Clinical Utility of TNF-based Isolated Limb Perfusion to achieve Limb Salvage

Dirk Jan Grünhagen

Clinical Utility of TNF-based Isolated Limb Perfusion to achieve Limb Salvage

The Rotterdam Experience

Klinische toepassing van geïsoleerde extremiteitsperfusie met TNF gericht op Extremiteitsbehoud

De Rotterdamse ervaringen

Proefschrift

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CHAPTER 1

General Introduction to the Thesis

INTRODUCTION

The technique of Isolated Limb Perfusion (ILP) was pioneered by Creech and Krementz at Tulane University in New Orleans [1] and first applied in a patient with extensive melanoma metastases on the lower limb. The principle idea was that direct administration of a chemotherapeutic agent to the area to be treated by intra-arterial infusion would sort out in the maximum effect. However, venous return should be blocked to prevent the agents from entering the systemic circulation. The method of ILP was thus developed to be able to achieve regional concentrations of chemotherapeutic agents that are 15 to 25 times higher than can be reached with systemic administration, but without the systemic side-effects [2].

A perfusion circuit is achieved by canulating the major artery and vein of a limb and connect these catheters to a pump oxygenator, thus creating extracorporeal circulation (figure 1). Further isolation of the limb from the corporeal circulation is guaranteed by the use of a tourniquet just proximal to the catheter tips. This revolutionary technique enables a locoregional therapy approach to tumours located in the extremity that fail other treatment options. Since the report of its effect in the treatment of melanoma in mice [3], and because of the favourable local toxicity profile of the drug, melphalan (L-phenylalanine mustard) is used as the standard chemotherapeutic agent in the ILP setting. Melphalan is commonly used in doses of 10 mg/L perfused tissue for a leg and 13 mg/L for an arm [4]. As cancer cells were known to be selectively susceptible to high temperatures, hyperthermia was introduced in the ILP setting [5]. The now most commonly used technique of mild hyperthermia for ILP was described shortly hereafter [6]. A new era in the treatment of extremity cancer began in 1988, when the cytokine Tumour Necrosis Factor-alpha (TNF) was introduced in ILP as not only tumour cells, but also the tumour vasculature, became target for therapy [7].

TNF was isolated as an endogenous factor, especially active in inflammation, with necrotizing ability on tumour cells [8]. Later studies revealed that TNF has a dual mechanism of action: high-dose TNF has a direct cytotoxic impact to tumour cells [9], but more importantly the TNF effect on the so called tumour associated vasculature induces a rapid change in tumour morphology [10]. These observations led to high expectations on this cytokine in anti-tumour therapy, but systemic application was very disappointing. TNF turned out to be a potent mediator in septic shock and therefore the systemic side-effects (acute drop in vascular resistance leading to low blood pressure rates, fever etc.) were the major factors hindering systemic application of this cytokine [11]. The maximum tolerated dose of TNF in humans turned out to be 10

to 50 times lower than needed for anti-tumour effects in murine models [12], so that systemic, but also intralesional administration of TNF was not clinically applicable.

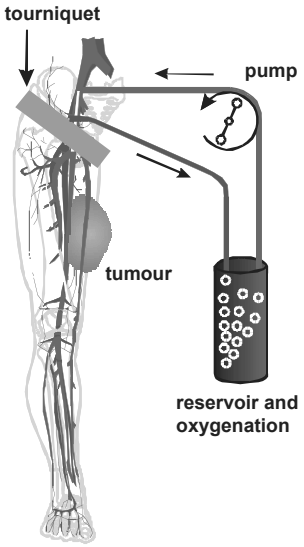

Figure 1: Isolated Limb Perfusion Circuit

However, the concept of ILP combines the advantages of the TNF anti-tumour activity with the avoidance of systemic effects. The cytotoxic effects of TNF are known to be enhanced in hyperthermic conditions [13] and with the addition of alkylating chemotherapeutics [14], both prerequisites already existing in the ILP model. The antivascular effects of TNF (a haemorrhagic necrosis of the tumour as a result of destruction of the tumour associated vasculature) were demonstrated in both histopathologic and angiographic studies [15, 16]. The application of TNF in the ILP setting was further studied in the preclinical setting. It turned out that for effective ILP a minimum duration of ILP of 30 minutes is required. The perfusion temperature should be above 38°C for maximum effect, but should not exceed 42°C in order to avoid unacceptable toxicity to the normal tissue [17]. TNF was shown to act synergisticly with melphalan by enhancing its uptake in tumour cells (but not in normal tissue) six fold [18]. Hypoxia, although enhancing the effect of melphalan activity and TNF activity alone, did not further enhance the synergistic effect of the combination of TNF and melphalan [17]. These studies form the basis of the clinical application of TNF-based ILP in both melanoma and sarcoma patients.

ILP FOR MELANOMA

Since the introduction of the ILP principle, melphalan ILPs were performed with very satisfactory results in melanoma patients, taken into account the disappointing response rates of this tumour on systemic chemotherapy. However, since TNF is applied in ILP, it has been used increasingly in combination with melphalan for the treatment of melanoma in-transit metastases (IT-mets). An early report on TNF-based ILPs from 4 centers in Europe showed a significant increase in CR rate up to 90% compared to a 52% CR rate after ILPs in these centers with melphalan alone [19]. Both clinical observations and an interim analysis of a randomised controlled trial performed in the USA [20] gave indications that the benefit of TNF as an adjunct to melphalan is especially appreciable in patients with large lesions. The special indication for ILP with both TNF and melphalan (TM-ILP) is therefore the patient with bulky melanoma IT-mets or the patient that has failed prior ILP with melphalan alone.

In our institution, 100 consecutive TM-ILPs for melanoma were performed between 1991 and 2003. **Part I** of this thesis describes the results of these 100 procedures. We determine prognostic factors for response, recurrence and survival and then focus on the very specific population of patients qualifying for a repeat ILP for recurrence of IT-mets after previous ILP, either with TNF and melphalan or with melphalan alone.

ILP FOR SOFT TISSUE SARCOMA

Soft Tissue Sarcoma (STS) of the limb is the second major indication for TM-ILP. In contrast to high response rates in melanoma patients, the results of melphalan-alone perfusions in STS patients were uniformly disappointing [21, 22]. The initial study of TNF in ILP however reported not only high response rates in melanoma patients, but also in 4 patients with advanced extremity STS [7]. This led to multicenter studies evaluating response- and limb salvage rates using TNF-based ILPs with or without Interferon-gamma, showing consistently overall response rates of 75- 85% and limb salvage in a similar percentages of the patients [16, 23]. These results led to the approval of TNF in Europe [24]. At present, TNF-based ILP for extremity STS is used as induction biochemotherapy to obtain local control and to make limb-sparing surgery possible.

The database of the Erasmus MC - Daniel den Hoed Cancer Center on TM-ILP for STS contains 217 procedures performed between 1991 and 2003 and is the largest single-

institution database worldwide. In **part II** of this thesis, the outcome of the patients with locally advanced extremity STS is studied. We identify prognostic factors for response and (recurrence-free) survival and we explore the possible application of TM-ILP in specific STS patient categories that are typically difficult to treat with limb-sparing surgery: patients who present with multifocal STS and patients who are previously treated with high-dose radiotherapy on the affected limb. A third patient category is formed by patients with desmoid tumours or aggressive fibromatosis as this type of STS can cause locally disabling disease but does not have the propensity to develop systemic metastases. ILP can provide shrinkage of the tumours, which can make a resection possible and can thus provide long-lasting local control.

GENERAL QUESTIONS IN ILP

The development of TM-ILP has led to several questions regarding the clinical use of this treatment modality. It is well accepted that patients with locally advanced disease of the limb, both melanoma and STS, are candidates for ILP, as the local situation in the extremity often prompts for treatment. On the other hand, both melanoma and STS are tumours with poor prognosis once the disease has spread systemically. This last patient category, staged as stage IV in the AJCC classifications, can however qualify for ILP as the local situation is disabling to an extent that the quality of life in the terminal life phase is severely threatened. This discussion is based both on patient-related arguments (whether the patient should be offered such an invasive treatment with possibly lethal systemic side-effects), but has also an economic part, since TM-ILP is a costly procedure. These last two issues can possibly be partly overcome by a reduction of TNF in TM-ILP. In **part III** of this thesis we give an answer to these clinical questions by retrospectively investigating our database on all TM-ILPs in Rotterdam. This will give the introduction to the general discussion on the indications and results of TNF-based Isolated Limb Perfusion.

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PART I

TNF-based ILP for Melanoma Patients

CHAPTER 2

100 Consecutive Isolated Limb Perfusions With TNF-α and Melphalan in Melanoma Patients with Multiple In-Transit Metastases

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ABSTRACT

Objective

The aim of this study is to describe the experience with 100 TNF-based isolated limb perfusions (ILP) for locally advanced melanoma and to determine prognostic factors for response, time to local progression and survival.

Methods

One hundred TNF-based ILPs were performed between 1991 and 2003 in 87 patients for whom local control by surgery of in-transit melanoma metastases was impossible. In total, 62 iliac, 33 femoral and 5 axillary ILPs were performed in mild hyperthermic conditions with 2 to 4 mg of TNF and 10 to 13 mg melphalan/L limb volume.

Results

Overall response was 95%, with 69% complete response, 26% partial response and 5% no change. Complete response rate differed significantly for patients with IIIA disease versus IIIAB and IV. Local and systemic toxicity was mild to moderate in almost all cases, with no treatment-related death and one treatment-related amputation. Five-year overall survival was 32%; local progression occurred in 55% after a median of 16 months. In complete response patients, 5-year survival was 42% with local progression in 52% at a median of 22 months. Response rate and survival were significantly influenced by stage of disease; (local progression free) survival was influenced by response rate.

Conclusions

TNF-based ILP results in excellent response rates in this patient population with unfavourable characteristics. Response on ILP predicts outcome in patients and reflects aggressiveness of the tumour.

INTRODUCTION

In-transit metastases (IT-mets) occur in approximately 5% to 8% of the patients with high-risk melanoma. The management of IT-mets remains a challenge because it is dictated by the biologic behaviour of melanoma, especially in terms of number and size of the lesions [1]. Simple wide surgical excision is often possible if IT-mets are limited in size and number, but fails when interval periods between new lesions are short, when numerous or bulky lesions are present and when various treatment modalities, e.g. radiotherapy, have preceded surgery. Apart from local control, melanoma is virtually refractory to all systemic treatments. Therefore, various locoregional approaches have been proposed and investigated. Isolated limb perfusion, developed in 1958 by Creech and Krementz [2], is the most effective regional treatment modality, as it achieves tissue concentrations in the affected limb of the chemotherapeutic agents that are more than 20 times higher than what can be achieved systemically [3]. Melphalan (L-phenylalanine mustard, L-PAM) has been used as standard drug over the years due to its efficacy and toxicity profile [4]. Melphalan-based ILP for melanoma IT-metastases is associated with Complete Response (CR) rates of 40% to 50% and overall response rates of 75% to 80% [1]. Hyperthermia may increase the response rates somewhat, but at the cost of increased locoregional toxicity. Large melanoma lesions are difficult to eradicate because of poor and inhomogeneous drug uptake as with soft tissue sarcomas. Therefore, ILP programs with melphalan alone have been abandoned for treating irresectable soft tissue sarcomas [5]. The application of Tumour Necrosis Factor alpha (TNF) [6] changed this situation dramatically because very large tumours were now observed to respond very well [7], which led to successful multicenter trials in Europe and the approval of TNF-α for the treatment of irresectable extremity soft tissue sarcomas [8].

TNF has also been used increasingly in combination with melphalan for the treatment of melanoma IT-mets in ILP. An early report on TNF-based ILPs from 4 centers in Europe showed a significant increase in CR rate up to 90% compared with a 52% CR rate after ILPs in these centers with melphalan alone [9]. The success of TNF in the treatment of large soft tissue sarcomas made the investigators aware early on that TNF also improved results in particular in melanoma patients with bulky lesions. Importantly, this clinical observation of efficacy in large tumours has been explained by observations in our laboratory in the experimental isolated perfusion setting in rats with advanced limb tumours. In contrast to the poor drug uptake of melphalan after an ILP with melphalan alone by these large tumours, it was shown that TNF increased the uptake of melphalan selectively in the tumour by a factor 3 to 6 in comparison to an ILP with melphalan alone [10].

We report here on our experience with 100 TNF-based ILPs in patients with multiple melanoma IT-metastases. We have analysed the data in this large group to determine prognostic factors for response such as stage of disease, size and number of lesions, local recurrence-free interval, and efficacy after failing other treatments.

PATIENTS AND METHODS

Patients

Between 1991 and 2003, 100 TNF and melphalan (TM)-ILPs were performed in 87 patients with multiple in-transit melanoma metastases in the limb. Demographic data, disease presentation at time of ILP and ILP characteristics were obtained from a prospectively maintained database. Patients were staged according to the MD Anderson staging system [11] and presented with stage IIIA disease in 45, stage IIIAB in 41 and stage IV in 14 cases. In all cases, local control by simple surgical excision was impossible due to bulky disease, which is illustrated by the size of the tumours (48 < 40 mm, 52 ≥ 40 mm) and the number of lesions (<10 lesions in 43 patients, 10-50 lesions in 32 and >50 lesions in 25 patients). Besides surgical excision of resectable tumours, 59 patients did not undergo other treatment modalities prior to ILP, whereas 41 patients were previously treated with systemic chemotherapy, radiotherapy, ILP with any chemotherapeutic, immunotherapy or a combination of the above-mentioned modalities. Patient and tumour characteristics are summarized in table 1.

Treatment

All patients underwent an ILP via the axillary (*n*=5), iliac (*n*=62) or femoral (*n*=33) approach. The method of ILP is described in detail previously [8]. In short: isolation of the limb is achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, ligation of collateral vessels and application of a tourniquet proximal to the site of perfusion. Once tissue temperature has reached 38°C, recombinant TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) is administered via the arterial line in a dose of 2 to 4 mg. Tissue temperatures are stabilized between 38°C and 39.5°C and leakage monitoring is performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit [12]. After 30 minutes, melphalan (L-PAM, Alkeran, Wellcome Ltd., London, UK) is added to the perfusate in a dose of 10 mg/L for leg and 13 mg/L for arm perfusions. In 23 ILPs, performed between 1991 and 1994, interferon γ (IFN) was added to the schedule according to trial prescriptions consisting of the subcutaneous injection of 0.2 mg IFN on days –2 and –1 prior to

Table 1

Patient and tumour characteristics of 87 patients undergoing 100 TM-ILPs			
Characteristics		**No. of patients**	
Sex	Female	60	(69%)
	Male	27	(31%)
Age (years), median+range		62	(25-90)
Stage	IIIA	45	
	IIIAB	41	
	IV	14	
Location	Arm	5	
	Leg	95	
No. of tumours	<10	43	
	10-50	32	
	>50	25	
Size of largest lesion (mm)	<40 mm	48	
	≥ 40 mm	52	
Prior treatment	None	59	
	XRT	4	
	CT	8	
	ILP	21	
	Immuno	3	
	Combination	5	

TM-ILP=isolated limb perfusion with tumour necrosis factor and melphalan XRT=radiotherapy, CT=chemotherapy, ILP=isolated limb perfusion, Immuno=immunotherapy

the ILP and the injection of 0.2 mg IFN during the ILP procedure into the arterial line prior to the administration of TNF. Median dose of melphalan was 89.2 mg (mean 94.5, range 39-140), median dose of TNF was 4 mg (mean 3.69, range 2-4) and all 23 IFN-ILPs were performed with 0.2 mg IFN. At the end of the perfusion period, a washout procedure using 2-4 L of a dextrane and/or electrolyte solution is performed. In patients undergoing an iliac perfusion, an iliac lymph node dissection is performed; an axillary lymph node dissection is performed in patients undergoing an axillary ILP. In patients with palpable nodal disease in the groin an ilio-inguinal lymph node dissection is performed in the same operative session as the ILP but before executing the ILP.

Evaluation of response and toxicity

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [13] in the following manner: (I) no reaction; (II) slight erythema or edema; (III) considerable erythema or edema with some blistering, slightly disturbed motility

permissible; (IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance; and threatening or manifest compartmental syndrome, and (V) reaction that may necessitate amputation. Response evaluation was performed 2 to 4 weeks and 8 weeks after ILP by clinical examination, and after that at 3-month regular intervals for the first 2 years and at longer intervals hereafter. Response rates were reported according to WHO criteria [14], in which complete response (CR) is the complete disappearance of all lesions and no new areas of disease appearing within the field of ILP. Partial response (PR) is defined as a reduction of 50-99% of the total tumour size; no change (NC) is recorded if <50% of the total tumour size responds.

Recurrence of tumour within the extremity after a CR, or progression of the lesions and the appearance of new lesions after a PR or after NC, is reported as local progression.

Statistical evaluation

Overall survival (OS) and time to local/systemic progression (TTLP/TTSP) were defined as time from ILP to death, local progression and systemic progression respectively and estimates were made using the method of Kaplan and Meier [15]. Disease free survival (DFS) is defined as time from CR to local progression, systemic progression or death, whichever occurs first. We evaluated the prognostic value of some baseline factors for these three endpoints (TTLP/TTSP and OS) with Cox regression. The hazard ratio belonging to each factor in table 3 is defined as the hazard of the second category divided by the hazard in the first category. We also evaluated the

Table 2

Local toxicity					
Wieberdink[12]	Grade I	Grade II	Grade III	Grade IV	Grade V
	15%	54%	27%	3%	1%
Systemic toxicity					
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Neurologic*	93%	7%	0%	0%	0%
Liver*	98%	0%	2%	0%	0%
Renal*	99%	0%	1%	0%	0%
Haematologic*	99%	0%	0%	0%	1%
	<38°C	38-39°C	39-40°C	>40°C, <24 hrs	>40°C, >24 hrs
Temp	37%	26%	31%	6%	0%
	Absent	Present			
Shock&	98%	2%			

Hrs=hours, *= WHO criteria[13], &= support of vasopressors needed

Table 3

Univariate analysis of clinical prognostic factors for CR achievement, local progression and survival			
Variable	**CR**	**Local progression**	**Survival**
	OR (p-value)	**HR (p-value)**	**HR (p-value)**
Sex (female* vs. male)	0.56 (0.20)	1.04 (0.91)	1.89 (0.02)
Age (≤ 60* vs. >60)	0.74 (0.50)	1.56 (0.11)	1.49 (0.11)
Location of lesions (three strata: Lower arm/leg* vs. Upper arm/leg[1] vs. Total limb[2])	0.68 (0.49)[1] 1.66 (0.35)[2]	0.82 (0.62)[1] 0.80 (0.49)[2]	1.04 (0.92)[1] 0.87 (0.64)[2]
Stage of disease (three strata: IIIA* vs. IIIAB[1] vs. IV[2])	0.37 (0.05)[1] 0.16 (0.006)[2]	1.54 (0.13)[1] 0.78 (0.69)[2]	1.96 (0.01)[1] 9.78 (<0.001)[2]
Size of largest lesion (Ø<4 cm* vs. ≥ 4 cm)	0.58 (0.21)	1.08 (0.78)	2.01 (0.005)
Number of lesions (<10* vs. ≥ 10)	0.64 (0.31)	2.85 (0.001)	0.96 (0.86)
IFN (no* vs. yes)	1.84 (0.27)	0.82 (0.52)	0.58 (0.08)
Prior ILP treatment (no* vs. yes)	1.30 (0.60)	1.34 (0.31)	0.57 (0.06)
Date of perfusion (1st 50 ILPs* vs. 2nd 50)	0.35 (0.02)	1.86 (0.02)	1.62 (0.07)
CR achieved (no* vs. yes)	n.a.	0.25 (<0.001)	0.27 (<0.001)

*=reference group, CR=complete response, OR=Odds Ratio, HR=Hazard Ratio, IFN=interferon gamma, n.a.=not applicable

prognostic value of some baseline factors on achievement of CR using logistic regression. The odds ratio of each factor is the odds of CR achievement in the second category divided by the odds of CR achievement in the first category. The prognostic factors that we included were: sex, age, site of tumour, stage of disease, size of largest lesion and number of lesions. This list represents all previously reported studies on prognostic factors after ILP [16-21]. After univariate analysis, all these factors were included in a multivariate model. We used a stepwise backward algorithm in order to exclude factors without prognostic value with a significance level of 5%. After obtaining the final model, we evaluated the additional prognostic value of complete response after perfusion. However, it must be noted that a prognostic model with response on perfusion applies only to patients *after* perfusion, whereas the model without response on perfusion can also be applied to patients before perfusion. All tests were done at a significance level of 5%.

RESULTS

Sixty female and 27 male patients with a median age of 62 years (mean 61, range 25-90) had multiple IT-mets in upper (*n*=5) or lower (*n*=95) extremity. A TM-ILP was performed to achieve local control in all 87 patients; 26 ILPs were performed in patients who had undergone one or multiple ILPs previously during the course

of their disease either in our institution (*n*=13, TM-ILPs) or in the referring hospital (*n*=13). In total, 100 TNF-based ILPs +/- IFN were performed in our institution.

Leakage and toxicity

There was no or minor leakage of the drugs in 93 ILPs (median leakage 0%). Eight ILPs had leakage percentages of 10% to 32%. Toxicity in these 8 cases was limited to transient hypotension in 2 patients for which vasopressor support was given; in 1 patient a grade IV leucopenia was observed that lasted only for 1 day and that did not need any type of intervention. Local toxicity after ILP was mild to moderate (Wieberdink grade II-III) in 81%. A Wieberdink I (no reaction) was seen after 15 procedures and in 3 patients a grade IV local toxicity occurred, with the need of performing a fasciotomy in 1 patient. One patient with IT-mets in the lower leg experienced extensive rhabdomyolysis of the upper leg necessitating an amputation. This reaction was classified as Wieberdink grade V (table 2).

Response rates and limb function

The overall response rate was 95%, with 69 CRs (69%), 26 PRs (26%) and 5 NCs (5%). The proportion of patients reaching CR presenting with stage IIIA disease (82%) differed from those presenting with stage IIIAB (63%) and stage IV disease (43%) (table 3). These differences showed a significant correlation between CR and stage of disease (IIIA vs. IIIAB, p=0.053; IIIA vs. IV, p=0.004; IIIAB vs. IV, p=0.184). Limb function was assessed in all 87 patients and was unaffected with respect to standard daily activities in 84 of them. One case of moderate function loss was recorded, and two amputations had to be performed: one because of a Wieberdink grade V local toxicity (see above) and 1 because of severe arteriosclerosis, which requested a below-knee amputation more than 1 year after the ILP.

Local progression

Local progression occurred after 55 ILPs (55%), at a median time of 16 months. Median TTLP of patients after CR was 22 months versus 6 months for patients after PR or NC (p<0.001) (figure 1). There was more rapid progression of disease in patients with a high (≥10) number of IT-mets (p=<0.001), consistent in both uni- and multivariate analysis (tables 3+4, figure 2). No difference in TTLP was observed for stage IIIA vs. stage IIIAB patients (p=0.12)

Systemic progression

During follow-up, systemic progression occurred after 71 ILPs. Median time to systemic progression (TTSP) was 14 months and differed significantly between stage IIIA (55 months) and stage IIIAB (9 months, p<0.001) patients (figure 3). Other prog-

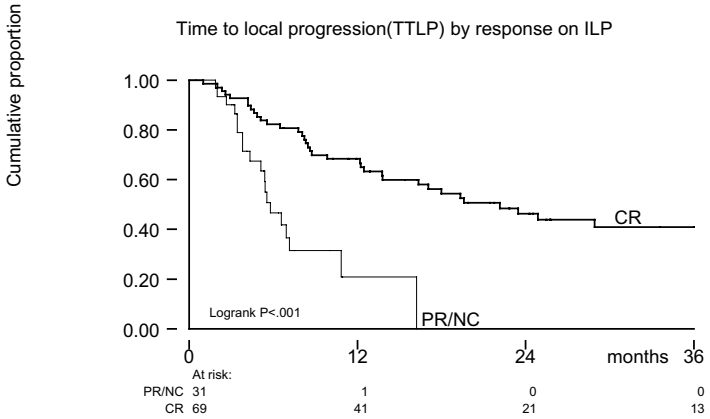

Figure 1: Time to local progression (TTLP) by response on ILP

X-axis: time in months, Y-axis: cumulative proportion, ILP=isolated limb perfusion, CR=complete response, PR/NC=partial response/no change

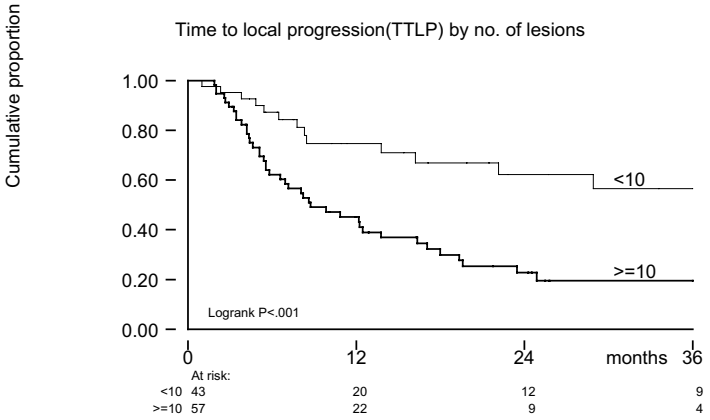

Figure 2: Time to local progression (TTLP) by no. of lesions

X-axis: time in months, Y-axis: cumulative proportion

nostic factors for systemic progression included sex ($p<0.001$), size of the largest lesion ($p<0.001$), prior ILP treatment ($p=0.003$) and ILP response ($p<0.001$). Prognostic factors for systemic progression were consistent with prognostic factors for survival

Survival

The overall actuarial 5-year survival rate was 32% (±5, SE); median survival was 25 months. Survival was influenced by stage of disease, sex, size of the largest tumour and previous ILP-treatment (table 3). After multivariate analysis, stage of disease and

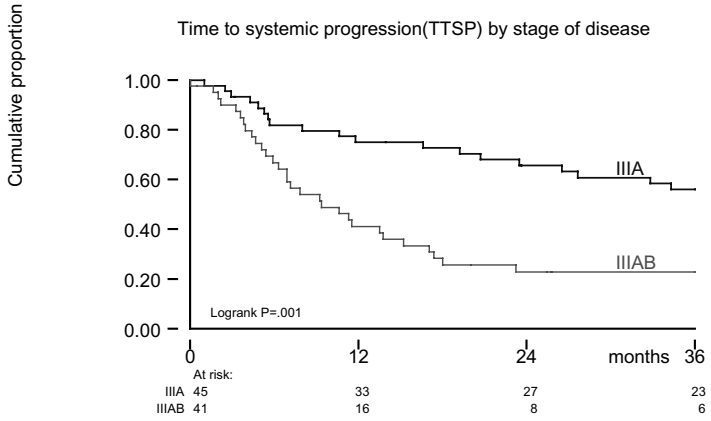

Figure 3: Time to systemic progression (TTSP) by stage of disease

X-axis: time in months, Y-axis: cumulative proportion, IIIA=stage IIIA disease, IIIAB=stage IIIAB disease, IV=stage IV disease

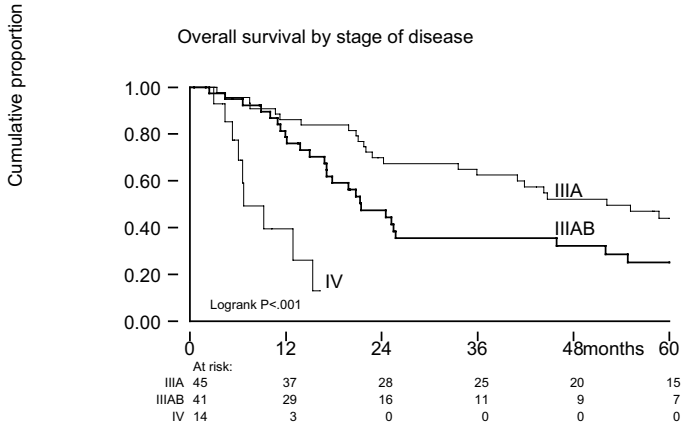

Figure 4: Overall survival by stage of disease

X-axis: time in months, Y-axis: cumulative proportion, IIIA=stage IIIA disease, IIIAB=stage IIIAB disease, IV=stage IV disease

age of the patient remained prognostic variables for survival. (Table 4, figure 4). Disease free survival was estimated for stage IIIA/IIIAB patients. At three years, DFS was 16% and was significantly different between stage IIIA (32%) and IIIAB (0%, p=0.008) patients.

When the achievement of CR after ILP was added to the prognostic factor analysis in the multivariate model, it showed to be of significance in TTLP (p<0.001) without influencing the prognostic value of number of lesions (p=0.001). For survival, the

Table 4

Multivariate analysis of clinical prognostic factors for CR achievement, local progression and survival prior to ILP						
Endpoint	**Variable**		**N**	**Haz ratio**	**p**	**95% CI**
CR	Stage of disease	IIIA*	45	1		
		IIIAB	41	0.375	0.053	0.139-1.013
		IV	14	0.162	0.006	0.044-0.598
Local progression	Number of lesions	<10*	43	1		
		≥ 10	57	2.854	0.001	1.539-5.293
Survival	Stage of disease	IIIA*	45	1		
		IIIAB	41	2.005	0.011	1.174-3.423
		IV	14	11.654	<0.001	4.640-29.272
	Age (years)	≤ 60*	47	1		
		>60	53	1.738	0.033	1.047-2.887

CR=complete response, ILP=isolated limb perfusion, Haz ratio=hazard ratio, CI=confidence interval, *=reference group

achievement of CR is of significant prognostic value, reducing the impact of age of the patients. Five-year overall survival for patients with a CR after ILP was 42% versus 5% for PR/NC-patients (p=<0.001) (Figure 5). Because stage of disease itself is a prognostic factor for response, the effect of response was evaluated for each stage of disease (table 5) and remained significant, especially in stage IIIA patients.

Influence of IFN, prior ILP treatment and tumour bulk

IFN-γ: No significant difference in CR-rate was found between patients receiving a TM-ILP with or without IFN (78% vs. 66% respectively, p=0.274). Neither could an

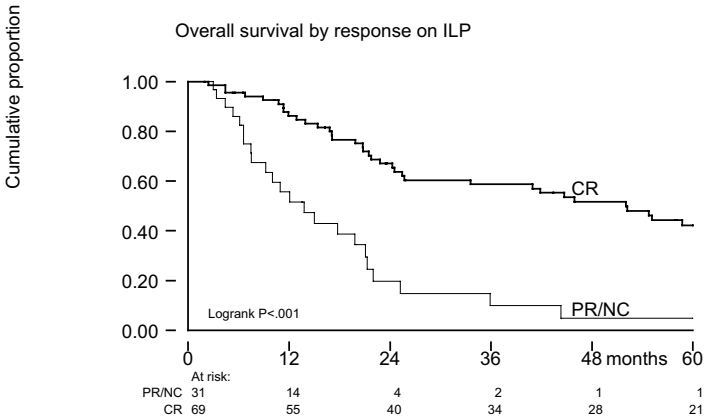

Figure 5: Overall survival by response on ILP

X-axis: time in months, Y-axis: cumulative proportion, ILP=isolated limb perfusion, CR=complete response, PR/NC=partial response/no change

Table 5

Influence of response rate on survival after ILP				
Stage of disease	**CR**	**N**	**Haz ratio**	**p**
IIIA	yes*	37	1	
	no	8	5.016	<0.001
IIIAB	yes	26	2.189	
	no	15	4.130	0.115
IV	yes	6	8.158	
	no	8	41.035	0.041

CR=complete response, Haz ratio=hazard ratio

Table 6

Influence of tumour bulk and stage of disease				
		1st 50 ILPs	**2nd 50 ILPs**	**p**
Size of largest lesion (Ø)	<4 cm	60%	36%	
	≥ 4 cm	40%	64%	0,016
Number of lesions	<10	50%	36%	
	10 to 50	34%	30%	
	>50	16%	34%	0,105
Stage of disease	IIIA	60%	30%	
	IIIAB	36%	46%	
	IV	4%	24%	0,002
CR rate		80%	58%	0,017
TTLP (median, months)		20	8	0,021
Survival (median, months)		45	21	0,066

additive effect of IFN be detected for TTLP (p=0.521) or for survival corrected for stage of disease (p=0.149).

Prior ILP: Prior ILP treatment, a typical indication for a repeat ILP with TNF, had no effect on CR-rate (p=0.601) or TTLP (p=0.312). The 26 patients who received multiple ILPs had a 5-year survival of 44% versus 28% for patients receiving a single treatment (p=0.059).

Tumour bulk: Because the experience with TNF-based ILP in soft tissue sarcomas clearly indicated that the addition of TNF induced impressive responses in large tumours in contrast to the experience with melphalan alone, we gradually changed our inclusion criteria for a TNF-based ILP and decided only to offer this treatment to patients with bulky disease or to patients that had failed previous perfusion(s). For

this reason, we retrospectively analysed the results of the first 50 ILPs versus the last 50. The tumour burden and stage of disease of the latter group differed significantly from the first (table 6). Both CR-rate (80% vs. 58%; p=0.017) and TTLP (20 months vs. 8 months; p=0.021) were significantly lower in the last 50 ILPs. Survival was also shorter in the latter group (45 vs. 21 months), but this difference just failed to reach statistical significance (p=0.066).

DISCUSSION

We report here on 100 TNF-based ILPs in melanoma patients out of a total of 350 TNF-based ILPs that we performed in our institution during the period from 1991 through July 2003. In patients with melanoma IT-mets, the procedure is associated with a close to 100% response rate in a patient population that has significantly shifted towards more bulky disease and pre-treatments over the years. This patient population is considerably less likely to respond to treatment than the patient population of "all patients that present with IT-mets", that we used to elect for a melphalan-only ILP in the past, even when IT-mets were limited in number and size. This single institution experience further underscores that in the expert setting, the procedure is very safe. TNF can be used in high doses without a single case of treatment-related mortality; there were no cases of grade 4 systemic toxicity except for a single case in which significant leakage occurred and leucocytes dropped below 1.0 for a single day. In such a setting, there is no need for other than standard cardiovascular monitoring peroperatively, no need for standard use of vasopressors, or for standardly having patients in the intensive care unit postoperatively [22].

The present series shows a CR rate of 69%, which is within the range of CR rates reported in previously published smaller series [6, 18, 23-25]. We identified stage of disease as a prognostic factor for response with the most prominent difference in IIIA versus IIIAB/IV patients, which is in line with the literature on melphalan-based ILP [16-19]. This probably reflects a difference in aggressiveness of melanoma biology in these patient categories, further exemplified by significant differences in survival. This hypothesis is sustained by the observation that during the course of melanoma progression, the ability of melanoma cells to express so-called death receptors diminishes [26]. TNF receptor and TNF-related Apoptosis Inducing Ligand (TRAIL), two examples of these receptors, play key roles in the acquired resistance of melanoma cells to undergo TNF-mediated apoptosis: whereas early-phase melanoma appears to utilize TNF pathways to undergo programmed cell death, late-phase melanoma does not [27].

Response on treatment therefore is an indicator of the tumour phenotype, which itself is of influence on patient survival.

Potentially it may also reflect a difference in immunocompetence between these patient categories that differ greatly because the absence of presence of lymph node metastases and/or visceral metastases. In our laboratory, we showed an attenuation of tumour response to ILP in leukocyte depleted rats [28], which corresponds well to the report on the role of an early and rapid invasion of tumour lesions after a TNF-based ILP by neutrophils [29]. Furthermore, we reported on the observation in tumour lesions in patients after ILP that eosinophils and macrophages ("melanophages") play an important role in the delayed-type reaction to TM-ILP [30].

Results of ILP treatment are often presented in overall response rates. Our prognostic factor analysis shows that especially the CR rate is of imminent importance, since it significantly affects both TTLP and survival. In fact, the marked difference in TTLP between CR and PR/NC patients identifies the period that the patient is without any need of locoregional treatment. We therefore specifically analysed the group of patients with IIIA/IIIAB disease and a PR after ILP, since these patients progress rapidly in the limb and therefore might need repeat locoregional treatment. Of these 23 patients, however, only 5 patients lived for more than 9 months without presenting distant metastases, and they received additional treatment (among which, 2 repeat ILPs). This indicates that response on ILP selects those patients with more aggressive nature of disease, which is sustained by the prognostic influence of response rate on overall survival, especially when corrected for stage of disease. The 42% 5-year survival rate in CR patients, which make up the large majority of patients (69%), is quite substantial and compares favourably with most commonly reported survival rates for this patient category (23-47%) [31]. This underscores the importance of the treatment and shows that it is a worthwhile intervention.

Patient selection

Since the introduction of TNF in the ILP setting, it has been discussed which patient subgroup would profit the most from addition of this cytokine [32]. The early experience of 4 European centers showed that the treatment of all patients with IT-mets, irrespective of number of tumours or size of the lesions, increased the complete response rate from 52% to 91% [9]. Yet the notion that the clearest benefit was seen in the patients population with bulky tumours that previously was known to be very poorly responsive to melphalan-based ILP was already obvious through our experiences in the multicenter trials in sarcoma patients since 1991, and it affected over the years the patient selection in our institution. We managed patients with

small tumours and a small number of tumours increasingly by repeated excisions and vaccine protocols and offered TNF-based ILP more and more exclusively to patients with a high tumour burden. In this patient category with highly unfavourable characteristics, analysed separately as 2nd 50 ILPs in our series, CR rate was still 58%. This is still superior to the response rate in the historical group of patients treated with melphalan alone without such unfavourable patient selection [9], and it is similar to the observations in the United States. In an interim analysis of a randomised trial by Fraker et al., TNF-based ILP showed to be of significant benefit in patients with a high tumour load, increasing the CR rate from 19% for melphalan-ILP to 58% in TM (+IFN)-ILP [33]. Moreover, Rossi et al. reported recently in a series of 20 melanoma patients with a high tumour burden a CR rate of 70% after TNF-based ILP [34]. We demonstrate in this series of patients that the median of TTLP of 16 months exceeds the median of TTSP of 14 months in the overall patient population. It underscores that systemic metastases present frequently in the stage IIIA and IIIAB populations and that an ILP should be reserved for patients who run out of simple management options such as excision of few lesions or participation in non-toxic vaccination protocols for (multiple) small lesions that do not present clear local morbidity. Those patients without local morbidity that still progress systemically can thus be spared the ILP intervention. Moreover, in case these patients respond to a vaccine, they most likely will do so also at systemic disease sites at the cost of little toxicity and no surgical intervention. This policy shift has occurred over the 12 year period described here and has led to a patient population with significantly more advanced disease locally in the second bracket of 50 ILPs (after 1996). It is important to realize that even in this patient population, the CR rate of 58% is still higher than the 52% CR rate in our prior experience with melphalan alone in patients with clearly less extensive disease [9].

A second indication for TM-ILP is failure after previous ILP-therapy [35]. In the present study, 26 ILPs were performed for recurrences in the limb after previous ILP treatment (13 ILPs after prior ILP elsewhere, 13 repeat TM-ILPs in our institution). The overall response rate of repeat ILP was 96% (CR 73%, PR 23%, NC 3%). This did not differ from the primary ILPs in our series and no increased toxicity was observed, as opposed to previous reports on the outcome of repeat melphalan-based ILP [36]. This observation underscores the efficacy of a TNF-based ILP in the repeat-ILP setting and thereby its indication.

Toxicity

Local toxicity after TNF-based ILP in our series was moderate to severe (grade III-IV) in 31 ILPs (31%), which compares with reported percentages in TNF or melphalan-

based ILP [37]. A reintervention by amputation or fasciotomy occurred in only two patients. Local toxicity is reported to be directly correlated with the incidence of long-term morbidity (tissue fibrosis, muscle atrophy and limb malfunction [38]). In our patient population however, the rate of limb function loss was markedly low, even in the 27 patients older than 75 years and in the 26 patients with multiple ILP treatments.

Systemic toxicity is directly correlated with leakage of the chemotherapeutics to the systemic circulation [8, 22, 39, 40]. In the present situation of leakage-free ILPs, the systemic toxicity is mostly limited to fever in the first 24 hours postoperatively, which can be easily avoided by the immediate postoperative application of indomethacin and/or paracetamol. Furthermore, the patients commonly show a period of 6 to 10 hours of a slightly elevated circulation due to a drop in peripheral resistance, which is compensated by a mild tachycardia and a small drop in blood pressure, easily managed by a generous intravenous fluid infusion policy, and does not require the standard use of vasopressors. The feared systemic adverse reaction of TNF - a systemic inflammatory response syndrome with a major drop in blood pressure that requires the administration of vasopressors - was not observed in any of our patients. Only in two patients, both with substantial leakage during the ILP, the blood pressure drop lead to the administration of vasopressors at a mild dose of 3-6µg/kg for a maximum duration of 36 hours. The absence of any major toxicity is due to a number of factors: first, adequate leakage control in virtually all patients; second, and this is of crucial importance, we have a policy of ample hydration of the patient during ILP and for the first 12-24 hours after ILP. This policy assures that all of our patients have adequate diuresis to keep the period of high circulating TNF levels post-ILP as short as possible [22]. Eight ILPs in our series had a leakage percentage above 10% (up to 32%), but in only one patient this lead to a systemic reaction by elevated liver enzymes, elevated urea and leucopenia, all easily managed with normal conservative measures. We do not use and do not advocate therefore the standard use of Swann-Ganz catheters, vasopressors or post-operative stay in the intensive care. In our experience, neither systemic nor local toxicity is significantly enhanced in TNF-based ILP compared with melphalan-based ILPs.

In conclusion, our results demonstrate the very high efficacy of TNF-based ILP in melanoma patients both in terms of local control of disease and of survival. Outcome is influenced by stage of disease, reflecting the aggressiveness of the melanoma. Complete response to ILP selects within each stage of disease those patients with relatively favourable characteristics. In our experience, the use of TNF increases the CR rate, especially in patients with high tumour burden and in those having failed

previous therapy. Local and systemic safety profile of the TNF-based ILP is so good that in the expert setting, a TNF-based ILP does not need a standard approach that differs from a melphalan-based ILP. The procedure should be considered in all cases of limb-threatening tumours or situations where simple surgical procedures to obtain local control fail. Currently, the procedure is the most efficacious one to obtain local control and achieve limb salvage in such conditions [41].

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CHAPTER 3

Efficacy of Repeat Isolated Limb Perfusions (ILP) with Tumour Necrosis Factor-α and Melphalan for Multiple In-Transit Metastases in Patients who failed prior ILPs

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ABSTRACT

Background

Isolated limb perfusion (ILP) is an effective treatment modality for multiple in-transit melanoma metastases confined to the limb. Recurrences after ILP, however, occur in approximately 50% of patients and are a challenge for further treatment. The efficacy of repeat ILPs to prolong local control in this patient category is evaluated in this article.

Methods

We used a prospective database in a tertiary referral center. Out of 100 ILPs with Tumour Necrosis Factor-α (TNF) and melphalan (TM-ILPs) in melanoma patients between March 1991 and July 2003, 25 repeat ILP procedures were performed in 21 patients in whom prior ILP treatment failed. All patients had bulky and/or numerous lesions and were treated with mild hyperthermic TM-ILP using 2 to 4 mg TNF and 10 to 13 mg/L of limb volume for the leg and arm, respectively.

Results

The complete response rate was 76%, a partial response occurred in 20% and no change was recorded in 4%. There was no difference in the complete response rate or local toxicity between first and repeat perfusions. Local recurrence occurred in 72%; the median time to local progression was 14 months. The five-year survival rate was 47%, which compares favourably with known survival rates of stage III A/AB patients. The median follow-up of the patients was 26 months.

Conclusions

Patients who experience treatment failure after previous ILP treatment respond very well to repeat perfusion, and prolonged local control can thus be obtained. The subgroup of patients qualifying for repeat ILP represents a relatively favourable biological behaviour of the melanoma.

INTRODUCTION

Recurrent melanoma of the limb remains a treatment challenge. Many options, from simple surgical excision to intralesional injections with cytokines to isolated limb perfusion (ILP), have been proposed. Depending on the size and number of the lesions, each modality is more or less suitable 1. ILP for melanoma has demonstrated to be very effective in patients with multiple metastases for which surgical excision is impossible. Melphalan-based ILP is associated with complete response (CR) rates of 40% to 50% and overall response rates of 75% to 80%. When Tumour Necrosis Factor-α (TNF) was introduced in the isolated limb perfusion model by Lejeune and Lienard in 1988 [2], CR rates of 80% to 90% were reported. The first report on the European multicenter experience with TNF-based ILP in melanoma patients revealed that a significant increase in CR rates was observed in the four participating centers: 91% with TNF and melphalan (TM)-ILPs compared with a 52% CR rate for melphalan-only-ILPs [3]. By this treatment, disease control in the limb can be obtained in patient categories with a poor prognosis quo ad vitam [4]. However, local progression in the limb after ILP with melphalan has been reported to develop in 46% to 54% of patients, and this rate was 55% in our own experience in 100 TM-ILPs, with predominantly high numbers of metastases, bulky disease, or both [5]. For this patient category, treatment options are limited. Some patients, however, qualify for re-treatment of the limb when treatment fails only locally without evidence of systemic metastases. Particularly for these patients repeat ILP might provide prolonged local control [6-8]. The use of TNF in the repeat ILP is hypothesized to improve response rates in patients who do not respond or respond insufficiently to melphalan-only-ILPs, but definitive prove is still lacking [7].

In this article, we report on 25 TM-ILPs for locally recurrent melanoma in patients who experienced treatment failure with previous ILP treatment, both with and without TNF. Special attention is given to local and systemic progression after repeat ILP and on the locoregional toxicity of the repeat procedure.

PATIENTS AND METHODS

Patients

Out of 100 TNF-based ILPs in 87 patients performed in our institution between 1991 and July 2003, 25 ILPs in 21 patients were repeat ILPs because of failure of previous ILP treatment for extensive in-transit melanoma metastases. Of these 25 ILPs, 12 were performed after failure of melphalan-based ILPs performed in referring hospitals, one was performed after a TM-ILP elsewhere, and 12 ILPs were second (n=10) or third (n=2) TNF-based perfusions performed in our own institution. Tumour stage was assessed according to the MD Anderson staging system: 7 stage IIIA patients (only in-transit metastases; no lymph node metastases or distant metastases), 15 IIIAB patients (both in-transit metastases and lymph node metastases, but no distant metastases) and 3 stage IV patients (IV: in-transit metastases and distant metastases). At the time of a second perfusion in our institution, progression of tumour stage had occurred in six patients. Local control of the in-transit recurrences by surgery was impossible due to numerous lesions (>10 lesions in 21 ILPs) and/or size of the lesions (>40 mm in 12 ILPs). Patient and tumour characteristics of the 25 repeat ILPs in 21 patients are listed in table 1.

Table 1

Patient and tumour characteristics of 21 patients undergoing 25 repeat TM-ILPs			
Sex	Female	15	
	Male	6	
Age (years), mean+range		60	(25-83)
Stage	IIIA	7	
	IIIAB	15	
	IV	3	
Stage shift	IIIA à IIIAB	4	
	IIIAB à IV	1	
	IIIA à IV	1	
No. of tumours	<10	4	
	10-50	9	
	>50	12	
Size of largest lesion (mm)	<40 mm	13	
	>40 mm	12	
Prior treatment (apart from ILP)	None	20	
	XRT	1	
	CT	2	
	Immuno	1	
	XRT + CT	1	

ILP = isolated limb perfusion, XRT = radiotherapy, CT = systemic chemotherapy, Immuno = immunotherapy

Treatment

All patients underwent an ILP of the lower extremity by an iliac (*n*=15), femoral (*n*=6) or popliteal (*n*=4) approach. The method of ILP has been described in detail previously [9,10]. In short, isolation of the limb is achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, ligation of collateral vessels and application of a tourniquet proximal to the site of perfusion. Once tissue temperature has reached 38°C, recombinant TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) is administered via the arterial line in a dose of 2-4 mg (mean 3.6 mg). Tissue temperatures are stabilized between 38°C and 39.5°C, and leakage monitoring is performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit [11]. After 30 minutes, melphalan (L-PAM, Alkeran, Burroughs Welcome, London, UK) is added to the perfusate in a dose of 10 mg/L for leg perfusions and 13 mg/L for arm perfusions. In six ILPs, performed between 1991 and 1994, interferon γ (IFN) was added to the schedule according to trial prescriptions, which consisted of the subcutaneous injection of 0.2 mg IFN on days –2 and –1 prior to the ILP and the injection of 0.2 mg IFN during the ILP procedure into the arterial line prior to the administration of TNF. The median dose of melphalan was 100 mg (mean 92.6, range 40-140), and all six IFN-ILPs were performed with 0.2 mg IFN. At the end of the perfusion period, a washout procedure using 2 to 4 L of a dextrane and/or electrolyte solution is performed. In patients undergoing an iliac perfusion, an iliac lymph node dissection is routinely performed. In patients with palpable nodal disease in the groin, an ilio-inguinal lymph node dissection is performed in the same operative session as the ILP: the iliac lymph node dissection prior to the ILP and the inguinal lymph node dissection immediately after the ILP.

Evaluation of response and toxicity

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [12]: (grade I) no reaction, (grade II) slight erythema or edema, (grade III) considerable erythema or edema with some blistering, slightly disturbed motility permissible, (grade IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance, and threatening or manifest compartmental syndrome, and (grade V) reaction that may necessitate amputation. Response evaluation was performed 4, 8 and 12 weeks after ILP by clinical examination, thereafter at regular 3-month intervals for the first 2 years and subsequently at longer intervals. Response rates were reported according to World Health Organisation criteria [13], in which CR is the complete disappearance of all lesions and no new areas of disease appearing within the field of ILP. Partial response (PR) was defined as a reduction of 50-99% of the total tumour size; no change (NC) was recorded if <50% of the

total tumour size responds. Recurrence of tumour within the extremity after a CR, or progression of the lesions and the appearance of new lesions after a PR or after NC, was reported as local progression.

Statistical evaluation

Estimates of survival and local/systemic progression were made using the Kaplan-Meier method and differences were evaluated using the log-rank test. P-values <0.05 were considered to be significant.

RESULTS

Of the 25 repeat ILPs performed in our institution, 19 (76%) resulted in a CR. With an additional 5 ILPs with a PR, total response rate was 96%. One patient (patient number 12) showed regression of the lesions of 20-25%, which was recorded as NC. There was no difference in the CR rate between repeat ILPs after prior treatment elsewhere (83%) and repeat TM-ILPs in our institution (69%; p=0.36). In the 12 patients who received multiple TM-ILPs in our institution, no difference in response rate was detected between first (70%) and second or third ILP (75%; p=0.58). Response rates for the different patient categories analysed are listed in table 2. Median time between first and repeat ILP was 25 months (range 3-76 months).

The median leakage of the repeat ILPs was 0% (range 0%-13%), leading to absence of severe (grade IV) systemic toxicity (table 3). Local toxicity was mild to moderate in all cases with Wieberdink grade I in 12%, grade II in 60% and grade III in 28% of the ILPs, and not a singe case of grade IV toxicity. No increase in local toxicity was observed in the patients receiving multiple TM-ILPs (table 4).

Table 2

Response Rates				
	Overall N=100	**Repeat** N=25	**Prior M-ILP** N=13	**TM-ILP repeats** N=12
CR	69/100=69%	19/25=76%	10/12=83%	9/13=69%
PR	26/100=26%	5/25=20%	2/12=17%	3/13=23%
NC	5/100=5%	1/25=4%		1/13=8%
Overall	94%	96%	100%	92%

M-ILP = melphalan-only isolated limb perfusion, TM-ILP = isolated limb perfusion with TNF and melphalan, CR = complete response, PR = partial response, NC = no change

Table 3: Local and systemic toxicity

Local toxicity					
Wieberdink	Grade I	Grade II	Grade III	Grade IV	Grade V
	12%	60%	28%		
Systemic toxicity					
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Neurologic*	96%	4%			
Liver*	100%	0%			
Renal*	100%	0%			
Haematologic*	96%	4%			
	<38°C	38-39°C	39-40°C	>40°C, <24 hrs	>40°C, >24 hrs
Temp	36%	24%	32%	8%	
	Absent	Present			
Shock&	100%	0%			

Hrs = hours, * = WHO criteria, & = support of vasopressors needed

Compared with our total database of TM-ILP for melanoma, consisting of 100 ILPs, CR rate of repeat perfusions did not differ significantly from the CR rate of patients undergoing only one ILP (76% vs. 68%, p=0.61). Local progression occurred after 18 repeat ILPs (72%). The median time to local progression (TTLP) was 14 months for repeat perfusions vs. 16 months for the overall population and 18 months for single ILPs (p=0.40; figure 1). Sixteen patients developed systemic metastases during the course of follow-up; 3 already had stage IV at time of repeat ILP. Time to systemic progression (TTSP) did not differ between patients receiving single (12 months) and multiple ILPs (15 months; p=0.27) [5]. In 3 patients (patients 1,2 and 5), repeat ILP (plus resection of a local recurrence on the lateral side of the proximal thigh in patient 1) contributed to sustained local control of disease in the leg. No systemic progression was observed in these patients during follow-up of 91, 129 and

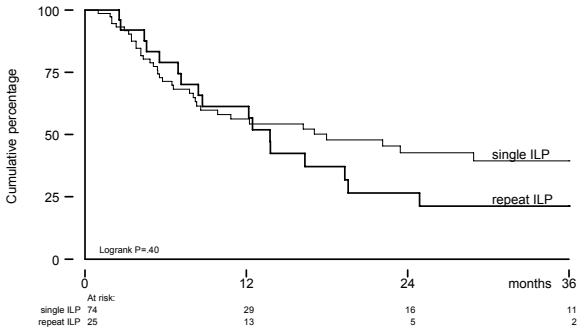

Figure 1: Time to local progression (TTLP) for repeat versus single isolated limb perfusions (ILPs).

X-axis: time in months counted from the last procedure

Y-axis: cumulative percentage

Table 4: Patient characteristics and treatment outcome

Pt no	Prior elsewhere	interval months	1st TM-ILP			interval	2nd TM-ILP			interval	3rdTM-ILP		
			level	resp	toxicity	months	level	resp	toxicity	months	level	resp	toxicity
1	yes	14	Fem	CR	2	25	Iliac	PR	2				
2	yes*	29	Poplit	CR	2								
3	no		Iliac	CR	3	11	Iliac	CR	2	26	Iliac	CR	2
4	no		Poplit	PR	2	19	Iliac	CR	3				
5	yes*	42	Fem	CR	2	18	Poplit	CR	2	42	Fem	CR	2
6	yes&	76	Fem	CR	3								
7	no		Iliac	CR	4	9	Fem	CR	3				
8	no		Iliac	CR	2	58	Poplit	PR	2				
9	yes	47	Iliac	CR	3								
10	yes	40	Iliac	CR	1								
11	no		Iliac	CR	1	9	Iliac	CR	2				
12	yes$	28	Iliac	NC	2								
13	yes	4	Poplit	CR	2								
14	yes#	28	Iliac	CR	1								
15	no		Iliac	CR	2	30	Iliac	CR	2				
16	no		Fem	PR	2	13	Iliac	PR	2				
17	yes	36	Iliac	CR	3								
18	yes	17	Iliac	PR	3								
19	no		Iliac	PR	3	15	Iliac	CR	3				
20	yes	5	Fem	CR	2								
21	yes[l]	3	Iliac	PR	1								

TM-ILP = isolated limb perfusion with TNF and melphalan, Toxicity = local toxicity (Wieberdink), Fem = femoral, Poplit = popliteal, Iliac = iliacal, CR = complete response, PR = partial response, NC = no change

* = previous ILP: M-ILP double schedule; & = adjuvant ILP; $ = prior TM-ILP; # = 2 previous ILPs: 1st adjuvant, 2nd cisplatin; [l] = 3 previous ILPs

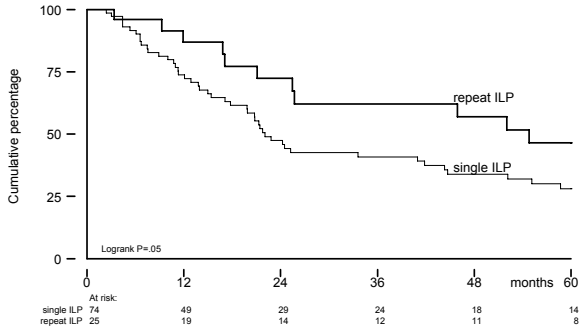

Figure 2: Overall survival for repeat versus single isolated limb perfusions (ILPs).
X-axis: time in months counted from the last procedure
Y-axis: cumulative percentage

72 months, respectively. The 21 patients who received multiple ILPs had a 5-year survival of 47%, compared with 28% for patients who received a single treatment (p=0.05; figure 2). The median follow-up was 26 months.

DISCUSSION

These results show that in a selected patient population of patients with local progression after previous ILP treatment, repeat TM-ILP is associated with excellent response rates (96%). The CR rate after TM-ILP was 76%, which compares favourably with the CR rate of single ILPs in our institution. This is in line with the scarce literature on the efficacy of repeat ILPs [6-8] and is indicative of the fact that a rather favourable group of patients, mostly patients with stage IIIA disease only and with a good response to the first ILP, are considered for repeat ILPs when they recur with in-transit metastases. The 5-year survival rate of 47% reported in this study compares favourably with recently published survival rates of patients with locally advanced melanoma (23%-47% [4]) and is again an indication of relatively mild biologic behaviour of the melanoma in this patient population.

The mechanism of recurrence after previous ILP treatment remains speculative. It is a common finding that in patients after a CR to the first perfusion, the new recurrent in-transit metastases arise at locations different from the original lesions that went into a CR after the initial ILP. Hypothetically this indicates that larger, well-vascularized lesions are completely eradicated but that dormant, (relatively) non-vascularized lesions are not eradicated by an ILP, because of inadequate drug exposure [14].

Patient selection for repeat ILP treatment is determined by the time to local progression after initial ILP and the absence of systemic metastases, unless the bulkiness of the disease is directly limb-threatening. Rapid disease progression after ILP is an indicator for aggressive disease and is therefore a relative contraindication for repeat ILP treatment, as the expected response rates are low [15]. The median interval between initial ILP treatment and repeat TM-ILP of 25 months, as indicated in table 4, is markedly longer than the median time to local progression both in this study after repeat ILP (14 months) and in our total database of 100 ILPs (16 months). In three patients (patients 13, 20 and 21) repeat TM-ILP was performed 3 to 5 months after failure of ILP with other chemotherapeutics in order to obtain sufficient response, and it resulted in 1 PR and 2 CRs. This shows that failure to respond to a melphalan-only ILP can be overcome by adding TNF, which in tumour models has been shown to enhance the uptake of melphalan by the tumour [16, 17]. This is in line with the observation that melphalan-only ILPs in soft tissue sarcoma patients are very poor, whereas TM-ILPs are associated with high response rates [3]. For our melanoma population we can conclude that, apart from the three patients with rapid failures after a melphalan-only ILP, the melanoma of patients qualifying for repeat treatment is relatively indolent. This observation is sustained by the significant difference in overall survival between repeat and single ILPs.

Whether to consider a patient for repeat ILP should be influenced by the stage of disease at first ILP. We found in our experience with 100 TM-ILPs that curves of TTLP and TTSP virtually overlap in stage IIIAB disease, whereas in patients with stage IIIA disease, the median TTSP was 55 months at a median TTLP of 18 months. This difference makes patients with stage IIIA disease with local progression much better candidates for repeat ILP than those with stage IIIAB disease [5].

We found toxicity after repeat TM-ILPs to be mild to moderate in all procedures. Compared with the first ILP, repeat TM-ILP did not increase local toxicity, in contrast to a previous report using melphalan alone [6]. In this report, however, more intensive schedules were used for the repeat ILP (higher tissue temperatures and ILPs at short intervals [6]. Other, smaller, studies do not report on increased local or systemic toxicity [7, 8]. On the basis of these data, we state that the use of mild hyperthermic TM-ILP for repeat perfusion is safe. The median leakage to the systemic compartment was 0% in these 25 repeat ILPs, just like in our overall experience with TM-ILP in over 350 patients. TM-ILP is a safe procedure, provided that adequate leakage monitoring is ensured and postoperative fluid management is generous to deal adequately with the TNF-mediated transient decrease in the peripheral vascular resistance after ILP [18, 19]. Moreover, the safety of the procedure is such that, in our opinion, there

should be no age limit. Especially in limb-threatening disease, limb salvage is of prime importance and this treatment should not be withheld from the elderly as we have demonstrated in our extensive experience in patients >75 years old [20].

In conclusion, repeat TM-ILP is a valuable treatment option in patients with local progression after previous ILP treatment. In patients with bulky or numerous lesions that cannot be managed with surgery, repeat TM-ILP provides excellent disease control. Especially patients with recurrences after ILP for stage IIIA disease and patients with a long interval between initial and repeat ILP, who constitute a subcategory of melanoma patients with relatively favourable characteristics, profit from this treatment. Moreover, patients with no response or rapid progression after a melphalan-only ILP can still respond with a CR to a TM-ILP.

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CHAPTER 4

Isolated Limb Perfusion for Melanoma Patients: a review of its indications and the role of Tumour Necrosis Factor-α

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ABSTRACT

Background

The treatment of melanoma in-transit metastases (IT-mets) can vary widely and is dependent on the size and the number of the lesions. When multiple, large lesions exist, Isolated Limb Perfusion (ILP) has established itself as an attractive treatment option with high response rates.

Methods

Review on the various methods of treatment of melanoma in-transit metastases, with a focus on Isolated Limb Perfusion. A Medline based literature search was performed for articles relating to this topic. Additional original papers were obtained from citations in those identified by the initial search. Indications and results are discussed and the extra value of Tumour Necrosis Factor (TNF) is evaluated.

Results

ILP with melphalan results in complete response rates of 40% to 82% and showed to be 54% in a large retrospective meta-analysis. The addition of TNF can improve these completes response rates (59%-85%) and although no data from randomised controlled trials are available, it seems of particular value in large, bulky lesions or in patients with recurrent disease after previous ILP.

Conclusions

TNF-based ILP has earned a permanent place in the treatment of patients with melanoma IT-mets. In patients with a high tumour burden, TNF-based ILP is the most efficacious procedure to obtain local control and to achieve limb salvage.

INTRODUCTION

Melanoma is the cancer type with the largest increase in incidence over the last decade, with estimated new cases in the USA increasing from <45.000 in 1999 [1] to nearly 60.000 in 2005 [2]. In The Netherlands, a recent calculation of the predicted incidence of melanoma reveals an increase of 99% from 2000 to 2015 [3]. The prognosis of melanoma patients varies widely and depends on the stage of disease [4]. In-transit metastases (IT-mets) of melanoma occur in 5-8% of high-risk patients and are categorised as stage IIIB or stage IIIC disease according to the most recent staging system of the American Joint Committee on Cancer (AJCC) [5]. IT-mets are cutaneous or subcutaneous deposits of melanoma between the primary melanoma site and the regional lymph node basin occurring in the course of lymphatic drainage. They reflect a disseminated stage of disease with corresponding 5-year survival rates of 25-30%, depending on associated lymph node metastases [4]. Besides the unfavourable prognosis, the occurrence of IT-mets forms a great discomfort for the patients, especially if numerous large lesions exist with ulceration of the tumours. The treatment of in-transit metastases of melanoma is therefore a challenge and is mainly dictated by the biology of the tumours in terms of size and number.

TREATMENT OPTIONS

Surgical excisions of IT-mets is chosen when size and number permits this approach. It must be realized that amputation is seldom if ever indicated and does not improve survival. [6]. Surgery should be radical, but no excisional margin is proven beneficial for these metastases, contrary to primary melanoma. The possibility of repeated excisions is completely dictated by the location, the size and for practical reasons the number of the lesions.

When numerous small lesions exist, local ablation by carbon dioxide laser therapy can be useful as it minimizes the injury of surrounding tissue and permits to treat multiple lesions in one session. Drawbacks are healing by secondary intention that can be lengthy and painful. Although initial results were reported to be adequate [7], the recurrence rate showed to be very high (7/15 patients with a local recurrence, disease-free interval 2-7 weeks) in another report [8]. This, together with the inability of the technique to treat lesions >1 cm in diameter, has limited the use of CO2 laser ablation to a very specific patient population.

Various other topical therapeutic options for IT-mets are studied over the last decades. Bacille Calmette-Guérin (BCG), Corynebacterium parvum, interferon (IFN) and interleukin-2 (IL-2) all were used to augment the autoimmune response, known to be existent in melanoma. Although a measurable antibody response could usually be achieved, this did not lead to satisfactory response rates. As multiple therapeutic sessions are needed and the injections are not free of toxicity, the use of intralesional therapy is largely abandoned and replaced by alternatives such as isolated limb perfusion. Truncal lesions however can be treated with one of these modalities. At present, electrochemotherapy (the use of bleomycin in combination with the application of electric current over cutaneous or subcutaneous lesions) has produced some promising results [9-11], but is not considered standard therapy.

Immunotherapy through vaccination is based on the same premise of augmenting the immune response to melanoma cells. Patients with IT-mets are attractive for these experimental protocols as the tumour material needed for vaccine development is easily accruable and subsequent biopsies for monitoring the vaccination effects can be taken from the residual lesions. Results of vaccination protocols however are up to know insufficient [12-14] and therefore vaccination is strictly experimental.

Although melanoma cells are relatively resistant to radiation, radiotherapy can be a useful treatment modality for obtaining local control in cutaneous melanoma at sites that would otherwise require complex surgical procedures. In gross disease in a relatively small total area, radiotherapy has proven to be effective in providing local control (in approximately 50% [15, 16]), which might even be increased by adding hyperthermia in centres with the expertise and equipment [17].

Systemic chemotherapy for metastatic melanoma has shown to be very disappointing due to the resistance of the tumour to cytotoxic drugs. Currently, no treatment schedule has proven to be superior in a randomised trial to the currently used dacarbazine (DTIC), especially in terms of local control combined with acceptable toxicity, which should be the aim of therapy in patients with melanoma IT-mets. As DTIC provides response rates of <20% with a median duration of response of 5-6 months [18], it should not be used for treatment of melanoma patients other than AJCC stage IV. Intra-arterial infusions of chemotherapeutic agents have been tried in order to achieve acceptable results without the complex setting of isolated limb perfusion. The initial results were rather disappointing with an overall response rate of 37% [19], but could be improved when a tourniquet was used for better occlusion [20].

ISOLATED LIMB PERFUSION

The technique of Isolated Limb Perfusion was pioneered by Creech and Krementz at Tulane University in New Orleans in 1958 [21] in order to achieve regional concentrations of chemotherapeutic agents that are 15 to 25 times higher than can be reached with systemic administration, but without the systemic side-effects [22]. Isolation of the blood circulation of the limb is achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, ligation of collateral vessels and application of a tourniquet proximal to the site of perfusion. The drugs can be introduced to the perfusion circuit once this isolation is secured. Melphalan (L-phenylalanine mustard) is the standard drug for reasons of its favourable local toxicity profile and its efficacy. Melphalan is commonly used in doses of 10 mg/L perfused tissue for a leg and 13 mg/L for an arm [23]. Tissue temperatures are stabilized and leakage monitoring is performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit [24]. Leakage monitoring is mandatory, especially when Tumour Necrosis Factor-alpha (TNF) is used in high doses. At the end of the 1 to 1.5 hour perfusion period, a washout procedure using an electrolyte solution is performed. In patients undergoing an iliac or axillary perfusion, the corresponding lymph node basin is dissected. In patients with palpable nodal disease in the groin an ilio-inguinal lymph node dissection is performed in the same operative session as the ILP but prior to executing the ILP. Response of the tumours on ILP is reported according to WHO criteria [25], in which complete response (CR) is the complete disappearance of all lesions and no new areas of disease appearing within the field of ILP. Partial response (PR) is defined as a reduction of 50-99% of the total tumour size; no change (NC) is recorded if <50% of the total tumour size responds. Acute local toxicity of the ILP procedure is classified according to Wieberdink et al. [26]: (I) no reaction, (II) slight erythema or edema, (III) considerable erythema or edema with some blistering, slightly disturbed motility permissible, (IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance, and threatening or manifest compartmental syndrome, and (V) reaction that may necessitate amputation.

ISOLATED LIMB PERFUSION FOR MELANOMA

Since the introduction of ILP almost half a century ago, many modifications have been applied in order to improve tumour response on ILP. These modifications have led to an improved insight in the optimal temperature, the optimal drugs and the optimal indications for the procedure.

Hyperthermia

Temperature of the perfused tissue is important in more than one way: the temperature of the skin has to be warmed during perfusion in order to prevent vasoconstriction in the cutis and subcutis. Especially in superficial IT-mets, application of a warm water mattress can improve the local drug delivery as the uptake of the drug by in-transit metastases in vivo has proven to be two times higher at 39.5°C than at 37°C [27]. The second reason for hyperthermia is the idiosyncratic sensitivity of tumour cells to heat. Moreover, hyperthermia can improve the uptake of the drug in tumour cells, especially at temperatures >41°C [28, 29]. Normothermic ILP-schedules, known to lead to an overall response rate of 65% [30] (table 1), are now largely abandoned for the

Table 1: The efficacy of melphalan-based ILPs

Efficacy of Melphalan-ILP								
ILP strategy	**Procedures**	**Results**				**Reference**		
	N	**CR%**	**PR%**	**OR%**	**Med mts**	**Year**	**Author**	**#**
Normothermia								
37-38°C	58	41	24	65	6	1994	Klaase	30
Mild hyperthermia								
39-40°C	23	65	26	91	ns	1983	Lejeune	37
	22	82	18	100	ns	1985	Minor	38
	67	ns	ns	78	ns	1990	Skene	39
	35	60	34	94	ns	1990	Kettelhack	40
	103	52	25	77	14	1995	Lejeune	41
	103	76	23	99	10	1996	Lingam	42
Borderline true hyperthermia								
40-41°C	32	56	25	81	ns	1985	Vaglini	33
	26	81	0	81	ns	1985	Storm	34
	85	40	42	82	ns	1995	Bryant	35
	105	73	13	86	10	1997	Thompson	36
True hyperthermia								
41.5-43°C	11	64	27	91	>6	1992	Kroon	32
	128	46	40	86	ns	1998	Di Filippo	31
Isolated limb infusion (ILI)								
37-38°C	128	41	44	85	16	2002	Lindner	49
Double perfusion schedules								
Double ILP, NT	42	76	14	90	5	1993	Klaase	52
Double ILP, THT	17	65	29	94	ns	2003	Noorda	53
Double ILI	47	41	47	88	18	2004	Lindner	51

ILP = isolated limb perfusion, CR = complete response, PR = partial response, OR = overall response (= CR+PR), Med mts = median duration of response in months, ns = not stated, ILI = isolated limb infusion, NT = normothermia, HT = hyperthermia

above-mentioned reasons. However, hyperthermia is associated with increased local toxicity. Tissue temperatures of 41.5-43°C during ILP can yield high response rates [31], but the local toxicity of these procedures led to major complications and even amputation of the perfused limb [32] (table 1). The use of true hyperthermia should therefore be avoided. The range from 39-41°C can be divided in mild hyperthermia (39-40°C) and borderline true hyperthermia (40-41°C). The latter is associated with high overall response rates of about 80%, but also with significant toxicity [33-36] (table 1). These observations make that mild hyperthermia for ILP is used as a compromise between response and toxicity [37-42] (table 1).

Failure of prophylactic ILP and ILP with other chemotherapeutics

The efficacy of melphalan ILP in eliminating melanoma IT-mets in the limb combined with the concept that melanoma IT-mets commonly arise from primarily clinically undetectable lymphogenic tumour deposits present at the time of primary treatment, give rise to the premise that prophylactic ILP of high-risk melanoma patients can prevent locoregional recurrence in the limb and might even influence survival. The first retrospective studies on this subject indeed showed improved outcome of patients with a high-risk primary melanoma after ILP treatment [43]. Although several retrospective studies have since confirmed these results, it was not until 1998 that the shortcoming of retrospective studies could be clearly demonstrated in the first well-designed prospective randomised trial on prophylactic ILP. This large, multicenter trial with 832 evaluable patients and a median follow-up of 6.4 years showed that although a small effect on locoregional recurrence could be observed (reduction of IT-mets development from 6.6 to 3.3%, reduction of regional lymph node metastases from 16.7 to 12.6%), there was no benefit on time to systemic metastases or on survival. As the procedure was accompanied by considerable morbidity and costs, the authors conclude that prophylactic ILP cannot be recommended as an adjunct to standard surgical excision in high-risk primary melanoma patients [44].

The same discussion can be held upon the issue of prophylactic ILP after excision of symptomatic IT-mets. Theoretically, more benefit of a prophylactic procedure can be expected because of the fact that in these patients, the melanoma has already shown a propensity to develop locoregional recurrences. Although retrospective studies point out in the direction of improved locoregional control and even survival, the single randomised phase III trial on this subject with 69 patients and a mean follow-up of 39 months showed no impact on overall 5-year survival of prophylactic ILP (44% vs. 39% for surgical excision only), despite a slightly improved disease-free interval. For the same reasons as mentioned above, the authors of this study

conclude that routine use of prophylactic ILP in melanoma patients with IT-mets is not to be recommended [45].

Several attempts have been made to improve the response on ILP by using other cytostatic drugs than melphalan. Commonly used drugs in the treatment of systemically metastasised melanoma, either alone or in a combination schedule, are dacarbazine and cisplatinum. Therefore, amongst others, these drugs were tested in the ILP-setting but no drug or drug combination used for melanoma patients has proven to achieve results superior to melphalan [23, 46].

Technical modifications: Isolated Limb Infusion and double perfusion schedules

Isolated Limb Infusion (ILI) was designed by Thompson et al. in 1994 as a simplification of the ILP procedure [47]. The procedure is comparable with ILP, but the catheters are placed percutaneously via the contralateral groin using the common Seldinger technique under radiological imaging. The catheter tips are placed (for ILI of the lower limb) in the popliteal artery and vein just proximal to the knee, which at the same time is one of the main disadvantages of ILI: only lesions at the distal two thirds of the limb are effectively treated in contrast to ILP that can treat lesions all the way up to the groin. After general anaesthesia and systemic heparinisation, a pneumatic tourniquet is positioned at the desired level and temperature probes are placed. ILI is currently performed with melphalan (5-10 mg/L limb tissue) and actinomycin D (50-100 µg/L limb tissue) added to 400ml of heparinised saline that has been warmed to 40°C. The chemotherapeutics are infused into the isolated limb via the arterial catheter and subsequently continuously circulated by withdrawing blood from the venous catheter and re-infuse it with a syringe arterially. A blood warmer is incorporated in the circuit to warm the infusate to 41°C before it is re-infused. This procedure is repeated for 30 minutes, after which the limb is flushed with saline, the tourniquet deflated and the catheters removed. Reported response rates of ILI are comparable to ILP with melphalan only [48]. In a prospective study of 128 patients, ILI showed to be able to achieve a CR-rate of 41% and a PR-rate of 44% [49] (table 1). Thus, ILI is a minimally invasive procedure with less complex precaution measures in the operation room than ILP and with significant cost-reduction. It is effective, easily repeatable and more widely applicable than ILP and can therefore be the standard treatment for a certain high-risk patient population with distal IT-mets [50].

Another modification of the ILP-procedure has been the use of a double perfusion schedule. The premise that repeated administration of chemotherapeutic agents is more effective than single use, is adopted from the systemic chemotherapy situation and is based on the idea that residual tumour cells after the first treatment may be

eliminated and that partially damaged tumour cell after first exposure may be more vulnerable to chemotherapy on repeated exposures. Double perfusion schedules were tested both for ILI and ILP. In ILI, the double infusion protocol (median interval between the treatments 4.2 weeks) did not improve CR-rate, OR-rate or duration of response [51]. In the ILP situation, the double perfusion schedule (interval 3-4 weeks, 2nd perfusion with reduced melphalan dose, normothermic ILP) led to higher CR-rates than single-ILP [52], but the median duration of response was not altered. Fractioning of melphalan administration and the use of true hyperthermia have been used in a double perfusion schedule where a true hyperthermic perfusion with no chemotherapeutic agents was followed by a normothermic melphalan-ILP one week later. The achieved response rates with this protocol are high, probably due to the synergistic effect of hyperthermia and melphalan, whereas due to the fractioning, no increased toxicity was observed compared to single ILP [53].

TUMOUR NECROSIS FACTOR-ALPHA

Probably the most influential adjustment of ILP has been the introduction of Tumour Necrosis Factor-alpha (TNF) by Lejeune and Lienard in 1988. TNF was isolated as an endogenous factor, especially active in inflammation, with necrotising ability on tumour cells [54]. Later studies revealed that TNF has a dual mechanism of action: the direct cytotoxic impact of high-dose TNF to tumour cells certainly plays a role in anti-tumour activity [55], but more importantly the TNF effect on the so called tumour associated vasculature induces a rapid change in tumour morphology characterised by haemorrhagic necrosis [56]. These observations led to high expectations on this cytokine in anti-tumour therapy, but systemic application, amongst others in melanoma patients [57], was very disappointing. TNF turned out to be a potent mediator in septic shock and therefore the systemic side-effects (acute drop in vascular resistance leading to low blood pressure rates, fever etc.) were the major factors hindering systemic application of this cytokine [58]. The maximum tolerated dose of TNF in humans turned out to be 10 to 50 times lower than needed for anti-tumour effects in murine models [59], so that systemic, but also intralesional administration of TNF was not clinically applicable. The concept of ILP combines the advantages of the TNF anti-tumour activity with the avoidance of systemic effects. Moreover, the cytotoxic effects of TNF are known to be enhanced in hyperthermic conditions [60] and with the addition of alkylating chemotherapeutics [61], both prerequisites already existing in the ILP model. The re-introduction of TNF in anti-cancer therapy by application in the ILP protocol combined optimal activity with minimal toxicity [62].

TNF as a synergetic mediator in ILP

The mechanism of action of TNF in ILP has extensively been studied in a pre-clinical rat model, mainly in a sarcoma bearing rat model as used in our laboratory [63], but recently also in a rat model with human melanoma xenograft [64]. A very small direct cytotoxic effect of TNF was observed in osteosarcoma bearing rats [65], but far more important are the indirect effects of TNF. It was demonstrated that addition of high-dose TNF to the perfusate results in a 4 to 6 fold increase in uptake of the cytostatic drug by tumour cells. This increased uptake was tumour-specific (no increased uptake in normal tissue), thus emphasizing the selective action of TNF on the tumour-associated vasculature [66, 67]. Moreover, the TNF-effect on the tumour vasculature induced microvascular damage by loss of endothelial cohesion [68] leading to haemorrhagic necrosis. This might result in a delayed effect of TNF to tumour regression [69]. Speculations exist on a third role of TNF in its synergistic working mechanism: exposure to TNF might lead to an immediate drop in interstitial pressure in the tumour [70].

Important lessons from the laboratory are that the temperature of the perfusate, analogous to the situation in melphalan-only ILPs, is of imminent importance. We demonstrated that true hyperthermia (42-43°C), though resulting in slightly improved response rates, was associated with very severe damage to the normal tissues, requiring amputation of the limbs. ILP at room temperature resulted in complete loss of anti-tumour activity. Therefore, mild hyperthermia seems essential in TNF-based ILP (TM-ILP) for obtaining optimal response with minimal toxicity [71]. Although hypoxia and acidosis are known to increase the response rates in melphalan-only ILPs [64], we could not demonstrate a beneficial effect of hypoxia in TM-ILP [71].

The dose of TNF in ILP to exert its optimal effect was studied in the rat model. In a de-escalation study, it was shown that a fivefold reduction of the standard used 50 µg to 10 µg did not alter the response rates significantly. Further reduction to 2 µg, however, resulted in complete loss of additional benefit of TNF over melphalan alone [71]. Both in vitro and in vivo studies report of a synergistic activity of Interferon-γ (IFN) on TNF [72, 73] by increasing the number of TNF-receptors on malignant cells. In our rat ILP model, response rates were only slightly better (IFN added to TM-ILP resulted in an increase in CR rate of 23% and in Overall Response of 16%, results not significant), but at the cost of increased toxicity [74]. Both the question of TNF dose and IFN addition were tested in the clinical situation and discussed later.

Table 2: The efficacy of TNF-based ILPs

Efficacy of TNF and melphalan (+interferon) ILP								
ILP strategy	**Procedures**	**Results**				**Reference**		
	N	**CR%**	**PR%**	**OR%**	**Med mts**	**Year**	**Author**	**#**
Mild hyperthermia								
39-40°C	19	89	11	100	>8	1992	Lienard	[62]
	44	90	10	100	>18	1993	Lejeune	[75]
	11	64	0	64	ns	1994	Vaglini	[76]
	58	88	12	100	26	1995	Eggermont	[77]
	26	76	16	92	ns	1996	Fraker	[78]
	64	73	22	95	14	1999	Lienard	[79]
*	20	70	25	100	10	2004	Rossi	[80]
	90	59	ns	ns	16	2004	Noorda	[81]
	100	69	26	95	16	2004	Grünhagen	[82]

TNF = tumour necrosis factor, ILP = isolated limb perfusion, CR = complete response, PR = partial response, OR = overall response (= CR+PR), Med mts = median duration of response in months, ns = not stated

*: low-dose TNF: 1 mg

CLINICAL APPLICATION OF TNF-BASED ILP

After the initial report of the clinical application of TNF setting by Lienard [62], many studies have been performed that all report excellent response rates of melanoma IT-mets on TM-ILP [62, 75-82] (table 2). These results seem far better than the results obtained with melphalan-only ILP, as the response rates of these studies are all superior to the response on melphalan-ILP, which showed to be around 54% in a large meta-analysis of retrospective series [46]. The early experience of 4 European centres showed that addition of TNF to the ILP schedule in patients with IT-mets, irrespective of number of tumours or size of the lesions, increased the complete response rate to 91% [83]. Recently, the American College of Surgeons Oncology Group (ACOSOG) randomised phase III trial of ILP with melphalan, with or without TNF, in patients with localised advanced extremity melanoma was prematurely closed to patient accrual as an interim analysis showed that response on TM-ILP was not significantly better than the response achieved the melphalan alone arm. The only reported results of this trial from an earlier interim-analysis revealed that although overall no beneficial effect of TNF could be established, in a subgroup of patients with bulky melanoma the CR rate on TM-ILP was 58% compared with 19% for melphalan-only ILPs [84]. These observations were recently sustained by Rossi et al. who found a 70% CR-rate (and 25% PR) in a homogeneous group of bulky melanoma patients treated with (low TNF dose) TM-ILP [80]. The tumour bulk of the melanoma IT-mets can indeed be crucial

in appraising the TNF effect, as these large, sarcoma-like lesions benefit the most from the destruction of tumour associated vasculature by TNF. The inhomogeneous drug uptake of Soft Tissue Sarcomas was once the reason for abandoning melphalan-ILP as a treatment option for these irresectable tumours [85]. The application of TNF changed this situation dramatically as very large tumours were now observed to respond very well [86], which led to successful multicenter trials in Europe and the approval of TNF-α for the treatment of irresectable extremity soft tissue sarcomas [87]. Unfortunately, TNF is not clinically available in North America because the patent and licensing rights are in the hand of a different company and thus the registration file has not been presented to the FDA. Based on the presently available evidence, we recommend the use of TNF in ILP for bulky melanoma IT-mets [82].

The role of IFN and the optimal TNF dose

Most of the TM-ILP studies include at least a number of patients in whom IFN was added to the perfusion schedule. It consists of the subcutaneous injection of 0,2 mg IFN on days –2 and –1 prior to the ILP and the injection of 0.2 mg IFN during the ILP procedure into the arterial line prior to the administration of TNF and is based on the presumed synergy between TNF and IFN. Its role has been evaluated in a randomised phase II trial that showed only a marginal improvement of outcome for the combination with TNF [79], which is in line with the above-mentioned preclinical studies.

The optimal TNF dose in ILP for the synergistic effect with melphalan has never been properly determined. However, both preclinical [71] and clinical studies [80, 88, 89] suggest that TNF dose reduction to 1 mg might be as effective as the now standard used TNF dose of 3 mg for arm- and 4 mg for leg-ILPs. We recently performed a retrospective, non-randomised study of 64 ILPs performed with reduced-dose TNF (16 melanoma ILPs) demonstrating no effect of TNF dose reduction on response, disease free survival or overall survival [90]. This suggests that the presently standard TNF dose might be higher than necessary to achieve maximum synergy between TNF and melphalan.

Prognosis after TM-ILP

Although TM-ILP can achieve excellent response rates in patients with melanoma IT-mets, the nature of the disease determines that the patients in this very unfavourable population often experience local recurrence of disease in the limb. Reported recurrence rates after ILP are around 50% [36, 82, 91, 92] and as mentioned in table 2, occur after a median time of 1-1.5 years after ILP. The prognostic factor for local recurrence in the limb is the total number of IT-mets present at the time of ILP [82]. The 5 years

survival of patients after TM-ILP was 32% in our series and was only influenced by stage of disease and age of the patient [82]. An important observation is that after an initial CR to ILP, melanoma recurrences were usually not at the site of tumour deposits that had been clinically apparent at the time of ILP but at other sites in the same limb [36]. This indicates that ILP is effective in tumour deposits large enough to have own tumour associated vasculature, but fails to be active against single tumour cell deposits. This might be the explanation for the failure of prophylactic ILP and the high recurrence rate after therapeutic ILP and gives support to the anti-vascular tumour activity of TNF. The management of limb recurrences after ILP is essentially the same as for IT-mets in general: local excision if technically feasible, but repeat ILP in extensive disease [92]. Response rates of repeat ILP are comparable to those achieved after single perfusion [93-95] and thus, repeat ILP can provide prolonged local control in this patient category. We recently found that the 5-year survival rate after repeat ILP in our institution is 47% [95], which compares favourably to known survival percentages of patients with melanoma IT-mets [5] and might be indicative of a relatively favourable biologic behaviour of the melanoma in patients that qualify for a repeat ILP procedure. A repeat ILP procedure can thus be worthwhile and is indicated both for recurrences after previous TM-ILP and after failure of ILP with other chemotherapeutics [94].

Although we know that the prognosis for melanoma patients with stage IV disease (systemic metastases present) is inevitably fatal, usually within one year, some patients have such disabling local disease in the limb that ILP can be considered. The response percentages of ILP in stage IV disease are significantly lower than generally achieved with TM-ILP [79, 82, 91], presumably due to the aggressive biologic behaviour of the tumour in these disseminated patients. Nevertheless, local control of the limb is the goal of treatment in these patients during the short remaining life period. This can be provided by TM-ILP, which makes this procedure a valuable palliative treatment option in stage IV melanoma patients [96, 97].

TM-ILP is a safe procedure

Whether TNF increases local toxicity compared to a melphalan-only ILP is still under debate. Initial reports explicitly state that no increased local toxicity was observed in a triple drug (TNF, melphalan, IFN) regimen [62, 98]. However, in a TNF dose escalation study with TNF doses up to 6 mg, Fraker et al. report that local toxicity was significantly related with TNF dose [78]. The authors speculate that the reason for this observation is the increased uptake of melphalan in the tissue, as experimental ILPs with TNF only, did not reveal local toxicity at all. Melphalan dose (total dose, peak concentration, concentration at equilibrium and area under curve) is a major

determinant of acute local toxicity [99, 100]. The one study specifically addressing the local toxicity of TM-ILP, showed a significant increase of local toxicity with TNF compared to melphalan-only ILPs [101]. The confounding factor here however was that the patients in that study participating in the TNF-ILP protocol were, due to trial prescriptions, exposed to higher temperatures during melphalan circulation. This difference might be partly the explanation of the observed difference in toxicity. Minimizing local toxicity is essential as it has shown to be directly correlated with the incidence of long-term morbidity (tissue fibrosis, muscle atrophy and limb malfunction) [102]. At present, TM-ILP is performed under mild hyperthermic conditions with gradual administration of melphalan instead of bolus injection. No difference in acute local toxicity, nor in long-term morbidity exist between TM-ILP and melphalan-only perfusions [81].

Systemic toxicity is directly correlated with leakage of the chemotherapeutics to the systemic circulation [87, 103-105]. In the present situation of leakage-free ILPs, the systemic toxicity is mostly limited to fever in the first 24 hours postoperatively, which can be easily avoided by the immediate postoperative application of indomethacin and / or paracetamol. Furthermore the patients commonly show a period of 6-10 hours of a slightly elevated circulation due to a drop in peripheral resistance, which is compensated by a mild tachycardia and a small drop in blood pressure. Essential in the management of this circulatory change is a policy of ample hydration of the patient during ILP and for the first 12-24 hours after ILP. This policy assures to have adequate diuresis in all patients in order to keep the period of high circulating TNF levels post-ILP as short as possible [103]. Standard use of vasopressors is not recommended. A further reduction of systemic toxicity by reducing the TNF dose could not be demonstrated [90].

TM-ILP is a safe procedure, both in terms of local and systemic side effects. It can be easily performed in elderly people [106, 107] and can be repeated if necessary without increasing toxicity [94, 95].

CONCLUSION

TNF-based ILP has earned a permanent place in the treatment of patients with melanoma IT-mets, albeit for a selected patient population. Patients with only few, small IT-mets should be treated locally: radical surgery if feasible, carbon dioxide laser therapy if necessary. ILP with melphalan has proven to be effective in patients with multiple IT-mets. If lesions are small and it is not likely that tumour-associated

vasculature has been formed, the additional effect of TNF is on theoretical ground negligible. In these cases, especially if lesions are located on the distal two-thirds of the limb, ILI is a very good alternative. In patients with high tumour burden however, TM-ILP seems superior to any other technique. Although a costly procedure, it can be performed safely and leads to excellent response rates in patients with limb-threatening and socially disabling disease. At present, TM-ILP is the most efficacious procedure to obtain local control and achieve limb salvage in this patient category [108].

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PART II

ILP for Soft Tissue Sarcoma Patients

CHAPTER 5

Outcome and Prognostic Factor Analysis of 217 Consecutive Isolated Limb Perfusions with Tumour Necrosis Factor-α and Melphalan for Limb-threatening Soft Tissue Sarcoma

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ABSTRACT

Background

Extensive and mutilating surgery is often required for locally advanced Soft Tissue Sarcoma (STS) of the limb. As it has become apparent that amputation for STS does not improve survival rates, the interest in limb-preserving approaches has increased. Isolated Limb Perfusion (ILP) with TNF and melphalan is successful in providing local tumour control and enables limb-preserving surgery in a majority of cases. Here we report on the mature largest single-institution experience with 217 consecutive ILPs for STS of the extremity.

Methods

Prospectively maintained database at a tertiary referral center. From July 1991 – July 2003, 217 ILPs were performed in 197 patients with locally advanced STS of the extremity. ILPs were performed at mild hyperthermic conditions with 1 to 4 mg of TNF and 10 to 13 mg/L limb-volume melphalan for leg and arm perfusions, respectively.

Results

Overall response rate was 75%. Limb salvage was achieved in 87% of the perfused limbs. Median survival post-ILP was 57 months and prognostic factors for survival were Trojani grade of the tumour and ILP for single versus multiple STS. The procedure could be performed safely with a peri-operative mortality of 0.5% in all patients with no age-limit (median age 54 years, range 12-91 years). Systemic and locoregional toxicity were modest and easily manageable.

Conclusions

TNF+M-based ILP can provide limb salvage in a significant percentage of patients with locally advanced STS and has therefore gained a permanent place in the multimodality treatment of STS.

INTRODUCTION

In the United States, 8680 new cases of Soft Tissue Sarcomas (STS) are diagnosed annually, and approximately 60% of these tumours occur in the extremity [1]. Although the propensity of the tumour to develop systemic metastases is the primary cause of the high disease-specific mortality rate (up to 50%), the extremity tumours are often large at time of presentation, causing a local management problem [1]. The local situation in the limb may require extensive and mutilating surgery (±radiotherapy), which can cause severe disability of the limb, while in some 10% of cases amputation may be inevitable. With the emergence of evidence that amputative surgery does not improve survival [2-4], the tendency to perform more and more limb-preserving surgery has led to the exploration of Isolated Limb Perfusion (ILP) as a procedure enabling limb salvage at time of surgery in cases of primarily unresectable STS.

ILP was fist described by Creech et al in 1958 [5] and is since then primarily used with melphalan as the cytostatic drug in patients with melanoma in-transit metastases. In contrast to high response rates in melanoma patients, the results in STS patients were uniformly disappointing [6, 7]. The introduction of Tumour Necrosis Factor-α (TNF) in ILP by Lejeune and Lienard changed this situation dramatically as they reported not only high response rates in 13 melanoma patients, but also in 4 patients with advanced extremity STS [8]. This led to multicenter studies evaluating response and limb salvage rates using TNF-based ILPs with or without Interferon-gamma, showing consistently overall response rates of 75% to 85% and limb salvage in a similar percentages of the patients [9, 10]. These results led to the approval of TNF in Europe [11]. At present, TNF-based ILP for extremity STS is used as induction biochemotherapy to obtain local control and to make limb-sparing surgery possible. Here we report on the mature results of the largest single center experience of 217 consecutive ILPs with TNF and melphalan (TM-ILPs) for locally advanced STS.

PATIENTS AND METHODS

Patients

From July 1991 – July 2003, 217 TM-ILPs were performed in 197 patients for locally advanced STS. Fourteen patients underwent a second ILP after recurrence of the tumour in the limb; 2 patients had 3 ILP procedures. In 4 patients (1 with Stewart-Treves lymphangiosarcoma, 2 with Kaposi sarcoma and 1 with neurofibrosarcoma), a second ILP procedure was performed on the other leg because of bilateral disease. Thus, a total of 201 limbs were treated with an ILP. There were 101 men and 96

Table 1: Patient and tumour characteristics of 217 TM-ILPs in 197 patients

Patient and tumour characteristics of 217 TM-ILPs in 197 patients			
		N	**%**
Gender	female	108	50
	male	109	50
Age	≤ 50	93	43
	> 50	124	57
Size	< 5 cm	67	31
	5 – 10 cm	58	27
	> 10 cm	92	42
Trojani Grade	1	41	19
	2	60	28
	3	116	53
Site	upper arm	31	14
	lower arm	29	13
	upper leg	108	50
	lower leg	49	23
Histology	Liposarcoma	31	14
	Synovial Sarcoma	34	16
	MFH	34	16
	Leiomyosarcoma	18	8
	Desmoid/Agg Fibro	12	6
	St-T/Kaposi sarcoma	23	11
	Other (16 tumour types)	65	30
Primary/recurrent	primary	132	61
	recurrent	85	39
Previous treatment	None	161	74
	XRT	34	16
	CT	21	10
	ILP	11	5
Single/multiple	single	153	71
	multiple	64	29
Post- ILP treatment	None	145	67%
	XRT	59	27%
	CT	10	5%
	XRT + CT	3	1%

TM-ILP = isolated limb perfusion with TNF and melphalan, MFH = malignant fibrous histiocytoma, Agg Fibro = aggressive fibromatosis, St-T = Stewart-Treves lymphangiosarcoma, XRT = radiotherapy, CT = chemotherapy

women with a median age at time of ILP of 54 years (range 12-91 years). Patient and tumour characteristics are summarized in table 1. All patients were candidates for amputation of the limb as resection of the tumour was impossible, or only possible at the cost of severe functional morbidity, due to either fixation of the tumour to neurovascular structures or bone, multifocality of the tumour or location of the tumour in a previously irradiated area without the possibility to perform a complete radical resection. Median follow-up of all patients was 22 (range 0.1-130) months.

Treatment

Patients underwent an ILP via the axillary (N=25), brachial (N=35), iliac (N=94), femoral (N=38) or popliteal (N=25) approach. ILP technique has been described previously [9, 10]. Briefly, recombinant human TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) and the cytostatic drug melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK), obtained as a sterile powder, were dissolved aseptically using solvent and diluents (Burroughs Wellcome Ltd., London, UK). Isolation of the blood circuit of a limb was achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, and application of a tourniquet to compress the remaining collateral vessels. TNF was injected as a bolus into the arterial line provided limb tissue temperature had reached 38°C. Melphalan was administered after 30 minutes at limb temperatures between 38 and 39.5°C. The administration of melphalan changed during the studied period from injection as a bolus (1991-1996) to infusion by pump over a period of 20 minutes (1996-present), because of reports that melphalan peak concentration is correlated with regional toxicity [12]. ILP consisted of a 90-minutes perfusion with 1 to 3 mg (arm) or 1 to 4 mg (leg) TNF, and a 10-mg/l (leg) or 13-mg/l (arm) volume of melphalan at mild hyperthermia (tissue temperatures of maximally 39.5°C in the leg and 38.5°C in the arm). Median dose of melphalan was 70 mg (mean 73.3, range 0-160); median dose of TNF was 4 mg (mean 3.5, range 1-4). In the first 25 ILPs, performed between 1991 and 1994, interferon γ (IFN) was added to the schedule according to trial prescriptions consisting of the subcutaneous injection of 0,2 mg IFN on days –2 and –1 prior to the ILP and the injection of 0.2 mg IFN during the ILP procedure into the arterial line prior to the administration of TNF. During the procedure, continuous leakage monitoring was performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected into the perfusion circuit. At the end of the ILP, the limb was washed out with at least 1 L (arm) up to 4 L (iliac perfusion) of physiologic saline solution and 6% dextran 70 (Macrodex Pharmacia, Uppsala, Sweden). ILPs were performed under general anaesthesia and normally took 2.5 to 4 hours. Median hospital stay of the patients was 8 days (mean 12, range 2-136).

Response evaluation and toxicity

Clinical response evaluation was performed 2, 4, 8 and 12 weeks after ILP and hereafter every 3 months for the first year both by clinical examination and by MRI (after 4-6, 8-12 weeks after ILP and hereafter every 3-6 months) [13], and reported according to WHO-criteria. In 130 patients, histological response could be assessed and in these patients response rates were adjusted if the pathological response (complete response (CR) if 100% necrosis, partial response (PR) if 50-99% necrosis and no change (NC) if <50% necrosis) differed from clinical response. New lesions or growth of the tumour at first response evaluation is reported as progressive disease (PD), and if occurring during follow-up as local progression.

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [14]: (I) no reaction, (II) slight erythema or edema, (III) considerable erythema or edema with some blistering, slightly disturbed motility permissible, (IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance, and threatening or manifest compartmental syndrome, and (V) reaction that may necessitate amputation. Systemic toxicity is reported according to WHO criteria.

Statistical evaluation

Overall survival (OS) and time to local/systemic progression (TTLP/TTSP) were defined as time from ILP to death, local progression and systemic progression respectively and estimates were made using the method of Kaplan and Meier. We evaluated the prognostic value of some baseline categories on overall response (CR or PR) achievement, using the Fisher's exact test for univariate analysis and logistic regression for multivariate analysis. Logrank test and Cox regression were used for TTLP, TTSP and OS. Based on previous reports on prognostic factors in patients with extremity STS [15, 16], we chose to evaluate gender and age of the patient, size, Trojani grade [17] and histology of the tumours and presentation with recurrent disease at time of ILP. We added previous irradiation therapy and presence of multifocal tumours to these baseline categories, as these conditions are often present when ILP treatment is considered. After univariate analysis all these factors were included in a multivariate model. We used a stepwise backward algorithm in order to exclude factors without prognostic value starting with the factor with the highest p-value, until $p<0.05$. All tests were done at a significance level of 5%.

RESULTS

Tumour response

Of 217 TM-ILPs for STS, a clinical complete response was obtained in 38 ILPs (18%), PR in 111 ILPs (51%), NC in 62 (29%) and PD in 4 (2%) ILPs. Clinical response was not assessed in 2 patients (1%, 1 patient died shortly after ILP, 1 reason unknown). In 130 patients (60%), ILP had made a complete resection of the tumours possible (71% in patients with single tumours, 34% in patients with multiple tumours, figure 1). In these patients (and in a minority of patients, especially with multiple sarcomas, in whom a core biopsy was performed to assess histological response), final outcome was adjusted according to the necrosis percentage found on histological evaluation. Final outcome therefore was: 56 CR (26%), 106 PR (49%), 49 NC (23%) and 5 PD (2%), resulting in an overall response percentage of 75%. One patient died 3 days after ILP so final response to ILP could not be assessed. On univariate analysis, the presence of multiple tumours (p=0.006) and ILP for Stewart-Treves lymphangiosarcoma or Kaposi sarcoma (p=0.002), were both related with a significantly better response rate (table 2). Only the latter remained statistically significant in multivariate analysis. There were no histological tumour types that did not, or significantly worse, respond to ILP. After ILP (± tumour resection), 72 patients received adjuvant therapy consisting of radiotherapy in 59 patients, systemic chemotherapy in 10 patients and a combination of both in 3 patients.

Table 2: Univariate analysis of clinical prognostic factors for CR/PR achievement, local progression, systemic progression and survival

Univariate analysis of clinical prognostic factors for CR/PR achievement, local progression, systemic progression and survival				
Variable	**CR/PR**	**Local progression**	**Systemic progression**	**Survival**
Gender	NS	NS	NS	NS
Age	NS	NS	NS	NS
Size of the tumour	NS	p<0.001	NS	NS
Trojani grade	NS	NS	p=0.001	p=0.020
Histology	p=0.041*	p<0.001	p=0.006	NS
Primary / Recurrent	NS	p=0.009	NS	NS
Previous XRT	NS	p=0.011	NS	NS
Single / Multiple tumours	p=0.006	p<0.001	NS	NS

CR = complete response, PR = partial response, XRT = radiotherapy, NS = not significant

* Overall, histology is a borderline significant prognostic factor for response (p=0.041), but Stewart-Treves lymphangiosarcoma / Kaposi sarcoma is associated with higher response rates (p=0.002, see text)

Limb function

Limb function of the 201 perfused limbs was assessed in 194 cases (97%) and was without functional loss in 145 limbs (72%), mildly disturbed in 14 (7%) and severely diminished leading to the use of crutches in 9 limbs (4%). An amputation could not be avoided in 26 perfused limbs (13%). Fifteen patients had an insufficient clinical response (PD/NC) and underwent immediate amputation. Notably, histological examination of the amputated limb showed PR in 3 of these patients. Nine amputations had to be performed due to rapid progression (2-9 months) after ILP: 5 after PR and 4 after CR. One patient with a histological PR but with sufficient local control of a synovial sarcoma of the lower leg developed a late local progression after 36 months necessitating amputation. One other patient had to undergo a late amputation: delayed resection of a 100% necrotic malignant fibrous histiocytoma of the lower leg caused osteomyelitis because of a period of inadequate soft tissue coverage of the bone resulting in a pathological fracture 17 months after ILP. Two patients had to undergo leg amputation during the follow-up period due to pre-existing vascular disease. Although the ILP-procedure might have altered the course of the vascular disease in these patients, these amputations are not considered Wieberdink V local toxicity or tumour-related amputations for analysis in this study.

Leakage and toxicity

Leakage of TNF and melphalan, as is reflected by the leakage of radioactively labelled albumen to the systemic circulation, was absent or minor (<10%) in 192 ILPs (88%). Median leakage was 0%, mean 2.6%. Six procedures were complicated by significant leakage of >20% (21-23-25-29-34-64%), but no serious systemic toxicity occurred in these patients and none of the patients required intensive-care stay of more than 24 hours. The 64% leakage in 1 ILP was attributable to snapping of the tourniquet 35 minutes after administration of TNF, just after adding melphalan to the perfusate. All other high-leakage ILPs were terminated when leakage exceeded 20%, but melphalan circulation time was at least 45 minutes in all these cases.

Local toxicity of the procedure was absent or mild (Wieberdink I-II) in 165 cases (76%), Wieberdink III in 45 patients and Wieberdink IV in only 4 patients. No treatment-related amputation had to be performed. In 3 patients local toxicity could not be assessed (1 rapid amputation due to PD, 1 rapid death and 1 reason unknown). Systemic toxicity was restricted to a transient rise in core temperature >40˚C in 8 patients, lasting over 24 hours in 1 patient. No toxic shock-like syndrome necessitating the use of vasopressors occurred. One patient, a 91-year old patient with significant arteriosclerosis and an excessively large high-grade liposarcoma of the

leg, developed a thrombosis of the mesenteric artery and died 3 days post-ILP (perioperative mortality 0.5%).

Local progression

Local progression of STS in the limb was observed after 57 ILPs (26%). If progression occurred, median time to local progression was 8.9 (range 1-54) months. Of these local failures, 28 occurred after resection of the tumour remnants post-ILP and are therefore true local recurrences (28 in 130 resections: 22%). Twenty-nine patients developed new lesions after ILP, or late regrowth of the known lesion(s), but in these patients the tumour was not resected after ILP (29 in 87 cases: 33%). The implications on local control of the management of the tumours post-ILP (resection, post-ILP irradiation) are outlined in figure 1.

Univariate prognostic factors for developing a local recurrence included size and histology of the tumour, ILP for recurrent STS, previous radiotherapy and the presence of multiple tumours. Prognostic factors for local progression after multivariate analysis were: tumour type (synovial sarcoma, malignant fibrous histiocytoma, leiomyosarcoma and Stewart-Treves/Kaposi associated with higher local recurrence rates) and multiple sarcomas at presentation ($p<0.001$). Prognostic factors are listed in tables 2 and 3.

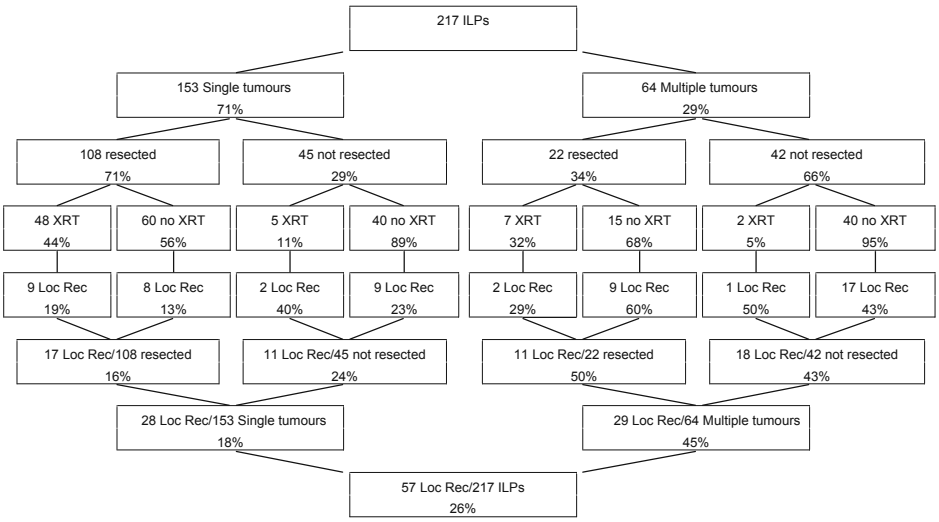


Figure 1: Flow-chart of management after ILP and its implications on local control

ILP = isolated limb perfusion; XRT = radiotherapy; Loc Rec = local recurrence

Table 3: Multivariate analysis on local progression, systemic progression and survival

Multivariate analysis on local progression, systemic progression and survival							
Variable		**Local progression**		**Systemic progression**		**Survival**	
		HR	**p**	**HR**	**p**	**HR**	**p**
Age	≤ 50			1			
	> 50			0.6	0.039		
Size of the tumour	≤ 5 cm			1			
	5 – 10 cm			1.5	NS		
	≥ 5 cm			2.4	0.005		
Trojani grade	1			1		1	
	2			2.1	NS	2.5	NS
	3			3.2	0.030	3.6	0.003
Histology	Lipo	0.8	NS	0.5	NS		
	Synovial	4.9	0.002	1.4	NS		
	MFH	5.0	0.001	0.7	NS		
	Leio	4.6	0.004	0.7	NS		
	Desmo	0.7	NS	0.0	*		
	St-T / Kaposi	2.9	0.036	0.2	0.005		
	Other	1		1			
Single / multiple	single	1		1		1	
	multiple	4.6	<0.001	2.4	0.001	1.7	0.015

* = Perfect prediction: no systemic progression in desmoid tumours

HR = hazard ratio, Lipo = liposarcoma, Synovial = synovial sarcoma, MFH = malignant fibrous histiocytoma, Leio = leiomyosarcoma, Desmo = desmoid/aggressive fibromatosis, St-T = Stewart-Treves lymphangiosarcoma, up = upper, low = lower, NS = not significant

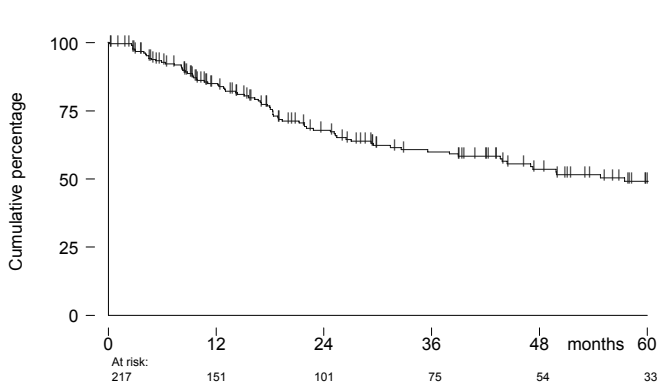
Systemic progression

We observed systemic metastases after ILP in 92 patients (42%). In those patients who developed systemic metastases, they became manifest after a median period of 4.6 (range 0-80) months. Notably, 34 patients (16%) had stage IV disease (metastases present) at time of ILP. On univariate analysis, Trojani grade (p=0.001) and histology (p=0.006) were significant prognostic factors for the development of systemic metastases. On multivariate analysis, age of the patient, size, grade and histology of the tumour and the presence of multiple tumours all were significant prognostic factors (tables 2 and 3).

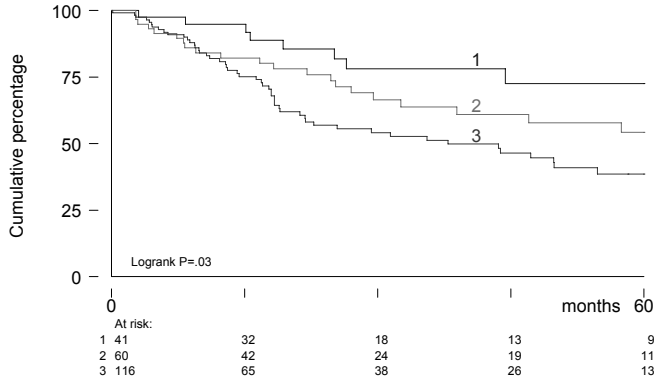
Survival

Overall, 5-years actuarial survival rate was 49%; median survival was 57 months. Survival after ILP for extremity STS is shown in figure 2. The prognostic factor for overall survival after ILP was Trojani grade (both on uni- and multivariate analysis, figure 2 b). Despite the fact that patients with multiple tumours had lower, but not

a) Total (X-axis: time in months; Y-axis: cumulative percentage)



b) By Trojani grade (X-axis: time in months; Y-axis: cumulative percentage)



c) By single/multiple tumours (X-axis: time in months; Y-axis: cumulative percentage)

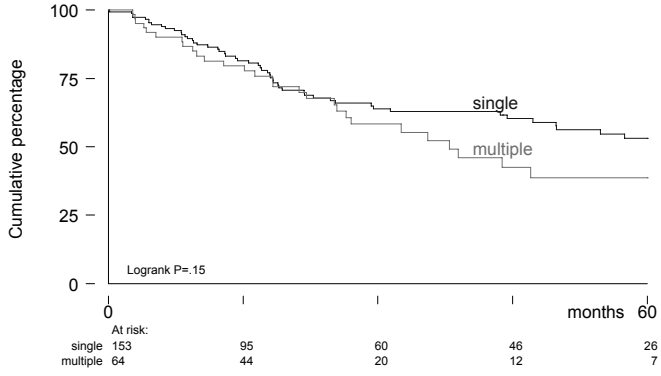


Figure 2: Overall Survival

statistically significant lower, survival rates than patients with single tumours (53% vs. 39% at 5 years, p=0.152), this factor showed to be significant on multivariate analysis (p=0.015, tables 2 and 3, figure 2 c).

DISCUSSION

The currently presented study of 217 TM-ILPs in a single center setting shows that limb salvage can be achieved in a large percentage of patients by combining induction biochemotherapy with marginal resection of the tumour. Even by ILP alone, significant tumour response rates can be obtained providing long-lasting local control in patients with large extremity STS and a limited life expectancy.

Response and limb function

The overall response rate in this series of 75% is in range with previous reports on TM-ILPs for STS with response rates varying from 63% to 91% [9, 10, 18-20]. With only 26 amputations in 201 perfused limbs, the limb salvage rate was 87%, which compares to smaller series reported in the literature. This is in fact an important observation, as we know that a large proportion of the patients will eventually develop systemic metastases and succumb to their disease. However, the local problem of a large tumour in the extremity can be managed with ILP, even if no resection is performed or if response is not complete. As TNF acts on tumour associated vasculature by destroying the vessels [21] and increasing the uptake of melphalan in the tumour in the preclinical tumour models [22], one could expect that response rates would be better in well-vasularised (high-grade) and large tumours. We could not demonstrate such a preferential effect in this study, nor could we identify a negative effect in tumours that lack extensive vascularisation. The only prognostic factors for response were ILP for multiple tumours and ILP for Stewart-Treves lymphangiosarcoma or Kaposi sarcoma. As these factors are highly related, only the latter remained statistically significant on multivariate analysis. We know from previous reports that response rates in these tumours indeed are very high (87-100% [23, 24]), presumably as these are small and highly vasularised tumours, and therefore we speculated that the clinical situation in these tumours more resembles the melanoma- than the STS-situation [25].

Progression and survival

Local tumour recurrence in this study occurred in 26%, which again is in range with results from the literature on ILP, ranging from 11% to 45% [9, 10, 18-20]. This is slightly higher than the 10% to 20% local recurrence rate reported in the literature for all STS of the extremity [26-28], supposedly because a large percentage of the patients qualifying for ILP presents with recurrent disease (39% in this study), which is a known adverse prognostic factor for local recurrence [29]. Systemic metastases developed in 42% of the patients and the actuarial 5-years overall survival rate was 49%. We could determine Trojani grade and leiomyosarcoma and synovial sarcoma as prognostic factors for systemic recurrence and Trojani grade as prognostic factor for survival,

which is virtually equal with known data [15, 16]. The fact that size of the tumour is not a prognostic factor for survival in our study, but on multivariate analysis presentation with multiple tumours is, reflects the high number of patients presenting with small but numerous tumours who specifically are candidates for ILP as primary resection is not feasible in these patients.

ILP with consecutive limb-sparing surgery is considered to be the alternative for amputation in this study population. This is based on the insight that limb-preserving surgery is equal to amputation in terms of survival [2-4], although the local recurrence rate of 10% to 20% [26-28] after limb-sparing resection is obviously higher than when an amputation is performed. There still is a debate whether local recurrence of STS is a determinant of overall survival, with studies that do not find a statistically significant effect [2, 29] and studies that do [27, 30]. Although the authors of the latter studies argue that patients who develop a local recurrence need aggressive treatment, a study from Memorial Sloan-Kettering Cancer Center showed that even in a patient category presenting with recurrent disease, amputation only improves local control but not survival [31]. We therefore claim that ILP is justified as a treatment option allowing limb-preservation in both primary and recurrent STS.

As ILP was applied to make limb-sparing surgery possible in the majority of patients in this study, its effect should be compared to other neo-adjuvant treatment regimens. The impact of induction systemic chemotherapy on the resectability of STS was recently studied at the MD Anderson Cancer Center [32]. Although a response of the tumour was observed in 43% of the patients, only 13% of the population (consisting of 65% extremity STS and 35% retroperitoneal STS patients) showed a radiographically documented response sufficient to reduce the extent of the operation. Notably, none of the extremity STS patients scheduled for amputation could be treated with limb-sparing surgery after neo-adjuvant systemic chemotherapy [32]. These results are sustained by a randomised study on neo-adjuvant chemotherapy for "high-risk" adult STS (150 patients, 82% extremity STS). In none of the 9 patients scheduled for amputation in this study, neo-adjuvant chemotherapy could provide limb salvage [33]. Systemically administered chemotherapy does have the advantage of a possible systemic effect on distant (micro-)metastases, although a large meta-analysis of doxorubicin-based adjuvant chemotherapy only showed a reduction in time to recurrence (both local and distal), but no significant effect on survival [34]. ILP with doxorubicin or melphalan alone in advanced STS has failed to demonstrate adequate activity in studies performed in the Netherlands [35] and in a recent study performed at MD Anderson [36] and is not recommended. Presumably the poor drug uptake in large tumours without the use of TNF is the cause of this failure [11, 37].

Preoperative radiotherapy also has the possible advantage of reducing the tumour to a size making resection possible. To our knowledge, no data on this issue exist to date. Preoperative radiotherapy has shown in a randomised trial to be as effective as postoperative irradiation in terms of progression-free survival, but is associated with higher wound-complication rates [38]. Chemoradiotherapy, a combination of preoperative (intra-arterial or intravenous) chemotherapy and radiotherapy, has shown to provide excellent local control rates and improved overall survival with acceptable toxicity both in small exploring studies [39] and in a comparison with a historical control group [40]. However, this treatment option remains investigational and the results of randomised trials are awaited. Moreover, to compare chemoradiotherapy with ILP is difficult, as the tumours are primarily resectable in the first, whereas they are generally not in the second.

As TM-ILP can be of particular value in the palliative treatment of patients with metastatic disease and a rapidly growing tumour threatening the limb, it is of eminent importance that the procedure is safe and without severe side effects. Systemic toxicity is directly correlated with leakage of TNF (and melphalan) to the systemic circulation [41-43]. In the present time of leakage-free ILPs, it should not be necessary to use vasopressors in order to keep the blood pressure at adequate levels to counteract the systemic inflammatory response syndrome that can occur when significant levels of TNF reach the systemic circulation. Ample hydration and adequate diuresis in order to keep the levels of circulating TNF after ILP low, should prevent systemic toxicity even in high-leakage ILPs [43]. The procedure can be safely performed in patients with advanced age [44] and the median hospital stay of 8 days shows that the procedure is relatively mild to undergo. This is also underlined by the peri-operative mortality-rate of 0.5%.

The results obtained in the 217 consecutive TM-ILPs described here, underline that TNF-based isolated limb perfusion can play a major role in the treatment of limb-threatening extremity STS. TM-ILP can provide excellent local control and a high rate of limb salvage. Therefore, TNF+M-based-ILP has gained a permanent place in the multimodality treatment of locally advanced extremity STS and is currently available in some 40 referral centers in Europe.

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CHAPTER 6

Isolated Limb Perfusion with TNF and Melphalan Prevents Amputation in Patients with Multiple Sarcomas in Arm or Leg

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ABSTRACT

Background

Treatment for extremity soft tissue sarcoma (STS) shifted in recent years from amputation to local wide excision combined with irradiation. For multiple sarcomas, this limb-sparing approach is often not possible. To avoid amputations, isolated limb perfusion (ILP) with tumour necrosis factor-α (TNF) and melphalan is an attractive treatment option for patients with multiple extremity sarcomas.

Methods

We investigated a prospective database at a tertiary referral institute. From July 1991 to July 2003, out of 217 ILPs, 64 ILPs were performed for either multifocal primary sarcomas or multiple sarcoma recurrences in 53 patients. All ILPs were performed under mild hyperthermic conditions by using 1 to 4 mg TNF and 10 to 13 mg/L limb volume for leg and arm perfusions, respectively.

Results

The overall response was 88%, with 42% complete response, 45% partial response, 11% no change and 2% progressive disease. This response rate is significantly better than our experience in 153 locally advanced single STS cases (88% vs. 69%). The toxicity of the procedure was mild to moderate in almost all cases; no treatment-related amputation had to be performed. The time to local recurrence was 29 months and differed significantly between multiple primary and multiple recurrent STS. The 5-year survival rate was 39%. Limb salvage was achieved in 45 (82%) of 55 treated limbs.

Conclusions

In a group of patients who are uniformly candidate for amputation, ILP can achieve limb salvage in approximately four out of five patients. Because this treatment option provides excellent local control, it should be considered before an amputation is planned.

INTRODUCTION

Of the 7800 new cases of soft tissue sarcoma (STS) diagnosed in the USA each year, approximately 4700 occur in the extremities [1]. Tumours are often large at the time of diagnosis. Treatment options for locally advanced extremity STS may consist of an amputation or an extensive limb-sparing surgical procedure followed by radiation therapy. This combination may mutilate and compromise limb function considerably. Since the application of tumour necrosis factor (TNF)-α in combination with melphalan in the isolated limb perfusion (ILP) setting, a new limb-salvage strategy has emerged. Multicenter trials in Europe have established high response rates and limb salvage rates in the management of limb-threatening STS, which led to the approval of TNF for this indication in Europe [2, 3]. Subsequent single center reports on TNF-based (TM-)ILP have reported response rates varying from 63-92 % and limb-salvage rates ranging from 58% to 85% [4-8]; this is very much in line with the 76% response rate and 71% limb-salvage rate as achieved in the multicenter pivotal trials setting [9].

Multiple sarcomas in the extremities are very rare and are usually uniformly treated by amputation of the limb [10, 11]. Reports on the surgical management of these patients are scarce because surgical options are usually limited to amputation of the limb. Limb salvage has come to the forefront in the management of all patients with extremity STS in light of the data that have shown that this approach has not influenced survival outcome adversely [12-14]. Because amputations are very rarely performed for patients with melanoma in-transit metastases, we have adopted a limb-salvage approach in patients with multiple sarcomas by using TM-ILP. In this report, we describe our unique experience with 64 TM-ILPs in the management of in 53 patients with multiple limb sarcomas.

PATIENTS AND METHODS

Patients

Out of 217 TM-ILPs for STS performed in the Daniel den Hoed Cancer Center between 1991 and July 2003, 64 ILPs were performed on 53 patients with multifocal sarcoma. Two patients, one patient with Kaposi sarcoma and one patient with Stewart-Treves lymphangiosarcoma, underwent ILPs on both legs, thus making the total number of limbs 55. There were 29 women and 24 men with a median age of 61 years (range 20-88 years). Nine ILPs (14%) were performed in patients with stage IV disease (systemic metastases were present). Histological subtyping of the tumours

Table 1

Tumour characteristics of 64 TM-ILPs for multiple STS						
Histology type	**Multifoc Primary**	**Multiple Recurrent**	**Grade 1**	**Grade 2**	**Grade 3**	**Total**
Liposarcoma	1	1	0	1	1	**2**
Synoviosarcoma	1	4	1	3	1	**5**
MFH	0	7	0	2	5	**7**
Leiomyosarcoma	1	5	0	0	6	**6**
Angiosarcoma	2	3	2	1	2	**5**
Fibrosarcoma	0	1	1	0	0	**1**
Clear cell sarcoma	0	4	0	1	3	**4**
Neurogenic sarcoma	0	1	0	1	0	**1**
Hemangiopericytoma	1	2	1	0	2	**3**
Osteosarcoma	0	1	0	0	1	**1**
Kaposi sarcoma	5 (3)	0 (2)*	4	1	0	**5**
Extraskeletal Ewing sarcoma	1	0	0	0	1	**1**
Stewart-Treves	16 (7)	0 (9)*	7	9	0	**15**
Aggressive fibromatosis/Desmoid	0	7	7	0	0	**7**
Total	**28**	**36**	**23**	**19**	**22**	**64**

*= Kaposi sarcomas and Stewart Treves lymphangiosarcomas are considered primary sarcomas for evaluation (explained in text).
MFH = malignant fibrous histiocytoma, Multifoc = multifocal

and classification of tumour grade according to Trojani [15] et al. are listed in table 1. In total, 28 ILPs were performed on patients with multifocal primary STS, and 36 ILPs were performed for multiple recurrent STS. A patient was regarded as having multifocal primary STS when presenting with multifocal disease without previous surgical resection of STS in the limb. Multiple recurrent STS was defined as the occurrence of multifocal recurrences of STS after previous surgery (with or without irradiation) for the primary tumour. Recurrences of Kaposi sarcoma and Stewart-Treves lymphangiosarcoma were also considered multifocal primary tumours when they "recurred" after ILP, because of the nature of the disease dictated that no prior complete surgical resections had preceded ILP. Twenty-six patients had undergone other previous treatments for their STS. These treatments consisted of irradiation (n=11), systemic chemotherapy (n=4), isolated limb perfusion (n=8, [7 in our institution, 1 elsewhere]), a combination of irradiation and systemic chemotherapy (n=1) and a combination of ILP and irradiation (n=2). All patients were candidates for amputation because primary surgical resection was impossible because of the multifocality of the tumours.

Treatment

Patients underwent an ILP via the axillary (*n*=13), brachial (*n*=4), iliac (*n*=28), femoral (*n*=14) or popliteal (*n*=5) approach. ILP technique has been described previously [2, 3]. Briefly, recombinant human TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) and the cytostatic drug melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK), obtained as a sterile powder, were dissolved aseptically using solvent and diluents (Burroughs Wellcome Ltd., London, UK). ILPs were performed under general anaesthesia and normally took 2.5 to 4 hours. Isolation of the blood circuit of a limb was achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, and application of a tourniquet to compress the remaining collateral vessels. ILP consisted of a 90-minutes perfusion with 1 to 3 mg (arm) or 3-4 mg (leg) TNF, and a 10 mg/L (leg) or 13 mg/L (arm) volume of melphalan at mild hyperthermia (tissue temperatures of maximally 39.5°C in the leg and 38.5°C in the arm). Median dose of melphalan was 60 mg (mean 68.7 mg, range 14-140 mg); median dose of TNF was 4 mg (mean 3.3 mg, range 1-4 mg). TNF was injected as a bolus into the arterial line provided limb tissue temperature had reached 38°C. Melphalan was administered after 30 minutes at limb temperatures between 38 and 39.5°C. During the procedure, continuous leakage monitoring was performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit. At the end of the ILP, the limb was washed out with at least 1 L (arm) up to 4 L (iliac perfusion) of physiologic saline solution and 6% dextran 70 (Macrodex; Pharmacia, Uppsala, Sweden).

Response evaluation and toxicity

Clinical response evaluation was performed 2, 4, 8 and 12 weeks after ILP and thereafter every 3 months for the first year both by clinical examination and by magnetic resonance imaging (4-6 and 8-12 weeks after ILP and thereafter every 3-6 months) [16, 17], and reported according to World Health Organisation (WHO) criteria. Histological response could be assessed in 22 cases after a median interval of 3 months (range 1-17 months) and was obtained in 14 cases by a biopsy of responding lesions and in 8 patients by evaluation of the resection specimen of tumour remnants that had become resectable. In these patients, the final outcome was adjusted if the pathological response (necrosis percentage: complete response (CR) if 100% necrosis, partial response (PR) if 50-99% necrosis and no change (NC) if <50% necrosis) differed from the clinical response. Of the eight patients with completely resected disease, seven received adjuvant radiotherapy after resection because of high tumour grade or narrow resection margins. Other post-ILP treatment consisted of radiotherapy (*n*=2) or chemotherapy (*n*=4).

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [18]: grade I, no reaction; grade II, slight erythema or edema; grade III, considerable erythema or edema with some blistering, slightly disturbed motility permissible; grade IV, extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance and threatening or manifest compartmental syndrome; and grade V, reaction that may necessitate amputation. Systemic toxicity wass reported according to WHO criteria.

Statistical evaluation

Estimates of overall survival (OS) and time to local or systemic progression (TTLP and TTSP, respectively) were made according to the method of Kaplan and Meier. The 64 multiple ILPs were compared with the group of single-sarcoma ILPs in our ILP database, and multifocal primary sarcomas were compared with multiple recurrences of STS because of the expected difference in behaviour, both by using the log-rank test. As desmoid tumours and aggressive fibomatosis are tumour types known to be locally aggressive but to have benign systemic behaviour, survival and progression were separately calculated with exclusion of these tumour types. Comparison between two groups was tested using the Fisher's exact test or the Wilcoxon trend test if appropriate. P-values <0.05 were considered statistically significant.

RESULTS

Response

A clinical CR after ILP in 64 patients with multiple sarcomas was observed in 38%. PR occurred in 47%, NC in 14% and progressive disease in 2%. In eight patients with a limited number of tumours (*n*=2-5), the post-ILP response was nearly complete, and this made the tumour remnants resectable. In five patients, no more vital tumour

Table 2

Response rates			
	All multiples	**Multifocal primary STS**	**Multiple recurrent STS**
	N=64	N=28	N=36
CR	42%	61%	28%
PR	45%	36%	53%
NC	11%	4%	17%
PD	2%		3%
Overall	**87%**	**96%**	**81%**

STS = soft tissue sarcoma, CR = complete response, PR = partial response, NC = no change, PD = progressive disease

cells were found in the resected tumour remnants. In another 14 responding patients a biopsy of (some of) the lesions was performed for response assessment, mainly to distinguish between PR and CR. Of these 14, 9 had >50% to 100% necrosis. The final outcome, therefore, was: 42% CR, 45% PR, 11% NC and 2% progressive disease. Compared with our ILP experience in single STS, the outcome of TM-ILP in multiple sarcomas is significantly better (total response rate 88% vs. 69%, p=0.005). There was no statistically significant difference in overall response among high-, intermediate- and low-grade tumours (trend test p=0.402). Response rates of multiple STS were analysed separately for multifocal primary sarcoma and multiple recurrences and were shown to be 96% in multiple primary tumours and 81% in multiple recurrences (p=0.070). Response rates for each group are listed in table 2.

Progression and survival

The local recurrence rate was 45% (29 local recurrences in 64 ILPs); the median TTLP was 29 months. This was significantly lower than TTLP after ILP for single sarcomas (median TTLP >129 months, p<0.001, figure 1). For the patients who developed

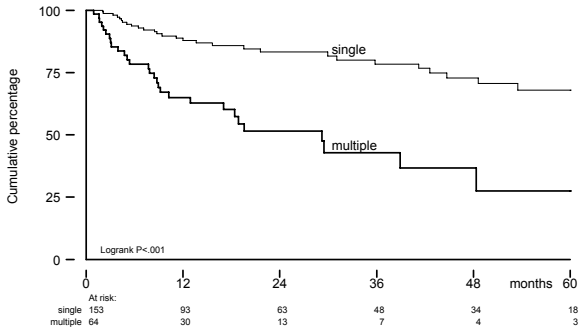

Figure 1: Time to local progression (TTLP) for single versus multiple sarcomas
X-axis: Time (months); Y-axis: Cumulative percentage

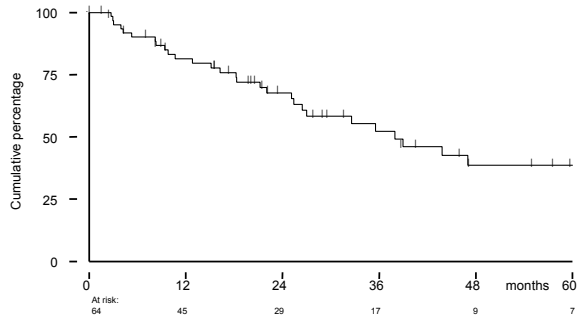

Figure 2: Overall survival (OS)
X-axis: Time (months); Y-axis: Cumulative percentage

Table 3: Local and systemic toxicity

Local toxicity					
Wieberdink	Grade I	Grade II	Grade III	Grade IV	Grade V
	12	37	15		
Systemic toxicity					
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Neurologic*	60	4			
Liver*	64				
Renal*	64				
Haematologic*	63	1			
	<38°C	38-39°C	39-40°C	>40°C, <24 hrs	>40°C, >24 hrs
Temperature	32	22	9	0	1
	Absent	Present			
Shock[&]	64				

Hrs = hours, * = WHO criteria, [&] = support of vasopressors needed

a local recurrence, the median time to this event was 8 months (vs. 13 months in single sarcoma patients, p=0.074). No significant difference in TTLP could be detected between multifocal primary (20 months) and multiple recurrent (39 months) STS (p=0.605). Systemic progression occurred in 28 patients; the median TTSP was 67 months. There was no statistically significant difference in TTSP for multiple versus single STS (p=0.711) or for multifocal primary versus multiple recurrent STS (p=0.105). Actuarial 5-year (OS) after ILP for multiple STS was 39% (figure 2), which is lower than but not significantly different from the 53% 5-year survival rate for single STS (p=0.152). No significant difference in OS could be detected for multifocal primary versus multiple recurrent STS (p=0.164). However, analysis excluding aggressive fibromatosis/desmoid tumours (all seven multiple recurrent STS) revealed a significant difference in both TTSP (p=0.021) and OS (p=0.030) between multifocal primary and multiple recurrent STS. Median follow-up of the patients in this study was 22.2 months (range 0.2-129 months).

Toxicity and limb function

Local toxicity was mild (grade I-II) to moderate (grade III) in all ILPs. Systemic toxicity was absent or mild: only one patient had a fever >40°C for >24 hours (table 3). Limb function of the 55 limbs was available in 53 cases and was perfect in 38 limbs, mildly disturbed in 4 cases and moderately disturbed leading to the use of crutches in 1 patient. An amputation could not be avoided in 10 perfused limbs, thus leading to a limb salvage percentage of 82%.

DISCUSSION

Our experience with TNF-based ILP for limb salvage in the treatment of multiple STS of the extremities is the largest experience reported to date and demonstrates the efficacy of this approach. In patients with multifocal limb-threatening STS, ILP with TNF and melphalan can prevent amputation in the vast majority of the perfused limbs. Response rates are excellent and local control is obtained for a median of 29 months.

The overall response rate of 88% of multiple sarcomas in this study compares to previously reported response rates in the literature of 63% to 91% with TNF and melphalan [2, 4, 6, 8], and is considerably better than that reported in ILP with only chemotherapeutics [19-22]. The overall response rate is in line with the results obtained by TM-ILP in a small series of 13 patients reported by Lev-Chelouche et al. [23].

Compared with our own experience in 153 single-STS ILPs, the overall response rate was significantly better. This can be attributed mainly to the high CR rate in multifocal primary STS of 61%. Because the nature of multifocal primary STS such as Kaposi sarcoma and Stewart-Treves lymphangiosarcoma is comparable to melanoma in terms of the number of lesions and the approach of post-ILP resection, we compared the response rates of multiple primary STS with our experience in 100 TNF-ILPs for melanoma [24]. The CR rate in melanoma (69%) is markedly higher than can be achieved in all STS patients (single and multiple tumours combined), but the CR rate of 61% in our series of multifocal primary STS was virtually the same as the high CR rate in melanoma. Also, the overall response rates in melanoma (95%) and in multifocal primary STS (96%) were practically identical. Moreover, the relatively short TTLP in multifocal primary STS of 20 months also compares to our melanoma experience (median TTLP 16 months). The behaviour of the tumours and the response on ILP of multifocal primary STS therefore matches more with melanoma than with single sarcomas, and this is further supported by the previously reported excellent response rates after ILP of 100% for Kaposi sarcoma [25] and 87% for Stewart-Treves lymphangiosarcoma [26].

Desmoid tumours and aggressive fibromatosis in this study all presented as multiple recurrences of STS after previous surgical resections. Recurrent STS is a known adverse prognostic factor for both systemic recurrence and disease-specific survival [14]. However, in desmoid tumours, systemic metastases do not occur [27]. Excluding these tumour types in progression and survival analysis in this study indeed revealed a worse outcome for multiple recurrent STS compared with multifocal primary tumours.

The local recurrence rate of 45% is very high in comparison to previously reported data in single STS, both after surgery [28] (19%) and after ILP [2,3] (22%). Because a large proportion of the presently studied patient presented with recurrent disease, which is known to be an independent adverse prognostic factor for local recurrence [14], this high local recurrence rate is to be expected. Still, local control of disease could be achieved for a significant period (median TTLP 29 months) and amputation could be avoided in 82% of the perfused limbs.

Local toxicity of the procedure was mild to moderate in all cases and did not seem to be influenced by repeated ILPs or by previous radiotherapy. This is in accordance with previous observations in our institute in 26 patients treated with ILP after previous irradiation [29].

In the present series of patients with difficult-to-treat tumours, these observations show that TM-ILP can provide limb salvage in approximately four out of five patients. Because no treatment other than surgical excision has proven to be effective in extremity STS and because this option is not applicable in patients with multiple STS, TM-ILP has to be considered before scheduling a patient for amputation.

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CHAPTER 7

Isolated Limb Perfusions with Tumour Necrosis Factor and Melphalan for Locally Recurrent Soft Tissue Sarcoma in Previously Irradiated Limbs

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ABSTRACT

Background

Recurrent extremity soft tissue sarcoma (STS) in a previously operated and irradiated area can usually be managed only by amputation. Tumour Necrosis Factor-α (TNF) -based isolated limb perfusion (ILP) is an established alternative to achieve limb salvage but is assumed to require sufficient vasculature. Because radiotherapy is known to destroy vasculature, we wanted to evaluate retrospectively whether the outcome of ILP in patients with radiotherapy for their primary tumour nonetheless showed a benefit from TNF treatment.

Methods

We consulted a prospective database of TNF-based ILPs at the Erasmus MC - Daniel den Hoed Cancer Center in Rotterdam. Out of 342 TNF-based ILPs between 1991-2003, 30 ILPs were performed in 26 patients with recurrent STS in the irradiated field after prior surgery and radiotherapy. Eleven patients (42%) had multiple tumours (*n*= 2->20). All patients were candidates for amputation.

Results

We observed 6 complete responses (20%), 15 partial responses (50%), no change in 8 patients (27%), and progressive disease in 1 patient (3%). The median duration of response was 16 months (range 3 - >56 months), at a median follow-up of 22 months (range 3 - > 67 months). The local recurrence rate was 45% in patients with multiple tumours and 27% in patients with single tumours. Ten patients (35%) died of systemic metastases. Limb salvage was achieved in 17 patients (65%). Regional toxicity was limited and systemic toxicity minimal.

Conclusions

TNF-based ILP can avoid amputations in the most patients with recurrent extremity STS in a prior operated and irradiated field.

INTRODUCTION

The approval by the European Medicine Evaluation Agency of Tumour Necrosis Factor-α (TNF) in combination with melphalan in the setting of isolated limb perfusion (ILP) for the treatment of locally advanced extremity soft tissue sarcomas (STS), on the basis of the excellent limb salvage results of multicenter trials in Europe, has added an important treatment modality to avoid limb amputations [1-4]. Patients with local recurrences in the limb after prior surgery and high dose radiotherapy and patients with multiple sarcomas are usually all candidates for amputation of the extremity.

In the early experience with the TNF-based ILP program for irresectable extremity STS, much attention was given to the post-ILP necrosis of the foot of a particular patient treated by TNF-based ILP after prior resections, high dose radiotherapy and prior ILP with cisplatin [5]. It was then speculated that the high dose radiotherapy might have resulted in damage to the vasculature of the foot. This might have rendered it susceptible to the toxic effects of the combination of high-dose TNF and melphalan, thus leading to complete necrosis of the healthy tissues, in contrast to the usually selective toxic effects on the tumour vasculature only. This observation led to hesitation to offer TNF-based ILP to patients when a local recurrence presented in a high-dose irradiated area. In an attempt to avoid amputation, we decided to offer TNF-based ILP in all cases where amputation was the only option, regardless of prior treatments. We performed 30 ILPs in 26 patients with tumour recurrences in irradiated limbs. Here we report on this unique experience.

PATIENTS AND METHODS

Between 1992 and 2003, 26 patients with recurrent STS initially treated with surgery and radiotherapy, were treated with ILP with melphalan and TNF. All patients were considered candidates for amputation at the time of referral because of extensive and/or irresectable disease. Irresectability was defined as the impossibility to perform an oncologically justified surgical resection without substantial function loss of the limb or complete loss of the limb.

The technique of the ILP with recombinant TNF and melphalan is in detail described elsewhere [2]. Briefly, recombinant human TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) and the cytostatic drug melphalan, obtained as a sterile powder (100 mg) were dissolved aseptically using solvent and diluents (Burroughs

Welcome, London, UK). ILPs were performed with patients under general anaesthesia and normally took 2.5 to 4 hours. Isolation of the blood circuit of a limb was achieved by clamping the major artery and vein and by applying a tourniquet to compress the remaining collateral vessels. Perfusion was performed at the axillary, brachial, iliac, femoral, or poplitial level. ILP consisted of a 90-minutes perfusion with 1.5 to 3 mg (arm) or 2-4 mg (leg) of TNF and a 10 mg/l (leg) or 13 mg/l (arm) volume of melphalan at mild hyperthermia. Maximum tissue temperatures were 39.5°C in the leg and 38.5°C in the arm. The composition of the perfusate was as follows: the priming volume of 700 to 850 ml consisted of 400 to 500 ml blood (50% RBCs, 50% plasma), 200 to 400 ml 5% dextran 40 in glucose 5% (Isodex; Pharmacia, Uppsala, Sweden), 10 to 30 ml 8.4% sodium bicarbonate, and 0.5 ml of 2500 to 5000 IU heparin. TNF was injected as bolus into the arterial line provided that the limb tissue temperature was greater than 38°C. Melphalan was administered 30 minutes later at limb temperatures between 38°C and 39.5°C. At the end of the ILP, the limb was washed with 1 L (axillary) to 4 L (iliac perfusion) of physiologic saline solution and 6% dextran 70 (Macrodex; Pharmacia, Uppsala, Sweden).

Evaluation of response and toxicity

Tumour response was assessed at least twice between 4 and 12 weeks after perfusion. Complete response was defined as the disappearance of all measurable disease in the limb for more than 4 weeks, partial response was defined as tumour size regression by greater than 50% for more than 4 weeks, no change was defined as regression of less than 50% or progression of less than 25% for longer than 4 weeks, and progressive disease was defined as more than 25% disease progression. In patients in whom a resection of residual tumour, necrotic tissue mass, or both was performed after the ILP, a histological response rate was assessed by determining the percentage of necrosis. Clinical responses were standardized according to World Health Organization (WHO) criteria.

Regional toxicity was graded according to Wieberdink et al. [6]: grade 1, no toxicity; grade 2, redness and slight edema; grade 3, considerable edema or erythema with some blistering; grade 4, extensive epidermolysis or obvious damage to the deep tissues causing definite functional disturbances, or threatening or manifest compartmental syndrome; and grade 5, reaction requiring amputation. Systemic toxicity was graded according to Eastern Cooperative Oncology Group/World Health Organisation criteria.

RESULTS

Over the past 10 years, more than 340 patients with advanced melanoma or sarcoma were treated by using ILP with melphalan in combination with TNF in the department of surgical oncology at the Erasmus MC - Daniel den Hoed Cancer Center. Twenty-six of these patients were previously treated with surgery and high dose radiotherapy (50-70 Gy) for their primary sarcoma and developed one or more irresectable local recurrences. One patient had known distant metastases at the time of treatment. Patient and tumour characteristics are listed in tables 1 and 2. The group consisted of 15 men and 11 women, with a median age of 50 years (range 21-84 years, mean 52 years). The median interval between treatment of the primary tumour and recurrence of disease was 19 months and ranged from 0 to 156 months.

In all patients, systemic toxicity after ILP was mild to moderate and was easily manageable. More than 10% leakage was measured in five patients during perfusion, but this was without significant toxicity. When postoperative hypotension occurred, it responded immediately to fluid administration, and none of the patients required circulatory support with vasopressors. One patient experienced a fever higher than 40°C after the ILP; this, however, was reversible within 24 hours. No patients endured neurotoxicity after the ILP.

A major tumour response was seen after 21 (70%) of 30 perfusions, with a partial response in 15 patients (50%) and a complete response in 6 patients (20%). Nine perfusions (30%) were not followed by any objective tumour response; four of these patients (patients 3, 4, 22, and 24) underwent an amputation of the limb. Two patients died of systemic disease with tumour present in the limb (patients 1 and 12). Patient 8 had a mixed response to the first perfusion, with 4 of 6 tumours showing a complete response but 2 tumours not responding at all, thus resulting in a no change score. The two remaining tumours responded well to the repeated perfusion scheduled 13 months after the first ILP. However, the patient relapsed within 3 months after the second perfusion and eventually underwent an amputation. Patient 10 responded insufficiently on the first perfusion but showed a partial response after a second ILP, 7 months later. Because of a local recurrence, an amputation had to be performed after 10 months. In patient 20, the clinical response was insufficient for a partial response score. However, the tumour had shrunk enough to allow resection. Histological responses could be established in 11 patients who were treated with an additional tumour resection. In three patients, these responses showed much more necrosis in the tumour remnant than was clinically expected (patients 11, 16, and 26).

Table 1: Characteristics of 26 recurrent sarcoma patients treated with ILP (N=34) after radiotherapy treatment.

Pt no	sex	age	no of tumours	site	histology	grade	1st-2nd rec	p/r interval (months)
1	M	72	1	Upper leg	Fibro	2	R	19
2	F	46	2	Wrist	Synovio	1	RR	156
3	F	63	1	Lower leg	MFH	3	RR	22
4	M	44	1	Lower arm	MFH	3	R	39
5	M	74	1	Lower arm	MFH	3	R	33
6	F	27	2	Lower leg	Kaposi	2	RR	14
7	M	48	1	Upper leg	MFH	3	R	156
8	F	81	4	Lower leg	MFH	2	R	28
		82	6	Lower leg + Ankle	MFH	3	RR	9
9	M	72	3	Fos pop	MFH	3	R	12
10	F	50	1	Lower arm	MFH	2	R	38
		50	1	Lower arm	MFH	2	R	-
11	F	33	6	Upper leg	Leio	3	RR	9
		34	2	Upper leg	Leio	3	RR	6
12	M	22	1	Upper arm	Schwan	1	R	24
13	F	84	3	Lower leg	MFH	3	R	19
14	M	41	1	Lower leg	Synovio	2	RR	24
15	M	72	1	Upper leg	Schwan	2	R	26
		75	1	Upper leg	Schwan	3	RR	30
16	M	25	1	Fos cub	Lipo	3	R	14
17	F	21	10	Upper + Lower leg	Ag fibro	1	RR	9
18	M	72	3	Upper leg + Fos pop	MFH	2	RR	0
19	M	33	3	Upper leg + Fos pop	Desmo	1	RR	12
20	F	33	1	Fos pop	Desmo	1	RR	14
21	M	20	8	Upper + Lower leg	Ag fibro	1	RR	0
22	M	40	1	Fos cub	Synovio	3	R	32
23	F	54	3	Upper leg + Fos pop	Neuro	2	R	98
24	F	53	1	Upper arm	MFH	3	RR	6
25	M	72	1	Lower arm	Fibro	3	RR	93
26	M	79	1	Lower arm	Pleio	3	RR	2

Abbreviations : F = female ; M = male ; Histology : explained in tekst ; P/R = primary /recurrence ; R = recurrence

Table 2: Additional characteristics of 26 recurrent sarcoma patients treated with ILP (N=34) after radiotherapy treatment.

Pt no	type ILP	Local Toxicity (Wieberdink)	Clinical outcome	Histology (% necrosis)	Final outcome	Duration of response (months)	Limb salvage	D/A	Follow-up (months)
1	Iliac	3	NC	-	NC	-	Yes	DOD	17
2	Brach	3	PR	-	PR	2	No	A, NED	59+
3	Pop	2	NC	<50 (NC)	NC	-	No	A, NED	49+
4	Brach	2	NC	10 (NC)	NC	-	No	A, NED	52+
5	Brach	2	CR	-	CR	43	Yes	DU	56
6	Pop	2	PR	-	PR	19 (+)	Yes	DOD	19
7	Iliac	2	PR	-	PR	9	Yes	DOD	30
8	Pop	2	NC	-	NC	9			22
	Fem	2	CR	NA	CR	2	No	DOD	
9	Iliac	2	PR	-	PR	9	Yes	DOD	22
10	Brach	3	NC	-	NC	-			67+
	Brach	2	PR	>50 (PR)	PR	5	No	A, NED	
11	Iliac	2	PR	100 (CR)	CR	8			44
	Iliac	2	PR	NA	PR	17	Yes	DOD	
12	Axil	1	NC	<10 (NC)	NC	-	Yes	DOD	20
13	Pop	2	PR	>50 (PR)	PR	9	No	DU	26
14	Fem	1	PR	>50 (PR)	PR	57+	Yes	A, NED	57+
15	Iliac	1	CR	-	CR	44+			44+
	Iliac	1	PR	-	PR	10+	Yes	A, NED	10+
16	Brach	1	PR	100 (CR)	CR	12+	Yes	AWD	12+
17	Iliac	3	PR	-	PR	30+	Yes	A, NED	30+
18	Fem	1	PR	-	PR	21+	Yes	A, NED	21+
19	Iliac	2	PR	-	PR	16+	Yes	A, NED	16+
20	Fem	2	NC	NA	NC	15+	Yes	A, NED	15+
21	Iliac	3	PR	-	PR	9+	Yes	A, NED	9+
22	Brach	2	NC	<50 (NC)	NC	-	No	DOD	22
23	Iliac	1	CR	100	CR	22+	Yes	A, NED	22+
24	Axil	2	PD	-	PD	-	No	A	3+
25	Brach	1	PR	-	PR	26+	Yes	A, NED	26+
26	Brach	1	NC	>50 (PR)	PR	3	No	A, NED	14+

Abbreviations : Brach = brachial ; Axil = axillary ; Iliac = iliacal ; Fem = femoral ; Pop = poplitial ; CR = complete response ; PR = partial response ; NC = no change. PD = progressive disease ; NA = not available ; NED = no evidence of disease. DOD = died of disease ; DU = death unrelated to disease ; AWD = alive with disease

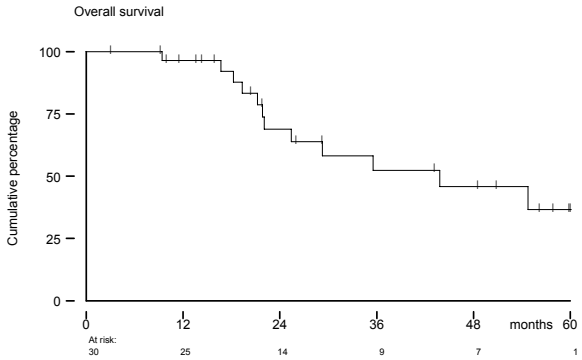

Figure 1: Overall survival of 26 patients after isolated limb perfusion for previously irradiated recurrent sarcoma
X-axis: Time (months); Y-axis: Cumulative percentage

In 17 (65%) of 26 patients, limb salvage could be achieved. The duration of response was confined to the time from ILP until the first evidence of local recurrent disease and varied from 2 to 57 months (and ongoing), with a median of 12 months (mean 17 months). The median follow-up of patients was 22 months (range 3 to >61 months, mean 29 months).

In 9 patients (11 perfusions), the tumour recurred locally after ILP: in 4 patients (4 ILPs) with a single tumour (24%) and in 5 patients (7 ILPs) with multiple tumours (54%). An amputation had to be performed to achieve local control in five patients, whereas in three patients no amputation was performed because of the poor short-term prognosis due to systemic metastases. In one patient (patient 5), a local recurrence occurred 43 months after the perfusion, and it could primarily be resected. This patient died one year later as a result of lung carcinoma. Nine patients developed systemic metastases after ILP, of which they died. Overall survival is shown in figure 1. There was no significant difference in response between low-grade or high-grade tumours. There was no correlation between tumour size and subsequent tumour response.

DISCUSSION

Our experience with 30 TNF-based ILPs in 26 patients with irresectable extremity STS recurrences in limbs previously treated with surgery in combination with high-dose radiotherapy shows that even in this patient population, limb salvage can be achieved in most patients. Moreover, limb salvage was achieved in 65% of the patients even though multiple recurrences were present in 42% of the patients. Furthermore, we have shown that TNF-based ILP in previously highly irradiated limbs is not associated with an increased local toxicity or complication rate.

In general, the management of extremity sarcomas has moved away from ablative surgical procedures toward function-preserving surgery, which is often being combined with radiotherapy. As a result, more patients now incur locally recurrent sarcoma arising in previously irradiated areas. Amputation might be the most effective treatment option in this selected group of patients with local recurrences, but although this will improve local disease control it does not affect overall survival rates [7-10]. Because amputation implies a significant decrease in quality of life, treatment modalities that guarantee preservation of the extremity and good limb function have become more important. Surgical resection of recurrent tumour in a previously irradiated field is often impossible because it usually requires the resection of all tissue exposed to a high radiation dose of 60 to 70 Gy. This can sometimes be handled by free transfer of vascularised tissue, but in most cases will require amputation.

Comparing our experience with data from the literature is difficult because comparable series of patients with recurrent STS in an irradiated field are not at hand. Moreover, 42% of patients in our series had multiple tumours, and these cases are usually not present in series that discuss the application of single uses or combinations of re-operation and/or re-irradiation of the recurrent sarcoma. Thus, comparison with data from the literature is limited to cases with single tumour recurrences after prior surgery in combination with radiotherapy.

The results after re-irradiation are reported by Essner et al. [11] in a group of 32 patients who received a second course of radiation for STS. In this group, 84% of patients showed benefit from preoperative radiotherapy in combination with subsequent surgery. Local excision of recurrent tumours followed by a second course of postoperative radiation resulted in a local failure in 8 (57%) of 14 patients and could not be recommended as a valuable therapy.

The use of external beam therapy is restricted to patients with large tumours lying at least partially outside the previous treatment volume. When STS recur in a previously irradiated area, further external beam radiation is often not possible. Here brachytherapy allows a radiotherapeutic alternative in an attempt to reduce the risk of further local recurrence [12]. Pearlstone et al. [13] reported on 26 patients who underwent resection and peri-operative brachytherapy in conjunction for recurrent STS. At a median follow-up of 16 months, they reported a 5-year local recurrence-free survival rate of 52% and a 33% disease-free survival. This experience shows that in a series with single tumour recurrences, the local control rate is still far from optimal. In addition, 15% of all patients experienced major wound complications that warranted re-operation. Another study by Nori et al. [14] describes 40 patients

treated with brachytherapy, with a 5-year local control rate of 68% and a 12.5% severe wound complication rate. Catton et al. [15] advocate combined conservative surgery with re-irradiation as the primary salvage therapy for patients who experience treatment failure with combined therapy and who are suitable for conservative re-excision. In this highly selective patient population, local control for patients treated with conservative excision without radiation was only 36%, compared with 100% for conservative surgery with re-irradiation. A very high proportion of patients (60%) experienced post-radiation complications. Obviously, this patient population, eligible for conservative surgery does not even resemble the patient population with single tumour recurrences that we have treated with TNF-based ILP, let alone the patients with multiple tumours.

Up to now, trials with systemic neo-adjuvant chemotherapy have failed to achieve any significant improvement in the survival of patients with primary or recurrent STS [16]. According to our findings, there are no data available in the literature up to now that describe the use of chemotherapy alone or in combination with the previously mentioned therapies for recurrent sarcoma in previously irradiated areas.

Regarding locoregional toxicity, no enhanced toxicity was observed as compared with TNF-based ILP in patients without prior surgery in combination with radiotherapy. In essence, TNF-based ILP has no increased regional toxicity over ILP with melphalan alone [17]. Regarding systemic toxicity, no toxicity of importance was observed in this patient population. This was the case in patients without significant leakage as well in the few patients with significant leakage during the perfusion and is in line with earlier reports on our experiences in these patients [18, 19]. This underscores our opinion that TNF-based ILP is safe and should be considered in all patients with limb-threatening tumours, irrespective of age, number of tumours or prior therapies [4, 20-22].

In conclusion, our experience clearly demonstrates that extremities should not be amputated without consideration of TNF-based ILP for limb salvage. In the described patient population with the extremely unfavourable characteristics of (multiple) limb-threatening sarcoma recurrences in an irradiated field after prior surgery and radiotherapy, the achievement of a 65% limb salvage rate clearly shows the efficacy of the TNF-based ILP approach to avoid amputations in what are often considered to be lost cases.

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CHAPTER 8

TNF-based Isolated Limb Perfusion in Unresectable Extremity Desmoid Tumours

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ABSTRACT H8

Background

Desmoid tumours are soft tissue sarcomas with local aggressive behaviour and a high rate of local recurrence after treatment. Although they do not tend to metastasise systemically, the local aggressiveness can lead to situations in which limb-preserving surgery cannot be performed without severe disability. As Isolated Limb Perfusion (ILP) with TNF and melphalan has proven to be extremely effective in the treatment of soft tissue sarcoma, we studied its potential in locally advanced extremity desmoid tumours.

Methods

Prospectively maintained database in a tertiary referral centre. Between 1991 and 2003, 12 ILP procedures were performed in 11 patients for locally advanced desmoid tumours. Local surgical therapy with preservation of limb function was impossible in all patients due to large or multifocal tumours, multiple recurrences or extensive previous treatment. Perfusions were performed with 3 to 4 mg TNF and 10 to 13 mg/L limb volume melphalan form leg and arm perfusions, respectively.

Results

Overall response rate was 75%: two complete responses were recorded (17%) and seven patients had a partial response (58%). Amputation could be avoided in all cases. Local control was obtained after 10/12 ILPs and in the other two patients through repeat ILP and systemic chemotherapy, thus leading to an overall local control rate of 100%. Local toxicity was mild and systemic toxicity was absent in all patients.

Conclusions

ILP is a very effective treatment option in the multimodality treatment of limb desmoid tumours. It should be considered in patients with aggressive and disabling disease where resection without important functional sacrifice is impossible.

INTRODUCTION

Desmoid tumours are considered to be part of the soft tissue sarcoma (STS) family, although they do not tend to metastasise. The incidence is low, with an estimated three new cases per million per year, accounting for 3% of STS. Histologically, these tumours are fibroblastic proliferations arising from fascial or muscoloaponeurotic structures with benign histopathologic characteristics. However, when we focus on the extra-abdominal desmoids, they are also known as aggressive fibromatosis, which describes the aggressive local behaviour of the tumour with infiltration of surrounding tissue leading to pain, deformity and severe disability. Surgery is the mainstay primary treatment for desmoids, but despite adequate margins, extremely high local recurrence rates are reported of up to 77% at 10 years of follow-up. This behaviour leads in some cases to situations where surgery cannot be performed with preservation of a normal function of the limb. Several therapies can then be considered, ranging from radiotherapy to systemic chemotherapy, hormone therapy or just observation. As Isolated Limb Perfusion (ILP) with Tumour Necrosis Factor-α (TNF) and melphalan has shown to be an effective treatment option in the multimodality-treatment of STS, this study was undertaken to assess the possible role of ILP in treating limb-threatening desmoid tumours.

PATIENTS AND METHODS

Patients

Between 1991 and 2003, out of 217 ILPs for soft tissue sarcoma of the limb, 12 ILPs in 11 different patients (7 females, 4 males) were performed for limb-threatening desmoid tumours. Median age of the patients at ILP was 32 years (mean 30.2 years, range 15-46 years). All patients had very large tumours, 10 ILPs were performed for recurrent disease and in seven patients the tumours were multifocal (n=3->20). In four patients, previous irradiation had taken place. Patient and tumour characteristics are listed in table 1. Local control through surgery was impossible in all cases, unless severe disabling operations or even amputations were performed.

Treatment

Patients underwent an ILP via the axillary (n=1), brachial (n=1), iliac (n=6), femoral (n=2) or popliteal (n=2) approach. ILP technique has been described previously [1, 2]. Briefly, recombinant human TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) and the cytostatic drug melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK), obtained as a sterile powder, were dissolved aseptically using

Table 1: Patient / tumour characteristics and outcome

Pt no	M/F	Age	No Tum	Site	Size (cm)	P/R/RR	PR-int (months)	Prev Treat	Type ILP	Clin Out	Res	Fin Out	Hosp Days	Adj Treat	TTLP (months)	FU (months)
1	F	38	8	Lo leg	16X12	R	33	None	Iliac	PR	Y	PR	14	None	29	98
	F	40	3	Tot leg	20X15	RR	32	None	Iliac	PR	N	PR	15	None	-	64
2	F	24	1	Up arm	5X5	P		None	Axil	PR	Y	PR	8	None	-	61
3	F	28	15	Tot leg	10X4	RR	72	None	Iliac	PR	N	PR	21	None	-	32
4	M	46	1	Ankle/foot	5X3	R	8	None	Poplit	NC	Y	NC	12	CT	6	77
5	M	15	1	Lo arm	5X4	P		None	Brach	NC	Y	NC	5	None	-	33
6	F	36	>20	Ankle/foot	14X16	RR	19	None	Poplit	CR	N	CR	17	None	-	16
7	F	21	10	Tot leg	5X15	RR	9	XRT	Iliac	PR	N	PR	7	None	-	30
8	M	33	3	Up leg/knee	20x8	RR	12	XRT	Iliac	PR	N	PR	5	None	-	16
9	F	31	1	Lo leg	8x3	RR	4	None	Fem	CR	N	CR	8	None	-	10
10	F	33	1	Knee	4x3	RR	14	XRT	Fem	NC	Y	NC	8	None	-	15
11	M	20	8	Tot leg	8x12	RR	0	XRT	Iliac	PR	N	PR	8	None	-	9

Pt no = patient number, M = male, F = female, P = primary, R = recurrent, RR = re-recurrent, PR-int = interval to recurrence, Prev Treat = previous treatment, ILP = isolated limb perfusion, Clin Out = clinical outcome, Res = resected, Fin Out = final outcome, Hosp Days = days of hospital stay, Adj treat = adjuvant treatment, TTLP = time to local progression, FU = follow-up, Lo = lower, Up = upper, XRT = radiotherapy, Axil = axillary, Brach = brachial, Iliac = iliacal, Fem = femoral, Poplit = popliteal, CR = complete response, PR = partial response, NC = no change, Y = yes, N = no, CT = chemotherapy

solvent and diluents (Burroughs Wellcome Ltd., London, UK). Isolation of the blood circuit of a limb was achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, and application of a tourniquet to compress the remaining collateral vessels. TNF was injected as a bolus into the arterial line provided limb tissue temperature had reached 38°C. Melphalan was administered after 30 minutes at limb temperatures between 38°C and 39.5°C. The administration of melphalan changed during the studied period from injection as a bolus (1991-1996) to infusion by pump over a period of 20 minutes (1996-present), because of reports that melphalan peak concentration is correlated with regional toxicity [3]. ILP consisted of a 90-minutes perfusion with 3 mg (arm) or 4 mg (leg) TNF, and a 10 mg/L (leg) or 13 mg/L (arm) volume of Melphalan at mild hyperthermia (tissue temperatures of maximally 39.5°C in the leg and 38.5°C in the arm). Median dose of Melphalan was 75 mg (mean 78.9 mg, range 25-121 mg); median dose of TNF was 4 mg (mean 3.8 mg, range 3-4 mg). In the first ILP for desmoid tumour, interferon γ (IFN) was added to the schedule according to trial prescriptions consisting of the subcutaneous injection of 0.2 mg IFN on days –2 and –1 prior to the ILP and the injection of 0.2 mg IFN during the ILP procedure into the arterial line prior to the administration of TNF. During the procedure, continuous leakage monitoring was performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit. At the end of the ILP, the limb was washed out with at least 1 L (arm) up to 4 L (iliac perfusion) of physiologic saline solution and 6% dextran 70 (Macrodex Pharmacia, Uppsala, Sweden). ILPs were performed under general anaesthesia and normally took 2.5 to 4 hours. Median hospital stay of the patients was 8 days (mean 11 days, range 5-21 days).

Response evaluation and toxicity

Clinical response evaluation was performed 2, 4, 8 and 12 weeks after ILP and thereafter every 3 months for the first year both by clinical examination and by MRI (4 to 6 and 8 to 12 weeks after ILP and hereafter every 3 to 6 months) [4, 5], and reported according to WHO-criteria. In 2 patients, histological response could be assessed by resection of the tumour remnants and in these patients response rates were adjusted if the pathological response (complete response (CR) if 100% necrosis, partial response (PR) if 50-99% necrosis and no change (NC) if <50% necrosis) differed from clinical response. New lesions or growth of the tumour at first response evaluation is reported as progressive disease (PD), and if occurring during follow-up as local progression.

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [6]: (I) no reaction, (II) slight erythema or edema, (III) considerable erythema or

Table 2: Dose and toxicity

Patient no	Leakage (%)	Local Toxicity (Wieberdink)	Limb function	Systemic toxicity	TNF dose (mg)	Melphalan dose (mg)
1	0	3		None	4	121
	9,3	2	Perfect	None	4	121
2	0	2	Perfect	None	3	35
3	0	3	Perfect	None	4	120
4	0	2	Mildly disturbed	None	4	50
5	0	2	Perfect	None	3	25
6	3	1	Mildly disturbed	None	4	35
7	0	3	Perfect	None	4	80
8	0	2	Perfect	None	4	120
9	0	2	Mildly disturbed	None	4	70
10	0	2	Perfect	None	4	60
11	0	3	No further functional loss	None	4	110

No = number, TNF = tumour necrosis factor, IFN = interferon

edema with some blistering, slightly disturbed motility permissible, (IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance, and threatening or manifest compartmental syndrome, and (V) reaction that may necessitate amputation. Systemic toxicity is reported according to WHO criteria.

RESULTS

Overall response rate of the 12 performed ILPs for desmoid tumours was 75% with 2 complete responses (17%) and seven partial responses (58%). In three patients, no response could be observed. Resection of the tumour was possible in five patients. In three patients with a single desmoid that did not respond to ILP (or insufficient for a PR score), a resection was performed accepting the functional loss that was associated with the resection in one patient (patient no. 4). No patient received adjuvant radiotherapy, neither after ILP alone nor after resection. Amputation was avoided in all patients. Partial responses usually were long lasting and no further surgical procedures were indicated in these patients as the biologic activity of the aggressive fibromatosis died out. Results of ILP are shown in table 1. Local control

of the tumour was obtained in 10/12 perfusions (83%). In 2 patients, the tumour recurred in the limb after 29 and 6 months, respectively. A second perfusion produced long-lasting local control in the first patient. The second patient received systemic chemotherapy (methotrexate and vinblastine), which led to prolonged local control as well. Thus, during a median time of follow-up of 31 months (mean 38 months, range 9-90 months), local control was maintained in all patients. Toxicity of the procedure was mild: local toxicity was limited to four patients having a Wieberdink grade III reaction. No systemic toxicity occurred. Limb function and toxicity of the procedure are listed in table 2.

DISCUSSION

The results of this study show that ILP is effective in obtaining local control in patients with locally advanced desmoid tumours. When primary resection of the tumour is impossible due to multifocality or previous therapies, excellent response on ILP can provide relief of symptoms. When the tumour is very large, adjacent to viable structures or in the vicinity of joints, ILP can provide tumour shrinkage to an extent that resection becomes a realistic option. Even a minor response, insufficient for a PR score as seen in three of the patients in the present series, can allow the surgeon to resect without severe morbidity.

It is hard to find comparable series of patients in literature, as all patients in the present study had unresectable disease. Lev-Chelouche at al. explored the use of ILP in six patients and reported a response rate of 83% [7]. The only other report specifically dealing with patients in whom primary resection was impossible, is published by Lewis et al. [8]. They followed 22 patients that had disease that was not resectable without amputation: seven patients underwent amputation, nine patients received radiotherapy and/or systemic therapy (chemotherapy, hormonal therapy, non-steroidal anti-inflammatory drugs or a combination), and six patients received no treatment at all. Of the nine patients receiving non-surgical therapy, only three (33%) showed a partial response. However, the most striking observation of this study was that none of the patients in whom no amputation was performed initially, required subsequent amputation during follow-up [8]. This finding confirmed the results of an early study by Rock et al., who followed 68 desmoid patients with observation only and found that 60 of those patients remained stable and even that a shrinkage of the tumour occurred in six patients [9]. All reports regarding response to therapy of the desmoid tumours, including the occasional reports on chemotherapy [10], hormonal therapy [11] but also the present study, should be viewed in this light.

Surgery is regarded the cornerstone in therapy for primary desmoid tumours. As disease-specific death in desmoid patients is virtually negligible, especially in limb tumours, the primary goal of therapy is obtaining local control. The prognostic factors for local control after surgery are still under debate. Complete resection of the tumour certainly is the goal at surgery, but where in some reports a positive resection margin is a prognostic factor for local recurrence [12-15], in others this is not [16-18]. The same discrepancy in literature exists regarding the prognostic value of presentation with primary versus recurrent disease on relapse-free survival. Most reports indicate multiple recurrences as a negative prognostic factor [12, 13, 17, 18], but some do not [14]. Presentation with multiple tumours seems to be of negative prognostic value, but only small series exist [12]. Adjuvant radiotherapy is recommended regardless of margin status [15], only after incomplete resection [12-14, 17, 19], or is not especially recommended as in some series the selective use of adjuvant radiation did not effect the rate of local recurrence for margin-positive nor for margin-negative resections [16, 18]. All in all, radiotherapy seems to increase the rate of local control after surgery, when selectively applied in patients with positive resection margins.

Many authors recommend radiotherapy as a primary treatment option in patients with unresectable disease [13, 17, 19]. Spear et al. reported a series of 15 patients treated with radiotherapy alone. In six of them the tumour was located in the extremity and 10 presented with recurrent disease. Although the overall local control rate was 93%, 1 patient ultimately required below-the-knee amputation [13]. Ballo et al. showed five relapses in 21 patients treated with radiotherapy alone (seven extremity, eight recurrent disease), thus a local control rate of 76%. A review by Nuyttens et al. combining the results of 22 studies including the two above-mentioned, revealed that in 102 patients treated with radiotherapy alone (all locations, 26 recurrent disease), local control rate was 78% [15]. The present study shows that ILP provides local control in 83% of a patient population with very unfavourable characteristics since only 17% presented with primary desmoids, whilst 83% presented with recurrent disease, 58% presented with multifocal tumours and 33% had received surgery and prior radiotherapy. Long-term complications of radiotherapy are fibrosis, paresthesias, edema and fracture [15, 17]. Fibrosis, paresthesias and edema are also seen in ILP and were noted in 3 patients in our study leading to mild disturbance of limb function. The main advantage of ILP compared with radiotherapy in the treatment of desmoid tumours, however, is the ability to apply different treatments after ILP. When a tumour recurs after ILP, a second ILP can provide prolonged local control as is shown in patient 1 in this study. When resection is performed after ILP and for some reason the resection margin is positive, adjuvant radiotherapy can be applied. Radiotherapy can only be applied once in sufficient dose to the same area. The impact of this

is reflected in the overall local control after multimodality treatment in this study, which reaches 100%.

In conclusion, ILP is a very effective treatment option in patients with desmoid tumours where resection without important functional sacrifice is impossible. It seems more effective than systemic treatment and has important advantages over radiotherapy. As observation is a realistic option in patients with unresectable disease, ILP should be reserved for symptomatic patients and for tumours that might be resectable after sufficient shrinkage.

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CHAPTER 9

Tumour Necrosis Factor-α-based Isolated Limb Perfusion for Soft Tissue Sarcoma

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INTRODUCTION

In the management of locally advanced extremity soft tissue sarcomas limb salvage has become all the more important in the light of evidence that amputations do not improve survival rates in patients with large (>5 cm) deep-seated high-grade sarcomas. Several studies have shown that marginal excisions with a high risk for local recurrence do not influence survival significantly [1-4]. Of the 8680 new cases of STS diagnosed in the USA each year, about 5200 occur in the extremities and tumours are often large at the time of diagnosis [5]. Treatment options for locally advanced extremity STS may consist of an amputation or a limb-sparing extensive surgical procedure followed by radiation therapy. This combination may mutilate and compromise limb function considerably. Preoperative therapies to improve limb salvage rates have been propagated. Preoperative radiotherapy alone or in combination with intra-arterial or intravenous chemotherapy has been reported to improve resectability rates of extremity soft tissue sarcomas [6-8]. Amputation may also be avoided and local control may be improved by combining a marginal resection with brachytherapy to the tumour bed [9]. Isolated limb perfusion is another strategy to deal with locally advanced soft tissue sarcomas, and can also be applied in case of multifocal primary or multiple sarcoma recurrences in limbs, thereby expanding the patient population that can successfully be treated.

TNF- BASED ISOLATED LIMB PERFUSION FOR STS

Inadequate results in STS with ILP with chemotherapeutic drugs only

In contrast to the efficacy of melphalan-based ILP in patients with multiple in-transit melanoma metastases, results with ILP with melphalan, doxorubicin and a variety of other drugs for large soft tissue sarcomas were disappointing. After studies in the seventies and eighties with poor response rates, ILP for advanced STS was largely abandoned [10-14]. Only recently, this was supported by a study from the MD Anderson Cancer Center that found no objective response and massive toxicity after ILP with doxorubicin alone [15]. The reported studies are listed in table 1.

Results with ILP with TNF + melphalan trials leading to approval of TNF

Thanks to the pioneering work of Lejeune and Lienard, this situation changed dramatically with the application of high-dose TNF in the ILP setting [16]. TNF-based ILP has been established as a highly effective new method of induction biochemotherapy in extremity soft tissue sarcomas with a 20% to 30% complete response (CR) rate and about a 50% partial response (PR) rate [17-22]. On the basis of results in a

Table 1: ILP with cytostatic drugs only for locally advanced STS

Drugs	# Pts	CR	PR	NC/PD	Limb Salvage	Year	Author	Ref #
Melphalan/Act-D/NH_2	17	0%	35%	65%	NS	1977	Krementz	[10]
Melphalan/Act-D/NH_2/Various	51	6%	12%	82%	NS	1985	Muchmore	[11]
Cisplatin	17	0%	18%	82%	NS	1988	Pommier	[12]
Melphalan/Doxorubicin	13	7%	0%	93%	61%	1989	Klaase	[13]
Doxorubicin	22	0%	74%	26%	91%	1994	Rossi	[14]
Doxorubicin	14	0%	0%	100%	25%	2004	Feig	[15]

#Pts = number of patients, CR = complete response; PR = partial response; NC = no change; PD = progressive disease; Ref# = reference number, Act-D = Actinomycin-D, NH2 = Nitrogen mustard, NS = not stated

multicenter program in Europe, TNF was approved and registered in Europe for the sarcoma indication in 1998 [22]. The European TNF/ILP assessment group evaluated 246 patients with irresectable STS enrolled in 10 years in four studies. All cases were reviewed by an independent review committee and compared with conventionally treated patients (often by amputation) of a population based Scandinavian STS database. In short: there were 246 patients with locally very advanced disease: primary sarcomas in 55%, local recurrent sarcomas in 45%, multifocal primary or multiple local recurrences in 22 %. Overt concurrent metastatic disease in 15%. Tumours >10 cm in 46%. Grade III tumours in 66%. Previous radiotherapy in 13%, chemotherapy in 15%. Patients underwent 1 ILP (222 patients) or 2 ILPs (24 patients) of 90 minutes at 39°C to 40°C with 2 to 4 mg TNF + melphalan (10-13 mg/L limb volume). The first 56 patients also received IFNγg. A delayed marginal resection of the tumour remnant was usually (76%) done 2 to 4 months after ILP. Major responses were seen in 56.5% to 82.6% of the patients, thus making resection of the sarcoma possible.

Limb salvage was achieved in 74% to 87% in these 4 studies and in 71% of the 196 patients who had been classified by the independent review committees as cases that normally could only have been managed by amputation (87%) or by functionally debilitating resection + radiotherapy (13%). Comparison with the survival curves based on a matched control study with cases from the Scandinavian Soft Tissue Sarcoma Databank showed that TNF had no negative effect on survival (p=0.96). It was concluded that the application of TNF in combination with melphalan in the setting of isolated limb perfusion represents a new and successful option in the management of irresectable locally advanced extremity soft tissue sarcomas [22] (table 2).

Confirmatory single-center studies

Lejeune et al. [23] reported a 17% CR and 64% PR rate in 22 STS patients treated for limb-threatening STS tumours, and achieved limb salvage in 77% of the patients. A

Table 2: Study reports of TNF-based ILP for Irresectable Soft Tissue Sarcomas

Drugs	# Pts	CR	PR	NC/PD	Limb Salvage	Year	Author	Ref #
TNF+Melphalan¶	8	100%[1]	0%	0%	64%	1993	Hill	[17]
TNF+IFN+Melphalan	55	18%[1]	64%[1]	18%[1]	84%	1996	Eggermont	[18]
		36%[2]	51%[2]	13%[2]				
TNF+Melphalan	10	70%[1]	20%[1]	10%[1]	89%	1996	Santinami	[19]
TNF±IFN+Melphalan	186	18%[1]	57%[1]	25%[1]	82%	1996	Eggermont	[20]
		29%[2]	53%[2]	18%[2]				
TNF±IFN+Melphalan	35	37%[2]	54%[2]	9%[2]	85%	1997	Gutman	[21]
TNF±IFN+Melphalan	246	28%[3]	48%[3]	24%[3]	76%	1999	Eggermont	[22]
	196	17%[3]	48%[3]	35%[3]	71%[4]			
TNF+Doxorubicin	20	26%[5]	64%[5]	10%[5]	85%	1999	Rossi	[27]
TNF±IFN+Melphalan	22	18%[2]	64%[2]	82%[2]	77%	2000	Lejeune	[23]
TNF±IFN+Melphalan	55	NS	NS	NS	84%	2001	Hohenberger	[24]
TNF±IFN+Melphalan	49	8%[2]	55%[2]	37%[2]	58%	2003	Noorda	[25]
TNF±IFN+Melphalan	217	18%[1]	51%[1]	31%[1]	75%	2006	Grünhagen	[26]
		26%[2]	49%[2]	25%[2]				
TNF + Melphalan[6]¶	72	49%	17%	34%	84%	2005	Bonvalot	[29]
		35%	22%	43%				
TNF+Doxorubicin¶	21	5%[1]	57%[1]	38%[1]	71%	2005	Rossi	[28]
		55%[5]	35%[5]	10%[5]				
TNF±IFN+Melphalan¶	48	38%[2]	31%[2]	29%[2]	85%	2005	Grünhagen	[30]

#Pts = number of patients, CR = complete response; PR = partial response; NC = no change; PD = progressive disease; Ref# = reference number, TNF = tumour necrosis factor, IFN = interferon gamma, ¶ = low dose (<4 mg for leg-ILP, <3 mg for arm-ILP), NS = not stated

[1] = Objective Clinical Response Rate By WHO criteria.

[2] = CR: clinical CR or 100% necrosis; PR: clinical PR or >50-99% necrosis

[3] = CR only recognized by EMEA (European Medicine Evaluation Agency) when histopathology showed 100% necrosis.

[4] = Independent committee recognised 196 patients as pure amputation candidates.

[5] = No clinical response data; CR: >90% necrosis; PR: radiological and/or histopathological >50% necrosis.

[6] = Different scoring system: upper panel: CR/PR: loss of vasculature on Ultrasound/MRI; lower panel CR: >90% necrosis on histopathology

similar limb salvage rate of 84% and excellent functional results were reported from the Berlin team regarding their experience in a series of 55 patients [24]. The Amsterdam group reported somewhat less favourable results in their experience in 49 patients (overall response rate 63%). The limb salvage rate of 57% was felt to reflect the selection of patients with particularly unfavourable characteristics [25]. We recently reported the single-center study of the Rotterdam group on STS patients, in which we found an overall response rate of 75% and were able to achieve limb salvage in 87% of the patients [26]. All in all, these studies on the overall STS population show that overall response on TM-ILP varies between 63% to 91% and limb salvage that ranges from 58% to 94%. These studies are listed in table 2.

A point of discussion that has been studied in overall populations is whether high doses of TNF (3-4 mg) are necessary or whether lower doses (1 mg) suffice. An early clinical report by Hill and co-workers suggested that low doses of TNF (up to 1 mg) were sufficient, as in eight STS patients eight complete responses were observed [17]. The small study sample, the concomitant use of high doses of corticosteroids and the fact that a different type of TNF was used did not allow for definitive conclusions. After the Italian studies on ILP with low-dose TNF in combination with doxorubicin [27, 28], the French group reported recently on their experience in 72 patients. In a randomised phase II trial, utilizing various doses of TNF ranging from 0.5 mg to 4 mg, they observed a 35% CR rate and an overall limb salvage rate of 84%. No significant differences between the various TNF dosage groups were observed [29]. Our own experience on low-dose ILP for STS confirms these data [30], although we know from our laboratory data that at very low TNF dose, all effect is lost [31]. Low-dose TNF studies are listed in table 2.

Results with TNF + doxorubicin

Very similar results have been obtained by Italian perfusion groups with the drug doxorubicin in combination with TNF. Interestingly, similar response and limb salvage rates are achieved while using lower doses of only 1 mg TNF instead of the usual doses of 2-4 mg used in combination with melphalan [27, 28] (table 2). The perfusions were performed at much higher temperatures (40°C-41°C), which is associated with higher locoregional toxicity. Grade IV locoregional toxicity was reported in 25% as opposed to only 5% in the large TNF+melphalan series [18, 22]. We found that with melphalan ILPs, grade IV toxicity was clearly related to tissue temperatures of above 39°C when melphalan was administered [32]. Therefore we have only allowed for tissue temperatures to rise to 39°C after melphalan has been added to the perfusion circuit the last eight years and have hardly seen any cases with grade IV toxicity since, without a drop in response rates [22, 26]. Most likely therefore the higher regional toxicity in the Italian experience with doxorubicin is primarily related to the hyperthermia, although doxorubicine may be responsible in part.

REPORTS ON SPECIAL PATIENT CATEGORIES

The results on TM-ILP in special STS patient categories are reported in table 3.

Patients with overt metastatic disease

Patients with overt metastatic disease and a limb-threatening tumour are a special category where tumour control can be relatively easily achieved, and thereby an

Table 3: Study reports of TNF-based ILP for special STS patient categories

Drugs	# Pts	CR	PR	NC/PD	Limb Salvage	Year	Author	Ref #
TNF±IFN+M metastatic	9	44%[2]	33%[2]	22%[2]	89%	1998	Olieman	[33]
TNF+M multiple	13	38%[2]	54%[2]	8%[2]	92%	1999	Lev-Chelouche	[37]
TNF+M desmoids	6	33%[1]	50%[1]	17%[1]	100%	1999	Lev-Chelouche	[40]
TNF+M kaposi sarcoma	5	20%[1]	80%[1]	0%[1]	80%	1999	Lev-Chelouche	[35]
TNF±IFN+M Stewart Treves	16	56%[2]	31%[2]	13%[2]	80%	2002	Lans	[36]
TNF±IFN+M >75yrs	29	38%[2]	38%[2]	76%[2]	76%	2003	Van Etten	[42]
TNF±IFN+M multiple	64	42%[2]	45%[2]	13%[2]	82%	2005	Grünhagen	[38]
TNF±IFN+M in irradiated field	29	20%[2]	50%[2]	30%[2]	65%	2005	Lans	[39]
TNF±IFN+M metastatic	37	16%[2]	68%[2]	16%[2]	97%	2006	Grünhagen	[34]
TNF±IFN+M desmoids	12	17%[2]	58%[2]	25%[2]	100%	2005	Grünhagen	[41]

#Pts = number of patients, CR = complete response; PR = partial response; NC = no change; PD = progressive disease; Ref# = reference number, TNF = tumour necrosis factor, IFN = interferon gamma, M = melphalan

[1] = Objective Clinical Response Rate By WHO criteria.

[2] = CR: clinical CR or 100% necrosis; PR: clinical PR or >50-99% necrosis

Special patient categories:

Metastatic = patients with metastatic disease at presentation

Multiple = patients with multifocal disease

Desmoids = patients with desmoid tumours / aggressive fibromatosis

Stewart Treves = patients with Stewart Treves lymphangiosarcoma

>75 = patients older than 75 years

In irradiated field = patients with tumour located in previously irradiated area

amputation avoided, by a palliative TNF-based ILP. A 77% response rate with a 89% limb salvage rate was reported in nine such cases [33], and we observed in 37 cases a 84% response rate and a 97% limb salvage rate [34]. This demonstrates that TNF-based ILP is an extremely attractive option in these patients.

Patients with multiple tumours in the extremity

Patients with multifocal primary tumours, such as Kaposi sarcomas [35], multiple lymphangio-sarcomas (Stewart-Treves Syndrome) [36], or with multiple primaries of various histologies or multiple recurrences after prior surgery [37], all present very difficult problems where TNF-based ILP is the ideal alternative to amputation. Remarkably good results have been reported. We observed after 16 ILPs in 10 patients with Stewart-Treves Syndrome a 87% reponse rate and a 80% limb salvage rate. In our overall experience with patients with multiple tumours, we observed after 64 ILPs a 77% response rate and a limb salvage rate of 82% [38]. These results indicate that TNF-based ILP is very effective in this patient population.

Patients with recurrent tumours in an irradiated field

We performed 29 ILPs in 26 such patients. In contrast to the believe that recurrent tumours in an irradiated field are unlikely to respond, we observed a response rate of 70% and a limb salvage rate of 65% [39], indicating that also in this very difficult patient category a TNF-based ILP is an attractive treatment option.

Patients with desmoid tumours and other histologies

Patients with desmoid tumours / aggressive fibromatosis often present with recurrent disease that is very difficult to treat surgically, with or without radiotherapy. Lev-Chelouche et al. and our own study both report on very similar results in six and 12 such cases. Response rates were 83% and 75 % respectively and the limb salvage rate was 100% in both reports, demonstrating the utility of the procedure [40, 41].

Elderly patients > 75 years old

A very important message is given by the report on the Rotterdam experience with 50 TNF-based ILPs in patients older than 75 years with limb-threatening tumours. Results were very favourable in the 34 perfusions for limb-threatening sarcomas, with a 38% CR and a 38% PR rate, achieving limb salvage in 76% of the patients. Equally good results were seen in 16 perfusions for bulky melanoma in-transit metastases in the elderly, as they resulted in a 75% CR and 25% PR rate. The procedure was proven safe in the elderly with the high reward of limb salvage, which is of overriding importance in this age group as amputations lead to loss of independency in the lives of the elderly [42]. Moreover, we reported on the absence of toxicity in patients without leakage and the relatively easy management and relative lack of toxicity in patients with high leakage of TNF during ILP [43, 44]. TNF-based ILPs are not associated with more regional toxicity than melphalan-based ILPs and do have less regional toxicity than doxorubicin-based ILPs [32].

TNF-based ILP active in many histologies

Since the tumour vasculature is the target of TNF and of the TNF+chemotherapy combination, it can be expected that this treatment is effective against a wide variety of tumour types as long as there is a well-developed vascular stromal component to the tumour. T.his is indeed the case. Apart from activity in some 20 different histological types of soft tissue sarcoma and activity in melanoma [45-49], the efficacy of TNF + melphalan ILP has also been demonstrated in various skin tumours [50] and bony sarcomas [51].

VASCULOTOXIC MECHANISM OF TNF+CHEMOTHERAPY

The target of TNF is the tumour-associated vasculature. This common denominator in all the well-vascularised tumours makes the use of TNF very attractive and explains its efficacy in combination with chemotherapy across all these different histologies. The selective destructive effects of TNF-ILP on tumour-associated vessels have been illustrated in previous publications by means of pre- and post perfusion angiographies [18]. Moreover, in sarcoma patients' magnetic resonance spectrometry studies we have clearly shown that the metabolic shut-down of the tumour is virtually complete within 16 hours after the perfusion, confirming the likelihood of TNF-α mediating its most important effects on the vasculature of the tumour [52]. At the histopathological level, we have also studied these intravascular effects such as thrombocyte aggregation, erythrostasis, endothelial and vascular destruction already in the early and late stages after ILP [53, 54].

NEW INSIGHTS THROUGH LABORATORY MODELS

To create further insight in the mechanisms underlying the positive results obtained with ILP in humans, we developed extremity perfusion models using the BN175 non-immunogenic fibrosarcoma in Brown Norway rats and the ROS-1 osteosarcoma in WAG rats. In both models, we could demonstrate that the tumour cells were resistant to TNF in vitro and that ILP in vivo with TNF alone had no major impact on tumour growth. In both models, a strong synergistic anti-tumour effect leading to CRs in some 60-70 % was observed after ILP with TNF+melphalan [55, 56]. TNF alone only caused some central necrosis and no regression of the tumour was observed as has been reported for the clinical setting as well. Histopathologically haemorrhagic necrosis was most prominent after ILP with both drugs. Early endothelial damage and platelet aggregation in the tumour vessels are observed after ILP with TNF + melphalan and this is believed to lead to ischemic (coagulative) necrosis, which is in line with observations in patients. Our observations confirm that TNF-α has its major effect on larger tumours, with well-developed vasculature in contrast to small tumours (diameter < 3 mm) that lack a developed capillary bed. TNF may exert its effect mainly through the neovasculature of the tumour, which is more abundant in large tumours. Moreover, there are distinct similarities between tumour stroma generation and wound healing and observations by us that sites other than the tumour (recent wounds or skin overlying tumours only when invaded by tumour), which undergo angiogenesis, also become necrotic after ILP with TNF + melphalan, but not after ILP with melphalan alone.

We have demonstrated a number of crucial elements in our rat tumour models identifying the mechanisms for the strong synergy between TNF and cytostatic drugs in ILP and have identified the prerequisites for an effective ILP:

Tumour vessel destruction

The vasculotoxic effects of the combination of TNF + melphalan leading to haemorrhagic and anoxic coagulative necrosis as described above.

Enhanced drug uptake by the tumour

We have recently demonstrated that the addition of high dose TNF to the perfusate results in a four- to sixfold increased uptake by the tumour of the cytostatic drug. For melphalan and for doxorubicin is was demonstrated that this uptake was tumour specific and that no increased uptake was noted in the normal tissues, thus emphasizing the relatively selective action of TNF on the tumour-associated vasculature [57]. This increase in concentration was also observed with doxorubicin [58], but not in its liposomal form [59]. Moreover, we have demonstrated that the effect correlates with the vascularity of the tumour. The more vascular the tumour, the better the synergistic effect between TNF and the chemotherapeutic agent [60]. Whether a TNF-mediated drop in interstitial pressure [61] in the tumour plays a role in this mechanism remains speculative.

Role of Leukocytes

We have shown that leukocytes play also an important role in the TNF-mediated anti-tumour effects. In rats that underwent total body irradiation and underwent an ILP at the time of absolute leukopenia, the anti-tumour effect of an ILP with TNF+melphalan was very similar to the effects of a perfusion with melphalan alone. In the leukopenic rat the TNF-effect was lost and the synergy between TNF and melphalan was no longer observed [62].

Dose range for TNF

We demonstrated that 10 micrograms of TNF (a fivefold reduction of the "standard dose of 50 microgram" was the threshold dose for activity of TNF in our rat tumour extremity perfusion model. At 2 micrograms, all TNF-effects were lost [31]. This finding would suggest that also in the clinical setting dose reduction without loss of activity could be explored as has also been suggested by the clinical results of various studies [17, 27-30].

Duration of ILP

As the pharmacokinetics of melphalan demonstrates that almost all melphalan uptake occurs over 20-30 minutes, the minimal duration for an effective ILP should be 30 minutes. Shorter perfusion times are associated with a drop in CR and PR rates whereas longer than 30 minutes ILPs do not seem to further improve the results [31].

Mild Hyperthermia

Temperatures of 38°C to 39°C were shown to be essential for obtaining a good anti-tumour response without damage to the normal tissues in the limb. True hyperthermia (42°C-43°C) resulted in an increase of CRs but was associated with very severe damage to the normal tissues. All anti-tumour efficacy was lost when perfusions were performed at room temperature [31].

Hypoxia

We demonstrated that hypoxia could enhance the anti-tumour effects of an ILP with either TNF alone or melphalan alone. Hypoxia did not further enhance the anti-tumour efficacy of an ILP with TNF+melphalan as the synergy between these two agents "overrided" any minor enhancement mediated by hypoxia [31].

Interferon-gamma

In spite of many reports of the synergy between IFN-gamma and TNF both in vitro as well as in vivo in murine tumour models, the role of IFN-gamma was not very strong in our rat models. We demonstrated that about a 10% increase in CR rate and an increase of about 20% in overall response rate was observed in our animal models [63], which resembles the situation in the clinic [49].

Idiosyncratic toxicity

Interestingly unexpected interactions may lead to idiosyncratic reactions between TNF and certain cytostatic agents. Actinomycin D is commonly used in combination with melphalan in the clinical setting. When investigating whether TNF would enhance the efficacy of actinomycin D, we discovered that it did in an idiosyncratic and nondiscriminative way. The combination was more effective against the tumour than TNF+melphalan, but this advantage was annulled by the toxicity of TNF+actinomycin D to the normal tissues, resulting in the amputation of all extremities in these animal models. We sent out a strong warning to the clinic not to use TNF in combination with actinomycin D [64].

Vasoactive drugs

Various vasoactive drugs have been and are being studied in our laboratory models. Nitric Oxide (NO) is an important molecule in the maintenance of both vascular tone and the integrity of the vascular wall and is highly produced in experimental and human tumours. We postulated that its inhibition could lead to hypoxia and an enhancement of the TNF-induced early vascular effects in the tumour. In our ILP BN175 rat model, we performed a response study with TNF in combination with the arginine analogues L-NAME and LNA, which inhibit NO synthase. In rats treated with TNF combined with L-NAME/LNA, important and immediate anti-tumour effects were observed and necrosis of the skin occurred at the tumour site. These effects are normally only observed when hypoxia or melphalan are added to TNF as described above. Typical TNF tumour response was observed when NO synthase was inhibited during ILP [65]. Other vasoactive drugs are histamine and IL-2. Both these drugs have shown to have a clear synergy with melphalan in our tumour models [66, 67]. These findings show the importance of agents that can change the pathophysiology of tumour vasculature and rheologic conditions and thereby can improve drug uptake in tumours. Moreover, this underlines the importance of investigating how to modulate tumour physiology and the potential that this approach has to improve efficacy of various standard agents.

CONCLUSIONS

Isolated limb perfusion methodology provides us an excellent tool in the clinic to obtain local control and to avoid amputations of limbs in patients with limb-threatening tumours. This has been largely achieved by the success of the antivascular TNF-based biochemotherapy in this setting. TNF, for the first time, has brought us an effective treatment against large, bulky tumours.

Moreover, isolated limb perfusion is a albeit somewhat exotic, but very interesting research model to develop and study agents that modify the pathophysiology of large tumours that blocks effective penetration of cytotoxic drugs into the tumour. We can now manipulate and study the tumour vascular bed in ways that will identify "new" drugs that can enhance the activity of "old" drugs. Moreover, it has proven to be a model system that may also facilitate the development of vector-mediated gene therapy and other innovative approaches.

Much of these developments have been initiated by the application of TNF in this setting. TNF-based isolated limb perfusion is a very successful treatment option to

achieve limb salvage in the management of advanced, multiple or drug-resistant extremity tumours. TNF-based ILPs are now performed in some 30 cancer centers in Europe with referral programs for limb salvage. TNF-based antivascular therapy of cancer is here to stay and its potential needs to be studied further [68]. Other drugs will follow and we may well learn through this model how to use them systemically more effectively as well.

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PART III

General Questions in Isolated Limb Perfusion

CHAPTER 10

TNF Dose Reduction in Isolated Limb Perfusion

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ABSTRACT

Background

Isolated Limb Perfusion with TNF and melphalan (TM-ILP) is highly effective in the local treatment of advanced sarcoma and melanoma of the limb. The optimal dose of TNF for this procedure is not well established. The aim of this study was to assess the efficacy and toxicity of TM-ILPs with reduced TNF dose.

Methods

Largest single institution prospective database on TNF-based ILP. Out of 339 TM-ILPs performed between 1991 and 2003, 64 procedures were performed with reduced TNF dose (<3 mg in arm perfusions, <4 mg in leg perfusions). Response rates and toxicity of the procedure and outcome of the patients are evaluated.

Results

Complete response in melanoma patients after reduced-dose ILP was 75% vs. 69% after standard-dose ILPs (overall response 94% vs. 95% respectively); overall response in non-melanoma patients was 69% (reduced) vs. 74% (standard). Response rates and outcome were comparable with the procedures performed with standard-dose TNF (p=NS for response, local/systemic progression and survival after multivariate analysis, both in melanoma and in non-melanoma patients). Systemic and local toxicity did not differ statistically between reduced- and standard dose TM-ILPs.

Conclusions

Provided doses at 1 mg or higher are used, TM-ILP with TNF dose reduction for both melanoma and non-melanoma patients seems to be as effective as the standard dose procedure in terms of response rate and patient outcome. Numbers to formally confirm or reject this hypothesis are too large for such a non-inferiority trial to be conducted in patients with these rare conditions.

INTRODUCTION

The use of Isolated Limb Perfusion (ILP) in the treatment of patients with extensive melanoma in-transit metastases and of patients with limb-threatening soft tissue sarcoma, has earned more and more appreciation in the last decade. Although the technique of ILP was described already in 1958 by Creech and co-workers [1] and used with great success in melanoma patients, patients with larger tumours (especially soft tissue sarcomas) did not profit from this technique because of poor and inhomogeneous drug uptake in these tumours. A breakthrough for this patient category came in the early 1990s when Tumour Necrosis Factor-α (TNF) was introduced as co-drug for ILP [2]. Excellent response rates could be obtained by this two-drug regimen [3]. This led to the approval of TNF in Europe for the treatment of irresectable extremity soft tissue sarcoma [4]. The use of TNF has some major implications for the centers in which ILP with TNF and melphalan (TM-ILP) is performed: the administration of TNF can lead to potentially lethal toxicity if there is important leakage of TNF to the systemic circulation. Moreover, the high cost of TNF and the fact that TNF is not commercially available worldwide, makes the procedure even more delicate. The approved dose of TNF used in the ILP procedure is 3 mg for ILP of the arm and 4 mg for ILP of the leg. As the experience with melphalan revealed that in regional application using ILP, drug concentrations 10 times higher than systemically tolerated were well tolerated in the limb, this same proportional translation was made for TNF [2]. This translation is not necessarily correct, as TNF in the ILP setting is known to be an enhancer of the melphalan effect (by immediate increased melphalan uptake in the tumour [5] and by eradication of the tumour associated vasculature) rather than having a direct anti-tumour effect itself. The optimal TNF dose in ILP for the synergistic effect with melphalan has never been properly determined. However, both preclinical [6] and clinical studies [7-9] suggest that TNF dose reduction to 1 mg might be as effective as the now standardly used TNF dose of 3 mg for arm- and 4 mg for leg-ILPs.

As in our institution over 300 TNF-based ILPs were performed since 1991, we retrospectively studied our procedures in order to assess whether ILPs performed with reduced dose TNF are comparable to the standard dose ILPs in terms of response to ILP, clinical outcome and local and systemic toxicity.

PATIENTS AND METHODS

Patients

Between 1991 and 2003, 339 TNF and Melphalan (TM)-ILPs were performed in patients with multiple in-transit melanoma metastases (*n*=99), soft tissue sarcomas (*n*=217) or other tumours (*n*=23) in the limb. Sixty-four ILPs were performed with reduced dose (<4 mg TNF for leg-ILPs, <3mg TNF for arm-ILPs). Dose reduction was applied in a non-randomised manner. Reasons for dose reduction were leakage problems prior to the administration of TNF especially when this happened in frail elderly patients with a cardiovascular compromised status. Moreover, the knowledge of the Italian experience with 1 mg TNF in combination with doxorubicin [10, 11] and the observations on the lack of impact of a four-fold TNF dose-reduction in our laboratory [6] influenced our attitude. Demographic data, disease presentation at time of ILP and ILP characteristics were obtained from a prospectively maintained database.

Treatment

All patients underwent an ILP of the upper (*n*=74) or lower (*n*=265) limb. The method of ILP is described in detail previously [3, 4]. In short: isolation of the limb is achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, ligation of collateral vessels and application of a tourniquet proximal to the site of perfusion. Once tissue temperature has reached 38°C, recombinant TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) is administered via the arterial line. Tissue temperatures are stabilized between 38-39.5°C and leakage monitoring is performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit [12]. After 10-30 minutes, Melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) is added to the perfusate in a dose of 10 mg/L for leg- and 13 mg/L for arm perfusions. The administration of Melphalan changed during the studied period from injection as a bolus (1991-1996) to infusion by pump over a period of 20 minutes (1996-present), because of reports that melphalan peak concentration is correlated with regional toxicity [13]. In the first 50 ILPs, performed between 1991 and 1994, interferon γ (IFN) was added to the schedule according to trial prescriptions. This consisted of the subcutaneous injection of 0.2 mg IFN on days –2 and –1 prior to the ILP and the injection of 0.2 mg IFN during the ILP procedure into the arterial line prior to the administration of TNF. At the end of the perfusion period, a washout procedure using 2-4 litres of a dextrane and/or electrolyte solution is performed. In patients undergoing an iliac perfusion for melanoma, an iliac lymph node dissection is performed; an axillary lymph node dissection is performed in patients undergoing

an axillary ILP. In melanoma patients with palpable nodal disease in the groin, an ilio-inguinal lymph node dissection is performed in the same operative session as the ILP but prior to executing the ILP.

Evaluation of response and toxicity

Clinical response evaluation was performed 2 to 4 weeks and 8 weeks after ILP by clinical examination, and after that at 3 months regular intervals for the first 2 years and a longer intervals hereafter. For non-melanoma ILPs, response evaluation by MRI was performed after 4 to 6 and 8 to 12 weeks after ILP, and thereafter every 3-6 months. Response rates were reported according to World Health Organisation (WHO) criteria [14]. Histological response could be obtained in 136 non-melanoma ILPs either by resection of the tumour remnant or by histological biopsy. In these patients, final outcome was adjusted if the pathological response (necrosis percentage: complete response (CR) if 100% necrosis, partial response (PR) if 50-99% necrosis, no change (NC) if <50% necrosis) differed from the clinical response. Recurrence of tumour within the extremity after a CR, or progression of the lesions and the appearance of new lesions after a PR or after NC, is reported as local progression.

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [15]: (I) no reaction, (II) slight erythema or edema, (III) considerable erythema or edema with some blistering, slightly disturbed motility permissible, (IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance, and threatening or manifest compartmental syndrome, and (V) reaction that may necessitate amputation. As Wieberdink grade I-II reactions are completely reversible local reactions with no implications on long-term outcome [16], and grade IV-V normally require surgical intervention in the postoperative period, these grades of local toxicity are combined for analysis.

Systemic toxicity is reported if any grade of neurogenic, hepatic, renal or haematologic toxicity as defined by the WHO [14] occurred, or if a drop in vascular resistance necessitated the use of vasopressors.

Statistical evaluation

Survival estimates of overall survival (OS), time to local progression (TTLP) and time to systemic progression (TTSP) were made using the method of Kaplan and Meier. We evaluated the prognostic value of some baseline factors for these three endpoints (TTLP, TTSP and OS) using the log-rank test. Because demographics were not balanced between the reduced dose and the normal dose group, a multivariate model using Cox regression was designed. This model includes for melanoma patients,

besides TNF dose, MD Anderson stage of disease, size of the largest lesion and age of the patient (known to be the prognostic factors for survival in ILP treatment for melanoma [17]). For non-melanoma patients, we included age of the patient, size of the tumour, recurrent disease at presentation and resection after ILP in the multivariate model [4, 18]. Difference between two binary variables was tested using the Fisher's exact test. Furthermore, a logistic regression was performed to evaluate the effect of dose reduction on toxicity with adjustment for diagnose and gender of the patient [19]. All tests were done at a significance level of 5%.

RESULTS

Sixty-four TM-ILPs with reduced TNF dose were performed in melanoma (*n*=16) or non-melanoma (*n*=48) patients. Median age of the patients treated with reduced dose ILP was 64 years (range 12-91 years); patients with normal dose ILP had a median age of 55 years (range 15-84 years, p=0.011). All patients were amputation candidates. Patient and tumour characteristics are listed in table 1.

Response rate and outcome

Melanoma patients

In 16 ILPs with reduced TNF dose in melanoma patients, CR rate was 75% versus 69% for ILPs with traditional dose (p=0.770). There was no statistical difference between 5-year local (30% vs. 30%), or 5-year systemic recurrence-free survival (22% vs. 29%) between the reduced dose and the normal dose ILPs. Overall 5-year survival rate was 16% for reduced dose patients versus 36% for patients treated with

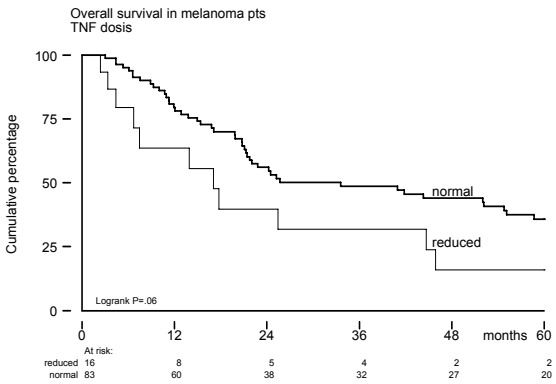

Figure 1: Overall survival in melanoma patients, reduced versus normal TNF dose
X-axis: Time (months); Y-axis: Cumulative percentage

Table 1

Patient and tumour characteristics						
		Melanoma			Non-melanoma	
		reduced n=16	normal n=83		reduced n=48	normal n=192
Gender	**F**	11	58		26	93
	M	5	25		22	99
Age	**≤ 60**	3	43		25	119
	>60	13	40		23	73
Site	**Arm**	1	4		13	56
	Leg	15	79		35	136
No of lesions	**1**	0	0		29	143
	2 - 10	6	37		9	31
	>10	10	46		10	18
Size largest lesion	**≤ 40 mm**	3	44			
	>40 mm	13	39			
Grade				**1**	7	32
				2	11	51
				3	24	101
				NA	6	8
Stage	**IIIA**	7	37	**Primary**	26	86
	IIIAB	7	34	**Recurrent**	16	74
	IV	2	12	**Metastasized**	6	32

standard dosage (hazard ratio 1.83, p=0.056) (table 2, figure 1). In multivariate analysis including MD Anderson stage, age of the patient and size of the largest lesion, the hazard ratio of OS of patients treated with TNF dose reduction with respect to standard dose patients was 1.42 (p=0.336), indicating that the shift in unfavourable prognostic characteristics in the low dose group is so important that the effect of dose is not an independent factor by itself and that the true p-value for dose effect is non-significant (p=0.336).

Non-melanoma patients

The response rate of 48 ILPs with dose reduction for non-melanoma patients was 38% CR, 31% PR, 25% NC, 4% PD and was not assessed in 1 patient. This differed from the response rates in normal dose ILPs, as the CR and PR rate in the latter group was 21% and 53% respectively (p=0.022). Overall response rate (CR+PR) did not differ statistically between the groups (p=0.468). Limb function could be assessed in 43/48 treated limbs; amputation could not be avoided in seven cases. No statistical difference could be detected between patients treated with normal and reduced

Table 2

Effect of TNF dose reduction on Response Rate and Outcome							
		Melanoma		p	Non-melanoma		p
		reduced n=16	normal n=83		reduced n=48	normal n=192	
Response	CR	12	57		18	41	
	PR	3	22		15	101	
	NC	1	4		12	46	
	PD				2	3	
	NA			**0,80**	1	1	**0,02**
TTLP	5-yrs	30%	30%	**0,94**	44%	59%	**0,27**
TTSP	5-yrs	22%	29%	**0,32**	45%	50%	**0,58**
Survival	5-yrs	16%	36%	**0,06**	36%	47%	**0,69**

CR = complete response, PR = partial response, NC = no change, PD = progressive disease, NA = not available, TTLP = time to local progression, TTST = time to systemic progression

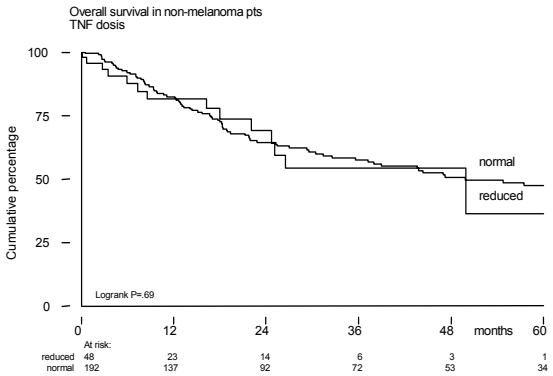

Figure 2: Overall survival in non-melanoma patients, reduced versus normal TNF dose
X-axis: Time (months); Y-axis: Cumulative percentage

dose TNF in terms of TTLP, TTSP or OS (table 2, figure 2). Adjustment for known prognostic factors for survival showed that although size of the tumour (HR=1.6 for tumours >10cm, HR=3.0 for tumours >20 cm), tumour recurrence at time of presentation (HR=2.2 for recurrent tumours, HR=0.45 for re-recurrent tumours) and resection of the tumour remnant after ILP (HR 0.58) all are significantly contributive, this had no influence on the lack of effect of TNF dose reduction on survival of non-melanoma patients in the multivariate model (HR=1.27, p=0.423).

Leakage and Toxicity

In 339 ILPs, 84 procedures had systemic leakage of 1% to 64%. Twenty-five ILPs were performed with systemic leakage between 10% and 20% without leading to severe

Table 3: Systemic and local toxicity

Systemic toxicity			
		Reduced (N=64)	**Normal (N=275)**
		N	**N**
Neurol	absent	61	255
	grade 1	2	18
	grade 2	1	1
	grade 3		1
Liver	absent	64	271
	grade 3-4		4
Renal	absent	64	274
	grade 3-4		1
Haem	absent	64	272
	grade 3-4		3
Shock	absent	63	272
	present	1	3
P-value		**0.62**	
Local toxicity			
Wieberdink	1-2	54	202
	3	8	64
	4-5	2	9
P-value		**0.14**	

Neurol = neurologic, Haem = haematologic

systemic toxicity. Six procedures were complicated by systemic leakage of >20% (21-23-25-29-34-64%). The procedure with 64% leakage was discontinued after 35 minutes, just after administration of the melphalan because the tourniquet snapped. The other 5 ILPs were terminated when the accumulative leakage passed 20%, but melphalan circulation time was at least 45 minutes in all procedures. No systemic toxicity could be detected in patients with these high-leakage ILPs. There was no difference in systemic toxicity between the normal and reduced dose ILPs (p=0.622). Local toxicity in 339 ILPs according to Wieberdink was grade I - II in 76%, III in 21% and IV -V in 3%. Overall, the Fisher's exact test for comparison between groups revealed a borderline difference in local toxicity in favour of the reduced dose procedures compared with the normal dose ILPs (p=0.135, table 3), which remained after adjustment for gender of the patient (odds ratio 2.0, p=0.065).

DISCUSSION

This retrospective non-randomised study of 64 ILPs performed with TNF dose reduction shows that both in melanoma and in non-melanoma patients, overall response rates are not affected. Toxicity of TM-ILPs in normal dose procedures is mild and easily manageable in virtually all cases and is not further reduced by the use of lower dose TNF.

Response

The use of TNF in melanoma ILPs is favoured in Europe since 1994, as a multicenter report on TM-ILPs showed a significant increase in CR rate up to 90% compared to the 52% CR rate in these 4 centers for melphalan-only ILPs [20]. Especially in bulky melanoma in-transit metastases, the use of TNF seems of extra value [21]. The presently reported CR rate of 75% for reduced-dose ILP in melanoma patients with bulky disease compares both to our own data on normal-dose ILPs and to data from the literature [17, 22, 23]. Importantly, it is far better than the reported CR rates for melphalan-only ILPs [24], indicating that the additional effect of TNF is still present at lower dose. These observations are sustained by the recent report of Rossi and co-workers, who find a CR rate of 70% in 20 melanoma patients treated with 1 mg TNF [8]. In non-melanoma patients with extensive limb disease unsuitable for resection, TM-ILP has proven to render excellent overall response and high limb salvage rates [3, 25, 26]. The overall response rate of 69% in this study is in line with these results and with the limited data available on low-dose TM-ILPs [7, 11]. The relatively high CR rate in the reduced dose population is partly explained by the fact that 5/16 ILPs for Stewart-Treves lymphangiosarcoma and 5/7 ILPs for Kaposi sarcoma, both tumours known to have better responses on ILP than other STS [27, 28], were performed with low-dose TNF. Reduction of the TNF dose in both melanoma- and non-melanoma-ILPs does not seem to have negative impact on response rates.

Toxicity

Systemic toxicity in a locoregional treatment procedure as ILP is, is determined by the amount of systemic leakage of the (bio-)chemotherapeutics to the systemic circulation. Although systemic leakage of melphalan should be avoided (systemic side effects consist of immediate postoperative nausea and vomiting and occasionally transient bone marrow depression [29]), systemic toxicity attributable to melphalan is often limited. Typical TNF related systemic toxicity consists of hypotension, fever and chills ("shock-like syndrome") [30] and occasionally of transient leukopenia, hepatobiliary or renal toxicity, or pulmonary complications. Because of these serious side effects at low dose already (maximum tolerated dose ± 400 μg/m^2 [31, 32]), systemic ad-

ministration of TNF has been abandoned. Leakage of this cytokine should therefore be minimized by adequate isolation of the limb and a thorough washout procedure, and should be carefully monitored during the procedure. Dose reduction of TNF in the ILP setting will only have an effect on systemic toxicity in case of significant leakage. At present, however, the ILP technique is capable of providing leakage-free procedures. Moreover, adequate fluid management in the immediate postoperative period shows to prevent TNF-related hypotension and makes vasopressor support virtually unnecessary. Systemic toxicity of TNF-ILP is therefore not essentially different from melphalan-ILPs [33], even in high-leakage ILPs [30], and is not likely to be further reduced by TNF dose reduction, as is confirmed by the data of the present study.

Local toxicity in the total of 339 ILPs performed in our institute was mild to moderate in virtually all cases, with only 2 treatment-related amputations. One case of Wieberdink V local toxicity necessitating an amputation concerned a patient with melanoma in-transit metastases of the lower leg who developed extensive rhabdomyolysis of the upper leg after a 4 mg TNF-ILP at the iliac level. The other patient underwent an ILP for Ewing sarcoma in the upper leg 10 days after systemic chemotherapy (DIME, 6th cycle) and developed extensive necrosis of the whole leg, most probably due to systemic vascular damage caused by the systemic chemotherapy. Whether TNF increases local toxicity compared to a melphalan-only ILP is still under debate. Initial reports explicitly state that no increased local toxicity was observed in a triple drug (TNF, melphalan, IFN) regimen [2, 34]. However, in a TNF dose escalation study with TNF doses up to 6 mg, Fraker et al. report that local toxicity was significantly related with TNF dose [23]. The authors speculate that the reason for this observation is the increased uptake of melphalan in the tissue, as experimental ILPs with TNF only revealed no local toxicity at all. The one study specifically addressing the local toxicity of TM-ILP, showed a significant increase of local toxicity with TNF compared to melphalan-only ILPs [19]. The confounding factor here however was that the patients in that study participating in the TNF-ILP protocol were, due to trial prescriptions, exposed to higher temperatures during melphalan circulation. This difference might in part be the explanation of the observed difference in toxicity. Second, the local toxicity as a whole was mild to moderate and no grade IV-V toxicity was recorded. These results compare with the data shown in the present study: local toxicity was higher in the standard dose ILPs compared to the ILPs performed with TNF dose reduction, although not statistically significant.

Optimal dose

The standard dose of TNF used in the ILP setting (4 mg for the leg, 3 mg for the arm) was originally chosen by Lienard and Lejeune based on the experience with melphalan that a 10-fold increase of the systemically tolerable dose could be safely used [2]. Ever since, the only clinical study about the TNF dose was the dose escalation study by Fraker et al. using 4 to 6 mg of TNF, showing no increased response but dose limiting local toxicity [23]. A dose finding study for TNF is never reported in humans, but there are some studies reporting excellent results with lower dose TNF [7-9], even for TNF doses as low as 125 µg [7]. A dose finding study performed in a rat model at our institution revealed that the TNF dose could be reduced five- to tenfold compared to the standard dose used in the rat without affecting the response rates [6]. However, further reduction of the TNF dose caused complete loss of the synergy of TNF and melphalan [6]. The present study was not designed to determine the optimal TNF dose, but the results confirm the conclusions of the above-mentioned studies that the standard dose of TNF used might be higher than strictly necessary. To definitely establish the optimal dose, a non-inferiority study would be the most appropriate, although we realize that due to the already low toxicity of ILP, this study would require such accrual that it will never be performed.

In conclusion, the ILP procedures performed in our institution with TNF dose reduction for both melanoma and non-melanoma patients seem to be as effective as the standard dose procedures. Each milligram of TNF reduced implies a cost reduction of €2300 for the entire procedure. We therefore state that the standard dose of 4 mg TNF for the leg and 3 mg for the arm might be higher than necessary for achieving the maximal synergy between TNF and melphalan. Lowering TNF dose reduces the costs of the ILP procedure and seems to affect local toxicity and is therefore preferable. Although the effect of lowering TNF dose on the survival of patients could not be fully addressed due to the non-randomised setting of this study, these data suggest that OS is not inferior to the survival rate of patients receiving normal dose TNF.

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CHAPTER 11

Palliative value of TNF-based Isolated Limb Perfusion in Metastatic Sarcoma and Melanoma Patients

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ABSTRACT

Background

Both patients with soft tissue sarcoma (STS) and patients with melanoma have limited treatment possibilities once the tumour has metastasised systemically. In patients with extremity STS or bulky melanoma in-transit metastases, the local tumour burden may be so problematic that, even in patients with systemically metastasised disease, an amputation may be inevitable. Isolated Limb Perfusion (ILP) has proven to be an excellent, local, limb-saving treatment option in patients with locally advanced extremity tumours. In this study, the authors investigated the palliative value of the ILP procedure to avoid amputation in patients who had stage IV STS and melanoma.

Methods

From 1991 to 203, of 339 tumour necrosis factor-α (TNF)-based ILPs, 51 procedures were performed for either stage IV STS (*n*=37 patients) or stage IV melanoma (*n*=14 patients). All patients underwent an ILP with TNF and melphalan of the upper (*n*=4 patients) or lower limb (*n*=47 patients) with 26 to 140 mg melphalan and 2 to 4 mg TNF.

Results

The overall response in patients with stage IV STS was 84%, and their median survival was 12 months after ILP. Limb salvage could be achieved in 36 of 37 patients, with 1 patient undergoing amputation due to treatment toxicity. In the patients with stage IV melanoma, the complete response rate was 43%. All melanoma patients preserved their limb during a median survival of 7 months.

Conclusions

TNF-based ILP is an excellent procedure that provided tumour control and limb salvage for the short survival of patients with metastasised, very bulky, limb-threatening tumours of the extremity.

INTRODUCTION

Soft Tissue Sarcoma (STS) has a high mortality rate of up to 50% due to the propensity of the tumour to develop distant metastases [1]. Patients with metastatic sarcoma (TNM stage IV) have a median survival of only 1,5 years [1, 2] and survival rates have not improved in the last decades [3]. For patients with non-metastasised STS, standard therapy consists of surgical resection of the tumour, often combined with radiotherapy. When large or multiple tumours make this treatment option impossible, Isolated Limb Perfusion (ILP) with Tumour Necrosis Factor-α (TNF) and melphalan (TM-ILP) has proven to be effective as a limb-salving therapeutic option [4-6].

Patients with distant melanoma metastases (stage IV) have very poor survival expectations, with a median survival that barely exceeds 1 year. In-transit metastases in the extremities occur in 5-8% of patients with high-risk melanoma, and these patients cannot be treated surgically. ILP has shown to be an excellent treatment option in this patient category [7, 8].

A minority of patients with stage IV STS or patients with stage IV melanoma may present with combined problems of systemic metastases and locally very advanced tumour (recurrences) or in-transit metastases. The local situation may be disabling and very difficult to handle because of the poor sensitivity of these tumours to systemic therapies. Radiotherapy options also may be absent or very limited in these patients because of prior treatments, the tumour size and the multiplicity of tumours in the extremity. In general, we offer these patients with severe local disability and no other palliative treatment options a TNF-based ILP. This study was undertaken to evaluate the value of the TM-ILP procedure in patients with stage IV STS and melanoma with a short life expectancy.

PATIENTS AND METHODS

Patients

Of 217 TM-ILPs for STS performed in the Daniel den Hoed Cancer Center between 1991 and July 2003, 37 patients were treated with systemically metastasised extremity STS. During the same period, 14 patients with American Joint Committee on Cancer / The University of Texas M. D. Anderson stage IV melanoma (distant metastases present) underwent an ILP for local control of disease in the limb. There were 14 females and 23 males in the STS group with a median age of 55 years (range 17-86 years), and the melanoma group consisted of 5 females and 9 males with a median

Table 1: Histological classification of 51 TM-ILPs for metastasised tumours

Histology	N
Synovial sarcoma	7
MFH / High grade pleiomorf sarcoma	5
Osteosarcoma	4
Liposarcoma	4
Clear-cell sarcoma	4
(Extraskeletal) Ewing sarcoma	3
Hemangiopericytoma	2
Other STS	8
Melanoma	14

MFH = malignant fibrous histiocytoma, STS = soft tissue sarcoma

Table 2: Patient and tumour characteristics of 51 TM-ILPs for metastasised tumours

Patient and tumour characteristics of 51 TM-ILPs for metastasised tumours					
		Stage IV sarcoma N=37	%	Stage IV melanoma N=14	%
Gender	F	14	38%	5	36%
	M	23	62%	9	64%
Age	≤60	24	65%	7	50%
	>60	13	35%	7	50%
Site	Arm	4	11%	0	0%
	Leg	33	89%	14	100%
Primary/Locally recurrent	Primary	18	49%		
	(Re)recurrent	19	51%		
Previous treatment*	None	16	43%	6	43%
	XRT	7	19%	3	21%
	CT	17	46%	4	29%
	ILP	2	5%	3	21%

* = 5 STS and 1 melanoma patients received both XRT and CT, 1 melanoma patient received both XRT and prior ILP.
F = female, M = male, XRT = radiotherapy, CT = chemotherapy, ILP = isolated limb perfusion

age of 61 years (range 25-78 years). Histological classification of the tumours is summarized in table 1. All patients with STS were candidates for amputation, because primary surgical resection was impossible either because of the size and location or because of the multifocality of the tumours. All patients with melanoma had large and multiple tumours in the limb (the size and number of the lesions prevented primary surgery), which caused severe local discomfort. In total, 51 patients underwent ILP for metastasised tumours. The patient and tumour characteristics are summarized in table 2.

Treatment

Patients underwent an ILP via the axillary (*n*=1), brachial (*n*=3), iliac (*n*=29), femoral (*n*=16) or popliteal (*n*=2) approach. The ILP technique has been described previously [5,9]. Briefly: recombinant human TNF (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) and the cytostatic drug melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK), which was obtained as a sterile powder, were dissolved aseptically using solvent and diluents (Burroughs Wellcome Ltd., London, UK). ILPs were performed under general anaesthesia and normally took from 2.5 hours to 4.0 hours. Isolation of the blood circuit of a limb was achieved by clamping and canulation of the major artery and vein, connection to an oxygenated extracorporeal circuit, and application of a tourniquet to compress the remaining collateral vessels. ILP consisted of a 90-minutes perfusion with 1 to 3 mg TNF (arm) or 3-4 mg TNF (leg), and a 10-mg/L volume (leg) or 13-mg/L volume (arm) of melphalan at mild hyperthermia (tissue temperatures of maximally 39.5°C in the leg and 38.5°C in the arm). The median dose of melphalan was 60 mg (mean 68.7 mg, range 26-140 mg); median dose of TNF was 4 mg (mean 3.3 mg, range 2-4 mg). In the melanoma group, all patients underwent lower extremity ILP and received a median dose of 90 mg melphalan (mean 90.4 mg, range 50-120 mg) and a median dose of 4 mg TNF (mean 3.7 mg, range 2-4 mg). TNF was injected as a bolus into the arterial line provided limb tissue temperature had reached 38°C. Melphalan was administered after 10-30 minutes at limb temperatures between 38°C and 39.5°C. During the procedure, continuous leakage monitoring was performed by using a precordial scintillation probe to detect leakage of radiolabelled albumen injected to the perfusion circuit. At the end of the ILP, the limb was washed out with at least 1 L (arm) up to 4 L (iliac perfusion) of physiologic saline solution and 6% dextran 70 (Macrodex; Pharmacia, Uppsala, Sweden). In patients with melanoma who underwent iliac perfusion, an iliac lymph node dissection was performed, and patients who had palpable nodal disease in the groin underwent an ilio-inguinal lymph node dissection in the same operative session as the ILP but prior to the ILP.

Response evaluation and toxicity

Clinical response evaluation in patients with STS was performed 2 weeks, 4 weeks, 8 weeks and 12 weeks after ILP and every 3 months thereafter for the first year both by clinical examination and by magnetic resonance imaging 4-6 weeks after ILP, 8-12 weeks after ILP and every 3-6 months thereafter. Response evaluation in patients with melanoma was performed 2-4 weeks and 8 weeks after ILP by clinical examination, and after that at regular 3- month intervals for the first 2 years and at longer intervals thereafter. Responses are reported according to World Health Organisation (WHO) criteria [10]. When histological response could be assessed in patients with STS, the

final outcome was adjusted if the pathological response (based on the percentage of necrosis: complete response (CR) if 100% necrosis, partial response (PR) if 50-99% necrosis, no change (NC) if <50% necrosis) differed from the clinical response.

Acute local toxicity of the ILP procedure was classified according to Wieberdink et al. [11], as follows: (grade I) no reaction, (grade II) slight erythema or edema, (grade III) considerable erythema or edema with some blistering, slightly disturbed motility permissible, (grade IV) extensive epidermolysis or obvious damage to the deep tissues, causing definite functional disturbance, and threatening or manifest compartmental syndrome, and (grade V) reaction that may necessitate amputation. Systemic toxicity is reported according to WHO criteria.

RESULTS

Response

STS

A clinical CR after ILP in 37 patients with metastasised extremity STS was observed in 8%. A PR occurred in 65%, NC was observed in 24% and clinical response was not determined in 1 patient (3%). Eighteen patients underwent resection of the tumour remnant after shrinkage, and patients with multiple tumours underwent a biopsy for response assessment (median time after ILP 3 months, range 0.4-7.4 months), which showed 100% necrosis in 4 patients, 50-99% necrosis in 12 patients and 0-50% necrosis in 2 patients. In 6 patients, histological response differed from clinical response (3 patients were reclassified from NC to a PR, and 3 patients were reclassified from a PR to a CR), resulting in a final 16% CR rate, a final 68% PR rate, and a final 16% NC rate. The overall response rate of TM-ILP in patients with stage IV sarcoma (84%) did not differ significantly from our ILP experience in treating patients with nonmetastatic disease (overall response rate 71%, p=0.11).

Melanoma

Among the patients with stage IV melanoma, a CR was observed in 6 patients (43%), a PR was observed in 7 patients (50%) and NC was observed in 1 patient (7%). The response in patients with stage IV melanoma differed significantly from the responses obtained in patients treated in our institution with stage IIIA disease (in-transit metastases only) or IIIAB disease (in-transit metastases and lymph node metastases; 84% and 63%, respectively, p-values=0.004 and 0.184, respectively).

Outcome and toxicity

Overall survival of the 37 patients with STS after ILP was 53% at 1 year and 15% at 2 years. The median survival after ILP was 12 months, and death was related to the disease in all cases. In patients with melanoma, the median time of survival was 7 months: Thirty-nine percent of the patients still were alive after 1 year, but no patients survived for 2 years. Local control of the disease for the full duration of life, which is the main objective of ILP for patients who have stage IV disease, was obtained in 33 patients with STS and in 11 patients with melanoma. In seven patients, continued control was not achieved: Four patients with STS and three patients with melanoma experienced local progression of the disease in the limb after an initial response. Because of their initial responses and the ongoing progression of their distant metastases, the local control achieved in these patients lasted long

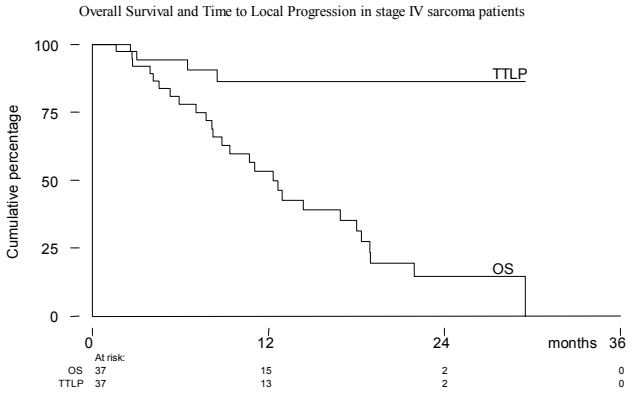

Figure 1: Overall survival (OS) and time to local progression (TTLP) after ILP in patients with stage IV sarcoma
X-axis: Time (months); Y-axis: Cumulative percentage

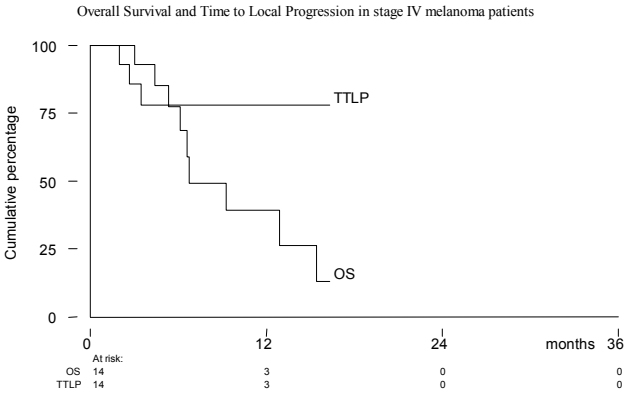

Figure 2: Overall survival (OS) and time to local progression (TTLP) after ILP in patients with stage IV melanoma
X-axis: Time (months); Y-axis: Cumulative percentage

Table 3: Local and systemic toxicity of 51 TM-ILPs for metastasised tumours

Local toxicity					
	Grade I	Grade II	Grade III	Grade IV	Grade V
Wieberdink	10	31	8	1	1
Systemic toxicity					
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Neurologic*	47	4	0	0	0
Liver*	50	0	1	0	0
Renal*	50	0	1	0	0
Haematologic*	49	1	0	0	1
	<38°C	38-39°C	39-40°C	>40°C, <24 hrs	>40°C, >24 hrs
Temperature	24	13	11	2	1
	Absent	Present			
Shock[&]	51				

Hrs = hours, * = WHO criteria[13], [&] = support of vasopressors needed

Table 4: Outcome of 51 patients after ILP for metastasised tumours

Outcome of 51 patients after ILP for metastasised tumours			
		STS	**Melanoma**
		N=37	**N=14**
OS	1-yr	53%	39%
	2-yrs	15%	n.a.
LPFS	2-yrs	86%	78%*
LS		96%	100%

STS = soft tissues arcoma, OS = overall survival, LPFS = local progression free survival, LS = limb salvage, n.a. = not applicable, * =estimated

enough to avoid a situation in which an amputation was required. Overall survival and time to local progression are shown in figure 1 (STS) and figure 2 (melanoma). The toxicity of TM-ILP was very mild, consistent with prior reports. Local toxicity was mild (grade 1-2) to moderate (grade 3) in 35 patients with STS who underwent ILP and in all patients with melanoma who underwent ILP. One patient developed pain in the lower leg without the symptoms of compartment syndrome (Wieberdink grade IV). In one other patient, there was extensive necrosis of the tumour after ILP that developed in a secondary infection. Eventually, an amputation was inevitable in the postoperative period, which is considered a grade V local toxicity of the ILP procedure. This was the only amputation that had to be performed in the described patient population. Limb function of the other 36 patients with STS was excellent in 27 patients; mildly disturbed in 5 patients, and severely affected (leading to the use of crutches) in 4 patients. All patients with melanoma had excellent limb function after perfusion. Systemic toxicity was absent or mild, with only 1 patient who had

STS developing a fever >40°C for >24 hours and with 1 patient who had melanoma developing a grade IV leucopenia, which lasted only for 1 day and that did not require any type of intervention (table 3). Median hospital stay for the 51 patients was 10 days. Outcomes of the patients are summarized in table 4.

DISCUSSION

The present data show that ILP can provide excellent local control and salvage of the limb in patients with stage IV STS or melanoma in combination with limb-threatening local tumours.

STS

The overall response rate (84%) in this study was relatively high compared both with data in literature (63-91% [5, 6, 12]) and with our own experience in nonmetastatic disease (71%). However, this high overall response rate was largely attributable to the proportion of patients who had a PR (68%). Especially in patients with metastatic disease, any response that leads to local control and prevents amputation is worth the effort in light of the limited life expectancy.

The median survival of the patients after ILP was 12 months. This corresponds well with data from other studies, which have reported a survival of 11-26 months [1, 2, 13, 14] in patients with metastatic disease, taken into account that there is a lead-time bias effect in the current study, because survival was calculated from the date of ILP.

Melanoma

The overall response rate in the 14 patients with stage IV melanoma was 93% with a CR rate of 43%. This is remarkably good for a stage IV melanoma population, because the results reported from ILP in patients with melanoma usually deal only with stage IIIA or IIIAB disease. The responses with TM-ILP achieved in our patients with stage IV melanoma who had bulky disease were as good as the reported results reported from melphalan-only ILPs in patients who had stage IIIA/IIIAB disease [15], but they were markedly lower than the results reported from TM-ILP in patients who had stage IIIA/IIIAB disease [8, 16, 17]. Reasons for this reduced response rate are unknown, but we speculated previously that in these patients with stage IV melanoma, all of whom had very bulky disease in the limbs, a reduced immunocompetence in stage IV melanoma may play a significant role, along with an increased aggressiveness of the melanoma itself [17]. However, further research will be needed to explain properly this decreased response rate, which has been found universally in this and

other (melphalan-based) studies [8, 18, 19]. Survival in patients with stage IV melanoma is extremely poor and reportedly is only 41-59% at 1 year, depending on the site of the metastases [20], which is in line with the 39% 1-year survival rate in this study, again taking into account the lead-time bias effect.

Toxicity

Systemic toxicity from TM-ILP virtually was absent in the procedures performed. This is a very important observation, because, in general, a palliative procedure should be relatively free of side effects. The dreaded, shock-like symptoms of (high-leakage) ILP with TNF could be avoided easily in the postoperative phase, because we know from the extensive experience in our institution that generous fluid management and the use of indomethacin in the immediate postoperative period prevents the symptoms of fever and the transient drop in blood pressure [21]. Local toxicity was severe (grade IV or V) in 2 patients in the study population and led to amputation of the limb in 1 of these patients. This is a severe, adverse, therapy-related effect, despite the fact that an amputation of the limb in this patient was inevitable if no treatment was offered. In our experience with TM-ILP in nearly 350 procedures for patients with melanoma and non-melanoma tumours, only 2 treatment-related amputations (Wieberdink grade V) had to be performed.

The rationale for palliative treatment of patients with stage IV disease by a locoregional treatment procedure deserves further discussion. For both systemically metastasised STS and melanoma, the reported effectiveness of systemic chemotherapy is very poor. In patients with STS, no effect of systemic chemotherapy on survival could be demonstrated either in the adjuvant setting after resection of the primary tumour [22], or in patients who already had metastatic disease [23]. In fact, a recent study from the Memorial Sloan-Kettering Cancer Center has shown that, despite improved insight into the tumour biology of sarcoma, the prognosis for patients with STS has not improved in the last 20 years [3]. For patients with melanoma, the same problem is encountered: although some treatment regimens seem to increase response rates, none of the treatment schedules has been able to prolong overall survival [24]. These data indicate that in both patients with STS and patients with melanoma who have distant metastases, the objective of treatment should be improving quality of life during the terminal phase. Previous studies have demonstrated that ILP could offer palliation in both patients with melanoma [25] and patients with STS [26] who have advanced disease. The 51 patients presented in the current study all had locally advanced tumour activity for which amputation of the limb was inevitable. Their pre-ILP limb function was very poor and especially in patients with melanoma, necrosis

of the tumours resulted in socially disabling conditions. TM-ILP preserved the limb in 50 of 51 patients with excellent limb function in 31 patients.

The currently series of 51 patients showed that TM-ILP can offer important benefits to patients with metastasised melanoma and sarcoma, with low treatment-related morbidity. When surgical resection of the tumours is impossible or is possible only at the cost of severe disability, a TNF-based ILP can offer limb salvage in 98% of the patients, which means important improvement in patient mobility during the last, often short, period of life. Therefore, we recommend considering TM-ILP for patients in this category as soon as the local situation of the limb prompts for action.

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CHAPTER 12

Summary and Conclusions

Samenvatting en Conclusies

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SUMMARY AND CONCLUSIONS

Isolated limb perfusion (ILP) with melphalan is effective in the treatment of small multiple melanoma in-transit metastases and is utilized widely for this indication. The technique achieves regional drug concentrations 15–25 times higher than systemic administration and is without systemic side effects [1, 2]. However, the treatment is much less effective against bulky melanoma metastases and has uniformly failed in the treatment of irresectable extremity soft tissue sarcomas. The addition of tumour necrosis factor-alpha (TNF-α) to this treatment approach has changed this situation dramatically and has greatly expanded the successful application of ILP. TNF-based ILP (TM-ILP) can avoid amputations regardless of the tumour size and type.

TNF-BASED ILP FOR MELANOMA PATIENTS

For patients with extensive melanoma in-transit metastases (IT-mets), treatment options are often limited. Surgical excision of IT-mets is chosen when size and number permits this approach. Treatment by carbon dioxide laser therapy or external beam radiotherapy is only possible in highly selected patient categories. Immunotherapy through vaccination has not yet produced satisfactory results and the response percentage of systemic chemotherapy for melanoma is such disappointing, that it should only be used in the palliative treatment of systemically metastasised patients [3]. It must be realized that amputation is seldom if ever indicated and does not improve survival [4]. Due to the lack of effective treatment options for patients with extensive melanoma IT-mets of the extremity, ILP is a very attractive treatment modality in this patient population. ILP with melphalan alone is reported to achieve about 50 % CR rate and 80% overall response rate [5]. The introduction of TNF-α in this setting led to an increase in CR rates to 70–90% and overall response rates to 95–100% [6]. These results encouraged us to start a series of TM-ILPs for patients with melanoma IT-mets.

In **chapter 2**, we report about 100 consecutive TM-ILPs in 87 melanoma patients using a 1–4 mg TNF-α dose. Of these, 45 ILPs were performed for stage IIIA (IT-mets without positive lymph nodes), 41 ILPs for stage IIIAB (IT-mets with positive nodes) and 14 ILPs for stage IV (IT-mets plus distant metastases) disease. Most patients had bulky disease (0–10 lesions in 43 patients, 11–50 in 32 patients, and over 50 lesions in 25 patients) and/or failed multiple prior treatments. In 21 patients, a second or third TM-ILP was performed because patients failed to respond to a prior M-ILP or TM-ILP. The overall response rate was 95% with 69 CRs (69%), 26 PRs (26%) and 5

stable diseases (5%). The CR rates differed significantly according to disease stage: 82% in Stage IIIA; 63% in Stage IIIAB and 43% in Stage IV. In 50% of patients with a CR, local recurrences occurred. Mean time to recurrence / progression in CR patients was 22 months (3–129+ months), versus 6 months in the other patients. There were 71 patients with metastatic disease at or after ILP and the mean time to distant metastases was 14 months. At a median follow up of 22 months (1–132+ months), overall 5-year survival was 32% and 10-year survival was 17%. The outcome of patients after ILP is determined by the stage of the disease, which is a reflection of the aggressiveness of the melanoma. However, within each stage of disease, a complete response on ILP selects those patients with relatively favourable characteristics. The local toxicity of the procedure was mild and no grade 3–4 systemic toxicity was observed, not even in the eight patients with high leakage (10–32%). The CR rate in the first 50 ILPs was 80% versus 58% in the last 50 ILPs (p=0.017). This difference in CR reflects a change in policy, whereby TM-ILP was offered only to patients with bulky disease and or multiple recurrences after multiple surgical interventions and/ or after vaccine-therapy trials, to which many patients were first entered. This patient category with highly unfavourable characteristics, still showed a superior response rate compared with the historical group of patients treated with M-ILP without such an unfavourable patient selection [6] and it is similar to the observations in the US in an interim analysis of a randomised trial by Fraker *et al.* [7]. Thus, TM-ILP results in high response rates in patients with melanoma IT-mets, and is of special value in patients with bulky disease.

In **chapter 3**, we report about our study of 25 ILPs with a 1–4 mg TNF-α for recurrences in the limb after previous ILP treatment. The overall response rate of repeat ILP was 96% (CR 76%, PR 20%) and there was no change in 4%. This did not differ from the primary ILPs in our series and no increased toxicity was observed. This observation underscores the efficacy of a TNF-based ILP in the repeat-ILP setting and thereby its indication for this difficult clinical problem.

Chapter 4 is an outline of possible treatment modalities in patients with melanoma IT-mets and determines the place of ILP in this gamut. For patients with extensive IT-mets of the limb that cannot be treated by local techniques, a regional technique is able to achieve drug concentrations in the affected limb that are sufficient to yield tumour response. It seems that patients with multiple small lesions can be treated effectively by melphalan-only techniques, whether that is Isolated Limb Infusion (ILI) for lesions in the distal part of the limb, or the traditional melphalan-only ILP. TM-ILP however, has shown to be more effective in terms of response rates. Due to the vasculotoxic effect of TNF, it is likely that large tumours with their own vasculature

profit the most from addition of this cytokine. Preclinical studies, various single-center reports, our own experience with 100 TM-ILP procedures for melanoma, and the interim analysis of a randomised controlled trial [7], all confirm this hypothesis. For patients with bulky melanoma metastases of the limb and those patients who failed prior ILP treatment, TM-ILP seems to be the most efficacious therapy to obtain local control and achieve limb salvage.

TNF-BASED ILP FOR SOFT TISSUE SARCOMA PATIENTS

Achieving limb salvage is a key element in the management of locally advanced soft tissue sarcomas (STS) of the extremities, because there is ample evidence that limb-preserving surgery is equal to amputation in terms of overall survival [8-10]. This improved the interest in neoadjuvant treatment options that could reduce the size of the tumours and hence make function-preserving surgery possible. In contrast to the efficacy of melphalan-based ILP for small melanoma in-transit metastases, results with ILP using melphalan, doxorubicin, or any other drug for large STS have been very disappointing. The introduction of TNF in the ILP setting in an initial report of 4 TM-ILPs in locally advanced STS changed this situation as it showed impressive and very rapid responses [11]. Multicenter trials confirmed that TNF-based ILP was a highly effective new method of induction biochemotherapy for extremity STS with a 20–30% complete remission (CR) rate, a ~ 50% partial remission (PR) rate and a limb salvage percentage of ~80% [12, 13]. Rotterdam was the leading institute of the pivotal multicenter trials of the application of TM-ILP for locally advanced STS.

In **chapter 5**, the 217 consecutive TM-ILPs in 197 patients for limb-threatening STS performed in Rotterdam between 1991 and 2003 are analysed. Primary sarcomas were perfused in 61% of patients, local recurrent sarcomas in 39%, multifocal primary or multiple local recurrences in 29%, and overt concurrent metastatic disease in 16%. Tumours >10 cm were found in 42% of patients; most tumours were high grade, with 81% showing grade II-III tumours. A major response after therapy was observed in 75% of the patients (26% complete responses, 49% partial responses). Limb salvage was achieved in 87%. Although on theoretical grounds it can be expected that high-grade tumours respond better to ILP, as they are usually more vascularized, this could not be confirmed in this study, and only ILP for Stewart-Treves lymphangiosarcoma or Kaposi sarcoma was associated with significantly better response. At a median follow-up period of 22 months (range 0.1-130+ months), actuarial overall 5-year survival was 49% with a median survival time of 57 months. Trojani grade and the presence of multiple tumours were negative prognostic factors for survival. The

excellent results of TM-ILP in locally advanced STS have established a permanent role of TM-ILP in the multimodality treatment arsenal for these tumours.

REPORTS ON SPECIAL PATIENT CATEGORIES

Patients with multiple tumours in the extremity

TNF-based ILP is the ideal alternative to avoid amputation in patients with multifocal primary tumours, who are difficult to treat, such as Kaposi sarcomas [14] and multiple lymphangiosarcomas (also known as Stewart Treves Syndrome) [15]. **Chapter 6** describes the experience of our group in patients of STS patients with multiple primaries of various histologies or multiple recurrences after prior surgery. Remarkably good results were reported, as we observed an 87% response rate and 82% limb salvage rate in 64 patients with multiple tumours. These results indicate that TNF-based ILP is very effective and can prevent amputation in this patient population.

Patients with recurrent tumours in an irradiated field

We performed 30 ILPs in 26 patients with recurrent tumours in an irradiated field, which is reported in **chapter 7**. In contrast to the belief that such tumours are unlikely to respond, we observed a 70% response rate and 65% limb salvage rate, indicating that in this very difficult patient category a TNF-based ILP approach is an attractive treatment option.

Patients with desmoids

Patients with desmoid tumours and aggressive fibromatosis often present with recurrent disease, which is very difficult to treat surgically with or without radiotherapy. In **chapter 8**, our group observed an overall response rate of 75 % and a limb salvage rate of 100%, demonstrating the utility of this procedure in achieving local control in this tumour type.

The results obtained in Rotterdam on TM-ILP for STS, both in the overall population and in patients with specific problems such as multifocality, previous radiotherapy and recurrent desmoid tumours, are compared to the literature in **chapter 9**. As TNF-α targets the vasculature-vasculature, which is a common denominator in all these tumour types, the use of TNF is very attractive and it explains its efficacy in combination with chemotherapy across these numerous histologies. No other neo-adjuvant treatment option in STS produces the response rates obtained with TM-ILP, which leads to the conclusion that a TM-ILP should be considered standard of care

in each patient with locally advanced STS where an amputation, or a resection with severe functional loss, is the only surgical option.

GENERAL QUESTIONS IN ISOLATED LIMB PERFUSION

An aspect that has been studied in overall populations is whether high doses of TNF-α (e.g. 3–4 mg) are necessary or whether lower doses (e.g. 1 mg) suffice. An early clinical report by Hill and co-workers suggested that low doses of TNF-α were sufficient [16]. The small study size, the concomitant use of high doses of corticosteroids, and the fact that a different type of TNF-α was used, meant that definitive conclusions were not possible. Confirmative reports came from the Italian studies of 1 mg TNF-α in combination with doxorubicin [17] and the French randomised phase II trial in 100 patients on four TNF-α doses (0.5, 1.0, 2.0 and 4.0 mg) [18]. **Chapter 10** describes our analysis of 240 ILPs in which the TNF-α doses varied between 1.0, 2.0, 3.0 and 4.0 mg. A reduction of TNF dose up to 1 mg is not correlated with an inferior response to therapy, nor does it affect toxicity rates. This is consistent with findings from our laboratory, which indicate that doses of 1 mg TNF-α are sufficient, but further dose reduction results in complete loss of activity [19].

In patients who have overt metastatic disease and a limb-threatening tumour a palliative TNF-based ILP could possibly induce tumour control and avoid amputation. In **chapter 11,** the results of 51 ILP procedures performed in patients with stage IV disease are presented. True local control was achieved in 33/37 STS patients and in 11/14 melanoma patients, and the remaining patients showed an initial response with ongoing progression of their metastatic disease. Only one amputation had to be performed in this patient category. Although this amputation was due to treatment toxicity, the overall toxicity of the 51 procedures was very low, both on the local and on the systemic level. This demonstrates that TNF-based ILP is an attractive option in these patients with a short life expectancy.

CONCLUSIONS AND FUTURE DIRECTIONS

ILP methodology provides us an excellent tool in the clinic to obtain local control and avoid amputations of limbs in patients with limb-threatening tumours. This has been largely achieved by the success of the antivascular TNF-based biochemotherapy in this setting. TNF-based ILP is a very successful treatment option to achieve limb salvage in the management of advanced disease and multiple or drug resistant

extremity tumours. TNF-based ILPs are now performed in 35 cancer centers in Europe with referral programs for limb salvage. TNFα-based ILP is a well-established treatment to avoid amputations and represents an important example of tumour vascularity-modulating combination therapy and should be offered in large volume tertiary referral centers. In the field of isolated perfusion, newly discovered vaso-active drugs await evaluation in preclinical research projects and future clinical trials.

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SAMENVATTING EN CONCLUSIES

Geïsoleerde extremiteitsperfusie (ILP) met melphalan is een effectieve behandeling voor multipele kleine melanoom in-transit metastasen en wordt hiervoor dan ook veelvuldig toegepast. De techniek maakt het mogelijk om locoregionaal een medicijnconcentratie te bereiken die 15-25 keer hoger is dan bij systemische toediening, zonder dat dit gepaard gaat met systemische toxiciteit [1, 2]. Toch is deze behandeling veel minder effectief gebleken voor grote melanoommetastasen en is voor de behandeling van irresectabele weke delen sarcomen van de extremiteit niet bruikbaar. Het toevoegen van tumor necrose factor-alpha (TNF-α) aan het perfusaat heeft hierin echter een dramatische verandering gebracht waardoor de succesvolle toepassing van ILP enorm kon worden uitgebreid. ILP met TNF (TM-ILP) kan amputaties voorkomen bij patiënten met tumoren van elke grootte en elk type.

ILP MET TNF VOOR MELANOOMPATIËNTEN

Voor patiënten met uitgebreide in-transit metastasen (IT-meta's) van het melanoom zijn de behandelingsmogelijkheden vaak beperkt. Chirurgische excisie van de IT-meta's wordt verricht als de grootte en het aantal dit toelaat. Een behandeling met CO2 laser therapie of met radiotherapie is alleen mogelijk bij een geselecteerde patiëntengroep. Immunotherapie in de vorm van vaccinatie heeft nog onvoldoende bevredigende resultaten opgeleverd en de responspercentages op systemische chemotherapie zijn dusdanig laag, dat dit alleen overwogen moet worden als palliatieve behandeling van systemisch gemetastaseerde patiënten [3]. Ook moet men zich realiseren dat amputatie van een extremiteit bij een melanoompatiënt zelden of nooit is geïndiceerd en niet tot een betere overleving leidt [4]. Door het gebrek aan effectieve behandelingen voor patiënten met uitgebreide IT-meta's van het melanoom op de extremiteit is ILP een erg aantrekkelijke behandelingsmodaliteit voor deze patiëntengroep. Van ILP met melphalan alleen zijn complete respons (CR) percentages bekend van rond 50% en totale respons percentages van 80% [5]. De introductie van TNF-α in het ILP model heeft deze percentages echter doen stijgen tot 70-90% voor CR en tot 95-100% voor de totale respons [6]. Deze resultaten hebben ons gestimuleerd om een serie behandelingen met TM-ILP te starten voor patiënten met IT-meta's van het melanoom.

In **hoofdstuk 2** beschrijven we de resultaten van 100 opeenvolgende TM-ILPs bij 87 melanoompatiënten waarbij TNF-α doseringen werden gebruikt tussen 1-4 mg. Van deze procedures werden 45 ILPs uitgevoerd voor stadium IIIA ziekte (IT-me-

ta's zonder positieve lymfklieren), 41 ILPs voor stadium IIIAB ziekte (IT-meta's met lymfkliermetastasen) en 14 ILPs voor stadium IV ziekte (IT-meta's plus afstandsmetastasen). De meeste patiënten hadden multipele grote afwijkingen (1-10 laesies bij 43 patiënten, 11-50 bij 32 patiënten en meer dan 50 laesies bij 25 patiënten) en/of hadden niet gereageerd op meerder voorafgaande behandelingen. Bij 21 patiënten werd een tweede of derde perfusie uitgevoerd omdat eerdere melphalan (M)-ILP of TM-ILP geen of onvoldoende effect had gehad. Het totale respons percentage was 95% met 69 CRs (69%), 26 partiële responses (PR, 26%) en 5 keer stabiele ziekte (5%). Het CR percentage verschilde significant per ziektestadium: 82% in stadium IIIA, 63% in stadium IIIAB en 43% in stadium IV. Bij 50% van de patiënten met een CR ontwikkelde zich een lokaal recidief. De mediane duur tot aan het ontwikkelen van een recidief respectievelijk progressie van de laesies was 22 maanden (3-129+) bij patiënten met een CR versus 6 maanden bij de overige patiënten. In totaal hadden of kregen 71 patiënten metastasen op afstand na een mediane duur van 14 maanden. Bij een mediane follow-up van 22 maanden (1-132+ maanden) bleek de 5-jaars overleving 32% en de 10-jaars overleving 17%. Het lot van patiënten na een ILP wordt bepaald door het stadium van de ziekte, hetgeen een weerspiegeling is van de agressiviteit van het melanoom. Toch selecteert een CR na ILP, binnen elk ziektestadium, die patiënten met relatief gunstige tumorkenmerken. De lokale toxiciteit van de procedure was mild, wat blijkt uit het ontbreken van graad 3-4 toxiciteit, zelfs bij de acht patiënten met veel (10-32%) lekkage. Het CR percentage in de eerste 50 ILPs was 80%, tegen 58% in de 2^{e} 50 ILPs (p=0.017). Dit is een gevolg van een beleidsverandering waardoor TM-ILP alleen werd aangeboden aan patiënten met zeer uitgebreide ziekte en/of multipele recidieven na eerdere chirurgische interventie en/of na vaccinatietherapie studies waaraan vele patiënten primair hadden deelgenomen. Deze patiëntencategorie met zeer ongunstige kenmerken bleek nog altijd te reageren op TM-ILP met betere responspercentages dan een historische groep patiënten die met M-ILP was behandeld en die deze ongunstige kenmerken miste [6]. Dit responspercentage is gelijk aan de resultaten die worden gemeld door Fraker *et al.* [7] in een interim-analyse van een gerandomiseerde studie in de Verenigde Staten. Concluderend resulteert TM-ILP in hoge responspercentages bij patiënten met melanoom IT-meta's, en is het van buitengewone waarde bij patiënten met uitgebreide ziekte.

In **hoofdstuk 3** melden wij de resultaten van onze studie van 25 ILPs met 1-4 mg TNF-α voor melanoomrecidieven in de extremiteit na eerdere ILP behandeling. Het totale respons percentage van de herhaalde ILPs was 96% (CR 76%, PR 20%) en 4% bereikte stabiele ziekte. Deze resultaten verschilden niet van de primaire ILPs in onze serie, noch bleek er sprake van verhoogde toxiciteit. Deze bevindingen

onderstrepen de effectiviteit van TM-ILP als herhalingsbehandeling en daarmee de indicatie voor dit lastige klinisch probleem.

Hoofdstuk 4 is een uiteenzetting van de mogelijke behandelingsvormen voor patiënten met melanoom IT-meta's en bepaalt de plaats van ILP in dit scala. Voor patiënten met uitgebreide IT-meta's van de extremiteit die niet met lokale behandelingen kunnen worden behandeld, blijkt deze regionale techniek in staat om medicijnconcentraties in het aangedane gebied te bewerkstelligen die hoog genoeg zijn om een tumor respons te induceren. Patiënten met multipele kleine laesies kunnen effectief worden behandeld met technieken die alleen melphalan gebruiken, of dit nu geïsoleerde extremiteitsinfusie (ILI) is voor laesies in het distale deel van de extremiteit, of de traditionele melphalan-ILP. TM-ILP heeft echter bewezen effectiever te zijn als het gaat om responspercentages. Doordat TNF toxisch is voor tumorvasculatuur is het aannemelijk dat juist de tumoren met een eigen vasculatuur het meest profiteren van de toevoeging van dit cytokine. Preklinische studies, meerdere single-center studies, onze eigen ervaringen met 100 TM-ILP procedures voor melanoom en de interimanalyse van een gerandomiseerde trial [7], bevestigen alle deze hypothese. Voor patiënten met uitgebreide melanoommetastasen in de extremiteit en voor patiënten die onvoldoende hebben gereageerd op eerdere ILP-behandeling, lijkt TM-ILP de meest effectieve therapie om lokale controle te verkrijgen en om de extremiteit te behouden.

ILP MET TNF VOOR WEKE DELEN SARCOOM PATIËNTEN

Extremiteitsbehoud is een belangrijke doelstelling van de behandeling van lokaal uitgebreide weke delen sarcomen (WDS) van de ledematen, gezien het ruim aanwezige bewijs dat extremiteitssparende chirurgie een even goede overleving oplevert als amputatie [8-10]. Dit heeft de interesse in neoadjuvante behandelingsmogelijkheden vergroot, die in staat zijn om tumoren te doen slinken en daarmee functiebehoudende chirurgie mogelijk te maken. In tegenstelling tot de effectiviteit van melphalanperfusies voor kleine in-transit metastasen van het melanoom, zijn de resultaten van ILP met melphalan, doxorubicine of willekeurig welk ander medicament zeer teleurstellend bij de behandeling van het WDS. De introductie van TNF in het ILP-model maakte een eind aan deze situatie door in een eerste studie van vier lokaal uitgebreide WDS patiënten een indrukwekkende en snelle respons te laten zien [11]. Multicentrische studies bevestigden dat ILP met TNF een zeer effectieve nieuwe methode van inductie biochemotherapie was voor het WDS van de extremiteit, met 20-30% complete respons (CR) percentages, ~50% partiële respons (PR) percentages

en extremiteitsbehoud in ~80% van de patiënten [12, 13]. Rotterdam was de initiatiefnemer van de eerste multicentrische studies naar de toepassing van TM-ILP voor het lokaal uitgebreide WDS.

In **hoofdstuk 5** worden de 217 opeenvolgende TM-ILPs bij 197 patiënten met extremiteitsbedreigend WDS geanalyseerd die van 1991 tot 2003 in Rotterdam zijn uitgevoerd. In 61% van de patiënten werd de perfusie uitgevoerd voor een primair sarcoom, in 39% voor een recidief, in 29% voor multifocale primaire tumoren of multipele lokaalrecidieven, en in 16% voor stadium IV ziekte (met afstandsmetastasen). Tumoren groter dan 10 cm waren bij 42% van de patiënten aanwezig; de meeste tumoren waren hooggradig met 81% graad II en III tumoren. Bij 75% van de patiënten kon een duidelijke respons op therapie worden bereikt (26% complete respons, 49% partiële respons). In 87% kon de extremiteit worden behouden. Hoewel op theoretische gronden van hooggradige tumoren verwacht mag worden dat ze beter responderen op perfusie omdat ze over het algemeen beter gevasculariseerd zijn, kon dit niet door deze studie worden bevestigd. Slechts ILPs voor Stewart-Treves lymfangiosarcomen en Kaposi sarcomen waren gerelateerd aan significant betere responspercentages. Gedurende een mediane follow-up van 22 maanden (0.1-130+ maanden) was de berekende totale 5-jaars overleving 49% met een mediane overlevingsduur van 57 maanden. De Trojani gradering van de tumor en de aanwezigheid van multipele tumoren bleken negatieve prognostische factoren voor overleving. De uitstekende resultaten van TM-ILP voor het lokaal uitgebreide WDS van de extremiteit maakt dat deze behandeling zich een permanente plaats heeft verworven in de behandeling van deze tumor.

STUDIES BIJ APARTE PATIËNTENCATEGORIEËN

Patiënten met multipele tumoren in de extremiteit

ILP met TNF is het ideale alternatief om amputatie te voorkomen bij patiënten met moeilijk behandelbare multifocale primaire tumoren zoals het Kaposi sarcoom [14] en multipele lymfangiosarcomen (beter bekend als het Stewart Treves Syndroom) [15]. **Hoofdstuk 6** beschrijft de ervaringen van onze groep bij WDS patiënten met multifocale primaire tumoren met verschillende histologische kenmerken en met multipele recidieven na eerdere chirurgische interventies. De gerapporteerde resultaten zijn opmerkelijk goed bij deze groep van 64 patiënten met multipele tumoren, bij wie een responspercentage werd gezien van 77% en een percentage extremiteitsbehoud werd bereikt van 82%. Deze resultaten laten zien dat ILP met TNF een zeer effectieve therapie is om amputatie te voorkomen bij deze patiëntenpopulatie.

Patiënten met recidief tumoren in bestraald gebied

Wij voerden 30 perfusies uit bij 26 patiënten met recidief tumoren in het bestralingsveld, zoals gepresenteerd in **hoofdstuk 7**. In tegenstelling tot de veronderstelling dat deze tumoren nauwelijks zouden reageren, vonden wij een responspercentage van 70% en een percentage extremiteitsbehoud van 65%. Dit laat zien dat voor deze zeer gecompliceerde patiëntencategorie een ILP met TNF een aantrekkelijke behandelingsmogelijkheid is.

Patiënten met Desmoïd Tumoren

Patiënten met desmoïd tumoren en agressieve fibromatose presenteren zich vaak met recidieven van de ziekte, die moeizaam te behandelen zijn door middel van chirurgie met of zonder radiotherapie. In **hoofdstuk 8** zag onze groep totale responspercentages van 75% en 100% extremiteitsbehoud in de groep van twaalf patiënten met deze aandoening. Dit demonstreert de bruikbaarheid van de ILP procedure om lokale controle te verkrijgen over deze tumoren.

De verkregen resultaten in Rotterdam met TM-ILP voor het WDS, zowel in de totale populatie als bij patiënten met specifieke problemen zoals multifocaliteit, voorafgaande radiotherapie en recidieven van desmoïd tumoren, worden vergeleken met de resultaten uit de literatuur in **hoofdstuk 9.** Omdat TNF-α aangrijpt op tumor vasculatuur, is het gebruik van TNF aantrekkelijk en verklaart het de effectiviteit in combinatie met chemotherapie voor al deze verschillende histologische subtypen. Geen andere neo-adjuvante therapie voor het WDS is in staat de responspercentages van ILP te evenaren. Dit leidt tot de conclusie dat TM-ILP als standaard therapie zou moeten worden beschouwd bij elke patiënt met lokaal uitgebreid WDS bij wie een amputatie, of een resectie met aanzienlijk functieverlies, als enige chirurgische optie wordt gezien.

ALGEMENE VRAGEN IN GEÏSOLEERDE EXTREMITEITSPERFUSIE

Eén van de aspecten die veelvuldig is bestudeerd als deelaspect in algemene studies, is of hoge doses TNF-α (3-4 mg) noodzakelijk zijn of dat lage doses (1 mg) volstaan. Een vroege klinische studie door Hill et al. suggereerde dat lage doses TNF-α voldoende zouden zijn [16]. Het feit dat het een studie betrof met kleine patiëntenaantallen, dat er gelijktijdig hoge dosis corticosteroïden werd toegediend en dat een variant type TNF-α werd gebruikt, maakt dat hier geen definitieve conclusies uit getrokken konden worden. Bevestigende berichten kwamen echter uit Italiaanse studies waarbij 1 mg TNF-α werd gebruikt in combinatie met doxorubicine[17], en de

Franse gerandomiseerde fase II studie bij 100 patiënten met vier verschillende doses TNF-α (0.5, 1.0, 2.0 and 4.0 mg) [18]. **Hoofdstuk 10** beschrijft onze analyse van 240 ILPs waarbij de dosis TNF varieerde tussen 1.0, 2.0, 3.0 en 4.0 mg. Een reductie van de TNF dosis tot 1 mg is niet gecorreleerd met een slechtere respons op perfusie, maar is ook niet van invloed op toxiciteit. Dit is consistent met onze bevindingen uit het laboratorium die aangeven dat een TNF-α dosis van 1 mg voldoende is, maar dat verdere reductie leidt tot een compleet verlies van effectiviteit [19].

Bij patiënten met gemetastaseerde ziekte en een extremiteitsbedreigende tumor zou een palliatieve ILP met TNF lokale tumorcontrole kunnen induceren en daarmee amputatie kunnen voorkomen. In **hoofdstuk 11** worden de resultaten van 51 ILP procedures gepresenteerd die werden uitgevoerd bij patiënten met stadium IV ziekte. Daadwerkelijke lokale controle kon worden verkregen bij 33/37 WDS patiënten en 11/14 melanoompatiënten, en in de overige gevallen werd een initiële respons gezien bij voortgaande progressie van de gemetastaseerde ziekte. Het bleek slechts voor één patiënt noodzakelijk om een amputatie uit te voeren. Hoewel deze amputatie wegens toxiciteit van de behandeling moest worden verricht, was de algemene toxiciteit van de 51 procedures zeer gering, zowel lokaal als systemisch. Dit toont dat ILP met TNF een zeer aantrekkelijke optie is voor de behandeling van deze patiënten met een korte levensverwachting.

CONCLUSIES EN VERWACHTINGEN VOOR DE TOEKOMST

De ILP methode verschaft ons een uitstekende mogelijkheid in de kliniek om lokale controle te verkrijgen en amputatie te voorkomen bij patiënten met extremiteitsbedreigende tumoren. Dit is grotendeels bereikt door de toepassing van antivasculaire biochemotherapie op basis van TNF-α in deze procedure. ILPs met TNF-α worden momenteel in 35 kankercentra in Europa uitgevoerd. ILP met TNF-α is een zeer succesvolle en inmiddels gevestigde behandelingsmogelijkheid om amputaties te voorkomen en vertegenwoordigt een belangrijk voorbeeld van therapie gericht op modulatie van tumorvasculatuur. De behandeling zou in tertiaire, hoog-volume verwijscentra aangeboden moeten worden. Op het terrein van de geïsoleerde perfusie wachten nieuwe vaso-actieve medicijnen op hun evaluatie tijdens preklinische onderzoeksprojecten en toekomstige klinische trials.

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NAWOORD

'The Rotterdam Experience' is de ondertitel van dit proefschrift en is tegelijkertijd een term die de sfeer weergeeft waarin dit proefschrift tot stand gekomen is. Vanaf het moment dat ik via een email aan Hans de Wilt solliciteerde naar een keuze co-assistentschap in de Daniel den Hoed Kliniek en deze mail binnen tien minuten positief werd beantwoord, zijn de gedrevenheid en het enthousiasme van het team chirurgische oncologie een buitengewone stimulans geweest. Niet alleen voor het schrijven van dit proefschrift, maar ook voor het uitoefenen van mijn werk als chirurg in opleiding. De energie en werklust van mijn promotor, professor Lex Eggermont, is inmiddels legendarisch en ligt ten grondslag aan alle artikelen die dit proefschrift vormen. Mijn co-promotor, Hans de Wilt, is met hetzelfde virus besmet en heeft de afgelopen jaren zowel op wetenschappelijk als op klinisch terrein op mij een bijzondere invloed gehad. Ook Bert van Geel en Kees Verhoef, co-auteurs van dit proefschrift, Marian Menke en alle fellows in de Daniel den Hoed Kliniek dragen bij aan de stimulerende omgeving. De statistische bewerkingen in dit proefschrift zijn gedaan door Wilfried Graveland, volledig in de Rotterdamse traditie: snel, accuraat en vrijwel altijd beschikbaar. Deze termen gaan zeker ook op voor het secretariaat chirurgische oncologie, met name Marja van Wijngaarden, Marinka Eijsberg en Nelly Haazebroek. Kortom: 'The Rotterdam Experience' staat voor enthousiasme en daadkracht. Het is een voorrecht om daar deel van uit te maken.

CURRICULUM VITAE

Dirk Jan Grünhagen, the author of this thesis, was born on May 12th 1977 in Eindhoven. He attended secondary school at the Lorentz Lyceum in the same city and started the study Medicine in Leiden in 1995. He matriculated in 2001 and obtained the medical degree in 2002 (cum laude). During his study, he participated in clinical research at the department of neonatology at the Leiden University Medical Center and the Juliana Children's Hospital (Prof. dr. F.J. Walther and Dr. A.J. de Beaufort) to investigate the effect of halogen spotlight phototherapy on the transepidermal water loss in preterm infants. After obtaining the medical degree, he became resident (not in training) at the Daniel den Hoed Cancer Center and started to investigate the clinical utility of isolated limb perfusion with TNF and melphalan, leading to this thesis. Since May 1st 2004, he is resident in training to become a surgeon at the Erasmus MC Rotterdam (educational heads Prof. dr. H.J. Bonjer and Prof. dr. J.N.M. IJzermans). This residency will be continued from May 2006 onwards at the IJsselland Hospital in Capelle aan de IJssel (educational head Dr. I. Dawson).

Dirk Jan Grünhagen, de auteur van dit proefschrift, werd op 12 mei 1977 geboren in Eindhoven. Hij volgde het VWO-gymnasium aan het Lorentz Lyceum in diezelfde stad en ving in 1995 de studie Geneeskunde aan in Leiden. Hij haalde het doctoraalexamen in 2001 en het artsexamen in 2002 (cum laude). Gedurende de studie deed hij onderzoek op de afdeling neonatologie van het Leids Universitair Medisch Centrum en het Juliana Kinderziekenhuis (Prof. dr. F.J. Walther en Dr. A.J. de Beaufort) naar de effecten van halogeen fototherapie op de vochtbehoefte van prematuur geboren neonaten. In aansluiting op het artsexamen volgde een assistentschap niet in opleiding in de Daniel den Hoed Kliniek en werd tegelijkertijd aangevangen met het onderzoek naar de klinische toepassing van geïsoleerde extremiteitsperfusie met TNF en melphalan dat tot dit proefschrift heeft geleid. Sinds 1 mei 2004 is hij in opleiding tot chirurg in het Erasmus MC Rotterdam (opleiders Prof. dr. H.J. Bonjer en Prof. dr. J.N.M. IJzermans). Deze opleiding zal vanaf mei 2006 worden voortgezet in het IJssellandziekenhuis in Capelle aan de IJssel (opleider Dr. I. Dawson).