Chapter 1

Introduction and scope of the thesis
General introduction

Soft tissue sarcomas (STS) comprise a heterogeneous group of malignant mesenchymal tumors, generally classified according to their resemblance to normal soft tissues. Currently, nineteen histological types and over 50 different subtypes of STS are being recognized.\textsuperscript{1,2}

Many of the histological types reveal different biological behavior, but even within a single histological group considerable divergence in the malignant potential has been noticed. During the last decade it has become evident that studies provide more insight when they are specified for histological type and grade.

STS represent 1\% of all adult malignancies.\textsuperscript{3} Seven percent of all malignancies in children are STS, with rhabdomyosarcoma as the most common type.\textsuperscript{4} A major difficulty in the management of STS is the low incidence of the individual histological types, as well as their insidious presentation. This hampers the acquisition of expertise in diagnosis and treatment of these tumors. Therefore, referral to specialized centers is greatly advocated. Careful diagnosis, disease staging and treatment planning by a multidisciplinary team of sarcoma specialists have improved the outcome for STS patients.\textsuperscript{5-10} Excellent local control can currently be achieved for most localized STS through surgery, with adjuvant radiotherapy in case of narrow resection margins, high-grade and large tumors. However, patients who are still treated outside specialized centers suffer from higher local recurrence rate and undergo more surgical procedures.\textsuperscript{6}

Despite adequate control of the primary tumor, roughly 30-40\% of patients progress to metastatic disease. A limited group of patients with pulmonary metastases can be cured with metastasectomy. For the vast majority of patients with disseminated disease, chemotherapy is the only tumor-directed therapy option. Unfortunately, the most active agents doxorubicin and ifosfamide have no curative potential in the metastatic setting. Therefore, knowledge of the mechanisms involved in chemotherapy resistance and the development of new treatment options are crucial for a more effective STS treatment.

One mechanism involved in resistance to chemotherapeutic agents is the expression of proteins by tumor cells that hamper the drugs to reach their target. Expression of P-glycoprotein (P-gp), multidrug resistance-associated protein-1 (MRP1) and lung-resistance related protein (LRP) is associated with cross-resistance to various anticancer agents. Efforts to
inhibit the function of these proteins in order to regain drug activity have largely failed in the clinical situation. Interestingly, a recent study demonstrated that a new generation P-gp/MRP1 inhibitor VX-710 could restore sensitivity to doxorubicin in STS.\textsuperscript{11}

An exciting topic in STS treatment is that of targeted drugs with direct access to the apoptotic machinery. Apoptosis is the complex process of cell death, started by an external stimulus and subsequently regulated by intracellular components. Cytokines of the tumor necrosis factor (TNF) family are very interesting, as some members have tremendous potential in inducing apoptosis of tumor cells.\textsuperscript{12} Moreover, the prototype TNF-\textgreek{a} has already demonstrated its anticancer activity, combined with melphalan, in locally advanced STS in the setting of hyperthermic isolated limb perfusion.\textsuperscript{13} Until now, toxic side-effects of TNF-\textgreek{a} have prevented it from systemic use. The recently identified tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in its native form appears advantageous, as normal tissues in non-human primates were spared at tumoricidal concentrations.\textsuperscript{14} At present, phase I studies with TRAIL are imminent.

A second alternative approach might be the molecular targeted drugs that inhibit receptor tyrosine kinase activity. Aberrant tyrosine kinase activity of tumor cells has been implemented in tumorgenesis and tumor progression. This is illustrated by the malignant gastrointestinal stromal tumors, the most common STS of the gastrointestinal tract, which often bear an activating mutation of exon 11 of the \textgreek{c-kit} gene. These tumors, while resistant to chemotherapy and radiotherapy, have a high response to the c-KIT inhibitor imatinib mesylate.\textsuperscript{15} Current studies focus on whether c-KIT tyrosine kinase might be involved in other STS as well, and on the feasibility of targeting other receptor tyrosine kinases.

The current surgical and radiation treatment of STS is well-defined and further progress with respect to limb saving and local control seems not realistic.\textsuperscript{16} The combined modality treatment of surgery and radiation is nowadays focused on diminishing treatment related events and long-term treatment-related morbidity. A better understanding of the tumor biology of STS, together with the development of new systemic treatment may improve of survival of patient with metastasized STS. The studies presented within this thesis were performed to gain insight in mechanisms of drug resistance in STS and to assess potential targets for new treatment options.
Chapter 1

Content of the thesis

The aim of the present thesis is:
1. To evaluate mechanisms involved in the biological behavior and responsiveness to chemotherapy on STS.
2. To assess potential targets in STS for new treatment modalities.

Chapter 2 provides a review on factors that are associated with the development of metastases in STS, as this is a major limiting factor in the treatment of STS. Current and evolving opportunities to treat metastatic disease are being discussed.

Chapter 3 presents the results of an immunohistochemical study on P-gp, MRP1 and LRP expression in 141 primary STS separated per histological (sub-)type and grade. Traditionally lumped together, marked differences in biological behavior exist within the group of STS. The results are discussed against the background of these differences.

Chapter 4 outlines the expression of P-gp, MRP1 and LRP in rhabdomyosarcomas, probed by immunohistochemistry. Rhabdomyosarcomas are the most common STS of childhood, but a limited number of cases occurs in adults. While in children the overall survival has been improved since the introduction of chemotherapy next to local treatment, prognosis for adults remains relatively poor. Therefore, it was hypothesized that MDR proteins were being relatively over-expressed in adult rhabdomyosarcomas.

Chapter 5 describes alterations in MDR protein expression in rhabdomyosarcomas after chemotherapy. The results were correlated with the gain in differentiation of rhabdomyosarcoma cells, as mediated by chemotherapy.

Chapter 6 present a study on the expression of MDR proteins in 35 primary STS, compared with that of their matching metastases. This study was performed because despite general impression that metastatic STS is incurable, to date it is still unknown whether metastatic lesions possess more resources of drug resistance.
Chapter 7 addresses the expression of P-gp, MRP1 and LRP in locally advanced STS before and after hyperthermic isolated limb perfusion with tumor necrosis factor-α (TNF-α) and melphalan. In vitro studies demonstrated a modulating effect of TNF-α on these MDR proteins. While TNF-α has been approved for the treatment of locally advanced limb STS, this perfusion setting offered a unique opportunity to translate results from in vitro studies to the actual clinical setting.

Chapter 8 describes the effects of doxorubicin, activated ifosfamide and TRAIL in rhabdomyosarcoma cells. TRAIL alone has demonstrated antitumor activity both in in-vitro and in-vivo experiments, but TRAIL-resistance has been encountered as well. Especially for TRAIL-resistant tumor cells, combination with conventional cytotoxic drugs might be of clinical value.

Chapter 9 reports on a clinicopathological assessment of 16 sarcomas, arising in a previously irradiated area. These so-called postradiation sarcomas are often difficult to treat surgically, while radiation treatment and chemotherapy are not feasible. A previous study mentioned the expression of KIT tyrosine kinase in two postradiation angiosarcomas. With the introduction of the KIT inhibitor imatinib mesylate for malignant gastrointestinal stromal tumors, KIT has come into view as a potential target for other tumors as well. Therefore, KIT expression was immunohistochemically assessed in these postradiation sarcomas, including angiosarcomas as well as other histological types. Additionally, the mutational status of exon 11 from the c-kit gene was analyzed, as the presence of exon 11 mutations has shown to be correlated to the effect of imatinib mesylate in malignant gastrointestinal stromal tumors.

Chapter 10 provides a summary of the studies covered by this thesis with final conclusions and ends with future perspectives in the treatment of STS.
Chapter 1

References