Chapter 1

General introduction
INTRODUCTION

Osteosarcoma is the most common primary malignant bone tumour in children and adolescents. The estimated incidence rate worldwide is 4/million/year with a peak incidence at the age of 15-19 years and a second, though small, peak in the elderly population. The male to female ratio is 1.4 : 1. Conventional osteosarcoma most commonly arises in the metaphyses of the long bones; preferential sites for osteosarcoma are the distal femur or proximal tibia (70%), followed by the proximal humerus (10%) and pelvis (7%). At older age, osteosarcoma follows a different pattern and occurs more often in the axial skeleton. Osteosarcoma in the elderly may develop as a sequel to irradiation for a prior malignancy or in skeletal areas of pre-existent bone disease. [1-4] The clinical presentation of osteosarcoma is highly unspecific; pain and swelling are common symptoms and are generally mild. Given the insidious course of symptoms, both patient-delay and doctor-delay can detain the diagnosis of osteosarcoma. Conventional radiography generally displays a bone mass with accompanying periosteal reaction, often seen with a surrounding soft tissue mass containing calcifications. An additional MRI can provide valuable information regarding the dimension of the tumour and the degree of infiltration into surrounding tissues. Ultimately, the diagnosis is formulated after biopsy and histopathologic evaluation of the tumour cells. The formation of new, immature bone (osteoid), both on X-ray and histologic examination, is pathognomonic for osteosarcoma. [4,5]

Osteosarcomas can be classified according to histologic subtypes and tumour location. One can distinguish between central (medullary) and surface (cortical or juxtacortical) tumours. The term “conventional osteosarcoma” refers to central, high-grade, osteoblastic osteosarcomas. Central osteosarcomas can also be of the chondroblastic, fibroblastic or teleangiectatic subtype. Small cell osteosarcomas and intra-osseous well-differentiated osteosarcomas also belong to the central osteosarcomas. Surface osteosarcomas include parosteal (juxtacortical) well-differentiated osteosarcomas, periosteal osteosarcomas and high-grade surface osteosarcomas. The histologic subtype is of influence on the behaviour of the tumour; small cell osteosarcomas have a more aggressive behaviour, fibroblastic and teleangiectatic tumours tend to show a better response to chemotherapy than do osteoblastic and chondroblastic osteosarcomas. [3,6-8]

Osteosarcoma typically is a complex tumour with numerous genetic and chromosomal aberrancies, however, there is no characteristic mutation or immunohistochemical marker that defines osteosarcoma. [5,7] The aetiology of osteosarcoma remains largely unknown, although there are several factors that are implicated in the development of osteosarcoma. First, there are a few common chromosomal abnormalities found in osteosarcoma that can consist of both partial or complete gains or losses. These include: gain of chromosome 1, loss of chromosome(s) 9, 10, 13 and/or 17, amplifications of chromosomes 8 and 12, partial or complete loss of chromosome 6. Rearrangements of chromosomes 11, 19 and 20
are also frequently encountered. [3,9-11] Second, certain rare syndromes are associated with an increased risk of osteosarcoma development, such as Bloom syndrome, Rothmund-Thomson syndrome and Li-Fraumeni syndrome. Germ line mutations of the most well known tumour suppressor genes, p53 and retinoblastoma (Rb), are reported to be involved in the pathogenesis of osteosarcoma. Combined mutations or inactivations of both genes are often encountered. Patients with hereditary retinoblastoma very regularly develop osteosarcoma as secondary tumours and have an increased risk of 500-fold compared to the general population. [11-14] Third, metabolic bone disease can predispose to osteosarcoma development. Patients with Paget’s disease have a risk of 1% to develop osteosarcoma, a risk that is 2500-fold higher than in the general population, the cause of which has not been elucidated. [12] Finally, there are external and environmental factors that can increase the risk of osteosarcoma development, including irradiation therapy for previous malignancies and exposure to the chemical compound of beryllium. [12]

At present, the standard treatment for high-grade osteosarcoma is a multidisciplinary effort by (paediatric) oncologists, orthopaedic surgeons, pathologists, rehabilitation physicians and specialised nurses and includes neoadjuvant multi-agent chemotherapy (including doxorubicin, cisplatinum, ifosfamide and methotrexate), radical surgery and postoperative chemotherapy. [3,5,15-17] Local control of the primary tumour is essential to obtain cure for osteosarcoma and therefore, in patients with axial and/or unresectable osteosarcoma, in whom local control is difficult to achieve, there is a high risk of progression, relapse and/or metastasis. In selected cases, radiotherapy could offer improved chances for survival. [1,18,19]

With the advent of chemotherapy, 5-year survival rates for osteosarcoma have improved over the past three decades from 12% in the 1980’s to 65% at the present moment for patients with localised disease. However, in the case of metastatic or recurrent disease, 5-years survival rates are reduced to approximately 20%, with a median survival of around 1 year. Currently, the only reliable prognostic factor for disease-free survival is the response to induction chemotherapy, assessed by immunohistochemical examination of the excision specimen of the primary tumour. Response rate is defined as the percentage of necrosis in the primary tumour; >90% necrosis is considered a good response. [1]

Metastatic disease is a major issue in the course of osteosarcoma. Osteosarcoma has a high tendency to metastatic spread and the absolute majority (80%) of metastases are pulmonary metastases. Most commonly the pulmonary metastases develop in the periphery of the lungs. Approximately 20-30% of patients present with metastasis at initial diagnosis and, additionally, in 40% of patients metastases occur at a later stage of their disease. Apart from lung metastases, metastasis to other skeletal locations is also common (20%). These bone metastases are generally not lethal, however, they account for considerable morbidity. Furthermore, the presence of bone metastases correlates to inferior survival outcomes.
Treating metastatic osteosarcoma remains a challenge and most deaths associated with osteosarcoma are the result of metastatic disease. In the case of metastatic disease, metastatectomy does yield improved survival and should therefore always be performed when feasible. [1,5,19-25]

AIMS AND OUTLINES OF THIS THESIS
Altogether, survival outcomes remain unsatisfactory for patients with osteosarcoma. A variety of combination therapy regimens and dose escalation of several therapeutics have not improved survival outcomes, [5,15-17] implying that the treatments we currently provide lack efficacy. This lack of treatment efficacy could be attributed to therapy resistance of the osteosarcoma cells.

The objective of this thesis is to define strategies to subvert or circumvent this relative resistance to therapy, thereby ultimately improving the efficacy of existing therapies.

Chapter 2 provides an introduction to molecular alterations in metastatic osteosarcoma (cells) that could potentially serve as therapeutic targets. Understanding the biology of osteosarcoma metastasis may uncover essential molecules or mechanisms for the survival of metastatic cells and interfering with these might elicit an anti-tumour effect. Therefore, a detailed study of the biological characteristics and behaviour of metastatic osteosarcoma cells may provide a rational basis for innovative treatment strategies. This chapter also provides an overview of (pre)clinical research efforts that were endeavoured upon in the past decade to exploit specific molecular pathways in metastatic osteosarcoma, in order to discover novel targets for treatment and to test their efficacy.

As stated above, therapy resistance of osteosarcoma cells can attribute to inferior treatment efficacy. Chapter 3 enlightens mechanisms of this therapy resistance within osteosarcoma cells and provides a framework to the following chapters in which we investigate various methods of targeted therapy to improve the efficacy of radiotherapy, doxorubicin chemotherapy and the delivery of therapeutics to osteosarcoma cells. Targeted therapy can be achieved either by selectively targeting intracellular proteins essential for tumours to survive, or by a targeted delivery of therapeutics to the tumour by directing therapy selectively to extracellular surface proteins or receptors on tumour cells.

In Chapter 4 we address the issue of resistance to radiotherapy in osteosarcoma. One strategy to enhance radiotherapy efficacy in patients with osteosarcoma is to push irradiated cells forward through the cell cycle using a small-molecule inhibitor drug. In doing this, DNA repair prior to cell division is hampered and the osteosarcoma cells are pushed into mitotic catastrophe. This results in a sensitisation of osteosarcoma cells to radiation therapy.

The issue of chemoresistance of osteosarcoma is addressed in Chapter 5. We hypothesise that to increase chemotherapy efficacy, essential survival pathways should be targeted
concomitantly with administering chemotherapy, thus tilting the intracellular balance to cell death rather than cell survival. Here, we describe the use of functional genomics to discover kinases that, upon silencing, enhance the sensitivity of osteosarcoma to doxorubicin treatment. After candidate selection, pharmacological studies are performed to confirm the initial findings.

Apart from shifting molecular balances within osteosarcoma cells to favour cell death after radiation and chemotherapy, another strategy to improve therapeutic efficacy could be to enhance the delivery of currently applied drugs to the tumour cells. In Chapter 6 we describe the identification of a suitable surface receptor on osteosarcoma cells that can be used as a receptor for targeted drug delivery. In this chapter, we select a proteomics approach to study the surface proteome of osteosarcoma cells compared to healthy bone cells. After candidate selection and confirmation of surface expression, we used specifically targeted adenoviral vectors to study the selective intracellular uptake of these moieties into osteosarcoma cells. Ultimately, the targeted delivery of drugs to osteosarcoma cells can lead to higher active doses at the site of the tumour and thus higher treatment efficacy, while sparing healthy tissues.

Chapter 7 summarises the preceding chapters and forms a general discussion to the topics covered in this thesis, addressing unexplored areas of research, implementation of our obtained knowledge into new treatment modalities for osteosarcoma, and perspectives for the feasibility of developing future cancer treatments in general, and, osteosarcoma in particular. Finally, future perspectives are formulated to conclude this work.

Chapter 8 provides a Dutch summary.