellen wisselen van IGF-1/IGF-1R naar IGF-2/IR-A signalering. Momenteel wordt een fase II studie met een tweevoudig blokkerend antilichaam uitgevoerd; we wachten gespannen op de resultaten.

PARPs zijn een familie van eiwitten die worden geactiveerd na DNA-schade. Als PARP is geïnactiveerd zijn cellen meer geneigd om in apoptose te gaan na DNA-beschadiging. Bij EWSR1-FLI1 positieve Ewing-sarcoom cellijnen en xenograften is het aangetoond dat deze erg gevoelig zijn voor PARP-1-remming. Sinds deze bevinding in 2012 zijn de resultaten van één klinische studie gepubliceerd. Helaas werd hierbij geen complete of gedeeltelijke reactie gezien. Preklinisch onderzoek suggereert dat betere resultaten kunnen worden verwacht bij combinatiebehandeling, maar dit moet getest worden in klinische studies.

Voor patiënten met een recidief of ongevoelig Ewing-sarcoom worden verschillende palliatieve chemotherapiebehandelschema's gebruikt. Deze patiënten worden in meerdere sarcoombehandelcentra in Europa behandeld met de combinatie van etoposide en carboplatin of etoposide en cisplatin. Er is geen literatuur gepubliceerd over de effecten van deze behandelcombinatie. In hoofdstuk 4 wordt een retrospectieve analyse van de overlevingsdata van deze patiënten beschreven. De gegevens van 107 patiënten werden verzameld: 61 waren behandeld met carboplatin-etoposide en 46 met cisplatin-etoposide. De gemiddelde overleving is 23 maanden voor beide groepen. Het responsepercentage in de carboplatin-etoposide groep was 51%, met een progressievrije overleving van 14,5 maanden. In de cisplatin-etoposide groep was het responsepercentage 29% met een progressievrije overleving van 6,3 maanden. Na vergelijking van de uitkomstgegevens van deze analyse met gepubliceerde gegevens van andere palliatieve behandelregimes kunnen we concluderen dat het een goede aanvullende behandeloptie is voor deze patiëntenpopulatie.

Het is ontdekt dat epigenetische veranderingen een belangrijke rol spelen bij het ontstaan en het verloop van kanker. Genexpressie wordt beïnvloed door deze veranderingen, zonder verandering van de nucleotidesequentie van het DNA. Gentranscriptie wordt gereguleerd door histone acetyltransferases (HATs) en histone deacetylases (HDACs) door modificatie van chromatin, welke een belangrijke rol speelt bij het inpakken van DNA. Op-regulatie van HDAC's is geassocieerd met het supprimeren van tumoronderdrukkende genen en oncogenese. Verschillende preklinische studies suggereren dat remming van HDAC een goede nieuwe behandeloptie is voor een Ewing-sarcoom. Er is bijvoorbeeld gevonden dat de expressie van EWSR1-FLI1 resulteert in een op-regulatie van HDAC en hierdoor een inactiviteit van de p53-route. In hoofdstuk 5 wordt een casus van een patiënt met een gemetastaseerd Ewing-sarcoom die is behandeld met een HDAC-remmer beschreven.

De patiënt had ziekteprogressie voor start van de behandeling en is drie keer per week behandeld met panobinostat mono-therapie. Na 20 dagen behandeling ontwikkelde de patiënt een laag trombocytengetal waarna de behandeling is gewijzigd naar drie keer per week om de week. Tweemaandelijke CT-scans toonden een stabiele ziekte voor de duur van 18 maanden. Klinische studies uitgevoerd voor andere solide tumoren laten zien dat combinatiebehandeling met chemotherapie de uitkomst mogelijk verbetert. Er moeten nieuwe klinische studies voor het zoeken naar synergistische combinaties van panobinostat met chemotherapie worden uitgevoerd.

Chondrosarcoomen zijn een meer heterogene groep van tumoren die de productie van chondroïde matrix gemeen hebben. Het betreft vooral volwassenen van 40 jaar en ouder. Het chondrosarcoom bestaat uit verschillende subtypes waarvan conventioneel de meest voorkomende vorm is een mesenchymaal, gededifferentieerd en heldercellig de meer zeldzame vormen.

Een chondrosarcoom is een erg zeldzame ziekte en als gemetastaseerde of inoperabele ziekte nog ongewoner. Hierdoor is de uitvoering van vroege fase klinische studies voor patiënten gediagnosticeerd met een chondrosarcoom erg moeilijk. Hoofdstuk 6 geeft een overzicht van alle klinische studies en retrospectieve studies gepubliceerd tussen 2000 en 2013. Totaal werden er 31 studies geïdentificeerd waarbij 1 of meer chondrosarcoom patiënten waren geïnccludeerd, 11 fase I, 11 fase II en 8 retrospectieve studies. Er zijn geen fase II studies die chondrosarcoom patiënten hadden geïnccludeerd gepubliceerd. Voor een lange tijd werd er gedacht dat een chondrosarcoom ongevoelig was voor chemotherapie en radiotherapie. Echter, retrospectieve studies laten zien dat dit niet correct is en dat deze patiënten mogelijk een overlevingsvoordeel hebben. Er moeten nieuwe studies worden uitgevoerd voor medicatie met een gegronde biologische rationale.

Voor patiënten met een gevorderd inoperabel chondrosarcoom blijft de verwachte overleving en het voordeel van behandeling op overleving onduidelijk. In hoofdstuk 7 zijn de gegevens van twee grote sarcoomcentra in Europa die deze patiënten behandelen retrospectief verzameld. Alle patiënten gediagnosticeerd met een inoperabel centraal chondrosarcoom in zowel het Rizzoli instituut in Bologna Italië of het Leiden universitair medisch centrum in Leiden Nederland tussen 1-1-1980 en 31-12-2011 zijn geselecteerd. Totaal zijn er 171 patiënten geïnccludeerd in deze studie. De algemene overleving voor alle patiënten was 48% na 1 jaar, 24% na 2 jaar, 12% na 3 jaar, 6% na 4 jaar en 2% na 5 jaar. Patiënten met alleen een lokale inoperabele ziekte hadden een significant betere algemene overleving vergeleken met patiënten met een gemetastaseerde ziekte. De algemene overleving was significant beter voor patiënten die behandeld werden met systemische therapie vergeleken met patiënten die geen behandeling ontvingen. Dit is

in lijn met de veranderende opinie en gepubliceerde preklinische en klinische studies dat chondrosarcoom patiënten voordeel hebben van systemische behandeling.

Vanuit eerdere studies was al duidelijk geworden dat voor patiënten gediagnosticeerd met een chondrosarcoom het histologische subtype een prognostische factor is voor algemene overleving. Echter, alle patiënten worden behandeld met hetzelfde behandelplan. Hoofdstuk 8 geeft een overzicht van patiënten met een inoperabel chondrosarcoom, de verschillende behandelplannen die zijn gebruikt en de uitkomstdata. Retrospectief zijn gegevens verzameld van patiënten behandeld in vier sarcoomcentra tussen 1980 en 2016. De gegevens van 112 patiënten zijn verzameld, 50 (45%) met een conventioneel, 25 (22%) met een mesenchymaal, 34 (30%) met een gedifferentieerd en 3 (3%) met heldercellig chondrosarcoom. Na eerstelijnsbehandeling werd een complete remissie volgens RECIST-criteria gezien. Zeven patiënten hadden een gedeeltelijke remissie; één patiënt gediagnosticeerd met een conventioneel, vier met een mesenchymaal en twee met een gedifferentieerd chondrosarcoom. Al deze patiënten waren behandeld met een op chemotherapie gebaseerd behandelplan. De gemiddelde progressievrije overleving en algemene overleving waren respectievelijk 11 en 87 maanden voor patiënten gediagnosticeerd met een conventioneel chondrosarcoom, 16 en 62 maanden voor een mesenchymaal, 15 en 32 maanden voor een gedifferentieerd en 10 en 16 maanden voor een heldercellig chondrosarcoom. Zoals in eerdere studies hadden de patiënten uit ons cohort met het conventionele of mesenchymale chondrosarcoom subtype een betere overleving dan patiënten met een gedifferentieerd chondrosarcoom. Hetzelfde behandelregime geeft verschillende uitkomsten voor de vier subtypes, wat er op wijst dat ze niet gezien moeten worden als dezelfde ziekte en niet op dezelfde manier moeten worden behandeld.

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Curriculum vitae

Annemiek Maria van Maldegem is geboren in Bergen op Zoom op 2 april 1981 en groeide op in Halsteren. In 2000 behaalde zij haar VWO-diploma aan het R.K. Gymnasium Juvenaat Heilig Hart te Bergen op Zoom. Hierna verhuisde zij naar Amsterdam voor haar studie Biomedische Wetenschappen waar zij in 2004 haar bachelordiploma behaalde en besloot om verder te gaan met de masteropleiding in de specialisatie oncologie. Voor haar afstudeerstage deed zij onderzoek naar visualisatie van HLA-DR/HLA-DO interacties in MHC-compartimenten en het effect van tetraspanin op MIIC-beweging in levende cellen op de afdeling tumorbiologie van het Nederlands Kanker Instituut te Amsterdam. In 2005 begon zij met de studie geneeskunde aan de Rijksuniversiteit Leiden waarvoor zij in 2010 haar artsexamen behaalde. Na haar opleiding vertrok zij voor 10 maanden naar Engeland om te werken bij de universiteit van Oxford als onderzoeksfellow. In 2011 en 2012 werkte zij als ANIOS interne geneeskunde bij respectievelijk het Alrijne ziekenhuis te Leiderdorp en het Albert Schweitzer ziekenhuis te Dordrecht. In 2013 is zij gestart met haar promotieonderzoek bij de afdeling medische oncologie in het LUMC. Per januari 2014 volgde zij de opleiding tot internist in het Albert Schweitzer ziekenhuis te Dordrecht en het EMC te Rotterdam. In 2018 begon zij met het aandachtsgebied ouderengeneeskunde in het EMC, de verwachting is dat zij dit in 2020 zal afronden.

Annemiek is getrouwd met Niels Konings en samen hebben ze een zoon, Simon.

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