Hyperthermic isolated limb perfusion is a major cancer operation, which can have systemic and local toxic side-effects. Since the introduction of hyperthermic isolated limb perfusion (HILP), indications for HILP treatment have expanded and new fields for its applicability are currently being explored. This makes it all the more important to be continuously informed about treatment-related toxicity and about the efficacy of the perfusion therapy with new chemotherapeutic agents and cytokines.

The first part of the introduction reviews the history of the technique of hyperthermic isolated limb perfusion, since its introduction in 1958 by Creech and colleagues. The underlying idea of HILP is to administer high doses of cytotoxic agents locally, with a maximum tumoricidal effect, without giving rise to systemic side-effects. In the second part of the introduction, the technique of HILP is described as well as some technical improvements, developed to optimize HILP treatment. The first cytotoxic drug used in HILP was the alkylating agent melphalan (L-phenylalanine mustard) and it has been most widely used in HILP treatment for extremity malignant melanoma. However HILP treatment for locally advanced extremity soft-tissue sarcoma (STS) with melphalan did not improve the local control rate and disease-free survival, when compared to other therapies. Other drugs used in HILP, such as doxorubicin, cisplatin, carboplatin or a combination of melphalan and dactinomycin were inferior to melphalan alone in HILP treatment for melanoma or STS. However, addition of the cytokine tumor necrosis factor-alpha (TNF-α) to melphalan meant a major breakthrough in HILP treatment for locally advanced extremity STS. TNF-α attacks tumor vascularization, which results in hemorrhagic necrosis of the tumor. The beneficial effect of adding interferon-gamma (IFN-γ) to HILP with TNF-α and melphalan is doubtful. The fourth part of the introduction describes the role of HILP in malignant melanoma treatment. HILP with melphalan is an established limb-saving treatment modality for local recurrence, in-transit metastases and satellites of malignant melanoma localized on the extremities,

whereas a favorable role in the adjuvant treatment of Stage I melanoma is questionable. HILP treatment for locally advanced extremity STS is described in the fifth part of the introduction. It was only after the addition of TNF-α to melphalan that HILP treatment resulted in high local response rates and high limb salvage rates.
The research questions which form the basis of this thesis, are formulated at the end of the introduction and are dealt with in detail in the next five chapters.

In **Chapter II** we describe a study on functional morbidity in patients treated according to the protocol of the European Organization for Research and Treatment of Cancer (EORTC). Patients with a high-risk for local recurrence (Stage I extremity melanoma with more than 1.5 mm Breslow thickness) were prospectively randomized over a group who received HILP treatment with melphalan followed by wide excision (WE, 3 cm margin) or WE alone. The research question was whether HILP with melphalan adds to short and long-term morbidity. Morbidity was evaluated on the basis of the length of hospitalization, postoperative pain, postoperative performance and the grade of perfusion toxicity. At 12-months follow-up, a diagnostic physical examination was performed to measure the mobility of the joints as well as the circumference and volume of the treated and untreated extremities. Eighty-three out of the 97 patients treated according to the EORTC protocol at the Groningen University Hospital could be evaluated. Age and sex distribution were comparable in the two treatment groups. Forty-six patients underwent HILP+WE and 37 patients underwent WE alone. There was no treatment-related mortality. Treatment-related complications were observed in two patients (1 urine retention and 1 wound dehiscence). For the leg, the period of hospitalization was an average of 1.9 days longer after HILP+WE, than after WE alone ($p=0.01$). This difference was absent for the arm. HILP generated mild local toxic reactions (grade 2, according to Wieberdink) in the upper extremity, with a

mean score of 2.1 (range 2-3) for the lower extremity. Obviously there were no toxic reactions after WE alone. At 12-months follow-up, the difference in morbidity between patients after HILP followed by WE and patients after WE alone had disappeared. Nevertheless, a number of subjective complaints were encountered in the HILP+WE group (e.g. pricking sensations or pain during changes in the weather). However, these complaints did not cause any functional morbidity. This study showed that HILP with melphalan did not cause long-term (functional) morbidity, except for some subjective complaints. A possible explanation for these complaints is fibrosis caused by perfusion. These findings are in contrast with those in another publication on this subject which mentioned 25% limitation of motion in the ankle joint after perfusion. One explanation could be that in Groningen, fasciotomy was always performed after HILP to prevent a (sub)clinical compartment syndrome, which may have prevented late fibrosis.

In **Chapter III** we present a study on angiographic changes in 25 patients treated for locally advanced extremity STS with HILP with TNF-α and melphalan. TNF-α targets tumor vascularization by causing selective changes in tumor-associated endothelial cells, whereas endothelial cells of normal tissue remain unaffected. Aim of the study was to assess whether this effect can be demonstrated angiographically and whether angiographic changes after HILP with TNF-α and melphalan were related to the histopathological response of locally advanced soft-tissue sarcoma to HILP treatment.

Angiography was performed before HILP with TNF-α and melphalan and after a median of 7 (range 4-14) weeks. After a median post-HILP period of eight weeks, the residual tumor mass was resected and examined histopathologically. The changes in tumor vascularization after treatment were scored and compared to the histopathological response. All baseline angiograms showed hypervascularity of the tumor. After HILP the angiographic findings were normal (NA) in 18 patients (72%)

and abnormal (AA) in 7 patients (28%). All the patients with NA showed a complete histopathological (pCR) response or a partial histopathological partial response with over 90% necrosis of the tumor (pPR>90%). In the 7 patients with AA, histopathological examination showed a pCR in 1 patient, 10% to 50% viable tumor volume in 4 patients and no histopathological response in 2 patients. Angiographic and histopathological classification showed good correlation ($p<0.001$). Post-HILP angiography provided an indication of the histopathological response that could be expected. This may be of value in determing the indication for a second perfusion treatment.

In **Chapter IV** the efficacy of adjuvant external beam radiotherapy (EBRT) is evaluated in terms of local disease control, limb-salvage and survival after HILP treatment with TNF-α and melphalan for locally advanced extremity STS. In addition, we addressed the question of whether EBRT after HILP adds to treatment-related morbidity.

When HILP with TNF-α and melphalan does not result in complete necrosis of a soft-tissue sarcoma and resection margins are close, external beam radiotherapy (EBRT) may be an adjuvant treatment modality. This study describes 34 patients with a locally advanced extremity STS, who underwent HILP with TNF-α and melphalan. Resection of the residual tumor mass was performed in the majority of patients after 8 weeks. Fifteen patients with histopathological viable tumor after resection received adjuvant 60-70 Gy EBRT (44%, HILP+EBRT group). Nineteen patients received HILP without adjuvant EBRT (56%, HILP alone group). Five patients in the HILP alone group also had distant metastases (15%) at the time of HILP and they received HILP with a palliative treatment intent. The limb salvage rate, treatment morbidity, local recurrence, regional and distant metastases were scored. During a median follow-up of 34 (range 8-54) months, limb salvage was achieved in 29 patients (85%): 14 patients after HILP+EBRT (93%) and 15 patients

after HILP alone (79%). None of the patients in the HILP+EBRT group developed local recurrence, whereas 5 patients in the HILP alone group did (26%) ($p<0.05$). Regional axillary or inguinal lymph node metastases were observed in 1 patient in the HILP+EBRT group (7%) and in 2 patients in the HILP alone group (14%). Distant metastases occurred in 4 patients (27%) after HILP+EBRT and in 4 patients (29%) after HILP alone with a curative intent. The mean morbidity (SOMA) score in both groups was 0.33 for skin and subcutaneous tissue. The SOMA scores for muscle and soft tissue were 0.34 (HILP+EBRT group) and 0.33 (HILP alone group) respectively. The results of this study demonstrate that adjuvant EBRT after HILP with TNF-α and melphalan and delayed tumor resection of locally advanced extremity STS is feasible and may increase local tumor control without increasing treatment-related morbidity.

In **Chapter V** we present the results of treatment with HILP with TNF-α and melphalan in 15 patients with locally advanced extremity squamous cell carcinoma or Merkel's cell carcinoma. Limb saving is sometimes impossible in these patients using conventional treatment modalities. The encouraging results of HILP treatment with TNF-α and melphalan in patients with extremity STS raised the question of whether this therapy might also be effective in the treatment of other locally advanced extremity tumors.
Fifteen patients with locally advanced primary, recurrent or metastatic extremity skin tumors (12 squamous cell carcinomas, 3 Merkel's cell carcinomas), underwent HILP with TNF-α and melphalan as a limb saving therapy. Six tumors were localized in the upper extremity (40%) and 9 in the lower extremity (60%). Treatment-related complications, limb saving rate, local recurrence, regional and distant metastases were scored during a median follow-up of 20 months. Nine patients showed a complete response to HILP treatment (60% CR, all histopathologically confirmed), 4 patients showed a partial response (27% PR, 1

histopathologically confirmed) and 2 patient showed no change in the resected tumor (14% NC, one histopathologically confirmed). There were 2 treatment-related complications (13%). Treatment-related mortality was 7%. The limb saving rate was 80%, while the local recurrence rate was 27% (local progression included). Regional lymph node metastases were observed in 13% and distant metastases in 14% of the patients treated with a curative intent.
A remarkably high limb saving rate and local control rate were achieved. Therefore, HILP with TNF-α and melphalan should be considered as a limb saving treatment modality option in patients with locally advanced extremity squamous cell or Merkel's cell carcinoma.

In **Chapter VI** the results and complications are described of HILP treatment with TNF-α and melphalan for locally advanced extremity STS in 9 patients who had regional or distant metastases at the time of HILP. The question was whether HILP with TNF-α and melphalan is worthwhile when used with palliative intent. The study group comprised 9 patients: 3 had regional and 6 had distant metastases at the time of the initial diagnosis of a locally advanced extremity STS. One patient had 2 perfusions, thus 10 perfusions were performed. Resection of the residual tumor mass, if possible, was performed 6-8 weeks after HILP treatment. Treatment-related morbidity, local recurrence and the limb saving rate were scored. During a median follow-up period of 9 (3-39) months, 6 patients died from metastatic disease. Treatment-related morbidity was observed in 3 out of the 10 perfusions (30%): superficial wound infection in 2 patients, and blow-out of the external iliac artery followed by iliac thrombosis in 1 patient. Two patients developed local recurrence after HILP and resection, while 1 patient showed local progression after 2 perfusions without resection. Limb saving was achieved in 8 patients (89%). The study results showed that HILP with TNF-α and melphalan for locally advanced extremity STS in patients with disseminated disease can be worthwhile as a palliative therapy.

Conclusions

I. HILP treatment with melphalan does not cause any additional long-term functional morbidity.

II. The response of STS to HILP with TNF-α and melphalan can be demonstrated with angiography.

III. Adjuvant EBRT after HILP with TNF-α and melphalan and delayed tumor resection of locally advanced extremity STS improves local tumor control, without increasing treatment-related morbidity.

IV. HILP with TNF-α and melphalan has proved to be useful in the treatment of locally advanced extremity squamous cell carcinoma and Merkel's cell carcinoma. HILP with TNF-α and melphalan should be considered as a limb saving treatment modality in patients with advanced extremity tumors that cannot otherwise be resected curatively.

V. HILP treatment with TNF-α and melphalan with a palliative intent can be worthwhile in patients with regional lymph node and distant metastases.

HILP = hyperthermic isolated limb perfusion
WE = wide excision
STS = soft-tissue sarcoma
EBRT = external beam radiotherapy
TNF-α = tumor necrosis factor-alpha
IFN-γ = interferon-gamma