

# TWO DECADES OF TUMOR NECROSIS FACTOR- $\alpha$ AND MELPHALAN BASED ISOLATED LIMB PERFUSION

Jan Deroose



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and melphalan based  
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de meeste woorden  
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## **Two Decades of Tumor Necrosis Factor- $\alpha$ and Melphalan Based Isolated Limb Perfusion**

**Jan Deroose**

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# Two Decades of Tumor Necrosis Factor- $\alpha$ and Melphalan Based Isolated Limb Perfusion

Twintig jaar geïsoleerde ledemaat perfusies met tumor necrosis factor- $\alpha$  en melphalan

Proefschrift

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# PART I

## Introduction and outline of the thesis



two decades of tumor  
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## Introduction

Isolated limb perfusion (ILP) finds its begin at the Tulane University in New Orleans in 1957 and was invented by Creech and Krentz.<sup>1</sup> They experimentally treated a 76-year old melanoma patient with extensively recurrent in-transit metastasis in the leg. A complete response was achieved resulting in a loco-regional disease free survival until he died at age of 92. The aim of this treatment modality was to administer high doses of drug direct to the tumor without inducing systemic side effects in order to prevent an amputation. On account of the unique method of isolating the circulation of the limb, cytostatica can be applied in an up to twenty-fold dose of what is tolerated systemically.<sup>2,3</sup>

Isolation of the targeted circuit is established by clamping the major artery and vein. Subsequently the vessels are canulated with catheters connected to an oxygenated extracorporeal circuit. Applying a tourniquet prevents systemic leakage via the collateral vessels. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored throughout the procedure. Melphalan (L-phenylalanine mustard) was the first agents used, given that animal experiments demonstrated its efficacy at cost of low local toxicity.<sup>4</sup> Growing evidence that cancer cells are sensitive to high temperature led to the successful application of hyperthermia in ILP.<sup>5</sup> However, high perfusate temperature is correlated with severe local toxicity.<sup>6,7</sup> Currently, ILP is performed under mild hyperthermic conditions (38C<sup>o</sup>-39C<sup>o</sup>).

Probably the most successful modification to ILP was the addition of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in the late eighties.<sup>8</sup> TNF- $\alpha$  is an endogenous cytokine, which plays an important role in the human immune system and is especially active in the inflammation cascade. The name of this cytokine emphasizes its necrotizing effect on tumor cells.<sup>9</sup> The strong antitumor effect of TNF- $\alpha$  is caused in two ways: I) High-dose TNF- $\alpha$  has a cytotoxic effect which plays a role in the antitumor activity;<sup>10</sup> II) Even more important is the effect of TNF- $\alpha$  on tumor vasculature leading to rapid change in tumor morphology characterized by hemorrhagic necrosis.<sup>11</sup>

TNF- $\alpha$  appeared not applicable for systemic therapy since the therapeutic dose exceeded the human tolerated dose largely.<sup>12</sup> However, ILP offered an opportunity to expose tumors to high concentrations of TNF- $\alpha$  without the systemic disadvantages. Not only the dual antitumor effect of TNF- $\alpha$  was hold responsible for the high response rates. It was shown in a rat model that TNF- $\alpha$  increases the uptake of melphalan selectively in the tumor by a factor 3 to 6.<sup>13</sup>

### **TNF- $\alpha$ based ILP in sarcoma patients**

Soft tissue sarcomas (STS) are a heterogeneous group of malignancies that arise from the mesenchymal stem cells. STS account for 1% of all malignancies and are associated with a disease specific death rate of 50%. Approximately 60% of STS occur in the extremities. These are often very large tumors at presentation requiring extensive surgery resulting in amputation in 10% of patients. With the emerging evidence that there is no survival benefit of amputation compared to radical resection accompanied by adjuvant radiotherapy,<sup>14, 15</sup> there has been increasing interest in limb sparing strategies like ILP.

In contrast to the high response rates in melanoma patients initial results of ILP in sarcoma patients were disappointing.<sup>16, 17</sup> Overall response rates were poor since bulky lesions showed an inhomogeneous uptake of melphalan. The introduction of TNF- $\alpha$  changed this situation dramatically with overall response rates up to 80% and similar limb salvage rates. These results led to multicenter studies and finally the approval of TNF- $\alpha$  in Europe.<sup>18-20</sup> Currently TNF- $\alpha$  and melphalan based isolated limb perfusion (TM-ILP) followed by radical resection and adjuvant radiotherapy is an established strategy for limb salvage in locally advanced extremity STS patients in Europe.

Long-term results of TM-ILP in extremity sarcomas will be discussed in **Chapter 1**. This section will focus on those patients with long-term disease free survival. Approximately 10% of all STS occur in the distal parts of the limb. Due to lack of readily expandable soft tissue achieving wide resection might be difficult. Furthermore blood vessels, nerves and tendons involved are important for normal function of hand or foot. Preserving those structures might compromise local control.<sup>21-23</sup> **Chapter 2** reports on a unique single center experience for TM-ILP in patients with STS in the most distal parts of the limb. The role of adjuvant radiotherapy (RTx) after TM-ILP in extremity STS patients is controversial. RTx after TM-ILP followed by resection is associated with superior local control,<sup>24</sup> However, it is a major contributing factor for short- and long-term local morbidity.<sup>25, 26</sup> **Chapter 3** tried to identify a specific group of patients that could be refrained from adjuvant RTx without loss in local control. **Chapter 4** debates the role of TM-ILP in locally advanced aggressive fibrosis patients on the basis of a retrospective multicenter series. **Chapter 5** describes the major changes and evolutions since the introduction of TNF- $\alpha$  in ILP and evaluates the TNF- $\alpha$  dose reduction effectuated in 2003.

### **TNF- $\alpha$ based ILP in melanoma patients**

Malignant melanoma incidence is raising fast across the world at an average of 2.7% over the last two decades.<sup>27, 28</sup> Local recurrence occurs in approximately 5%.<sup>29, 30</sup> In about 5%-8%

recurrence is in a pattern called in-transit metastases (IT-mets). IT-mets are cutaneous or subcutaneous melanoma cells trapped within the lymphatics between the primary tumor and the regional lymph node basin. IT-mets often precede systemic disease and are associated with poor survival.<sup>31</sup>

If not too numerous, surgical excision of IT-mets is treatment of choice. For smaller lesions too numerous for excision carbon dioxide laser therapy, intralesional injections and electrochemotherapy have been used, this with poor clinical results.<sup>32-37</sup> In case of superficial lesions RTx can be considered.<sup>38</sup> So far, systemic therapy has a limited role for IT-mets. Nevertheless, promising results were reported on systemic therapy in melanoma patients, in terms of mutation based targeted therapy,<sup>39</sup> as well as in terms of novel ways of modulation of the immunity system.<sup>40, 41</sup>

In case of extensive disease ILP is an attractive treatment modality that can improve local control markedly. Since its introduction melphalan was the most common cytostatic drug resulting in complete response rates of 40% to 50%.<sup>42</sup> The introduction of TNF- $\alpha$  in the early nineties led to a remarkable improvement of complete response rates up to 90%.<sup>8, 43, 44</sup> Despite the fact that ILP has been used for over 50 years, there is no widespread consensus about the anti-neoplasm agents that should be used. Particularly the use of TNF- $\alpha$  in melphalan based ILP is still under large debate.

Long-term results of TM-ILP as treatment modality for melanoma patients with extensive IT-mets of the limb will be discussed in **chapter 6**. The major evolutions and the effect on clinical response and local control of the dose reduction of TNF- $\alpha$  are topic in **Chapter 7**. The role of repeat ILP in recurrent melanoma IT-mets will be discussed in **Chapter 8**. An overview of literature on treatment modalities of melanoma IT-mets with a special focus on TM-ILP will be given in **Chapter 9**.

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# PART I

Tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion in soft tissue sarcoma patients



two decades of tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion

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# Chapter 1

**Long-term results on tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion in locally advanced extremity soft tissue sarcomas**

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## Abstract

### *Purpose*

Because there is no survival benefit of amputation for extremity soft tissue sarcomas (STS), limb sparing surgery has become the golden standard. Tumor size reduction by induction therapy to render non-resectable tumors resectable or facilitate function preserving surgery can be achieved by TNF- $\alpha$  and melphalan based isolated limb perfusion (TM-ILP). This study reports the long-term results of 231 TM-ILPs for locally advanced extremity STS.

### *Methods*

We analyzed 231 TM-ILPs in 208 consecutive patients (1991-2005), who were all candidate for functional or anatomical amputation for locally advanced extremity STS. All patients had a potential follow up of up to 5 years. TM-ILP was performed under mild hyperthermic conditions with 1-4 mg TNF- $\alpha$  and 10-13 mg/l limb volume melphalan. Almost all patients (85%) had intermediate-high grade tumors.

### *Results*

The overall response rate (ORR) was 71% (18% CR; 53% PR). Multifocal sarcomas had a significantly better ORR of 83% ( $p=0.008$ ). The local recurrence rate was 30% ( $n=70$ ) and was highest for multifocal tumors (54%;  $p=0.001$ ) and recurrences after previous radiotherapy (54%;  $p<0.001$ ). Five-year overall survival was 42%. Survival was poorest in patients with large tumors ( $p=0.01$ ) and with leiomyosarcomas ( $p<0.001$ ). Limb salvage rate was 81%.

### *Conclusion*

We demonstrated that TM-ILP results in a limb-salvage rate of 81% in patients with locally advanced extremity STS who would otherwise have undergone amputation. Whenever an amputation is deemed necessary to obtain local control of an extremity STS, TM-ILP should be considered.

## Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of rare malignancies. Approximately 60% of STS occur in the limb. These STS are often large at presentation and require extensive and mutilating surgery resulting in amputation in 10% of patients. With growing evidence that ablative surgery does not improve survival,<sup>1, 2</sup> interest in limb sparing treatment strategies has increased.

The successful introduction of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in isolated limb perfusion (ILP)<sup>3</sup> was followed by multicenter studies. These results led to the approval of TNF- $\alpha$  for ILP in Europe.<sup>4-6</sup> Because cytostatics alone are ineffective in the setting of ILP,<sup>7</sup> the addition of TNF- $\alpha$  is crucial for two reasons: (I) TNF- $\alpha$  increases melphalan uptake in the tumor significantly (4-5 fold),<sup>8</sup> (II) TNF- $\alpha$  leads to necrosis of endothelial cells<sup>9</sup> and pericytes of the tumor vasculature,<sup>10</sup> resulting in selective destruction of tumor vasculature.<sup>11</sup>

Currently, in Europe, in patients requiring functional or anatomical amputation for advanced extremity STS, TNF- $\alpha$  and melphalan based ILP (TM-ILP) is an established strategy for limb salvage. This study reports the long-term follow up of a large single center experience of 237 consecutive TM-ILPs for locally advanced STS.

## Patients and Methods

### *Patients*

Between 1991 and 2005, 237 TM-ILPs were performed in 214 patients with locally advanced STS. All patients that were considered for ILP were referred, because an anatomical or functional amputation was deemed necessary by the referring physician since tumor control could not be achieved with a local resection. Six patients were excluded from this analysis: three patients died shortly after ILP, so the response could not be assessed. One patient died of mesenteric thrombosis and one patient died of candida septicaemia. The third patient died at home within one month of surgery; cause of death was not established. None of these 3 patients had systemic leakage during ILP. Three patients were lost to follow-up, because they returned to their native country postoperatively.

Consequently, 231 TM-ILPs in 208 patients were analyzed. Thirteen patients underwent a second TM-ILP and two patients a third TM-ILP for recurrence. These patients all had multifocal STS (Stuart Treves). A bilateral perfusion of the legs was performed in two patients with Kaposi sarcoma, one patient with malignant peripheral nerve sheath tumor and one patient with Stewart-Treves lymphangiosarcoma. Thus, a total of 212 limbs were treated. Concurrent distant metastases were present at the time of 32 ILPs in 29 patients

<b>Table 1: Patient, tumor and ILP characteristics</b>		
	<b>n</b>	<b>%</b>
<b>Sex</b>		
female	101	49
male	107	51
<b>Age (years)</b>		
median (mean)	57 (55)	
range	12-88	
<b>Size</b>		
<5 cm	72	31
5-10 cm	37	16
>10 cm	122	53
<b>Trojani grade</b>		
grade I	34	15
grade II	57	25
grade III	140	60
<b>Site</b>		
upper arm	24	10
lower arm	36	16
upper + lower arm	6	3
upper leg	110	47
lower leg	51	22
upper + lower leg	4	2
<b>Histology</b>		
liposarcoma	36	16
synovial sarcoma	36	16
High grade pleiomorphic sarcoma notother	58	25
Leiomyosarcoma	19	8
Stuart treves/ Kaposi sarcoma	27	11
others (16 types)	55	24
<b>Primary vs recurrent</b>		
primary	134	58
recurrent	97	42
<b>Previous treatment</b>		
none	167	72
RTx	22	9
CTx	15	7
ILP	9	4
combination	18	8
<b>Unifocal/multifocal</b>		
unifocal	165	71
multifocal	66	29
<b>Type of ILP</b>		
Axillar	26	11
Brachial	40	17
Illiactal	95	41
Femoro-popliteal	70	31

ILP = isolated limb perfusion ; RTx = radiotherapy; CTx = chemotherapy

(14%). Demographic data, disease and ILP characteristics were recorded in a prospectively maintained database and are summarized in table 1.

### *Treatment*

The technique of TM-ILP has been described previously.<sup>5, 12</sup> Briefly, the procedure is performed under general anesthesia. After heparinization, a targeted blood circuit is isolated by clamping and canulation of the major artery and vein and connected to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels and prevents leakage. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored throughout the procedure.

A dose of 1-3 mg (arm) or 1-4 mg (leg) of recombinant TNF- $\alpha$  (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) was injected as a bolus once the temperature of the limb reached 38°C. Subsequently, 13 mg/L limb volume (arm) or 10 mg/L (leg) melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) was administered 30 minutes after the limb temperature reached 38 – 39.5°C. The median dose of TNF- $\alpha$  administered was 4.0 mg (range 1-4), while the median dose of melphalan was 70 mg (range 10-160). After 90 minutes of perfusion, the limb was flushed with 1 L (arm) to 4 L (Iliac perfusion) of saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden).

### *Response evaluation and toxicity*

Response was assessed after 2, 4, 8 and 12 weeks following ILP. Hereafter, evaluation took place three-monthly in the first year after TM-ILP and at longer intervals during further follow up. Evaluation consisted of clinical examination and magnetic resonance imaging (MRI) and was reported according to the World Health Organisation criteria<sup>13</sup>: complete response (CR), partial response (PR) and stable disease (SD). New lesions or growth of tumor are reported as progressive disease (PD) when established at the first evaluation and as local recurrence (LR) when established during further follow up. Local toxicity was scored according to the Wieberdink classification.<sup>14</sup>

### *Local resection*

Whenever the response of the STS to TM-ILP rendered a local resection possible, local resection was carried out approximately 12 weeks after TM-ILP. In each surgical specimen the margins were inked. A microscopically positive (R1) margin was defined as unequivocal tumor extension to the inked margin on permanent section. Margins with close proximity to the tumor, but no actual involvement of the inked margin, were considered microscopically negative (R0).

### *Radiotherapy*

In general, radiotherapy was used after post-ILP resections for single tumors. No radiotherapy was administered if patients had received radiotherapy at an earlier stage or preoperatively.

### *Statistical evaluation*

Time to local recurrence (TLR), distant metastasis free survival (DMFS) and overall survival (OS) were defined as time between ILP and local recurrence, systemic progression and death respectively. Estimates were made with Kaplan-Meier curves, using Log rank statistics. Prognostic value for clinical outcome of baseline characteristics was evaluated using logistic

binary regression analysis or Fisher's exact test for univariate analysis. Influence on TLR, DMFS and OS of these baseline characteristics was evaluated with Cox regression analysis and expressed in hazard ratios. Multivariate analysis was performed for those baseline characteristics that reached 10% significance in univariate analysis. A backward step algorithm was used in order to exclude factors without prognostic value. All tests were performed at a 5% significance level.

## Results

### *Patients*

All 208 patients had a minimal potential follow up of 5 years, resulting in a median potential follow up of 12 years (range 5-19). Because of the poor prognosis of these patients, median clinical follow up was 29 months (range 1-195).

Reason for TM-ILP was multifocality in 66 cases (29%). A total of 60 patients underwent TM-ILP for a unifocal tumor <10cm (26%). Although these tumors were relatively small, tumor control was only achieved by anatomical or functional amputation due to their location: 28 were located in a joint, hand or foot and 16 in the lower arm. In 14 cases nerve involvement was established and cortex was involved in two cases. There were 105 unifocal tumors larger than 10 cm (46%) of which 44 tumors were larger than 15 cm. Patients with tumors > 10 cm in lower leg and/or arms (n=26) need an amputation to achieve negative margins. The remaining patients needed an amputation because of location: joint (n=17), neurovascular bundles (n=16) and cortex involvement (n=2)

### *Response*

The overall clinical response rate (ORR) was 71% (n=163), reflecting a clinical CR after 42 TM-ILPs (18%) and PR after 121 TM-ILPs (53%). SD was found in 61 (26%) and PD in 7 cases (3%). Surgical resection of unifocal disease after ILP was performed in 109 patients. A radical resection (R0) was achieved in 63 patients (58%), while 46 patients (42%) had a R1 resection. Patients with multifocal disease had a higher ORR compared to patients with unifocal disease: 83% vs. 66% respectively (p=0.008, table 2). Clinical outcome was significantly better in patients with Stewart-Treves lymphangiosarcoma and Kaposi sarcoma (ORR=93%, p=0.02), whereas high-grade pleomorphic sarcoma not otherwise specified (HGPS) had a significant worse outcome (ORR 59%, p=0.02). After ILP, radiotherapy was administered to 72 patients (31%). After resection of unifocal disease after ILP, 55 patients (51%) received adjuvant radiotherapy.

**Table 2: Univariate analysis of prognostic values baseline characteristics for clinical outcome, local progression, systemic progression, disease free survival and overall survival**

Variable	Overall response OR (p-value)	Local progression HR (p-value)	Metastasis free survival HR (p-value)	Disease free survival HR (p-value)	Overall survival HR (p-value)
<b>Gender</b>					
Female* vs male	0.88 (0.65)	1.13 (0.60)	1.06 (0.80)	1.10 (0.60)	0.95 (0.77)
<b>Age</b>					
≤50* vs > 50 years	1.23(0.48)	1.62 (0.07)	<b>0.62 (0.03)</b>	0.99 (0.94)	1.19 (0.32)
<b>Size of tumor</b>					
≤5 cm* vs 5-10cm vs ≥10cm	1.43 (0.48) 0.61 (0.14)	0.71 (0.33) <b>0.54 (0.02)</b>	1.57 (0.20) <b>1.85 (0.02)</b>	1.26 (0.39) 1.12 (0.58)	1.55 (0.11) <b>1.67 (0.01)</b>
<b>Trojani grade</b>					
grade I* vs II vs III	1.56 (0.37) 0.85 (0.70)	0.76 (0.43) 0.67 (0.19)	1.71 (0.18) <b>2.11 (0.04)</b>	1.15 (0.63) 1.21 (0.46)	1.39 (0.77) <b>1.82 (0.02)</b>
<b>Histopathological type</b>					
Liposarcoma lipo vs else*	0.81 (0.58)	0.58 (0.13)	1.05 (0.85)	0.74 (0.21)	0.79 (0.33)
Synovial sarcoma syn vs else*	0.81 (0.58)	0.97 (0.92)	1.22 (0.49)	0.92 (0.75)	0.89 (0.62)
HGPS HGPS vs else*	<b>0.48 (0.02)</b>	1.31 (0.30)	0.85 (0.54)	1.05 (0.82)	1.05 (0.80)
Leiomyosarcoma Leio vs else*	1.18 (0.76)	1.30 (0.33)	<b>3.14 (&lt;0.001)</b>	<b>2.04 (0.01)</b>	<b>2.74 (&lt;0.001)</b>
St-T/Kaposi ST/Kaposi vs else*	<b>5.98 (0.02)</b>	<b>2.88 (&lt;0.001)</b>	0.66 (0.30)	<b>1.81 (0.02)</b>	0.91 (0.72)
Overall	<b>0.04**</b>	<b>0.002***</b>	<b>0.003***</b>	<b>0.01***</b>	<b>0.004 ***</b>
<b>Recurrent disease</b>					
Primary* vs recurrent	1.48 (0.18)	<b>2.25 (0.001)</b>	0.84 (0.43)	1.25 (0.23)	1.06 (0.74)
<b>Previous treatment</b>					
Previous RTx No RTx* vs RTx	1.08 (0.85)	<b>2.78 (&lt;0.001)</b>	1.00 (0.99)	1.51 (0.06)	0.98 (0.93)
Previous ILP No ILP vs ILP*	2.52 (0.15)	1.32 (0.49)	0.86 (0.71)	1.03 (0.93)	0.87 (0.67)
<b>Multifocality</b>					
Unifocal* vs multifocal	<b>2.63 (0.008)</b>	<b>4.48 (&lt;0.001)</b>	0.95 (0.85)	<b>2.17 (&lt;0.001)</b>	1.14 (0.49)
<b>Adjuvant Radiotherapy</b>					
No* vs yes	Not applicable	0.68 (0.14)	1.27 (0.29)	0.91 (0.63)	0.76 (0.13)

\* index  
 \*\* Chi-Square  
 \*\*\* Log rank  
 HR = hazard ratio, HGPS= High grade pleiomorphic sarcoma not other specified; St-T = Stewart-Treves lymphangiosarcoma; RTx = radiotherapy; ILP = isolated limb perfusion;

*Limb function*

Limb function could be assessed in 210 of 212 limbs treated (99%). No functional loss was seen in 64% of patients (n=134), impairment of limb function without need for crutches in 12% patients (n=25), whereas 5% (n=10) of patients needed crutches due to functional loss. An amputation could not be avoided in 41 patients (19%). Consequently, the limb salvage

rate was 81%. Median time between last ILP and amputation was six months (range 0-63). The most frequent reason for amputation was insufficient response to TM-ILP (n=20, 49%). Five patients (12%) had local recurrence within 1 year after TM-ILP. Late or persistent recurrence led to amputation in eight patients (20%). Impaired wound healing and necrosis resulted in four amputations (10%) while three amputations (7%) were related to pre-existent peripheral vascular disease. One amputation was performed because of local toxicity (Wieberdink V).

Patients treated with adjuvant RTx showed a limb salvage rate of 85% whereas those refrained from RTx had a limb salvage rate of 79% (p=0.31).

### *Toxicity and leakage*

There was mild toxicity (Wieberdink II) in 136 patients (59%) and considerable erythema or edema (Wieberdink III) in 44 patients (19%) after TM-ILP (table 3). Local toxicity resulting in damage to deep-seated soft tissues (Wieberdink IV) was observed in five patients, requiring amputation (Wieberdink V) in one patient 30 days after TM-ILP.

Systemic leakage of ILP was absent or minor (<10%) in 204 cases (88%). Moderate systemic leakage (10-20%) was observed during 22 ILPs (10%) and severe leakage (> 20%) in five procedures (2%). These five perfusions with leakage rates of 23% - 64% did not lead to post-operative complications. All 5 patients were discharged from the ICU within 24 hours. Systemic toxicity was confined to transient fever of >40 °C in eight patients, lasting for >24 hours in one patient. No toxic shock-like syndrome necessitating administration of vaopressors was observed.

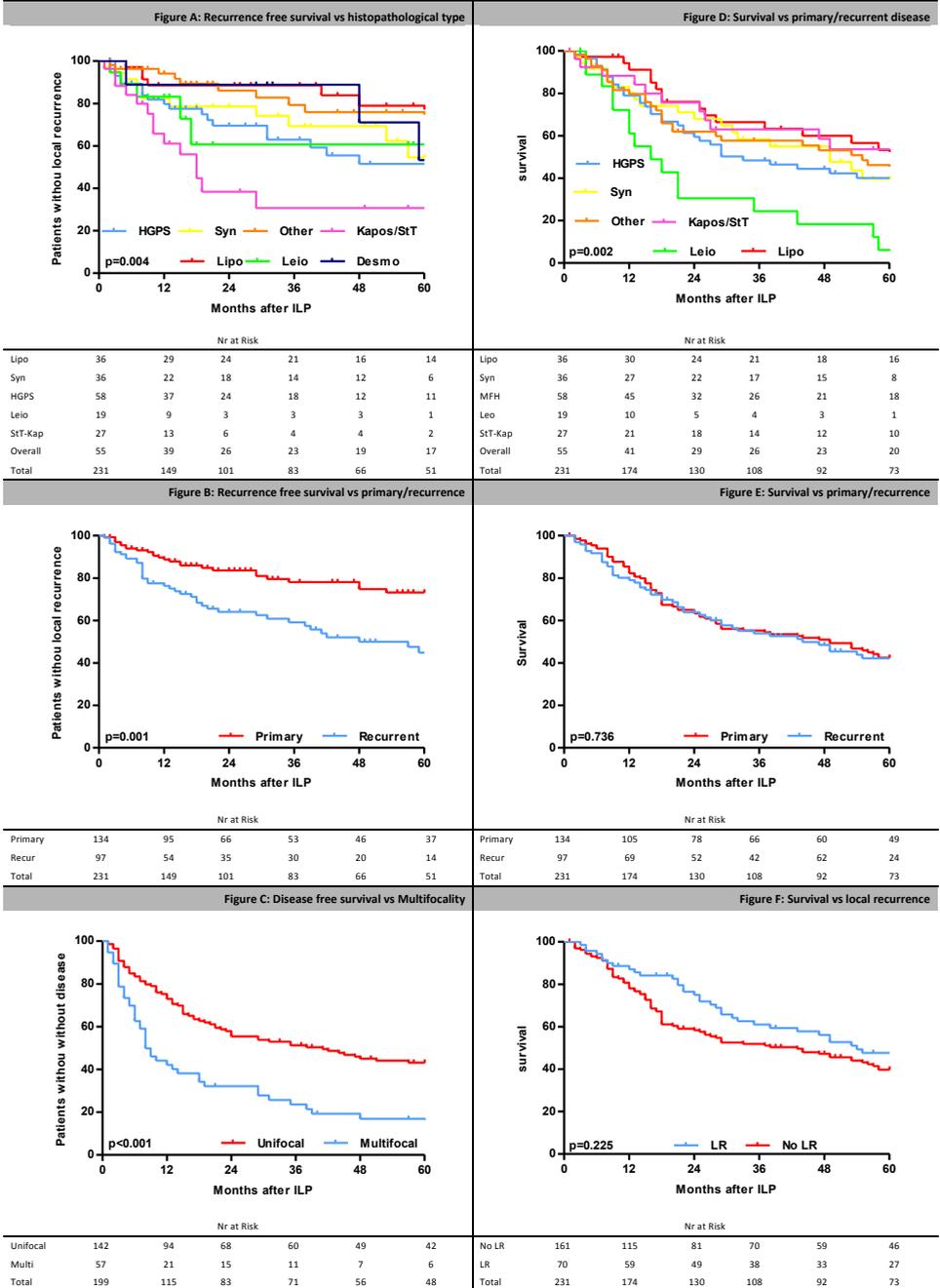
**Table 3: Wieberdink local toxicity stratified for type of ILP**

	W-I	W-II	W-III	W-IV	W-V	Total
<b>Type of ILP</b>						
Axillar	2 (8%)	18 (69%)	6 (23%)	-	-	26
Brachial	11 (28%)	19 (47%)	8 (20%)	2 (5%)	-	40
Iliacal	15 (16%)	56 (59%)	21 (22%)	2 (2%)	1 (1%)	95
Femoro-popliteal	18 (26%)	43 (61%)	9 (13%)	-	-	70
<b>Total</b>	46 (20%)	136 (59%)	44 (19%)	4 (2%)	1 (0%)	231
ILP = isolated limb perfusion						

### *Local recurrence*

Local recurrence was observed in 70 patients (30%). In case of local recurrence, median time to local progression (TLP) was 12 months (range 1-170). The one, three and five-year

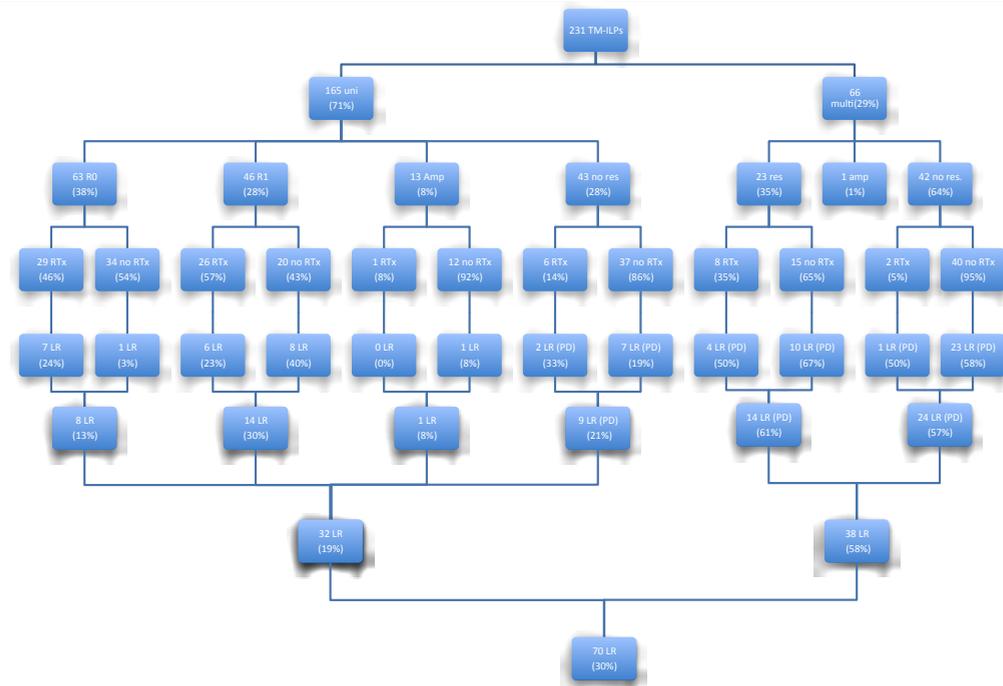
Figure 1: Survival curves



Lipo = liposarcoma; Syn = synovial sarcoma; HGPS= High grade pleiomorphic sarcoma not other specified; leio = leiomyosarcoma; desmo = desmoid; ST/Kap = Stuart Treves/Kaposi sarcoma; recur = recurrent disease; multi = multifocal; LR = local recurrence

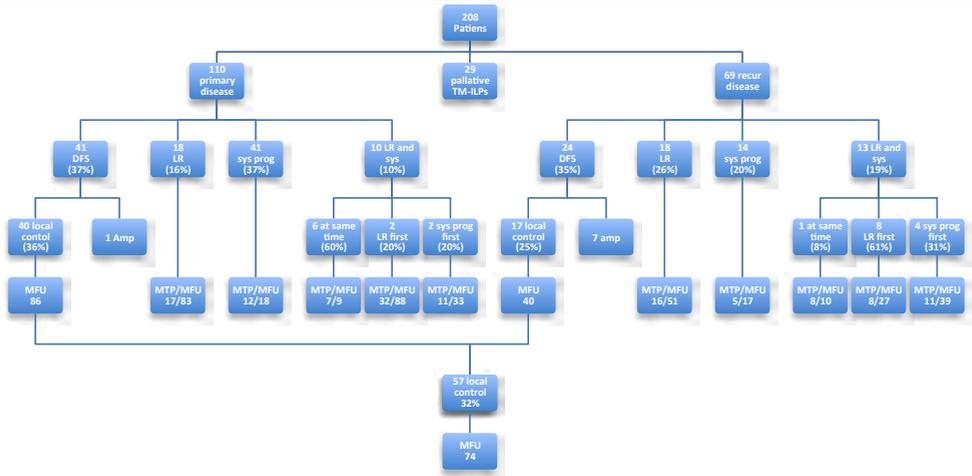
recurrence free survival (RFS) rate was 83%, 74% and 61%, respectively. Patients treated for unifocal disease had a 5-year RFS rate of 75%, while patients with multiple lesions had a 5-year RFS rate of 29% ( $p < 0.001$ ). Histopathological type of tumor (figure 1-A,  $p = 0.002$ ), tumors  $< 5$  cm ( $p = 0.02$ ) and patients previously treated with radiotherapy ( $p < 0.001$ ) had higher local recurrence rates. The 5 year RFS in patients treated for recurrent disease was 45%, compared to 73% in patients with primary disease (figure 1-B,  $p = 0.001$ ). In multivariate analysis, multifocality and previous radiotherapy remained significant negative prognostic factors for LR. Local recurrence had no significant correlation with OS (figure 1-F). Patients treated with adjuvant RTx showed 3-years local control rate of 75% compared to 66% for those refrained from RTx ( $p = 0.14$ ). Figure 2 displays the LR rate or progression of disease stratified for unifocal vs multifocal disease, resection, completeness of resection and adjuvant radiotherapy. Resection of residual tumor was not performed in 43 patients with unifocal disease for several reasons: 9 patients (21%) had a CR, 17 patients (40%) were treated with palliative intent, 3 patients (7%) developed systemic metastases shortly after ILP, 2 patients (5%) were treated with a second TM-ILP shortly after the first procedure. In one patient (2%), even exarticulation would not have resulted in complete removal of the tumor. In 11 patients, the reason for not performing a resection of the tumor is not known.

**prooFigure 2: Local recurrence stratified for multifocality, resection and adjuvant radiotherapy**



TM-ILP = TNF- $\alpha$  based isolated limb perfusion; uni = unifocal; multi = multifocality; amp = amputation; res = resection; RTx = radiotherapy; LR = local recurrence; PD = progressive disease

Figure 3: Disease free survival



TM-ILP = TNF- $\alpha$  based isolated limb perfusion; DFS = disease free survival; LR = local recurrence; sys prog = systemic progression; MFU = median follow up in months; MTP = Median time to progression in months

Ten patients (17%) developed systemic disease after a local recurrence (figure 3).

*Systemic progression*

Seventy-eight out of 179 patients (44%) treated with curative intent developed systemic disease. Metastases became apparent after a median period of 11 months (range 1-108). Five-year distant metastasis free survival (DMFS) was 55%. High-grade sarcomas (p=0.04), leiomyosarcomas (p<0.001), younger age (p=0.03) and tumors >10 cm (p=0.02) correlated with shorter DMFS. In multivariate analysis, leiomyosarcoma, size and age remained significant prognostic factors (table 4). After development of systemic disease, median survival was 8 months (range 0-71).

*Disease free survival*

One, three and five-year disease free survival for patients treated with curative intent was 64%, 43% and 36% respectively. Median time to any appearance of disease was 24 months. We separately analyzed patients with a first TM-ILP (n=208). Fifty-seven of these patients (32%) had no recurrence after TM-ILP with or without resection and with or without radiotherapy after a median follow up of 72 months (figure 3). Patients treated for primary disease had a disease free survival rate of 36% after a median follow up of 86 months.

**Table 4: Multivariate analysis of prognostic value of baseline factor on local progression, systemic progression and overall survival**

Endpoint	Variable		n	HR	p	95%-CI
Complete response (n=231)	Kaposi sarcoma / Stuart Treves	no	204	1		
		yes	27	5.98	0.02	1.38-26.00
Local progression (n=231)	Previous RTx	no	192	1		
		yes	39	2.33	0.002	1.38-3.93
	Multifocality	unifocal	165	1		
		multifocal	55	4.17	<0.001	2.56-6.78
Metastasis free survival (n=199)	Age	≤50	76	1		
		>50	123	0.51	0.003	0.33-0.80
	Size	<5cm	66	1		
		5-10cm	34	1.39	0.37	0.68-2.83
		>10 cm	99	2.00	0.01	1.17-3.39
	Leiomyosarcoma	no	181	1		
yes		18	3.96	<0.001	2.10-7.45	
Disease free survival (n=199)	Leiomyosarcoma	no	190	1		
		yes	18	2.24	0.05	1.28-3.94
	Multifocality	unifocal	142	1		
		multifocal	57	2.25	<0.001	1.55-3.26
Overall survival (n=231)	Size	<5cm	72	1		
		5-10cm	38	1.36	0.26	0.79-2.34
		>10 cm	121	1.72	0.01	1.14-2.58
	Leiomyosarcoma	no	221	1		
		yes	19	2.82	<0.001	1.67-4.75

HR = hazard ratio; CI = confidence interval; RTx = radiotherapy;

### Survival

Overall 5 and 10-year survival was 42% and 33% respectively. Median OS was 47 months. When stratified for Trojani grade, 5-year survival was 69% for patients with grade I tumors, 45% for grade II tumors and 34% for grade III tumors ( $p=0.05$ ). In univariate analysis, histopathological type of STS was correlated with OS (figure 1-D,  $p=0.004$ ). When subtypes were analyzed separately, leiomyosarcoma was associated with decreased survival (median

survival 18 months, 5-year OS 10%.  $p < 0.001$ ). Furthermore, size  $> 10\text{cm}$  was associated with shorter OS ( $p = 0.01$ , table 2). In multivariate analysis, size  $> 10\text{cm}$  and leiomyosarcoma remained significant unfavorable prognostic factors for OS (table 4).

## Discussion

STS of the limb are often large at presentation and require extensive and mutilating surgery, resulting in amputation in 10% of patients. Our tertiary referral center receives the majority of locally advanced extremity STS patients in the Netherlands, as well as a number of international referrals. This report presents our treatment results of a highly selected group of patients requiring functional or anatomical amputation to achieve local control. It is a mature report on the largest series of patients available. This series supports and confirms the role of TM-ILP as a limb salvage strategy in this patient group. Limb salvage was achieved in the majority of patients (81%) by TM-ILP followed by delayed local resection, and in some patients by TM-ILP alone. The small number of cases with local or systemic toxicity confirms that TM-ILP is a safe treatment modality.

The ORR in this study was 71%, which is comparable to response rates previously reported (63-96%).<sup>4, 6, 12, 15-19</sup> Our limb salvage rate of 81% was comparable to previous reports as well (66-89%).<sup>5, 6, 12, 15-20</sup> Although the majority of extremity STS patients will die of systemic disease, TM-ILP can often manage the local problem, even without resection or complete response and even in the presence of unfavorable prognostic factors such as high Trojani grade or large tumor.

The present data show a local recurrence rate of 30%, in line with reports in the literature (11-45%),<sup>4, 6, 12, 15, 18, 20-24</sup> and was slightly higher than recurrence rates reported for resected STS (10-27%).<sup>25-28</sup> Pisters et al.<sup>29</sup> reported a local recurrence rate of 17% in a large series ( $n = 1041$ ) of resected extremity STS. However, in our series the local recurrence rate may be increased by the large number of patients with multifocal STS (30%), who are excluded in most series. In patients with unifocal tumors, the recurrence rate in our group was only 19%. Furthermore, our series contained a relatively high number of patients with recurrent disease (44%), compared to other studies, while recurrent disease is a known adverse prognostic factor for local recurrence.

In contrast to previous results in the literature,<sup>29</sup> large ( $> 10\text{cm}$ ) tumors were a favorable prognostic factor for local control in our series. This may be explained by the fact that the group of small tumors ( $< 10\text{cm}$ ) included more patients with recurrent disease ( $p < 0.001$ , data not shown) as well as patients with multifocal disease ( $p < 0.001$ , data not shown). Both are associated with decreased local control. Size of tumor did not reach statistical significance in multivariate analysis on time to local recurrence.

In terms of overall survival, limb-preserving surgery after TM-ILP is equivalent to amputation. However, local recurrence is more common after limb-preserving surgery.<sup>26, 30</sup> There is an ongoing debate whether local recurrence may be associated with systemic progression and overall survival.<sup>1, 26, 31</sup> Some authors claim that local recurrence reflects the intrinsic aggressiveness of the tumor and therefore correlates with systemic progression.<sup>32, 33</sup> Our results show that only 17% of patients with local recurrence develops systemic disease and that there is no correlation between local recurrence and OS.

A limb salvage rate of 81% was achieved. Functional limb salvage was 64% whereas 17% of limbs showed impairment. The fact that 31 TM-ILPS were in patients with metastatic disease might slightly inflate the limb salvage rate since these patients showed very poor survival and therefore had a short follow up. On the other hand these patients suffered from a situation in which anatomical amputation was imminent and other options than ILP were not considered viable.

The beneficial effect of adjuvant radiotherapy after resection was first demonstrated by Rosenberg et al.<sup>1</sup> and is currently standard therapy. Subsequently, several studies reported the positive influence of adjuvant radiotherapy on local control after TM-ILP and resection.<sup>34, 35</sup> Therefore, the administration of radiotherapy after resection of extremity sarcomas is common practice.<sup>36</sup> Short- and long-term morbidity after radiotherapy however, is regularly seen.<sup>37, 38</sup> Recently Al-Refaie et al.<sup>39</sup> reported no beneficial effect on survival of adjuvant radiotherapy after resection for T1 low grade, as well as high grade extremity STS. However, studies on TM-ILP contain many patients with II-b tumors. Our group demonstrated that patients who underwent TM-ILP followed by radical resection and had >50% necrosis in the resection specimen had no local recurrence, regardless of the administration of adjuvant radiotherapy. We therefore suggested that this particular group can be spared adjuvant radiotherapy.<sup>40</sup>

Other preoperative down-staging regimes may also enable limb-sparing surgery. The possible role of induction chemotherapy was studied by the MD Anderson Cancer Center, but although a tumor response was observed in 43% of these patients, the therapy could only reduce the extent of operation in 13% and no scheduled amputation could be prevented.<sup>41</sup> Preoperative radiotherapy has not been studied as an induction therapy in patients with primary irresectable extremity STS. It has been shown to be as effective as postoperative radiotherapy in STS patients, but at the cost of higher wound complication rates.<sup>42</sup> Literature suggests that combined neoadjuvant chemoradiotherapy with limb salvage surgery is an option for treating patients with deep STS of the extremity. A series with a median tumor size of 15cm demonstrated a 22% partial response rate according to the RECIST criteria which is a promising result.<sup>43</sup> However, up to now this treatment option remains investigational and results from large studies have to be awaited.<sup>44</sup>

The present study reports the largest single center long-term series of TM-ILPs as a treatment modality for locally advanced extremity STS. This study does not claim an impact on survival, but confirms the role of TM-ILP as a safe and valuable strategy for limb salvage and local control.

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# Chapter 2

**Isolated limb perfusion with tumor  
necrosis factor- $\alpha$  and melphalan for  
the distal parts of the limb in  
soft tissue sarcoma patients**

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## Abstract

### *Background*

Approximately 10% of soft tissue sarcomas (STS) occur in the most distal parts of the extremities. The standard therapy is local excision with adjuvant radiotherapy, but achieving wide resection margins might be difficult in the distal parts of the limb. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and melphalan-based isolated limb perfusion (TM-ILP) is effective in locally advanced STS of the extremities. We report the results of TM-ILP for STS in the most distal parts of the limb.

### *Methods*

Between 1991 and 2009, 34 ILPs were performed in patients with irresectable STS of the wrist, hand, ankle or foot. Disease was unifocal in 21 (62%) patients.

### *Results*

Overall response rate was 71% (n=24). After a median follow-up of 34 (range 1-143) months the local recurrence rate was 32%. Amputation was unavoidable in four patients (13%), four other patients (13%) underwent a partial amputation of the hand or foot.

### *Conclusion*

With a limb salvage rate of 87%, TM-ILP is an effective treatment modality in patients with distal STS. In all patients with an indication for amputation surgery due to an STS in the distal part of the limb, TM-ILP should be considered.

## Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of rare tumors that account for approximately 1% of all malignancies. About 10% of all STS occur in the most distal parts of the extremity. Currently, STS are treated with wide surgical resection and adjuvant radiotherapy (RTx) <sup>1-3</sup> even when they are located in the distal parts of the limbs <sup>4</sup>. However, achieving wide excision margins in the distal part of the limb is difficult due to the lack of readily expandable soft tissue. In addition, blood vessels, nerves and tendons at risk are important for normal function of hand or foot. Preserving these structures results in preserved limb function, but positive margins are associated with high local recurrence rates <sup>5-7</sup>. Adjuvant RTx is associated with improved local control for extremity STS. Bray et al. reported minimal co-morbidity in a series of 25 resected STS in the hand treated with adjuvant RTx<sup>8</sup>. However, large series of extremity STS generally reported serious co-morbidity after RTx <sup>9-11</sup>. These factors make hand or foot-preserving surgery challenging in patients with locally advanced STS in the distal parts of the limbs.

Since amputation does not improve survival rates <sup>1, 2</sup>, interest in regional limb-saving treatment modalities for locally advanced extremity STS has increased. This has led to the successful introduction of isolated limb perfusion (ILP) with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and/or melphalan <sup>12</sup>. After multicenter studies reported overall response rates of 75-85%, this led to the approval of TNF- $\alpha$  and melphalan based ILP (TM-ILP) in Europe <sup>13,14</sup>.

In the absence of activity of cytostatics alone in the setting of ILP <sup>15</sup> the role of TNF- $\alpha$  is crucial because: TNF- $\alpha$  significantly increases melphalan uptake (4-5 fold) into tumor tissue <sup>16</sup>, and TNF- $\alpha$  leads to necrosis of endothelial cells <sup>17</sup> and pericytes <sup>18</sup> of the tumor vasculature, resulting in selective destruction of the tumor vasculature, as demonstrated in animal models and in patients <sup>19</sup>.

The present study reports on the results of TM-ILP for locally advanced STS in the most distal parts of the limbs.

## Patient and methods

### *Patients*

Between 1991 and 2009, 303 TM-ILPs were performed for extremity STS of which 34 were in 29 patients with STS of the wrist, hand, ankle or foot. All patients were candidates for disabling surgery or amputation, since a radical oncological resection was deemed impossible. Patient, tumor and perfusion characteristics were retrieved from a prospectively maintained database.

### *Isolated limb perfusion*

The technique of ILP has been described in detail elsewhere<sup>20</sup>. Briefly, the procedure is performed with patients under general anesthesia. After heparinization, the targeted blood circuit is isolated by clamping and cannulation of the major artery and vein, and connected to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels and prevents leakage. Using a precordial scintillation probe to detect technetium-labeled albumen, leakage is monitored during the whole procedure.

A dose of 1-3 mg (wrist or hand) or 1-4 mg (ankle or foot) of recombinant TNF- $\alpha$  (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) was injected as a bolus once the temperature of the limb reached 38°C. Subsequently, 13 mg/L (arm) or 10 mg/L (leg) melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) was administered 30 min after the limb temperature reached 38-39.5°C. The median dose of TNF- $\alpha$  administered was 3.0 (mean 2.76, range 1-4) mg, while the median dose of melphalan was 50 (mean 49, range 25-90) mg. After 90 min of perfusion, the limb is washed out with 1 L (arm) to 4 L (Iliac perfusion) of physiological saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden).

### *Response evaluation and toxicity*

Clinical evaluation was assessed 2, 4, 8 and 12 weeks after ILP. Hereafter, evaluation took place 3-monthly in the first year after ILP and at longer intervals during further follow-up. Evaluation consisted of clinical examination and magnetic resonance imaging (MRI) and was reported according to the World Health Organization (WHO) criteria<sup>21</sup>. Complete response was defined as a complete disappearance of the tumor lasting for at least 4 weeks; Partial response was defined as regression of the tumor of >50% for > 4 weeks, and no change was defined as  $\leq$ 50% regression of the tumor. New lesions or growth of the tumor was recorded as progressive disease if reported at first response evaluation and recorded as local recurrence when it was reported during further follow-up.

Local toxicity was defined according to Wieberdink et al.<sup>22</sup>: 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.

### *Local resection and radiotherapy*

A local resection was attempted whenever facilitated by the response of the tumor to TM-ILP. To define marginal status the criteria used by Pisters et al. were applied<sup>11</sup>. In each specimen the resection margins were inked. A microscopically positive (R1) margin was defined as an unequivocal tumor extension to the inked margin on permanent section. Cases

in which tumor was close to but did not reach the inked margin were considered to have microscopically negative margins (R0).

Whether or not patients would receive adjuvant RTx was decided during a multidisciplinary meeting with a surgical oncologist, a medical oncologist, a radiation oncologist, a pathologist and a radiologist.

### *Statistical evaluation*

Time to local or systemic recurrence and overall survival were defined as the time interval between TM-ILP and local recurrence, systemic recurrence and death (death due to disease, and death due to other causes), respectively, and estimates were made using the method of Kaplan Meier. To compare groups Chi-square and log rank tests were used. All tests were performed at a significance level of 5%.

## **Results**

### *Patients*

All patient characteristics are summarized in Tables 1 and 2. In total, 34 TM-ILPs were performed. One patient (pat no. 1) was treated three times for a Stuart Treves sarcoma of the hand. Two Kaposi sarcoma patients (pat no. 18 and 19) underwent a TM-ILP bilaterally; one patient was treated in both legs once, and the other patient had a left-sided limb perfusion once and a right-sided twice. As a result a total 31 limbs were analyzed in 29 patients. Sixteen patients (55%) were female. Median age at time of TM-ILP was 58 (mean 54, range 13-88) years. Five patients (15%) had a grade I sarcoma, 9 patients (26%) a grade II sarcoma, and 20 patients (59%) a grade III sarcomas. Adjuvant RTx was administered after 8 TM-ILPs (24%). Median follow-up was 34 (mean 41, range 1-143) months.

### *Clinical response*

The overall response rate was 71% (n=24). Eleven TM-ILPs (33%) resulted in a complete response and 13 TM-ILPs (38%) in a partial response. Stable disease was recorded after 10 TM-ILPs (29%).

In case of unifocal disease the overall response rate was 52% (of 21 patients) whereas a complete response rate of 10% (2 of 21 patients) was achieved. Resection of the tumor was deemed possible after 15 of 21 ILPs (71%) in patients with unifocal disease, resulting in a radical resection in 9/15 patients (60%). Limb-sparing resection was not performed in 6 patients with unifocal disease after ILP; one patient (pat no. 25) was treated in palliative setting and another patient (pat no. 21) was deemed a candidate for an amputation due to

**Table 1-a Patient overview (arms)**

Patient no	Age (Y)	Gender	Patholog. type	No tumor	Primary vs recurrence	Location	Trojani grade	Max dia (cm)	Clin res	Resection (% necrosis)	Adj RTx	LR (Mo)	Sys prog (Mo)	Amp (Mo)	Status (FU)
1	78	F	Stuart-T	>100	recurrence	hand	III	1	CR	no	no	19	no	no	DOC (47)
	80	F	Stuart-T	>100	recurrence	wrist & hand	III	1	CR	no	no	12	no	no	DOC (27)
	82	F	Stuart-T	>100	recurrence	wrist & hand	III	3	PR	no	no	no	no	no	DOC (2)
2	66	F	synovial	1	primary	hand	II	3	PR	no	no	no	no	no	LTFU(2)
3	86	F	spindle	1	primary	wrist	II	3	NC	R <sub>1</sub> (20%)	yes	no	no	no	NED (9)
4	25	M	epithelial	1	primary	wrist	III	3	NC	R <sub>1</sub> (50%)	yes	no	24	no	DOD (47)
5	62	F	epithelial	1	primary	hand	II	4	PR	R <sub>1</sub> (10%)	yes	no	no	no	NED (74)
6	20	M	epithelial	1	primary	finger	III	2	NC	R <sub>0</sub> (0%)	yes	no	no	dig III (1)	NED (74)
7	58	F	epithelial	1	primary	wrist	II	6	PR	R <sub>0</sub> (100%)	no	no	no	no	NED (41)
8	46	F	synovial	2	recurrent	hand	I	5	PR	no	no	1	no	hand (57)	NED (57)
9	28	M	angio	1	primary	hand	I	2	NC	R <sub>0</sub> (100%)	no	no	no	no	NED (6)
	37	M	epithelial	1	primary	hand	II	2	NC	R <sub>0</sub> (0%)	no	no	no	3 <sup>+</sup> + 4 <sup>+</sup> ray (4)	NED (20)

Adj RTx = adjuvant radiotherapy, Mo = months; FU = follow up; Stuart T = Stuart Treves sarcoma; agg fib = aggressive fibromatosis; CR = complete response; PR = partial response; NC = no change; DOC = died of other cause; LTFU = lost through follow up; NED = no evidence of disease; DOD = died of disease

Table 1-b Patient overview (legs)

Patient no	Age (Y)	Gender	Patholog. type	No tumor	Primary vs recurrence	Location	Trojani grade	Max dia (cm)	Clin res	Resection (% necrosis)	Adj RTx	LR (Mo)	Sys prog (Mo)	Amp (Mo)	Status (FU)
11	36	F	agg fib	1	recurrence	foot	I	16	CR	no	no	no	no	no	NED (143)
12	68	M	Kaposi	20-50	recurrence	foot	III	1	CR	no	no	no	no	no	DOC (7)
13	46	M	agg fib	1	recurrence	foot	I	5	NC	R <sub>1</sub> (0%)	no	5	no	no	NED (119)
14	69	M	exos	2	primary	foot	III	12	CR	no	yes	no	at LLP	no	AWD (22)
15	28	F	clear cell	1	recurrence	foot	III	5	NC	R <sub>0</sub> (100%)	no	no	no	no	NED (44)
16	32	F	synovial	1	recurrence	foot	III	2	CR	no	no	no	no	no	NED (10)
17	50	M	synovial	1	recurrence	foot	III	2	NC	R <sub>0</sub> (0%)	yes	57	no	Tr., MIT (57)	NED (90)
18	70	M	Kaposi	20-50	primary	foot (L)	III	4	CR	no	no	10	no	no	NED (90)
19	71	M	Kaposi	20-50	primary	ankle & foot (R)	III	3	CR	no	no	29	no	no	NED (81)
20	74	M	Kaposi	20-50	recurrent	foot (R)	III	4	CR	no	no	no	no	no	NED (48)
19	57	M	Kaposi	10-20	primary	foot (L)	III	4	PR	no	no	no	no	no	LTFU (1)
57	57	M	Kaposi	10-20	primary	foot (R)	III	7	PR	no	no	no	no	no	LTFU (4)
20	82	F	pleiomorphic	5	recurrent	ankle and foot	II	3	CR	no	no	3	3	AKA (3)	DOD (9)
21	87	F	leiio	1	recurrent	foot	II	7	PR	no	no	2	no	no	DOC (7)
22	88	M	angio	6	recurrence	foot	II	4	CR	no	no	14	no	no	DOC (20)
23	68	F	sarcoma NOS	1	primary	ankle and foot	III	6	NC	amp (30%)	no	no	1	UKA (2)	DOD (11)
24	26	F	synovial	1	primary	foot	II	5	NC	amp R <sub>0</sub> (0%)	no	no	13	1* + 2* ray (3)	DOD (24)
25	67	M	synovial	1	recurrence	foot	III	3	PR	no	no	no	at LLP	no	DOD (2)
26	13	F	synovial	1	primary	ankle	III	3	PR	R <sub>1</sub> (0%)	yes	35	42	UKA (37)	DOD (49)
27	23	F	clear cell	1	recurrent	foot	III	1	PR	R <sub>0</sub> (100%)	no	no	no	no	NED (44)
28	33	M	dermatofibr	1	recurrent	foot	I	4	PR	R <sub>0</sub> (90%)	no	no	no	no	NED (98)
29	31	F	synovial	1	primary	foot	III	10	PR	R <sub>1</sub> (25%)	yes	no	17	no	AWD (43)

Adj RTx = adjuvant radiotherapy; Mo = months; FU = follow up; agg fib = aggressive fibromatosis; exos = extra osseale osteosarcoma; NOS = not otherwise specified; CR = complete response; PR = partial response; NC = no change; amp = amputation; NED = no evidence of disease; AWD = alive with disease; LTFU = lost through follow up; DOD = died of disease; AKA = above knee amputation; UKA = under knee amputation

rapid progression, but refused treatment. One amputation was performed shortly after TM-ILP since the response was insufficient to enable a limb-sparing radical resection (pat no. 23). Two patients (pat no. 11 and 16) had a clinically complete response on MRI and one patient was lost to follow-up (pat no. 2). The median time interval between local resection and TM-ILP was 93 (range 46-260) days.

After ILPs performed in patients treated for multifocal disease (n=13) an overall response rate of 100% was achieved, which was significantly higher compared to the 52% overall response rate after ILPs for patients with unifocal disease (Table 3;  $p=0.003$ ). The complete response rate also showed a significant difference: in case of multifocality a rate of 69% was achieved whereas two patients with unifocal disease showed a rate of 10% ( $p<0.001$ ). Since the complete response rate is high and resection is often impossible in case of multifocal disease, there was no resection performed in patients with multifocal disease.

<b>Table 2: Characteristics of the patients, tumors and ILP characteristics</b>		
	<b>n</b>	<b>%</b>
<b>Gender</b>		
Female	16	55
Male	13	45
<b>Age (years)</b>		
Median (range)	58 (13-88)	
≤50	15	44
>50	19	56
<b>Location</b>		
Wrist or hand	12	35
Ankle or foot	22	65
<b>Size</b>		
< 5 cm	23	68
≥ 5 cm	11	32
<b>Type</b>		
Synovial	8	24
Kaposi/Stuart Treves	9	26
Epithelioid	5	15
Other (10 types)	12	35
<b>Trojani grade</b>		
Grade I	5	15
Grade II	9	26
Grade III	20	59
<b>Primary vs recurrent</b>		
Primary	17	50
recurrent	17	50
<b>Unifocal vs multifocal</b>		
Unifocal	21	62
Multifocal	13	38
<b>Type of ILP</b>		
Axillar	4	12
Brachial	8	23
Femoral	15	44
Popliteal	7	21

ILP = isolated limb perfusion

### *Limb function*

Limb function was assessed in all 31 limbs. There was no functional loss in 18 cases (58%) whereas impairment of the limb was reported in 9 cases (19%). Four cases of impairment were caused by partial amputation of the hand or foot. Two patients underwent a ray amputation, one patient in the hand (pat no. 10) and another patient in the foot (pat no. 24). Furthermore, a transmetatarsal (pat no. 17) and a finger amputation (pat no. 6) were performed. Other reasons for impairment of the limb were persistent edema (n=4) and nerve palsy (n=1). A limb amputation was unavoidable after 4 TM-ILPs (13%), therefore the limb salvage rate was 87%. Two patients (pat no. 8 and 26) required a limb amputation due to late recurrence, one patient (pat no. 23) underwent amputation because of lack of response on TM-ILP, another patient (pat no. 20) underwent above-knee amputation due to rapid recurrence.

### *Leakage and toxicity*

In this series there were no cases of high leakage procedures. In 3 cases there was 5% leakage (9%), which did not lead to any postoperative complication. No patients were admitted to the intensive care unit and no toxic-like syndromes necessitating vasopressors were reported.

Local toxicity was recorded as absent (Wieberdink I) after 10 procedures (29%), mild (Wieberdink II) after 19 procedures (56%), and considerable (Wieberdink III) after 5 procedures (15%). No cases of Wieberdink IV or Wieberdink V occurred.

### *Local and systemic progression*

Local recurrence occurred after 11 TM-ILPs (32%). The estimated median time to local

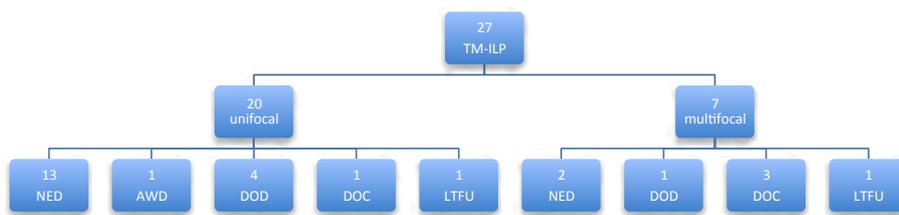
Endpoints	Unifocal	Multifocal	P-value
<b>Response</b>			
Complete response rate	10%	70%	<0.001
Overall response rate	52%	100%	0.003
<b>Local recurrence</b>			
Local recurrence rate	19%	54%	0.002
Estimated 1-y rec free	90%	59%	0.088
Estimated 3-y rec free	82%	18%	0.002
Estimated 5-y rec free	65%	18%	0.002
<b>Survival</b>			
Estimated 3-y survival	78%	54%	0.192
Estimated 5-y survival	58%	43%	0.274
Median survival	-	47 months	0.274

y = year; rec = recurrence

recurrence was 57 months. A 1 and 3 -year estimated recurrence-free interval was achieved in 79% and 59% of patients, respectively. In case of multifocality an estimated 18% of patients showed a 3-year recurrence-free interval compared to 82% for those with unifocal disease (Figure 2-a,  $p=0.002$ ). Table 3 summarizes data on local recurrence stratified for multifocality.

Systemic progression was reported after 6 TM-ILPs (19%). In case of systemic progression, median time to systemic progression was 15 months. In one patient systemic disease was preceded by local progression, in another patient systemic disease and local progression were diagnosed simultaneously. Four patients had systemic progression without evidence of local disease.

**Figure 1: Final outcome for patients treated with curative intent stratified for multifocality**

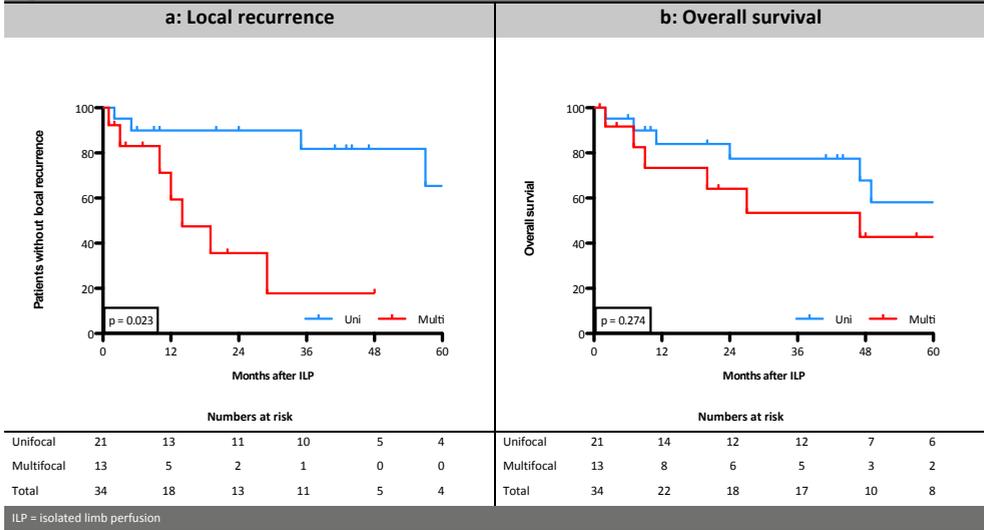


TM-ILP = TNF- $\alpha$  based ILP; NED = no evidence of disease; AWD = Alive with disease; DOD = died of disease; DOC = died of other cause; LTFU = lost to follow-up

### Overall survival

Actual 3 and 5-year overall survival rates were 72% (SE  $\pm 9\%$ ) and 52% (SE  $\pm 12\%$ ), respectively. A total of 10 patients (34%) died during follow-up; 6 of these patients died due to distant metastases. Two other patients (ID no. 14 and 29) were alive at the end of follow-up with systemic disease. A total of 15 patients (52%) were free of disease at the end of follow-up, with a median follow-up of 57 (range 6-143) months. Figure 1 shows the final outcome of all patients without distant metastases at time of TM-ILP stratified for multifocality. In the survival analysis, focality was not a prognostic factor for overall survival (Figure 2-b,  $p=0.274$ )

Figure 2: Data on unifocal vs multifocal local recurrence and overall survival



## Discussion

Patients with STS in the distal parts of the limb are difficult to treat due to difficulty in achieving radical local resection. This report describes patients requiring amputation or mutilating surgery, since an oncologically justified resection was deemed impossible or at the cost of severe co-morbidity. The study shows that TNF- $\alpha$  and melphalan-based ILP is an effective limb-saving treatment modality in patients with locally advanced STS of the distal parts of the limb. This is underlined by the favorable limb salvage rate (87%) of the patient population that was without evidence of disease at the end of a median follow-up of almost 5 years. The absence of systemic toxicity and the relatively mild local toxicity emphasize that TM-ILP is a safe and reliable treatment option.

The overall response rate was 71%, which is in accordance with earlier studies on TM-ILP for extremity sarcomas that reported response rates of 63-91%<sup>13, 14, 23-27</sup>. More recent response rates are slightly higher, i.e. Pennaccholi et al.<sup>28</sup> reported a rate of 96% (n=88), and Bonvalot et al.<sup>29</sup> reported an overall response rate of 79% (n=100). However, these latter data do not represent solely distally located sarcomas.

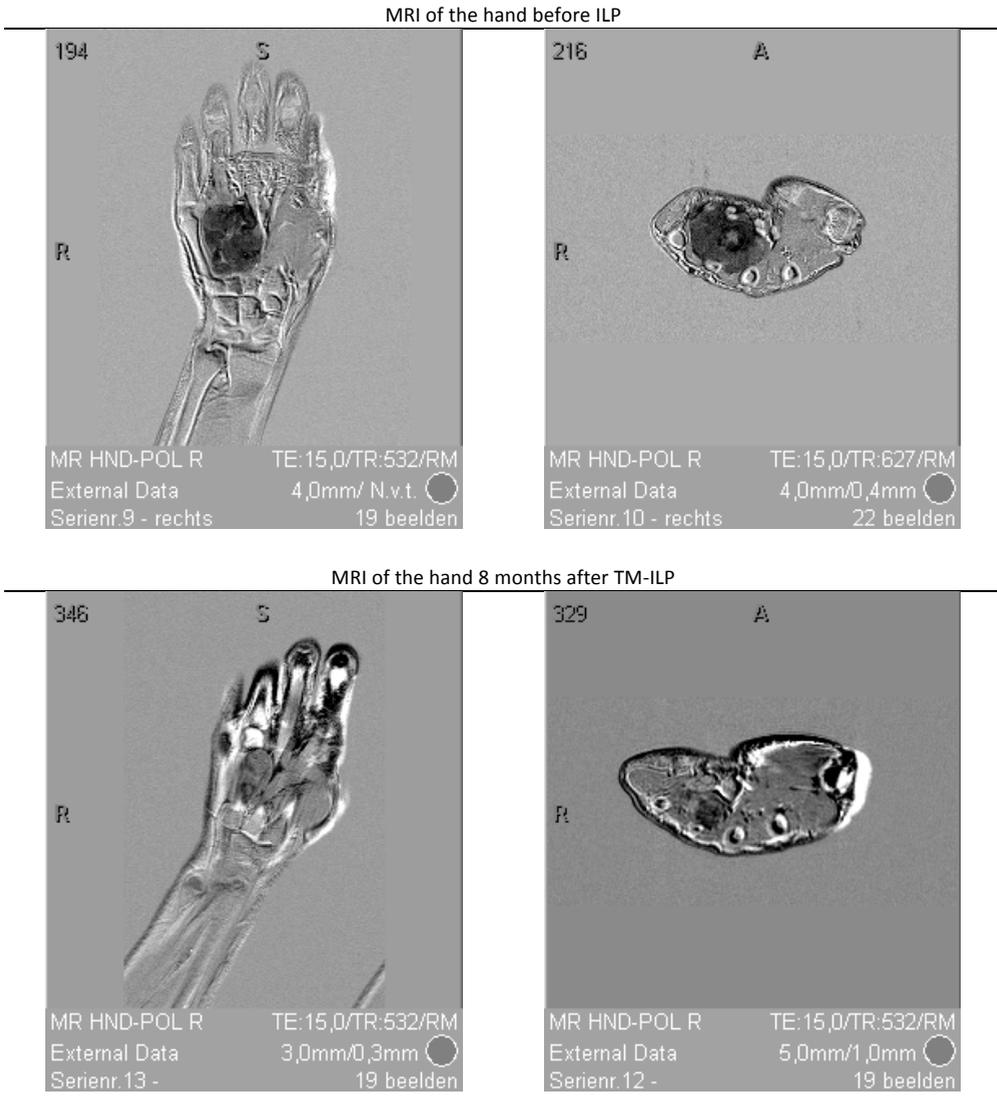
The limb salvage rate of 87% is in accordance with other reports on TM-ILP for locally advanced extremity STS (73-89%)<sup>13, 14, 24-30</sup> and also with other reports on wide resection of STS of the distal parts of the limb, with or without adjuvant RTx (83-90%)<sup>7, 31, 32</sup>. Our limb

salvage rate is substantially higher compared to that of Lin et al. who reported a rate of 73% in the largest series (n=115) of STS of the distal parts of the limb <sup>33</sup>.

In the present study local recurrence occurred in 32%, which is also in line with other reports on TM-ILP for extremity STS (11-45%) <sup>13, 14, 23-25, 27, 28, 34-36</sup>. Local recurrence rates on primarily resectable STS of hand and foot range from 21-41% <sup>4, 32</sup>, while Lin et al. reported a 3-year recurrence-free survival of 77% <sup>33</sup>. Pradhan et al. found a local recurrence rate of 32% and demonstrated significantly less frequent local recurrence after partial or complete amputation compared to wide excision <sup>7</sup>. In the present study no such correlation was found, which might be explained as follows: 1) due to shrinkage of the tumor an oncological radical resection becomes possible in patients with locally advanced tumors and hence an amputation can be avoided; Fig. 3 shows the shrinkage of a large epithelioid tumor in the hand (pat no. 5); and 2) Grabellus et al. hypothesized that after TM-ILP an improved margin status is achieved <sup>34</sup>. Our group demonstrated that after a histopathological response of >50% TM-ILP mediated necrosis, a local recurrence rate of 19% was observed in patients with an R1 resection <sup>37</sup>. This is a remarkable improvement compared to a large analysis (n=1,041) by Pisters et al. who reported a local recurrence rate of 40% in patients with an R1 resection <sup>38</sup>.

The value of adjuvant RTx for STS has been debated for many years. The beneficial effect of RTx for STS was first demonstrated by Rosenberg et al. <sup>1</sup>; thereafter several reports confirmed the favorable effect of RTx on local control as adjuvant of TM-ILP followed by resection <sup>35, 39</sup>. However, short and long-term morbidity after RTx is regularly reported <sup>9, 10, 40, 41</sup>. Our group demonstrated that patients who underwent TM-ILP with a radical resection and had >50% necrosis in the resection specimen showed no local recurrence, regardless of undergoing adjuvant RTx <sup>37</sup>.

The only goal of TM-ILP in extremity STS patients is to render irresectable tumors resectable. A possible alternative is preoperative radiotherapy, although this has not yet been studied in irresectable extremity STS. Preoperative radiotherapy appeared to be as effective as postoperative radiotherapy, but carries a greater risk of wound healing problems <sup>10</sup>. Induction chemotherapy showed a tumor response of 43%; however, this treatment modality could only reduce the extent of the operation in 13% and failed to avoid scheduled amputations <sup>42</sup>. Combined neoadjuvant chemotherapy with neoadjuvant radiotherapy might be an option in patients with deep extremity STS. A series with a median tumor size of 15 cm showed a 22% partial response rate according to the RECIST criteria, which is a promising result <sup>43</sup>. However, this treatment option remains investigational and results from large studies are awaited <sup>44</sup>.

Figure 3: Example of tumor response to TNF- $\alpha$  based isolated limb perfusion (ILP)

In the present study the 5-year overall survival was 52%, which is lower compared to data on outcome for hand or foot STS (67-87%)<sup>4, 7, 33</sup>. However, this difference should be interpreted with caution since all studies reported disease-specific deaths and censored those patients who died of other causes. The 5-year disease-specific survival of the present series was 66%, again, in line with other studies.

With an overall response of 71% resulting in a limb salvage rate of 87% this report demonstrates the efficacy of TNF- $\alpha$  based ILP in patients with STS of the distal parts of the limb. Local and systemic toxicity are limited which emphasizes the safety of this treatment

modality. We consider TNF- $\alpha$  and melphalan-based ILP the standard of care for patients with irresectable STS of the distal parts of the limb who would otherwise be a candidate for an amputation.

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# Chapter 3

## **Radiotherapy for soft tissue sarcomas after isolated limb perfusion and surgical resection: Essential in all patients?**

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## Abstract

### *Background*

Standard treatment for localized soft tissue sarcoma (STS) is resection plus adjuvant radiotherapy (RTx). In approximately 10% of cases, resection would cause severe loss of function or even require amputation because of the extent of disease. Isolated limb perfusion (ILP) with TNF- $\alpha$  and melphalan can achieve regression of the tumor facilitating limb saving resection. RTx improves local control but may lead to increased morbidity.

### *Methods*

In our database of over 500 ILP's, 122 patients with unifocal STS were treated by ILP followed by limb sparing surgery. All included patients were candidates for amputation.

### *Results*

Surgery resulted in 69 R0 resections (57%) and in 53 specimens (43%) resection margins contained microscopic evidence of tumor (R1). Histopathological examination revealed >50% ILP induced tumor necrosis in 59 cases (48%). RTx was administered in 73 patients (60%). Local recurrence rate was 21% after a median follow-up of 31 months (2-182). Recurrence was significant less in patients with >50% ILP induced necrosis versus  $\leq$  50% necrosis (7% versus 33%,  $p=0.001$ ). A similar significant correlation was observed for R0 resections versus R1 resections (15% versus 28%,  $p=0.04$ ). In 36 patients with a R0 resection and >50% necrosis, of which 21 were refrained from RTx, no recurrences were observed during follow up.

### *Conclusion*

In patients with a locally advanced primary STS, treated with ILP followed by R0 resection and with >50% ILP induced necrosis in the resected specimen, RTx is of no further benefit.

## Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of rare malignancies. In the United States, 8680 new cases of STS are diagnosed annually, of which approximately 60% are located in the extremities.<sup>1</sup> Soft tissue sarcomas are associated with early metastasis and a high disease specific mortality rate of approximately 50 percent. Moreover, STS of the extremity are often large at the time of presentation, making local resection and tumor control difficult.<sup>1</sup> Local control may require extensive surgery, resulting in loss of limb function or amputation in 10% of cases. Amputation however, is not associated with prolonged survival, because survival is determined by the occurrence of systemic disease.<sup>2, 3</sup> Since amputation has no beneficial effect on survival, a growing interest in limb salvaging techniques has arisen. Isolated limb perfusion (ILP) is a technique that results in perfusion of a tumor with high concentrations of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and melphalan under hyperthermic conditions. Following the introduction of the technique in the early nineties, Eggermont et al. reported encouraging results in a large European multicenter study.<sup>4</sup> Subsequent studies showed high local response and limb salvage rates and acceptable local and systemic toxicity.<sup>5</sup>

Currently, the use of ILP followed by resection and radiotherapy is an established strategy for limb salvage in Europe. Long-term morbidity has been described after ILP.<sup>6</sup> A contributing factor to morbidity is adjuvant radiotherapy.<sup>6</sup> However, whether adjuvant radiotherapy is necessary for all patients treated with ILP remains unclear. The aim of this study was to identify a specific group of patients in which adjuvant radiotherapy was of no further benefit in tumor control. All patients in our hospital undergoing ILP for unifocal STS of the extremity followed by local resection were identified. Subsequently, we determined whether patients with a STS of the extremity may be spared adjuvant radiotherapy under specific circumstances.

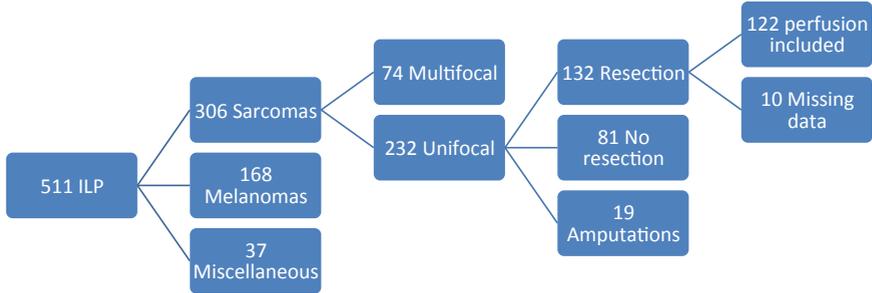
## Patients and methods

### *Patients*

From August 1991 to March 2009, 511 ILPs were performed in our hospital. These 511 cases comprised 168 melanomas, 306 STS and 37 miscellaneous malignancies (see figure 1).

ILP was the treatment strategy in 74 cases of multiple STS, while 232 patients were treated for unifocal disease. In this retrospective study we evaluated all consecutive 122 ILPs (in 120 patients) for unifocal STS followed by limb sparing surgical resection. The minimal TNM stage was IB, while the vast majority of STS was staged as IIB. Liposarcoma were most common

Figure 1: Inclusion flow chart



ILP = isolated limb perfusion

treated types of STS in our study of which 3 were well-differentiated, 2 were de-differentiated, 7 were pleiomorphic and 16 were of the myxoid subtype. The patient characteristics are summarized in table 1.

### Perfusion

The technique of ILP with TNF- $\alpha$  and melphalan has been described previously.<sup>4,5</sup> Briefly, the procedure is performed with patients under general anesthesia. After heparinization, a targeted blood circuit is isolated by clamping and cannulation of the major artery and vein and connection to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels and prevents leakage. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored for the length of the procedure. Patients underwent ILP via the axillary (n=9), brachial (n=19) iliac (n=54), femoral (n=24) or popliteal (n=16) approach.

A dose of 1 to 3 mg (arm) or 1 to 4 mg (leg) of recombinant TNF- $\alpha$  (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) was injected as a bolus once the temperature of the limb reached 38°C. Subsequently, 13 mg/L (arm) or 10 mg/L (leg) melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) was administered 30 minutes after the limb temperature reached 38 – 39.5°C. The median dose of TNF- $\alpha$  administered was 4.0 mg (mean 3.2, range 1-4), while the median dose of melphalan was 75 mg (mean 79.8, range 18-160). Several studies reported of the successful use of reduced doses of TNF- $\alpha$  in ILP for STS with comparable local recurrence rates and no systemic toxicity.<sup>7,8</sup> Hereafter perfusion was executed with 1 mg TNF- $\alpha$  in the arm and 2 mg in the leg. Consequently, since 2005 the median dose of TNF- $\alpha$  has been 2.0 mg (mean 2.09, range 1-3). After 90 minutes of perfusion, the limb is washed out with 1 L (arm) to 4 L (Iliac perfusion) of physiological saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden). Patients were observed

<b>Table 1: Patients and tumor characteristics</b>		
	<b>n</b>	<b>%</b>
<b>Gender</b>		
female	55	45
male	67	55
<b>Age</b>		
≤50	56	46
>50	66	54
<b>Size</b>		
<5cm	20	16
5-10cm	34	28
>10 cm	68	56
<b>Trojani Grade</b>		
I	11	9
II	31	25
III	73	60
Missing	7	6
<b>Site</b>		
upper arm	8	7
elbow	5	4
lower arm	10	8
wrist or hand	5	4
upper Leg	46	38
knee	20	16
lower leg	22	18
ankle or feet	6	5
<b>Histology</b>		
liposarcoma	28	23
synovial sarcoma	24	20
HGPS NOS	16	13
leiomyosarcoma	12	10
other (16 types)	43	34
<b>Primary/recurrent</b>		
primary	102	84
recurrent	20	16
<b>Adjuvant RTx</b>		
no	49	40
yes	73	60

HGPS NOS = High grade pleomorphic sarcoma not otherwise specified; RTx = radiotherapy

on the ICU for 1 night. The median length of hospital stay was 8 days (mean 11, range 2-136). Clinical response was defined using clinical findings and magnetic resonance imaging (MRI). A complete response (CR) was defined as a complete remission of tumor tissue, while a partial response (PR) was defined as a 51%-99% remission. No change (NC) was defined as a 0-50% remission. Clinical evidence of new lesions or growth of the tumor was defined as progressive disease (PD).

### *Local control*

Whenever the response of the tumor to ILP appeared to facilitate a local resection without major limb function loss, a local resection was attempted. Local resection was performed at a median of 84 days (mean: 91, range 14-260) after ILP. Marginal status of the resection was defined using the criteria according to Pisters et al.<sup>9</sup> In each surgical specimen the peripheral

margins were inked. A microscopically positive (R1) margin was defined as unequivocal tumor extension to the inked margin on permanent section. Cases in which tumor was close to but did not reach the inked margin were considered to have microscopically negative margins (R0). The percentage of the tumor that was necrotic due to ILP was estimated by the pathologist by macroscopic inspection and microscopic confirmation. Two categories were defined: 51-99% necrosis of the tumor and 0-50% necrosis of the tumor.

### *Radiotherapy*

Adjuvant radiotherapy was administered to 73 (60%) patients, while 49 (40%) patients did not receive radiotherapy. Like all other decisions concerning STS, decisions on the subject of adjuvant RTx were made in a multi disciplinary board containing a surgical oncologist, a medical oncologist, a radiation oncologist, a pathologist and a radiologist. Before 1998, there was little evidence that adjuvant radiotherapy was beneficial after local resection of STS. Since Olieman et al.<sup>10</sup> reported improved outcome after adjuvant radiotherapy in 1998, our center has administered significantly more RTx ( $p=0.04$ ). Reasons for refraining to administer adjuvant radiotherapy after 1998 were summarized in table 2.

	<b>n</b>	<b>%</b>
Systemic disease	8	40
Joint/Acra	3	15
Age/comorbidity	2	10
Refused by patient	1	5
Reason not specified	6	30
<b>Total</b>	<b>49</b>	<b>100</b>

RTx = Radiotherapy

## **Results**

### *Clinical response*

The clinical response after ILP was complete in 5 cases (4%). PR was obtained in 80 patients (66%), NC in 35 patients (29%) and PD in 1 patient (1%). Clinical response was not assessed in 1 patient (1%) for unknown reason.

### *Local control*

Results are summarized in table 3. Local resection of the STS resulted in a R0 resection in 69 patients (57%), while 53 (43%) patients had a R1/R2 resection. The overall local recurrence rate was 21% (25 patients) after a median follow up of 31 months (mean 48, range 2-182), 5

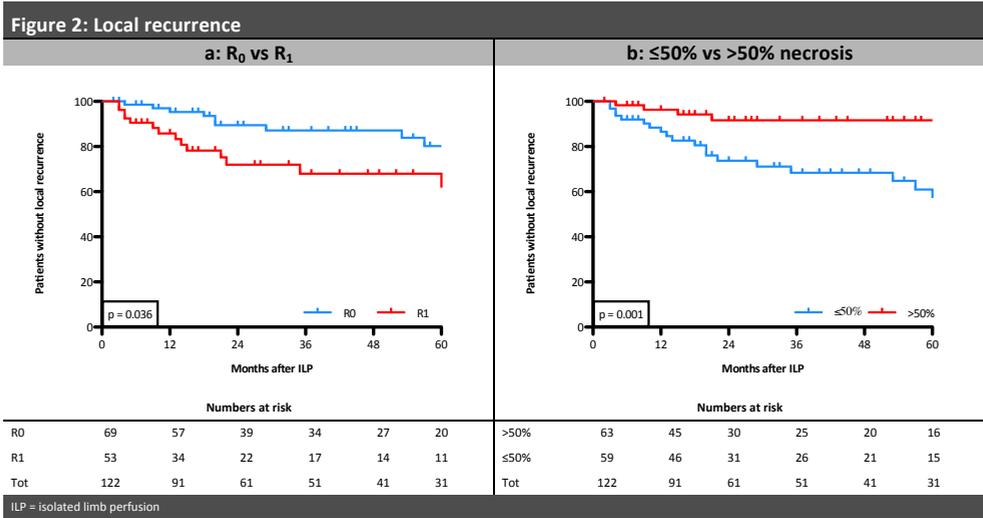
Table 3: Results for all STS				
R	Histology	n	Recurrence	Recurrence Rate
<b>no RTx</b>				
R0	≤50%	7	1	14%
R0	>50%	28	1*	4%
R1	≤50%	8	4	50%
R1	>50%	6	2	33%
<b>Total</b>		<b>49</b>	<b>8</b>	<b>16%</b>
<b>RTx</b>				
R0	≤50%	19	8	42%
R0	>50%	15	0	-
R1	≤50%	29	8	28
R1	>50%	10	1	10%
<b>Total</b>		<b>73</b>	<b>17</b>	<b>23%</b>
<b>Total</b>				
R0		69	10	15%
R1		53	15	28%
	≤50%	63	21	33%
	>50%	59	4	7%
<b>Total</b>		<b>122</b>	<b>25</b>	<b>21%</b>

\*One patient with local recurrence of a secondary diagnosed STS; RTx = radiotherapy

year recurrence free survival (RFS) was 75% ( $\pm$  5% SE). If recurrence occurred the median time to recurrence was 15 months (mean 27, range 3-170). After a R0 resection 5 year RFS was 80% while patients with a R1 resection had 5 year RFS of 72%. This difference reached significance ( $p=0.04$ , figure 2-a). The local recurrence rate was 15% in the R0 group (10 patients), while the R1 group had a local recurrence rate of 28% (15 patients). In 13 cases (11%) an amputation, after limb saving resection, was deemed necessary during follow up. The median time between limb-sparing resection and amputation was 29 months (mean 25, range 2-60). Consequently, the limb salvage rate was 89%.

Histopathological examination of the resection specimens revealed >50% ILP induced tumor necrosis in 59 cases (48%). In 63 (52%) cases the response to ILP was 50% or less. Patients with >50% necrosis in specimen after resection had 5 year RFS of 92% while patient with ≤50% necrosis had 5 year RFS of 61%. As can be seen in figure 2-b this difference was significant ( $p=0.001$ ). In the group with >50% necrosis of the tumor, the local recurrence rate was 7% (4 cases), compared to 33% (21 cases) in the group with ≤50% tumor necrosis. None of the patients were treated with adjuvant systemic chemotherapy or any other treatment modality in the time interval between ILP and resection.

Combining these results we could define a group of 43 patients (35%) with a R0 resection and >50% tumor necrosis, with a single case of local recurrence. This concerned a patient who was treated for a local recurrence. This female, aged 44, had no signs of residual vital tumor in the resected specimen. Since the tumor had been small and difficult to locate per-operatively, the completeness of the resection was deemed doubtful. Therefore,



radiotherapy was proposed, but refused by the patient. In retrospect, we believe that the tumor was not resected during surgery. None of the patients with a R0 resection and >50% necrosis who were treated for primary STS (n=36) developed local recurrence. Patients with a R0 resection but less than 50% necrosis of tumor (n=26) recurrence in 9 cases (35%). The local recurrence rate for patients treated with adjuvant RTx was 23% while patients not treated with RTx had local recurrence in 8 cases (16%). Whether patients with a R0 resection had radiotherapy was not based on main tumor characteristics (table 4).

### Discussion

Our results suggest that patients with a primary and unifocal STS of the extremity, in whom a R0 resection is achieved after successful ILP (induction of >50% necrosis), may not benefit from adjuvant radiotherapy. In these patients, the recurrence rate was 0%. Patients treated for recurrence of STS (n=7), who had a R0 resection and >50% tumor necrosis showed local recurrence in 1 case. The overall local recurrence rate in our series was 21%, which is in accordance with several other studies describing ILP as a limb saving strategy for STS (11-34%)<sup>5, 7, 10-16</sup>, while reported recurrence rates for primarily resectable STS range from 10% to 27%.<sup>17, 18</sup> A CR rate of 4% seems low compared to a rate of 28% in the multicenter trial published by Eggermont et al.<sup>15</sup> or in comparison with the latest studies, Bonvalot et al.<sup>7</sup> (30%; n=100), Grabellus et al.<sup>14</sup> (15%; n=47) and Pennachioli et al.<sup>16</sup> (41%; n=88). The discordance is due to selection bias since we only included patients with limb sparing surgery after ILP. The vast majority of patients with a MRI proven CR were not considered for resection and had clinical follow up for 10 years. Another bias was the exclusion of multifocal

tumors in general (Lev-Chelouche<sup>19</sup>, CR=38, n=53), or Stewart-Treves lymphangiosarcoma (Lans et al.<sup>20</sup>, CR=56%; n=16), which are known to respond well to ILP. With the introduction of the use of TNF- $\alpha$  in ILP there was a substantial improvement in treatment for non-resectable extremity STS.<sup>4, 5, 12, 21, 22</sup>

Nowadays ILP with delayed resection is an established strategy for limb salvage in Europe. Median time span between ILP was 84 days, which was within the range of previous reports (42-117 days).<sup>22, 23</sup> Completeness of resection is an important prognostic factor for local recurrence.<sup>14, 17, 18, 24-26</sup> After R0 or R1 resections in patients with over 50% of tumor necrosis a local recurrence rate of only 7% was observed. Patients with  $\leq$ 50% necrosis in resection specimen had recurrence in 33%. This highly significant difference suggest that the degree of ILP-mediated tumor necrosis might be an even stronger prognostic factor for local recurrence and support the findings of Grabellus et al.<sup>14</sup> that improved margin status is achieved after ILP. In patients with a R1 resection with > 50% necrosis 19% local recurrence was observed. This is a notable improvement in comparison with the large analysis (n=1041) of Pisters et al.<sup>18</sup> who reported of a local recurrence rate of 40% for R1 resection of extremity STS without ILP. Considering the larger proportion of large tumors (>10 cm; 25% vs. 56%) in our series this result get even more remarkable.

It could be argued that determining the degree of necrosis may not necessarily reflect a therapy effect but may be inherent tumor necrosis. Furthermore, macroscopic evaluation is a subjective and therefore imprecise factor. Considering the fact that tumor necrosis in untreated soft tissue sarcoma is an independent unfavorable prognostic factor it may be assumed that the necrosis observed in ILP-treated sarcomas is indeed therapy related and prognostically relevant. Secondly, considering the broad categories defined for tumor necrosis in grading systems (0%, <50% and >50%) it is highly unlikely that the degree of necrosis in single cases falls near the cut-off point. Furthermore, in our experience the pattern of necrosis in ILP-treated sarcomas, consisting of large confluent areas of necrosis, is different from the patch pattern of necrosis seen as spontaneous necrosis in untreated sarcomas. Finally the MRIs that were performed before ILP showed central necrosis in only 7 cases (12%) of 59 cases with >50% ILP induced necrosis.

The value of adjuvant radiotherapy after ILP and resection is still unclear. The beneficial effect of irradiation in limb saving surgery for STS was first demonstrated by Rosenberg et al.<sup>2</sup>. Despite reporting a significant decrease in STS local recurrence after irradiation therapy, Yang et al. concluded that in selected patients with low risk for recurrence irradiation should not be considered because of important life time risk for complications.<sup>27</sup> Other studies claimed that adjuvant radiotherapy in all cases had a significant positive influence in obtaining local control.<sup>10, 23</sup>

Complications of radiotherapy should not be underestimated. Hoven-Gonderie et al.<sup>28</sup> reported that two-thirds of all patients experienced serious late toxic problems after combined treatment for STS. Major problems with wound healing and continuous wound infections (8%-14%) are described in literature.<sup>29, 30</sup> Vascular damage (4-14%) is a common long-term complication of radiotherapy.<sup>6, 30, 31</sup> Kalman et al.<sup>32</sup> described 4 cases of long-term vascular side effects in the Axillary artery after mastectomy with adjuvant radiotherapy 10 to 27 years after treatment. Radiotherapy may cause neuropathy, especially when a boost is given<sup>28, 33</sup>.

Although radiation is effective in improving local control, several studies suggest that after margin free resection a subset of patients do not benefit from RTx<sup>14, 34, 35</sup>. In a prospective study Pisters et al.<sup>9</sup> reported that patients with a T1, R0 resected STS have acceptable long-term local control and may be spared the short- and long-term toxicity of radiotherapy.

In our series, patients were not randomized for adjuvant RTx so the conclusion based on our findings has to be read with caution. Furthermore, with 36 patients in 18 years the defined group that can be spared of RTx is relatively small. Further studies should be performed to confirm these findings. Nevertheless our results suggest that patients with a R0 resection combined with >50% ILP induced necrosis may be spared adjuvant RTx. The benefit of adjuvant radiotherapy after ILP followed by limb sparing surgery for this subset of patients seems limited. The added morbidity, the lack of survival benefit and the limited effect on local recurrence, should be discussed with these patients.

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# Chapter 4

## **Isolated limb perfusion using tumor necrosis factor- $\alpha$ and melphalan in patients with advanced aggressive fibromatosis**

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## Abstract

### *Introduction*

Aggressive fibromatoses (desmoid tumors) may be locally aggressive, but do not metastasize. Although a conservative approach is advocated for most patients, pain and functional impairment are indications for active treatment. Tumor necrosis factor- $\alpha$  based isolated limb perfusion (TM-ILP) is a limb saving treatment modality for soft tissue tumors. This study reports the results of TM-ILP treatment in patients with aggressive fibromatosis.

### *Methods*

Institutional databases of three European centers were searched. All patients that received TM-ILP treatment for aggressive fibromatosis between 1990 and 2012 were included. Before therapy, the patients were discussed in multidisciplinary tumor boards.

### *Results*

Twenty-five patients were treated with 28 TM-ILP treatments. The median age of the patients was 28 years, interquartile range (i.q.r.) 19-34 years and median hospital stay eight (7-12) days. Median follow-up was 84 (34-114) months. Complete response was achieved after two TM-ILP treatments, and partial response after 17 treatments in 16 patients. Stable disease was reported after eight treatments in seven patients, including a patient with stable disease after the first treatment and progression after the second TM-ILP. Toxicity was modest after most treatments; Wieberdink IV (extensive epidermolysis, and threatening or manifest compartmental syndrome) was seen after two TM-ILP treatments. Systemic leakage was reported after one treatment, but did not lead to systemic toxicity. Functional outcome was good: 16 patients had no physical limitations; six patients had some limitations but did not need medical aids. Amputation was prevented in all but three patients.

### *Conclusion*

Tumor necrosis factor- $\alpha$  based isolated limb perfusion was effective in patients with aggressive fibromatosis.

## Introduction

Aggressive fibromatosis or desmoid tumor is a rare benign soft tissue tumor that is localized throughout the body. Aggressive fibromatosis does not metastasize, but locally advanced or recurrent disease is frequently seen in tumors localized in the extremities<sup>1-3</sup>. Extra-abdominal aggressive fibromatosis is not related with mortality, unlike intra-abdominal tumors<sup>4</sup>. Current literature advocates a conservative approach for these benign tumors, due to compelling evidence of disease stabilization and spontaneous tumor regression in many patients<sup>5,6</sup>. Despite the benign nature, some tumors behave aggressively, leading to pain and functional impairment. In patients with severe impaired quality of life, a conservative approach may no longer be an option. The choice of treatment depends on many factors and as such, an algorithm was recently proposed<sup>6</sup>. Involvement or proximity of vital structures, for instance major nerves and vessels, may lead to mutilating surgery or even amputation. In these particular patients a limb saving strategy that result in relief of symptoms should be preferred.

In 1958 isolated limb perfusion was introduced as a treatment modality for extremity malignancies, such as locally advanced sarcomas and melanoma in-transit-metastases<sup>7</sup>. In patients with sarcoma this technique appeared ineffective when combined with cytostatics, but markedly gained in effectiveness in combination with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )<sup>8,9</sup>. Currently, TNF- $\alpha$  and melphalan based isolated limb perfusion (TM-ILP) is the standard of care in Europe for patients with limb-threatening sarcomas and has shown to lead to limb salvage in up to 89 per cent of patients<sup>10</sup>.

In the present series, the data of three sarcoma centers of the European Organization for Research and Treatment of Cancer (EORTC) on patients with locally advanced aggressive fibromatosis treated with TM-ILP in order to avoid a limb amputation is reported.

## Patients and methods

### *Patients*

Consecutive patients with aggressive fibromatosis that underwent treatment with TM-ILP in the participating EORTC centers 1990-2012 were included in the study. Participating centers were: Gustave Roussy Cancer Campus, Villejuif-Paris, France, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy, and Erasmus Medical Center Cancer Institute, Rotterdam, the Netherlands.

Due to the benign tumor biology of aggressive fibromatosis, only patients suffering from intolerable pain or functional impairment are considered for surgical treatment in these institutes. The safety and efficacy of conservative treatment is presently being evaluated in

prospective trials in all three centers. For patients in the present study, a variety of treatments had previously been performed, and radical resection was deemed possible only with mutilating surgery or amputation. In these patients, TM-ILP treatment was considered. Prior to TM-ILP, all patients were discussed in a multidisciplinary tumor board.

### *Perfusion*

Isolated limb perfusion (ILP) technique has been described extensively<sup>8,11</sup>. The procedure is performed with patients under general anesthesia. After heparinisation, the targeted blood circuit is isolated by clamping and cannulation of the major artery and vein and connected to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels to prevent leakage. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored throughout the procedure. Tumor necrosis factor- $\alpha$  and melphalan are used and after 60-90 minutes of perfusion, the vascular system of the limb is rinsed from the active compounds and the circulation restored. The ILP-protocols in the contributing institutions had minimal differences. Details of the procedures have been described before<sup>9,12,13</sup>.

### *Response and toxicity*

Response was evaluated by clinical examination and magnetic resonance imaging (MRI) at 4-8 weeks after treatment with ILP and reported following World Health Organization (WHO) criteria<sup>14</sup>. Complete response (CR) was defined as complete disappearance of the tumor, partial response (PR) was a decrease by more than 50 per cent, whereas stable disease (SD) was recorded if neither the criteria for PR nor progressive disease were met. Progressive disease (PD) was defined as 25 per cent increase of tumor with initially no documentation of CR, PR or SD. In patients with incomplete radiographic measurements, response was based on available radiological information and clinical judgment.

Acute regional toxicity after perfusion was classified according to Wieberdink et al.<sup>15</sup>: (I) no reaction, (II) slight erythema or oedema, (III) considerable erythema or oedema with some blistering, slightly disturbed motility permissible, (IV) extensive epidermolysis, and threatening or manifest compartmental syndrome, and (V) reaction that may necessitate amputation.

Functional outcome was based on clinical assessment and categorized as perfect, impairment without the necessity of medical aids, and impairment with the necessity of medical aids or amputation.

## Statistics

Categorical data are presented with numbers and continuous data with median and inter quartile range (i.q.r.).

## Results

A total of 25 patients were treated with 28 TM-ILP treatments. The median age of the patients at the time of TM-ILP was 28 (i.q.r. 19-34) years and the median hospital stay after treatment was 8 (7-12) days. The majority of patients were females. The median follow-up was 84 (34-114) months. Baseline characteristics of the patients are summarized in Table 1. Five patients were treated for a primary tumor. Two of these patients had previously received systemic anti-inflammatory treatment for inoperable disease, but this did not lead to sufficient reduction or relief of complaints. The indication for choosing ILP as a primary treatment in the other three patients was bone involvement, sciatic nerve involvement and refusal of systemic treatment by the patient.

**Table 1: Baseline characteristics of patients with aggressive fibromatosis.**

		n
<b>Gender</b>	Male	8
	Female	17
<b>Age in years</b>	Median (i.q.r.)	28 (19-34)
<b>Localization</b>	Arm	4
	Leg	21
<b>Size in cm</b>	Median (i.q.r.)	12 (8.5-15)
<b>Number of tumors</b>	Single	14
	Multiple	11
<b>Tumor treated</b>	Primary tumor	5
	Primary recurrence	10
	Secondary recurrence	10
<b>Previous treatment</b>	None	3
	Surgery	7
	RTx	2
	Systemic treatment	2
	Surgery + RTx	3
	Surgery + RTx + Systemic treatment	4
	Surgery + Systemic treatment	4

i.q.r. = interquartile range; RTx = radiotherapy

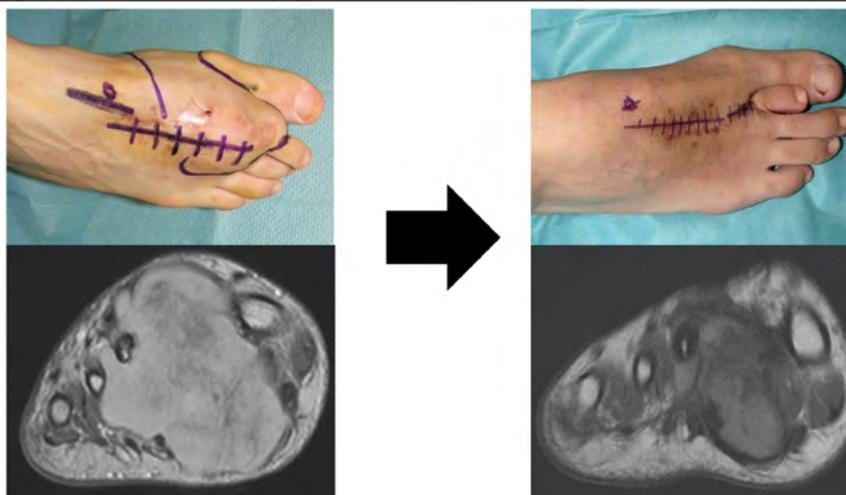
## Treatment outcome

Details on the treatments are illustrated in Table 2. Overall response was seen after 20 of 28 TM-ILP treatments. Complete response was achieved in two patients. Both patients underwent TM-ILP as a single treatment modality for recurrent disease, after previous surgical resections and for one patient also following radiotherapy. These two patients had sustained CR until end of follow-up at 94 and 36 months respectively.

	n	
<b>Type of ILP</b>	Axillar	3
	Brachial	1
	Iliacal	10
	Femoral	10
	Popliteal	4
<b>Response</b>	Complete	2
	Partial	18
	Stable disease	7
	Progression	1
<b>Wieberdink class</b>	I	4
	II	16
	III	6
	IV	2
<b>Hospital stay in days</b>	Median (i.q.r.)	8 (7-12)
<b>Treatment after ILP*</b>	None	21
	Resection	3
	Radiotherapy	2
	Amputation	3
<b>Local recurrence after ILP</b>	No	17
	Yes	11
<b>Limb function**</b>	Perfect	16
	Limited	6
	Amputated	3

i.q.r. = interquartile range; ILP= isolated limb perfusion  
 \*one patient underwent both amputation and radiotherapy  
 \*\*for patients at the end of follow-up

Partial response was recorded after 17 TM-ILP treatments in 16 patients. For 11 patients, TM-ILP led to control of disease and symptoms that was sustained until end of follow-up without need for additional treatment; an example is shown in Figure 1. Of the other five patients, one patient received adjuvant radiotherapy in order to achieve control of disease. Surgical resection of the residual disease was performed after the first TM-ILP in one patient presenting with recurrent disease. A second recurrence, which was treated with another TM-ILP at 32 months after the first treatment, resulted in PR and in disease control until the end of follow-up. Three patients with initial PR after TM-ILP treatment developed progression of remaining disease during follow-up. One of these patients had tumor deposits throughout one of the legs and had previous undergone extensive radiotherapy. The patient was included in a phase-I-study (GW786034) with treatment by paclitaxel and pazopanib. During this treatment the patient developed necrosis of the foot and an amputation below the knee was performed three years after TM-ILP. Several tumor deposits were still in situ and stable at the end of follow-up. The other two patients with progression received systemic treatment for the progressive disease, which lead to disease control. Stable disease was recorded after eight TM-ILP treatments in seven patients. Of these eight patients, the response to TM-ILP was sufficient to achieve disease control in four patients

**Figure 1: Patient with recurring aggressive fibromatosis on the right foot.**

Left hand side before isolated limb perfusion. Right hand side status 24 months after isolated limb perfusion  
 Upper panel = macroscopic appearance; lower panel = Axial view of contrast enhanced T1 weighted MRI

which was sustained in two of them until the end of follow-up. One of the patients with initial disease control developed tumor progression after 24 months and received systemic treatment. The other patient with initial disease control developed a new tumor lesion after 57 months, with stable disease of the primary tumor. After systemic treatment, both lesions remained stable until end of follow-up. In the last three patients with SD, the response was sufficient to perform limb-sparing surgery. One of these patients with 30 per cent tumor response after TM-ILP treatment had macroscopic negative margins after surgical resection (R1 resection). Four years after resection, progression of disease led to a second treatment with TM-ILP. After treatment, the leg was amputated above the knee due to healing problems. However, a new recurrence occurred in the stump, for which exarticulation of the hip was performed with postoperative adjuvant radiotherapy. At the end of follow-up, the patient was free of disease.

Another patient who underwent surgery with R1 resection after TM-ILP, developed recurrence which was successfully treated with chemotherapy.

In the last patient with SD, disease control was achieved after surgery (R1 resection). After 19 months, a second recurrence was diagnosed. Systemic treatment was ineffective, and a second TM-ILP was performed 56 months after the first TM-ILP. Due to continuous progression of disease, the patient underwent amputation above the knee and radiotherapy of the stump, after which disease stabilisation was obtained. The patient developed yet another recurrence which was treated with systemic therapy. This was the only patient with progressive disease at the end of follow-up.

### *Toxicity and function*

Local and systemic toxicity were modest. Wieberdink I was recorded after four procedures, Wieberdink II after 16 procedures and Wieberdink III after six procedures. More severe toxicity (Wieberdink IV) was seen after two TM-ILP treatments; one patient was treated with fasciotomy and necrosetectomy, and one patients with rapid disease progression with amputation.

Leakage of the perfusate to the systemic circulation was seen after one treatment. The leakage was conservatively managed and did not lead to systemic toxicity.

Functional outcome was good. Some 16 patients had no limitations in physical function; six patients had some limitations of the limb, but without the need of medical aids. Amputation could not be prevented in three patients.

### *Recurrence and survival*

Local recurrence or progression of disease after initial disease control was documented after 11 of the 28 TM-ILP treatments. Median time for tumor recurrence or progression was 27 (17-44) months. None of the patients died during follow-up.

## **Discussion**

This multicenter study indicates that treatment with TM-ILP may be a limb saving strategy for aggressive fibromatosis in patients in whom previous therapy had failed or surgical treatment would cause severe limb impairment. Tumor response was seen after 20 of 28 TM-ILP treatments, and amputation was avoided in all but three of the 25 patients.

A limitation of this study is the absence of pain scores before treatment, which is due to the retrospective study design. Although details are lacking, the indication for TM-ILP for each patient was carefully assessed during a multidisciplinary board, which, among other things, included evaluation of pain due to tumor growth.

Tumor behavior of aggressive fibromatosis varies greatly. At the time of diagnosis, a conservative approach with careful follow-up only is advocated in agreement with a recent study<sup>6</sup>. Even minor tumor progression in the absence of patient complaints may justify a conservative approach. Aggressive local tumor growth or intolerable pain are reasons for active therapy. In these patients, surgery is the mainstay of treatment, but adequate surgical resection might lead to severe impairment of the limb or even amputation. In these particular patients, treatment with TM-ILP might be successful and may achieve an excellent limb salvage rate as shown in this series.

Chemotherapy, non-cytotoxic systemic treatment and radiotherapy, are other options when the extent of disease excludes surgery as a primary treatment modality. Several different chemotherapy regimens have been proposed with varied response rates<sup>16</sup>. In an overview of

the French Sarcoma Group, 62 patients were analysed after treatment with various regimes<sup>17</sup>. The overall response rate was 21 per cent. The systemic side-effects of chemotherapy for a localized tumor disease warrant careful consideration before treatment. Less aggressive systemic treatment options are hormonal therapy, non-steroid anti-inflammatory drugs or the tyrosine kinase inhibitor imatinib. Response rates of approximately 50 per cent are reported, but only small groups of patients have been studied and, with limited follow-up time<sup>18,19</sup>. Side effects vary depending on the type of treatment, but often include gastro-intestinal complaints and fatigue. Long-term side effects are unknown and follow-up protocols are yet to be established.

Most of the experience with radiotherapy has been acquired in the adjuvant setting. Few reports discuss radiotherapy as single modality treatment for aggressive fibromatosis. Ballo et al.<sup>20</sup> showed a 5-year progression free survival of 69 per cent. A review<sup>21</sup> reported local tumor control rates of 78 per cent. The most compelling data are from a recent EORTC study<sup>22</sup>, which included 44 patients with inoperable or incompletely resected disease. All 44 patients received a dose of 56 Gy in 28 fractions, and had a median follow-up of 4.8 years. The 3-year local control rate was 81.5 per cent. Further response with tumor regression was seen after 3 years in three patients; two patients had CR and one patient PR. Acute grade 3 side-effect were limited to the skin, mucosa and pain whereas mild oedema was the late toxic effect in ten patients<sup>22</sup>.

In the present study, nine patients had received radiotherapy prior to treatment with TM-ILP, which excluding radiotherapy as a treatment option. For patients without previous radiotherapy, the authors of the present study advocate treatment with TM-ILP because of the potential for surgical resection, and radiotherapy, or even re-TM-ILP after the initial treatment. In areas that are not amenable for TM-ILP, such as the groin or buttocks, radiotherapy might be an attractive alternative for irresectable and limb-threatening tumors. Local and systemic toxicity was limited in the present series. The toxicity in these patients was comparable to the toxicity reported in previous series on nearly 400 TM-ILP treatments of extremity sarcomas<sup>9,10,12</sup>.

Patient selection is essential in the current era of tailored treatment strategies. Tumor necrosis factor- $\alpha$  based isolated limb perfusion is an aggressive treatment for this relatively benign disease. Patients who are considered for TM-ILP have tumors with aggressive tumor biology and severe symptoms.

Etiological studies have shown the importance of the Wnt-pathway and  $\beta$ -catenin in the development of aggressive fibromatosis<sup>23,24</sup>. Specific mutations on the CTNNB1-gene (encoding for  $\beta$ -catenin) have been associated with aggressive fibromatosis<sup>25-27</sup>. The precise mechanism and the different effects exerted by the specific mutations are not well understood. Several studies have demonstrated a predictive value of the mutations on the

risk of tumor recurrence following surgery<sup>28-31</sup>. Whether the mutational status of the tumor has an effect on the outcome after TM-ILP is unknown.

Tumor necrosis factor- $\alpha$  based isolated limb perfusion is an effective limb sparing technique to treat aggressive fibromatosis in selected patients. The treatment should be considered after failure of initial therapy and if surgery of recurrent or progressive disease would lead to functional loss or amputation.

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# Chapter 5

**Treatment modifications in tumor  
necrosis factor- $\alpha$  based isolated limb  
perfusion in patients with advanced  
extremity soft tissue sarcomas**

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## Abstract

### *Background*

Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and melphalan based isolated limb perfusion (TM-ILP) is an attractive treatment option for advanced extremity soft tissue sarcomas (STS). This study reports on a 20-years single center experience and discusses the evolution and changes in methodology since the introduction of TNF- $\alpha$  in ILP.

### *Patients and methods*

We performed 306 TM-ILPs in 275 patients with extremity STS. All patients were candidate for amputation or mutilating surgery in order to achieve local control. Clinical response evaluation consisted of clinical examination and magnetic resonance imaging. To evaluate the importance of TNF- $\alpha$  dose, treatment results of two periods (1991-2003 high dose (3-4mg) TNF- $\alpha$ ; 2003-2012 reduced dose (1-2mg) TNF- $\alpha$ ) were compared.

### *Results*

During the study period, more femoral perfusions were done instead of iliac perfusions. Reduction of TNF- $\alpha$  dose and reduction of total ILP time did not lead to different clinical response rates (70% and 69% for periods 1 and 2 respectively) or different local recurrence rates, but was associated with less local toxicity (23% and 14% for periods 1 and 2 respectively). Hospital stay was significantly reduced during the study period. There was an improved pathological response in the high dose TNF- $\alpha$  group without consequences for clinical outcome.

### *Conclusion*

TM-ILP remains a very effective treatment modality for limb threatening extremity STS. Moreover, reduction of dose and the growing experience in ILP led to less local toxicity and shorter hospital stay.

## Introduction

Soft tissue sarcomas (STS) are a heterogeneous group of rare tumors that arise from tissues of mesenchymal origin and account for approximately 1% of malignancies.<sup>1</sup> Approximately 60% of STS occur in the extremity and are often large at presentation, which may lead to extensive mutilating surgery or end up in amputation in about 10% of STS patients. With the emerging evidence in the 1980's that amputation had similar survival as radical resection,<sup>2-4</sup> interest in loco-regional treatment modalities such as isolated limb perfusion (ILP) increased. ILP with cytostatics alone is ineffective in treatment of large extremity STS.<sup>5</sup> The combination with high dose tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) demonstrated to result in high response and limb salvage rates in a number of multicenter studies.<sup>6, 7</sup> Further studies revealed that the role of TNF- $\alpha$  in ILP is crucial for a number of reasons: a) TNF- $\alpha$  increases melphalan uptake significantly (4-5 fold) in to tumor tissue.<sup>8</sup> b) TNF- $\alpha$  leads to necrosis of endothelial cells<sup>9</sup> as well as of the pericytes<sup>10</sup> of the tumor vasculature, resulting in a selective destruction of the tumor vasculature, as demonstrated in animal models as well as in patients.<sup>11</sup>

Currently, TNF- $\alpha$  and melphalan based ILP (TM-ILP) is used in STS patients with irresectable disease in order to obtain local control and make limb-sparing surgery possible. Last 20 years, the procedure has undergone some significant alterations, which has led to perfusions that are nowadays well tolerated and safe. This study reports on a two-decade single center experience of TM-ILP in STS patients and will outline evolution and major changes since the introduction of TNF- $\alpha$  in ILP.

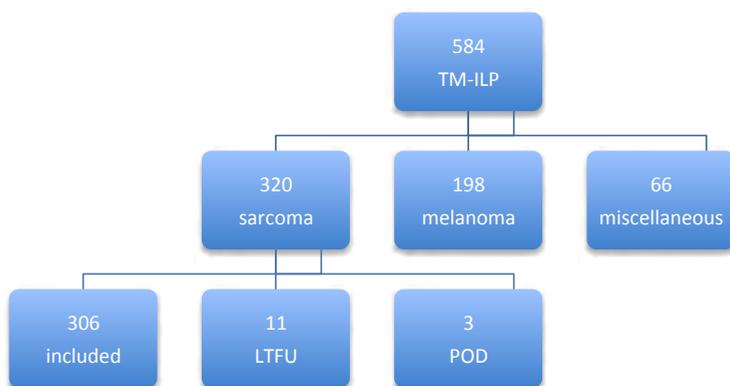
## Patients and methods

### *Patients*

Between 1991 and 2012 almost 600 TM-ILPs were performed in our institute of which 320 in STS patients (figure 1). All patients were candidate for an amputation or mutilating surgery, as a local radical resection of the tumor was impossible or only at cost of severe impairment of limb function. Due to perioperative death and insufficient data 306 perfusions were left for analysis.

Due to literature indicating that in sarcoma patients a lower dose of TNF- $\alpha$  might be as effective as a high dose, TNF- $\alpha$  dose was reduced in 2003 in our center<sup>12, 13</sup> from 3-4 mg to 2 mg for a lower limb perfusion and from 3 mg to 1 mg for an upper limb perfusion. High dose TNF- $\alpha$  perfusions between 1991 and 2003 (n=220) and low dose TNF- $\alpha$  perfusions between 2003 and 2012 (n=86) were compared for patient and tumor characteristics and outcome. Demographic data, disease and ILP characteristics were retrieved from a prospectively maintained database.

Figure 1: Inclusion flow chart



TM-ILP = TNF- $\alpha$  based isolated limb perfusion; LTFU = lost to follow up; POD = perioperative death

### Treatment

Technique of TM-ILP has been described previously.<sup>14</sup> Standard dose of recombinant TNF- $\alpha$  (Boehringer-Ingelheim GmbH, Ingelheim/Rhein, Germany) in the first decade of TM-ILP was 3 mg in the arm and 3-4 mg in the leg. TNF- $\alpha$  was injected as a bolus. Administration of melphalan changed during the studied period from injection as a bolus (1991 - 1996) to infusion by pump (1996-present). This because of reports that melphalan peak concentration is correlated with regional toxicity.<sup>15</sup> Currently a dose of 1 mg TNF- $\alpha$  in the arm and 2 mg in the leg is injected as a bolus once temperature is 38°C. After 10 minutes, 13 mg/L (arm) or 10 mg/L (leg) melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) is administered for 60 minutes. After 70 minutes of perfusion, the limb is washed out with 1 L (arm) to 4 L (Iliac perfusion) of physiological saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden). A precordial scintillation probe was used to detect technetium labeled albumen, leakage is monitored throughout the whole procedure.

### Response and toxicity

Clinical evaluation took place after 2, 4, 8 and 12 weeks after TM-ILP. Hereafter, evaluation was regularly assed at longer interval. Clinical response evaluation consisted of clinical examination and magnetic resonance imaging (MRI) and was reported according to the WHO response classification.<sup>16</sup> New lesions or growth of the tumor were reported as progressive disease (PD) when it was during first evaluation and as local recurrence (LR)

when it was during further follow up. Local toxicity was scored according to the classification by Wieberdink et al.<sup>17</sup>

### *Local resection*

Whenever response of the tumor to ILP appeared to facilitate local resection without major limb function loss, a local resection was attempted. Marginal status of resection was defined using the criteria according to Pisters et al.<sup>18</sup> In each surgical specimen peripheral margins were inked. A microscopically positive (R1) margin was defined as unequivocal tumor extension to the inked margin on permanent section. Cases in which tumor was close to but did not reach the inked margin were considered to have microscopically negative margins (R0). The pathologist estimated the percentage of the tumor that was necrotic due to ILP by macroscopic inspection and microscopic confirmation. Two categories were defined: No pathological response ( $\leq 50\%$  necrosis in resection specimen) and pathological response ( $> 50\%$  necrosis in resection specimen)

### *Statistical evaluation*

Time to local recurrence (TLR) and overall survival (OS) was defined as time between TM-ILP and local recurrence (LR) or death respectively. Estimates were made using the method of Kaplan and Meier. Prognostic value of baseline factors on clinical response, TLR and OS was evaluated using univariate binary logistic regression analysis and cox regression analysis and expressed in odds and hazard ratios, respectively. Factors reaching a 10% significance level were included in a multivariate model. A stepwise backward algorithm was used to exclude factors without significant influence. All tests were performed at a 5% significance level.

## **Results**

### *Patients*

A total of 306 TM-ILPs in 279 limbs and 275 patients were evaluated. Median age was 57 years (12-88; IQR 41-69). In the second study-period significant more patients  $> 50$  years of age were treated ( $p=0.039$ ). Thirty-seven TM-ILPs (12%) were performed despite evidence of distant metastases. There was no significant difference in percentage of palliative treatment over period (13% vs 9%,  $p=0.349$ ). The median follow up was 27 months (1-243; IQR 11-61). All characteristics on patient and tumor are summarized in table 1.

<b>Table 1: patient, tumor and ILP characteristics</b>				
	high dose 1991-2003	low dose 2003 -2012	Total 1991-2012	p
<b>Gender</b>				
Female	97 (48%)	30 (41%)	127 (46%)	0.309
Male	105 (52%)	43 (59%)	148 (54%)	
<b>Age</b>				
≤50 year	92 (42%)	25 (29%)	117 (38%)	<b>0.039</b>
>50 year	128 (58%)	61 (71%)	189 (62%)	
<b>Size</b>				
<5 cm	64 (29%)	36 (42%)	100 (33%)	<b>0.002</b>
5-10 cm	35 (16%)	22 (25%)	57 (18%)	
>10 cm	121 (55%)	28 (33%)	149 (49%)	
<b>Trojani grade</b>				
grade I	29 (13%)	9 (10%)	38 (12%)	0.139
grade II	37 (17%)	23 (27%)	60 (20%)	
grade III	154 (70%)	54 (63%)	208 (68%)	
<b>Site</b>				
upper arm	24 (11%)	9 (11%)	33 (11%)	0.904
lower arm	35 (16%)	12 (14%)	47 (15%)	
upper + lower arm	5 (2%)	1 (1%)	6 (2%)	
upper leg	104 (47%)	39 (45%)	143 (47%)	
lower leg	46 (21%)	23 (27%)	69 (22%)	
upper + lower leg	6 (3%)	2 (3%)	8 (3%)	
<b>Histology</b>				
Liposarcoma	33 (15%)	14 (16%)	47 (15%)	0.421
synovial sarcoma	32 (15%)	14 (16%)	46 (15%)	
Malignant fibrous histiocytoma	54 (25%)	27 (32%)	81 (27%)	
Leiomyosarcoma	18 (8%)	7 (8%)	25 (8%)	
desmoid/aggressive fibromatosis	10 (5%)	-	10 (3%)	
Stuart treves/ Kaposi sarcoma	20 (9%)	8 (9%)	28 (9%)	
others (16 types)	53 (24%)	16 (19%)	69 (23%)	
<b>Primary vs recurrent</b>				
Primary	123 (56%)	56 (65%)	179 (58%)	0.142
Recurrent	97 (44%)	30 (35%)	127 (42%)	
<b>Previous treatment</b>				
None	161 (73%)	62 (72%)	223 (73%)	0.056
RTx	22 (10%)	9 (10%)	31 (10%)	
CTx	13 (6%)	3 (4%)	16 (5%)	
ILP	5 (2%)	8 (9%)	13 (4%)	
Combination	19 (9%)	4 (5%)	23 (8%)	
<b>Unifocal/multifocal</b>				
Unifocal	159 (72%)	72 (84%)	231 (75%)	<b>0.031</b>
Multifocal	61 (28%)	14 (16%)	75 (25%)	

RTx = radiotherapy; CTx = chemotherapy; ILP = isolated limb perfusion

### Treatment

Patients underwent ILP via the axillary (n=33), brachial (n=53) iliac (n=112) or femoral (n=108) approach. A significant shift from iliacal ILPs towards femoral ILPs was established (p<0.001, table 2). The median length of hospital stay decreased in the second study-period for all types of TM-ILP (table 2, p<0.001).

### Clinical outcome

A clinical overall response rate (ORR) of 69% (n=212) was achieved (Table 3). Complete response (CR) was recorded after 53 TM-ILPs (17%). There was no significant difference in ORR between the defined study-periods (70% and 69% respectively, p=0.873).

	High dose (1991-2003)				Low dose (2003-2012)				Total (1991-2012)				P
	Ax	Br	Il	Fe	Ax	Br	Il	Fe	Ax	Br	Il	Fe	
<b>Type of ILP</b>													<b>&lt;0.001</b>
Axillary	24 (11%)				9 (11%)				33 (11%)				
Brachial	40 (18%)				13 (15%)				53 (17%)				
Iliacal	94 (43%)				18 (21%)				112 (37%)				
Femoral	62 (28%)				46 (53%)				108 (35%)				
<b>Dose (mg)</b>													
Median melphalan	37	33	110	60	40	40	100	60	39	33	110	60	
<b>Hospitalization</b>													
median days	7	6	11	8	5	5	6	6	6	6	10	7	

Ax = axillary; Br = brachial; Il = Iliacal; Fe = femoral; ILP = isolated limb perfusion

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**Table 3: Univariate and multivariate analysis of prognostic value of baseline factors on overall response, local progression and overall survival**

	Overall response OR (p-value)		Local progression HR (p-value)		Overall survival HR (p-value)	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
<b>Patient characteristics</b>						
Gender Female* vs male	0.79 (0.340)	-	1.10 (0.650)	-	1.09 (0.621)	-
Age ≤50* vs> 50 years	1.22 (0.436)	-	1.51 (0.071)	-	<b>1.42 (0.042)</b>	-
<b>Tumor characteristics</b>						
Size ≤5 cm* vs 5-10cm vs ≥10cm	1.26 (0.549)	-	<b>0.48 (0.026)</b>	-	<b>1.83 (0.018)</b>	<b>1.99 (0.007)</b>
Trojani Grade grade I* vs II vs III	0.79 (0.387)	-	<b>0.44 (0.001)</b>	-	<b>1.72 (0.009)</b>	<b>1.73 (0.008)</b>
Recurrent disease Primary* vs Recurrent	1.43 (0.403)	-	0.74 (0.462)	-	<b>1.94 (0.080)</b>	<b>1.91 (0.086)</b>
Number of tumors Unifocal* vs multi focal	1.78 (0.111)	-	1.26 (0.473)	-	<b>2.98 (0.001)</b>	<b>3.05 (0.001)</b>
	1.28 (0.332)	-	<b>2.36 (&lt;0.001)</b>	<b>1.61 (0.050)</b>	0.94 (0.716)	-
	<b>2.31 (0.011)</b>	<b>2.31 (0.011)</b>	<b>3.40 (&lt;0.001)</b>	<b>2.75 (&lt;0.001)</b>	1.11 (0.605)	-
<b>Perfusion characteristics</b>						
Dose of TNF-α Low dose* vs high dose	1.05 (0.873)	-	0.96 (0.880)	-	0.99 (0.978)	-

OR = Odds ratio; HR = hazard ratio; TNF-α = tumor necrosis factor-α

### Toxicity and leakage

Leakage appeared to occur more often in the high dose period. Twenty-five of 29 procedures with high leakage ( $\geq 10\%$  leakage) occurred during a high dose TM-ILP ( $p=0.072$ ). Iliacal procedures ended up with high leakage more often compared to femoral procedures. (prevalence: 13% vs 5%,  $p=0.024$ ).

Prevalence a high-grade local toxicity (Wieberdink III – Wieberdink V) was seen more often in case of high dose perfusions (23% vs 14% respectively,  $p=0.086$ ).

### Local recurrence and survival

Local recurrence or local progression (LR) occurred after 87 TM-ILPs (28%). In case of LR, median time to recurrence was 12 months (range 1-170). Estimated one- and three-year recurrence free survival (RFS) was 84% and 70%, respectively.

Five-year and ten-year overall survival (OS) rates were 45% and 34%, respectively. Median survival was 49 months. No statistical differences were observed in local recurrence rate or OS between the two study periods (table 3).

	TM-ILPs		Overall response			Local recurrence		
	Low dose	High dose	Low dose OR –rate	High dose OR –rate	p-value	Low dose 3-y RFS	High dose 3-y RFS	p-value
Arm (n=86)	22	64	73%	63%	0.385	33%	66%	0.200
Iliacal (n=112)	18	94	72%	71%	0.935	100%	78%	0.085
Femoral (n=108)	46	62	65%	74%	0.312	67%	65%	0.731
All ILPs (n=306)	86	220	69%	70%	0.873	66%	70%	0.949

TNF- $\alpha$  = tumor necrosis factor- $\alpha$ ; OR –rate = overall response rate; RFS = recurrence free survival

### Dose of TNF- $\alpha$

The dose reduction of TNF- $\alpha$  did not influence ORR, LR or OS (table 3). Sub analysis for perfusions in the arm ( $n=86$ ), iliacal perfusions ( $n=112$ ) and femoral perfusions ( $n=108$ ) was performed to determine any possible effect on clinical outcome caused by the dose reduction of TNF- $\alpha$  (table 4). When a low dose (1mg) of TNF- $\alpha$  was administered in the arm, an ORR of 73% was achieved compared to 63% after high dose perfusions (2-3mg,  $p=0.385$ ). In the leg there was distinguished between iliacal en femoral perfusion because of the substantial difference in perfusion volume between both approaches. In the leg a low dose perfusion was defined as 1-2 mg TNF- $\alpha$  used in the perfusions whereas a high dose was 3-4

Patients treated		Resection margins (% RO)			Pathological response (% >50 %)		
Low dose	High dose	Low dose	High dose	p-value	Low dose	High dose	p-value
38	109	45%	58%	0.164	26%	51%	0.007

RO = radical resection

mg TNF- $\alpha$ . A pathological response (>50% necrosis in resection specimen) was significantly less often observed after low dose TM-ILP (26% vs 51%, respectively;  $p=0.007$ , table 5). This significant worse pathological response was not of influence on resection margins. A univariate and multivariate analysis was performed with the same baseline factors as listed in table 3 and pathological response as dependent variable. Low dose vs high dose TM-ILP was the only significant covariate (OR = 2,96,  $p=0.009$ ; table 6).

Baseline factors	Pathological response OR (p-value)	
	Univariate	Multivariate
Gender	0.82 (0.544)	-
Female* vs male		
Age	1.64 (0.143)	-
$\leq 50^*$ vs > 50 years		
Size	2.30 (0.120)	-
$\leq 5$ cm* vs 5-10cm vs $\geq 10$ cm	2.20 (0.071)	
Trojani Grade	2.31 (0.183)	-
grade I* vs II vs III	2.34 (0.132)	
Recurrent disease	0.48 (0.098)	-
Primary* vs Recurrent		
Dose of TNF- $\alpha$		
Low dose* vs high dose	<b>2.96 (p=0.009)</b>	<b>2.96 (p=0.009)</b>

OR = Odds ratio; TNF- $\alpha$  = tumor necrosis factor- $\alpha$

## Discussion

The present study, reporting on a 20-year single center experience of TNF- $\alpha$  and melphalan based ILP, demonstrates that the experience of almost 600 TM-ILP's has led to some significant changes in the way we perform the perfusion procedure. Improved management of local toxicity, lowering of the TNF- $\alpha$  dose and the tendency to perform more distal ILPs, has led to a reduction in hospital stay. This improved safety is of special importance when ILP is considered in the elderly. In recent years, an older patient population was treated, as we showed that ILPs can be performed safely in the elderly population.<sup>19</sup>

Alterations, especially dose reduction of TNF- $\alpha$ , have not led to reduced clinical efficacy. A limb salvage rate of 79% was achieved in patients who were candidates for a limb amputation, which is in line with a recently published review.<sup>20</sup>

The mature data of this study are in line with results in the literature that TNF- $\alpha$  dose reduction has no significant effect on clinical outcome, nor on local recurrence rate.<sup>12, 13, 20</sup>

Dose reduction of TNF- $\alpha$  was of negative influence for pathological response. In our opinion, clinical outcome measures such as local control and overall survival are of more importance than pathological response. Although Bonvalot et al<sup>12</sup> did not find any correlation between pathological response and dose of TNF- $\alpha$  in their prospective trial, the present, albeit retrospective series, is based on a significantly higher number of patients.

The goal of ILP in unifocal disease is to enable a radical resection of a previously irresectable sarcoma. In our series, high or low dose TNF- $\alpha$  did not influence R0 resections (Table 5). This means that even the demonstrated reduced pathological response rate in the low-dose era, has no clinical impact. We previously demonstrated that local recurrences did not occur in patients who underwent TM-ILP followed by R-0 resection and had more than 50% necrosis in resection specimen, regardless of administration of adjuvant radiotherapy.<sup>21, 22</sup> Therefore, we suggested that this particular group can be spared adjuvant radiotherapy. If adjuvant RTx after resection is undesirable (e.g. in peri-articular areas) maximum pathological response may be pursued and in these cases, high-dose ILP can be considered. Of note, 64% of patients with unifocal disease underwent a resection, 54% of these resections was a R0 resection. So the prerequisites for withholding RTx are only met in a minority of patients. This has to be balanced against the observed higher toxicity in high-dose ILPs. Increased pathological response rate in the present study fits in the work on TM-ILP for melanoma in transit metastases published earlier by our group.<sup>23</sup> A complete response was significantly less often observed after a low dose iliacal TM-ILP.

It could be argued that determining the degree of necrosis may not necessarily reflect a therapy effect but may be inherent tumor necrosis.<sup>24</sup> Furthermore, macroscopic evaluation is a subjective and therefore imprecise factor. Considering the fact that tumor necrosis in untreated soft tissue sarcoma is an independent unfavorable prognostic factor it may be assumed that the necrosis observed in ILP-treated sarcomas is indeed therapy related and prognostically relevant. Secondly, considering the broad categories defined for tumor necrosis in grading systems (0%, <50% and >50%) it is highly unlikely that the degree of necrosis in single cases falls near the cut-off point. Furthermore, in our experience the pattern of necrosis in ILP-treated sarcomas, consisting of large confluent areas of necrosis, is different from the patch pattern of necrosis seen as spontaneous necrosis in untreated sarcomas.

Since Erasmus MC was one of the first centers to perform TNF- $\alpha$  based isolated limb perfusions patients from all over the world were treated in Rotterdam. Nowadays TM-ILP is a more common treatment modality, especially in Europe and performed in a sufficient number of tertiary referral cancer centers. Induction (chemo)radiotherapy is also used more

frequently. This explains the lower number of performed TM-ILP per year in the low dose period. Patient and tumor characteristics were roughly comparable over both periods. There were significant more small tumors treated in the low dose period. Size of tumor is not correlated with clinical outcome,<sup>25</sup> hence this finding will not interfere with our results. However it suggests that indication of preventing an amputation might have shifted towards a neoadjuvant therapy to facilitate an oncological resection without severe impairment of the limb. Overall this report describes retrospective analysed data so these results about the comparison between the low dose and high dose group should be read with caution.

Currently, iliacal TM-ILP is preserved for very proximal tumors. For all other tumors, adequate isolation of the limb and an adequate perfusion circuit can be achieved by application of a high tourniquet with femoral TM-ILP. The total volume of the perfused limb is significantly lower in the femoral approach, which leads to a lower dose of melphalan used and hence to a reduction in acute local toxicity.<sup>26, 27</sup> Another benefit of femoral TM-ILP over iliacal TM-ILP is the lower leakage. In this series there was a significant correlation between iliacal TM-ILP and incidence of high leakage. Usage of a tourniquet might be safer compared to an iliacal Esmarch band. The trend toward more femoral TM-ILPs led to a reduction of high leakage procedures. Even in the present situation of low-leakage ILPs, we believe that leakage monitoring is still mandatory. Early detection of minor leakage prompts alterations in either pump flow of the perfusion circuit or in systemic blood pressure, all to prevent the undesirable effects of systemic TNF- $\alpha$ . This early intervention upon detection of leakage by the precordial scintillation probe improves the safety of the procedure.

Local toxicity was lower in the second study-period with a trend towards significance ( $p=0.086$ ), which in our opinion can be explained by the TNF- $\alpha$  dose reduction and more distal perfusions.

With the growing insight and experience, the infrequent incidence of local toxicity, and the fact that major complications are seldom observed after TM-ILP, one can understand that the hospital length of stay has decreased over period. Moreover, observation for the first postoperative day on an intensive care unit is no longer part of our protocol. Together all these changes made, emphasize the major importance of high volume centers in order to warrant the presence of sufficient experience in this complicated treatment modality on a rare disease. The changes in protocol over time have not affected clinical outcome. The growing experience in our high volume center has improved safety.

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# PART II

## Tumor necrosis factor- $\alpha$ and melphalan based isolated limb perfusion in melanoma patients



two decades of tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion

de meeste woorden begrijp ik niet...



# Chapter 9

## **Long-term outcome of tumor necrosis factor- $\alpha$ based isolated limb perfusion for patients with melanoma in-transit metastases**

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## Abstract

### *Background*

Isolated limb perfusion (ILP) is an attractive treatment modality for in transit melanoma metastasis. The use of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in ILP is not uniformly accepted. We report on the long term results of adding TNF- $\alpha$  to standard melphalan based ILP (TM-ILP) as treatment modality in patients with melanoma in transit metastases.

### *Methods*

Demographic data, disease and ILP characteristics were retrieved from a prospectively maintained database between 1991 and 2005. ILP was performed in hyperthermic fashion with 1-4 mg TNF- $\alpha$ . With a median potential follow up of 13 years, response rates, time to local progression and disease specific survival were analyzed versus standard baseline factors.

### *Results*

A total of 118 TM-ILPs were analyzed in 105 patients. Fifty-four patients had stage IIIA disease, 50 stage IIIAB and 14 stage IV. An overall response of 93% was achieved. Complete response rate was 68% (n=80), partial response rate was 25% (n=30). Response rate was significant influenced by stage of disease (IIIA vs IIIAB, p=0.006). Complete response maintained till end of follow up in 35 patients (33%), in 12 other patients (11%) local control was achieved with 1 additional intervention. Local progression occurred in 66 patients (56%). Number of in transit metastases (p=0.008) and complete response after ILP (p<0.001) were strong prognostic factors for time to local progression. Five-year disease specific survival was 28%, survival was positive influenced by age, stage of disease, previous ILP and complete response after ILP.

### *Conclusions*

TM-ILP is a strong tool in obtaining local control in melanoma patients with in transit metastases. The application of TNF- $\alpha$  is a valuable addition to standard melphalan based ILP.

## Introduction

Cutaneous melanoma accounts for 75% of skin cancer deaths. Standard treatment is surgical excision with a safety margin some distance from the borders of the primary tumor. Local recurrence occurs as first event in disease progression in approximately 5%.<sup>1,2</sup> In about 5%-8% recurrence of primary melanoma is in a pattern called in transit metastases. In transit metastases are cutaneous or subcutaneous deposits of melanoma cells trapped within the lymphatics between the primary tumor and regional lymph node basin. When in transit metastases are limited in number and size simple surgical excision is the preferred treatment modality. For smaller lesions too numerous for surgical excision, other therapy options such as carbon dioxide laser therapy, intralesional injections, or electrochemotherapy have been used but with poor clinical response rates.<sup>3-8</sup> When lesions are superficial and in a relative small area, radiotherapy can be of value.<sup>9</sup> Systemic therapy for in transit metastases has a limited role. For patients with BRAF gene mutation an overall promising response rate with use of BRAF inhibitors in systemic fashion have been reported.<sup>10</sup> Hodi et al.<sup>11</sup> reported of prolonged survival after the use of ipilimumab in patients with stage III en stage IV melanoma disease. However, the role of such new agents in the treatment for in transit metastases is still unclear.

Isolated limb perfusion (ILP) is an attractive treatment modality for multiple melanoma in transit metastases.<sup>12,13</sup> melphalan is used in ILP as the standard chemotherapeutic drug with complete response rates of 40% to 50%<sup>14</sup>. However, response rates of melphalan-only ILPs are disappointing in bulky melanoma lesions, due to poor drug uptake by the tumor, similar to advanced soft tissue sarcomas.<sup>14,15</sup> The introduction of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in ILP led to a remarkable improvement of response rates for soft tissue sarcomas, especially for very large tumors.<sup>15,16</sup> Consequently, TNF- $\alpha$  added to a melphalan based ILP (TM-ILP), after the approval of TNF- $\alpha$  in Europe for locally advanced extremity soft tissue sarcomas,<sup>17</sup> has been used increasingly for advanced melanoma in transit metastases, but results have not been uniform.<sup>18,19,20</sup>

The aim of this study is to report on the long-term results of a large series TM-ILPs for extremity melanoma in transit metastases. Moreover we investigated significant prognostic factors for complete response rate, local progression and survival after isolated limb perfusion for in transit melanoma metastasis.

## Methods

### *Patients*

Demographic data, disease and isolated limb perfusion characteristics were retrieved from a prospectively maintained database between 1991 and 2005.

### *Isolated limb perfusion*

The technique of ILP with TNF- $\alpha$  and melphalan has been described previously.<sup>16</sup> Briefly, under general anaesthesia and after heparinization, a targeted blood circuit is isolated by clamping and cannulation of the major artery and vein and connected to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels and prevents leakage. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored throughout the procedure. Patients underwent TM-ILP via the axillary (n=6), iliac (n=69), femoral (n=33) or popliteal (n=10) approach.

A dose of 1 to 3 mg (arm) or 1 to 4 mg (leg) of recombinant TNF- $\alpha$  (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) was injected as a bolus once the temperature of the limb reached 38°C. Subsequently, 13 mg/L (arm) or 10 mg/L (leg) melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) was administered 10-30 minutes after the limb temperature reached 38 – 39.5°C. Temperatures for a perfusion of an arm were kept between 38 – 38.5°C and for perfusions of the leg between 39 – 39.5°C because a report by Vrouenraets et al.<sup>21</sup> demonstrated that tissue temperatures of 40°C were associated with an increased acute and long term local toxicity. The median dose of TNF- $\alpha$  administered was 4.0 mg (mean 3.5, range 1-4), while the median dose of melphalan was 90 mg (mean 88, range 39-140). After a total perfusion time of 70-90 minutes, the limb is washed out with 1 L (arm) to 4 L (Iliac perfusion) of physiological saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden). The median length of hospital stay was 10 days (mean 13, range 3-67).

### *Clinical evaluation*

Clinical response was evaluated 2 to 4 weeks and 8 weeks after isolated limb perfusion by physical examination. Hereafter evaluation took place 3-month regular in the first 2 years and at longer interval in further follow up. The last date of follow up date was the 31 of December 2009. Response rates were defined following WHO criteria,<sup>22</sup> in which complete response is complete disappearance of all lesions without evidence of new disease in the field of perfusion. Partial response is defined as a reduction of 50% to 99% of the total tumor size; and no change is <50% of reduction.

Local recurrence or appearance of new lesions in the field of perfusion after a complete response, as well as progression of disease in the treated limb after partial response or no change, was classified as local progression. Systemic progression was defined as any appearance of distant metastases.

#### *Evaluation of toxicity*

Acute local toxicity of TM-ILP was categorized according to Wieberdink et al.<sup>23</sup> (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.

#### *Statistical analysis*

Disease specific survival, time to local progression and time to systemic progression were defined as the time between TM-ILP and disease specific death, local progression and systemic progression respectively. Estimates were made according to Kaplan Meier and compared with the log rank score. The prognostic value for these endpoints of the baseline factors was evaluated with a univariate cox regression and expressed in hazard ratios (HR). Prognostic factors associated with complete response were examined with a logistic regression and expressed in odds ratios (OR). Multivariate regression analysis was performed with all variables having a significance level of 0.10. A stepwise backward algorithm was used at a level of 5% significance to exclude factors. Since patients with stage IV disease (n=14) have a very poor life expectancy (median survival 6 months) and underwent the ILP for appealing locoregional problems they have been excluded for multivariate regression analysis. Statistical significance was set to  $P < 0.050$ .

## **Results**

#### *Patients and disease characteristics*

Between 1991 and 2005, we performed 124 TM-ILPs in 111 patients with multiple melanoma in transit metastases in the limb. Six patients were excluded from analysis. One patient died 4 days after ILP, without any leakage of TNF- $\alpha$  nor locoregional or systemic toxicity due, to a myocardial infarction. Five foreign patients were lost for reliable response evaluation or follow up was too short (<12 months) for long-term analysis. Thus this study reports on 118 TM-ILP in 105 patients.

Table 1: Patient and tumor characteristics		
	n	%
<b>Gender</b>		
male	31	71
female	74	29
<b>Age (years)</b>		
median	64	
range	30-90	
<b>Breslow thickness (mm.)</b>		
median	2.89	
range	0.70-15	
missing	29	28
<b>Location primary</b>		
arm	3	3
leg	59	56
foot	35	33
back	4	4
unknown prim.	3	3
missing	1	1
<b>Median time to IT-mets (months)</b>		
median	18	
range	0-347	
<b>Location</b>		
arm	6	5
leg	112	95
<b>Number of lesions</b>		
<10	52	44
10-19	21	18
20-49	20	17
50-99	12	10
≥100	13	11
<b>Size largest</b>		
<40 mm	64	54
≥40 mm	54	46
<b>MD-Anderson stage</b>		
IIIA	54	46
IIIAB	50	42
IV	14	12
<b>Prior treatment</b>		
none	77	65
ILP	19	16
RTx	3	3
CTx	8	7
immuno	4	3
combination	7	6

IT-mets = in transit metastases; ILP= isolated limb perfusion; RTx = radiotherapy; CTx= chemotherapy; immuno = immunotherapy

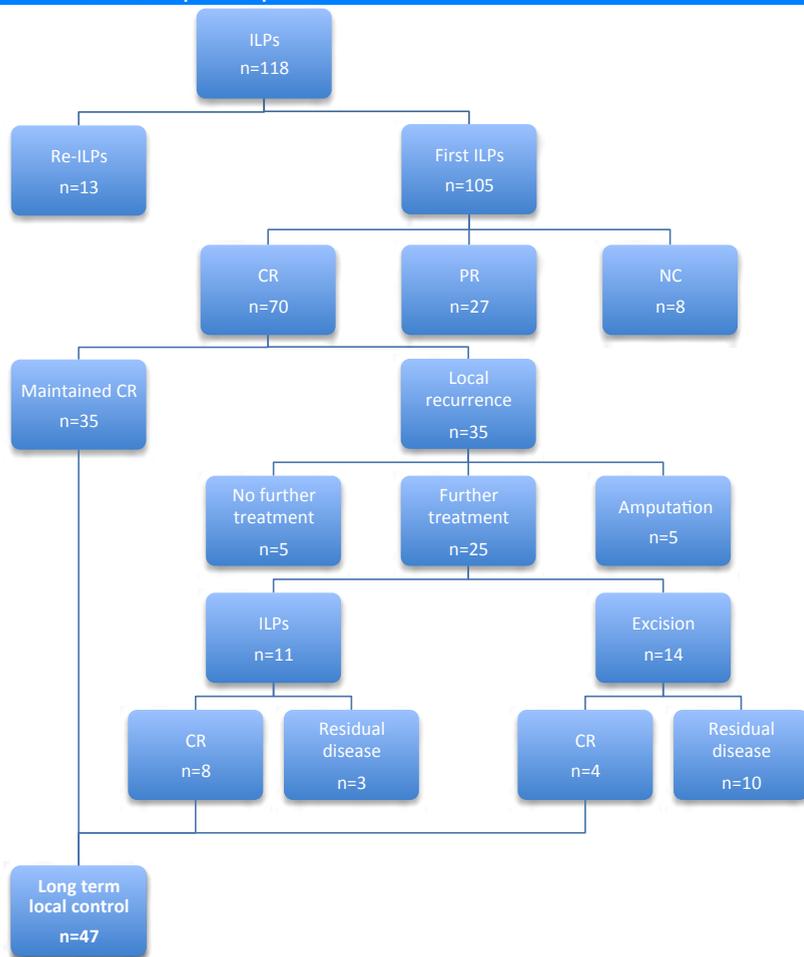
Median age of 64 years (range 30-90 years), 74 patients (71%) were female. Median Breslow thickness of the primary tumor was 2.88 mm (range 0.70-15 mm). Breslow data were missing in 29 patients (28%) due to varying reasons (unknown primaries, not measured or missing data). Thirteen patients were previously treated with ILP in their referral hospital,

which usually was with a melphalan based ILP. Nine patients were treated twice and two patients were treated three times with TM-ILP. Fifty-four ILPs (46%) concerned stage IIIA disease, 50 (42%) stage IIIAB and 14 (12%) stage IV. Time span between primary melanoma and diagnosis of in transit metastases ranged from 0 to 347 months. The number of in transit metastases varied from one to >99. All patient, tumor and TM-ILP characteristics are summarized in table 1.

*Clinical response*

Overall response was achieved after 110 TM-ILPs (93%), with 80 complete responses (68%), 30 partial responses (25%) and 8 no change (7%) with a median clinical follow up of 23

**Figure 1: Flow chart maintained complete response**



ILP = isolated limb perfusion; CR = complete response; PR = partial response; NC = no change

months (mean 37, range 2-144). Median potential follow up was 13 years (range 5-19). Stratified for stage of disease at patient presentation the proportion complete responses was 85% for stage IIIA, 58% for stage IIIAB and 36% for stage IV (IIIA vs. IIIB,  $p=0.006$ ; IIIA vs IV,  $p=0.001$ ; IIIAB vs IV,  $p=0.174$ ). Patients aged younger than 65 years had a complete response rate of 76% while older patients showed a complete response rate of 58% ( $p=0.037$ ). In multivariate analysis, stage of disease appeared to be the only analyzed significant prognostic factors for complete response.

These locally advanced cases led to limb amputation in 8 patients (8%), consequently limb salvage rate was 92%. Median time to amputation after first ILP was 17 months (mean 17, range 2-28). The majority of amputations were performed after local progression ( $n=5$ ), 2 cases of Wieberdink V toxicity were observed of which 1 case was after a repeat ILP performed in 2006. One patient underwent an amputation for arthrosclerosis, this despite a complete response.

We specially analyzed patients treated with TM-ILP for the first time in our hospital ( $n=105$ ) to determine the long-term local control rate after TM-ILP. Complete response was recorded in 70 cases (67%). Half of these patients ( $n=35$ ) were in remission till the end of follow up, while in the other half ( $n=35$ ) local progression occurred after a median time of 8 months (mean 11, range 2-59) (fig. 1). Patients with local relapse or progression were offered further treatment in 25 cases. In five patients this recurrence ended up with an amputation, five patients were diagnosed with systemic metastasis and were not offered large interventions because of short live expectancy. Further treatment ended up in eight complete responses after a second ILP and 4 radical surgical excisions, all without local recurrence. Three ILPs for recurrent disease were less successful: one patient in the ILP group had a partial response, the other two had recurrent disease with rapid onset. In patients treated with salvage surgery for recurrence after ILP, surgical excision was irradical in one patient and nine patients had local progression despite radical surgery.

As a result in 47 of the 105 patients (45%) long-term control was achieved after a median follow up of 33 months (mean 47, range 2-136), of which 15 patients (14%) had a follow up of over 5 years.

#### *Local progression*

Local progression occurred after 66 TM-ILPs (56%), at a median time of 13 months. Progression was less rapid in case of complete response after TM-ILP compared to those with a partial response or no change: 19 months vs. 6 months ( $p<0.001$ ). The number of lesions was a significant prognostic factor for time to local progression as well: when TM-ILP was given for  $\geq 10$  In transit metastases, median time to local progression was 10 months while patients with  $<10$  in transit metastases had median time to local progression of 28

**Table 2: Univariate analysis of prognostic factors for complete response, local and systemic progression and disease specific survival**

Variable	Complete response		Local progression		Systemic progression		Survival**	
	OR (p-value)	95% CI	HR (P-value)	95% CI	HR (P-value)	95% CI	HR (P-value)	95% CI
<b>Sex</b>								
female* vs male	0.57 (0.187)	0.25-1.31	0.98 (0.943)	0.56-1.72	<b>2.46 (&lt;0.001)</b>	1.47-4.13	<b>2.50 (&lt;0.001)</b>	1.59-3.93
<b>Age</b>								
<65 vs ≥65 years	<b>0.44 (0.039)</b>	0.20-0.96	1.25 (0.371)	0.77-2.05	1.60 (0.057)	0.99-2.58	<b>1.71 (0.019)</b>	1.09-2.66
<b>Breslow thickness</b> (in mm.)	0.92 (0.318)	0.78-1.08	1.01 (0.922)	0.90-1.12	1.10 (0.129)	0.97-1.25	1.10 (0.052)	1.00-1.21
<b>Interval prim vs IT-mets</b> ≤1* vs >1 year	0.98 (0.952)	0.45-2.14	0.77 (0.298)	0.47-1.26	1.42 (0.168)	0.86-2.34	1.32 (0.233)	0.84-2.09
<b>Location of lesions</b>								
Upper* vs. lower vs. total	0.43 (0.101)	0.15-1.18	1.10 (0.781)	0.58-2.09	1.34 (0.378)	0.70-2.56	1.40 (0.242)	0.80-2.45
<b>Number of lesion</b> <10* vs ≥10 lesions	1.22 (0.680)	0.47-3.19	0.74 (0.341)	0.40-1.37	1.21 (0.494)	0.70-2.10	1.09 (0.755)	0.65-1.83
<b>Size of largest lesion</b> <4* vs ≥4 cm.	0.79 (0.554)	0.37-1.71	<b>2.03 (0.008)</b>	1.20-3.43	1.08 (0.759)	0.67-1.74	0.88 (0.878)	0.57-1.36
<b>Stage of disease</b>								
IIIA* vs IIIAB vs IV	<b>0.24 (0.003)</b>	0.09-0.61	1.39 (0.201)	0.84-2.30	<b>2.74 (&lt;0.001)</b>	1.68-4.46	<b>2.42 (&lt;0.001)</b>	1.49-3.96
<b>Prior ILP</b> no* vs yes	<b>0.10 (0.001)</b>	0.03-0.37	0.82 (0.724)	0.29-2.36	0.67 (0.37)	0.43-1.36	<b>9.68 (&lt;0.001)</b>	4.81-19.49
<b>Interval IT-mets vs ILP</b> ≤6* vs >6 months	2.35 (0.116)	0.81-6.81	1.12 (0.687)	0.65-1.95	0.77 (0.37)	0.43-1.36	<b>0.63 (0.098)</b>	0.36-1.09
<b>CR achieved</b> no* vs yes	1.25 (0.590)	0.56-2.81	1.21 (0.466)	0.72-2.02	0.60 (0.050)	0.36-1.00	0.70 (0.141)	0.44-1.12
	NA	NA	<b>0.34 (&lt;0.001)</b>	0.20-0.58	<b>0.36 (&lt;0.001)</b>	0.21-0.61	<b>0.30 (&lt;0.001)</b>	0.19-0.48

\*Reference group  
 \*\*Disease specific survival  
 CR = complete response; OR = odds ratio; HR = hazard ratio; CI = confidence interval ILP = isolated limb perfusion; NA = not applicable

months (p=0.006). Stage of disease had no significant influence on time to local progression (IIIA vs IIIAB, p=0.325), nor had Breslow thickness of the primary lesion (p=0.852). Hazard ratios of all baseline factors tested in a univariate and multivariate cox regression analysis are summarized in table 2 and 3.

*Systemic progression*

Systemic progression was diagnosed in 63 patients (68%) during follow up at a median time of 19 months. Patients with stage IIIA disease had a significant longer time to systemic

**Table 3: Multivariate analysis of prognostic factors for complete response, local and systemic progression and disease specific survival**

Endpoint	Variable	n	OR/HR	p	95% CI
<b>Complete response</b>	Stage	IIIA	54	1	
		IIIAB	50	0.24	0.003
<b>Local progression</b>	Number of IT-mets	<10	43	1	
		≥10	61	2.09	0.008
<b>Systemic progression</b>	Sex	Female	80	1	
		Male	24	2.25	0.006
	Age	<65	57	1	
		≥65	47	1.97	0.007
	Stage	IIIA	54	1	
IIIAB		50	2.29	0.002	1.37-3.83
<b>Disease Specific Survival</b>	Age	<65	57	1	
		≥65	47	2.03	0.006
	Stage	IIIA	54	1	
		IIIAB	50	3.47	<0.001
	Previous ILP	No	80	0	
Yes		24	0.25	0.002	0.18-0.68

CR = Complete response; prog = progression; OR = odds ratio; HR = hazard ratio; CI confidence interval; IT-mets = in transit metastasis; ILP = isolated limb perfusion

progression compared to patients with stage IIIA disease, 55 months vs 11 months ( $p < 0.001$ ).

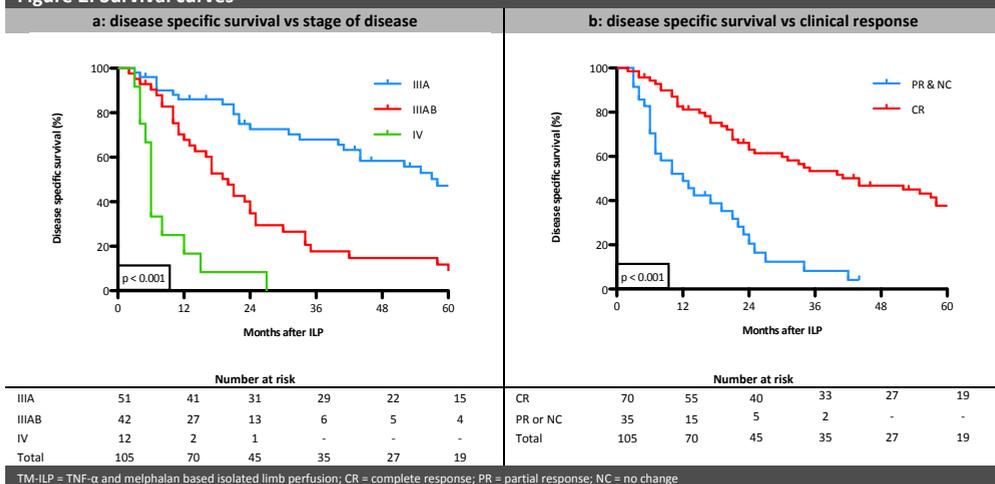
Median time to systemic progression in case of complete response after TM-ILP was 32 months, median time to systemic progression in the partial response or no change group was 7 months ( $p < 0.001$ ). Sex ( $p < 0.001$ ), and size of the largest lesion ( $p = 0.023$ ) were prognostic base line factors reaching significance in univariate cox regression (table 2). In multivariate regression, sex ( $p = 0.006$ ) and stage of disease ( $p = 0.002$ ) remained significant, whereas age became a significant prognostic variable ( $p = 0.007$ , table 3).

### Survival

The 5 and 10-years disease specific survival was 27% and 16%, respectively; median disease specific survival time was 24 months. In univariate analysis disease specific survival was influenced by sex ( $p < 0.001$ ), age ( $p = 0.019$ ), Breslow thickness ( $p = 0.052$ ), size of the largest lesion ( $p < 0.001$ ), stage of disease (IIIA vs IIIAB,  $p < 0.001$ ; IIIA vs IIIAB,  $p < 0.001$ ), and complete response after TM-ILP ( $p < 0.001$ ) (table 2). In multivariate analysis age and stage of disease remained significant while ILP in medical history became a favorable prognostic value (table 3).

Patients presenting with stage IIIA disease had 5 year disease specific survival of 47% with a median disease specific survival of 58 months compared to 12% with a median disease specific survival of 20 months in the stage IIIAB group ( $p < 0.001$ ). In case of stage IV, no patient survived for 3 years (fig 2-a). Ten-year disease specific survival was 31% for patients with stage IIIA disease against 4% for stage IIIAB. Complete response was a strong predictor for survival as well. Median disease specific survival time after complete response was 44

Figure 2: Survival curves



months, 5 year disease specific survival was 38%. In case of partial response or no change median disease specific survival was 12 months ( $p < 0.001$ , fig 2-b).

### *Leakage and toxicity*

No or only minor leakage ( $\leq 10\%$ ) occurred in 112 TM-ILPs (95%), median leakage was 0%, range 0-25). In 6 patients leakage was over 10% with limited toxicity of which two patients experienced transient hypotension that could be treated with vasopressors. One patient had a grade IV leucopenia that lasted for 1 day, which did not need any intervention. Three patients did not experience any inconvenience of the  $>10\%$  systemic leakage.

Local toxicity was not observed (Wieberdink I) or mild (Wieberdink II) after 71% of ILPs ( $n=84$ ) and considerable (Wieberdink III) after 25% of TM-ILPs ( $n=30$ ). Three patients (3%) experienced deep damage of tissues (Wieberdink IV) after TM-ILP. In one patient (1%) an amputation (Wieberdink V) was performed 2 months after TM-ILP. Initially toxicity after this perfusion was rather mild. Nevertheless the increasing reaction resulted in severe necrosis of muscles.

## **Discussion**

The overall response rate of 93% (110 out of 118 TM-ILPs), a maintained complete response of 45% (47 out of 105 patients had a complete response till end of follow up with a median of 33 months) with limited toxicity proves that TM-ILP is an effective treatment option in melanoma patients with numerous and bulky in transit metastases.

Present results are in accordance with previously published studies with shorter follow up.<sup>15, 19, 24-29</sup> More recently Alexander et al.<sup>30</sup> reported a complete response rate of 69% on a mixed series of ILPs with melphalan alone or in combination with TNF- $\alpha$  in a more favorable population (68% stage IIIA disease, which is a highly significant prognostic factor for clinical outcome). Rossi et al.<sup>20</sup> reported a 60% complete response rate for TM-ILP and 42% complete response rate for melphalan based ILP in a patient population more comparable to ours (49% stage IIIA). This difference in complete response rate between anti-neoplasm agents appeared significant ( $p=0.05$ ).

Patients with local progression after TM-ILP could be offered re-treatment leading to 12 cases of maintained remission. In total, 47 patients (45%) showed long term control (fig 1). Sanki et al.<sup>28</sup> reported of a comparable maintained loco-regional control rate of 42% in a series with a more favorable population (56% stage II or stage IIIA disease) that underwent ILP with various cytotoxic drugs (majority of ILPs with melphalan and actinomycin-D) whereas Rossi et al.<sup>20</sup> showed a complete response till end of follow up without any additional intervention of 35%.

Local progression rate was 56% with a median time to local progression of 13 months, which is within the range of previous reports (9-17 months).<sup>20, 28, 30</sup> Median follow up might appear lower than expected which can be explained by the poor life expectancy in this study population. A total of seven patients were lost though follow up the first five years of follow up.

Despite the fact that ILP has been used for over 50 years, there is no widespread consensus about the anti-neoplasm agents that should be used. In the last decades, many retrospective reports were published, however, there still is uncertainty about the use of TNF- $\alpha$  in ILP for patients with melanoma in transit metastases. The only randomized controlled trial by Cornett et al.<sup>18</sup> reported of increased local and systemic toxicity while no beneficial effect of TNF- $\alpha$  was observed (complete response rate 26% for TM-ILP vs. 25% for melphalan based ILP). The methods and conclusions of this publication were subject of several criticisms;<sup>31</sup> (I) Clinical response was assessed after 3 months which is an uncommon endpoint since a substantial proportion of complete responses is reached between 3 to 6 months after ILP, consequently a very low complete response rate was achieved. (II) The authors reported very lacunary on patients and tumor characteristics differences between the two study arms. (III) The true indication, bulky disease was not analysable. Therefore conclusions on this trial should be read with caution.

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# Chapter 7

**20 Years experience of tumor necrosis factor- $\alpha$  based isolated limb perfusion for in-transit melanoma metastases: Tumor necrosis factor- $\alpha$  dose matters**

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## Abstract

### *Background*

Approximately 5-8% of melanoma patients will develop in-transit metastases (IT-mets). Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and melphalan based isolated limb perfusion (TM-ILP) is an attractive treatment modality in melanoma patients with multiple IT-mets. This study reports on a 20 years experience and outlines the evolution and major changes since the introduction of TNF- $\alpha$  in ILP.

### *Methods*

A total of 167 TM-ILPs were performed in 148 patients, between 1991 and 2009. TM-ILPs were performed at high doses of TNF- $\alpha$  (3-4 mg) from 1991-2004 (n=99) and at low doses of TNF- $\alpha$  (1-2 mg) from 2004-2009 (n=68) under mild hyperthermic conditions (38 – 39.5°C.). melphalan doses were unchanged at 10-13mg/L (leg and arm, respectively). Characteristics for the 167 ILPs were: 81 stage III-B, 65 stage III-C, 21 stage IV disease.

### *Results*

The overall response rate was 89% (n=148). (CR=61%; PR=28%). CR rates correlated with stage (p=0.001) and with high dose vs low dose TNF- $\alpha$  (70% vs 49%; p < 0.006). High dose TNF- $\alpha$  prolonged local control (median 16 months vs 11 months; p=0.076). Survival was not influenced by TNF- $\alpha$  dose. CR-after ILP and number of lesions also correlated with local progression free interval. Overall survival did correlate with stage of disease (p<0.001), size of the lesions (p=0.001), and a CR (p<0.001).

### *Conclusion*

This two-decade single center experience demonstrates that TM-ILP is safe and effective treatment modality for melanoma patients with multiple IT-mets. Higher dose of TNF- $\alpha$  was associated with significantly higher CR-rates and prolonged local control without an effect on overall survival.

## Introduction

Malignant melanoma incidence is rising rapidly. In 2008 there were approximately 62000 new cases of primary melanoma in the United States,<sup>1</sup> of which approximately 50% were extremity melanoma. In 5% to 8% of cases, melanoma patients will develop in-transit metastasis (IT-mets). As regional recurrence often precedes systemic disease, amputative surgery is in general no longer practiced, although old series of radical surgery have demonstrated that some patients with IT-mets confined to the limb can be cured.<sup>2,3</sup> Simple surgical resection may suffice for incidental and low numbers of IT mets, but in case of rapid recurrences and multiple IT-mets, isolated limb perfusion (ILP) provides an attractive treatment option that can improve local control markedly and thereby quality of life.

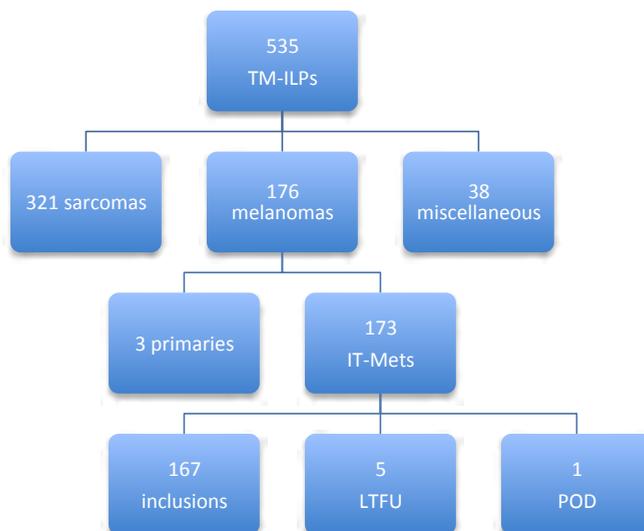
ILP, developed by Creech et al.<sup>4</sup>, achieves a twentyfold higher concentration of chemotherapeutic drugs when compared to systemic therapy.<sup>5</sup> Melphalan based ILP (M-ILP) has been the standard treatment and has been reported to achieve overall CR rates in the range of about 50%.<sup>6</sup> In general large IT-mets showed a poor response and inhomogeneous uptake comparable with locally advanced soft tissue sarcomas (STS). The introduction of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) changed this situation dramatically. Large tumors now reacted very well to ILP.<sup>7</sup> This led to a successful multi-center trial in Europe and the approval of TNF- $\alpha$  based ILP (TM-ILP) for irresectable extremity soft tissue sarcomas (STS).<sup>8</sup> Similar encouraging results were reported for the use of TNF- $\alpha$  in ILP for melanoma patients.<sup>9</sup> Preclinical<sup>10</sup> and clinical<sup>11-13</sup> studies suggested that a reduction of the dose of TNF- $\alpha$  to 1mg for the arm and 2 mg for the leg might be as effective as the higher doses. Therefore we changed TNF- $\alpha$  doses from 4mg to 2 mg for ILP of the leg and from 3 mg to 1 mg for an ILP of the arm starting in 2004. This study reports on our twenty-year experience, analyzed the determinants of response and toxicity in patients with multiple melanoma IT-mets of the limb and outlines the evolution and major changes since the introduction of TNF- $\alpha$  in ILP.

## Patients and methods

### *Patients*

Between 1991 and 2009, 173 ILPs were performed in patients with extensive melanoma IT-mets in the limb. For five patients clinical data were insufficient because they came from abroad and did not have adequate follow up in our center. One patient died 4 days after ILP without any leakage of TNF- $\alpha$  due to a myocardial infarction (mortality: 0.6%). Thirteen

Figure 1: Inclusion flow chart



TM-ILP = TNF- $\alpha$  based ILP; IT-Mets = in-transit metastasis; LTFU = Lost trough follow up; POD = Perioperative death

patients underwent ILP twice due to recurrence. Three patients underwent 3 perfusions. As a result 167 ILPs in 148 patients were included for analysis (fig. 1).

Due to publications in literature indicating that in sarcoma patients a lower dose of TNF- $\alpha$  might be as effective as high dose, we lowered the dose of TNF- $\alpha$  in 2004 in our center<sup>11, 14</sup> from 3-4 mg to 2 mg for a lower limb perfusion and from 3 mg to 1 mg for an upper limb perfusion. High dose TNF- $\alpha$  Perfusions between 1991 and 2004 (n=99) and low dose TNF- $\alpha$  perfusions between 2004 and 2009 (n=68) were compared to each other. All demographic data, disease presentation and ILP characteristics were retrieved from a prospectively maintained database.

### Treatment

The technique of ILP with TNF- $\alpha$  and melphalan has been described previously.<sup>15, 16</sup> Briefly, the procedure is performed with patients under general anesthesia. After heparinization, a targeted blood circuit is isolated by clamping and cannulation of the major artery and vein and connected to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels and prevents leakage. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored for the length of the procedure. The standard dose of TNF- $\alpha$  in the first decade was 3 mg for the arm and 4 mg for the leg. Currently, a dose of 1 mg in the arm or 2 mg in the leg of recombinant TNF- $\alpha$  (Boehringer Ingelheim GmbH,

Ingelheim/Rhein, Germany) is injected as a bolus once the temperature of the limb reached 38°C. Subsequently, 13 mg/L (arm) or 10 mg/L (leg) melphalan (L-PAM, Alkeran, Burroughs Wellcome Ltd., London, UK) was administered 30 minutes after the limb temperature reached 38 – 39.5°C. The doses of melphalan were not changed during the last two decades and have been standardized for over 40 years. After 90 minutes of perfusion, the limb is washed out with 1 L (arm) to 4 L (Iliac perfusion) of physiological saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden).

### *Response and toxicity*

Clinical response was obtained 2-4 weeks and 8 weeks after ILP. Afterwards follow up was three monthly in the first 2 year after ILP and at longer intervals hereafter. Response rates were defined according to WHO criteria.<sup>17</sup> Toxicity after ILP was classified following Wieberdink et al.<sup>18</sup>

### *Statistical evaluation*

Overall survival (OS) and time to local or systemic progression (TLP/TSP) were defined as time between ILP and death, local progression or systemic progression respectively. The end of follow up was defined as the last visit to the outpatient clinic. On the first of January 2011 the community death register was consulted to determine OS. Estimates were drawn using the Kaplan and Meier method.<sup>19</sup>

Prognostic value of baseline factor as used in previous literature,<sup>16, 20-28</sup> was evaluated for three endpoints (TLP/TSP/OS) using Cox regression and was expressed in hazard ratios. Prognostic value of the same factors for CR was determined using logistic regression and analogously expressed in odds ratio. Multivariate analysis was performed with all factors that reached 10% significance in univariate analysis. A stepwise backward algorithm was used to exclude factors without significant prognostic value. To compare baseline factors within the two groups a student-t test was used. All tests were done at a significance level of 5%.

## **Results**

### *Patients*

In total 167 TM-ILP were analyzed in 148 subsequent patients. Median age of patients was 65 years (range 25-93), 103 patients (70%) were female. Median follow up was 20 months (range 1-130). Disease staging was according to the AJCC staging system,<sup>29</sup> which resulted in 81 patients (48%) with stage III-B, 65 patients (39%) with stage III-C and stage IV in 21 cases

<b>Table 1: Patient and tumor characteristics</b>				
	<b>High-dose (1991-2004)</b>	<b>Low-dose (2004-2009)</b>	<b>Total (1991-2009)</b>	<b>p</b>
<b>Sex</b>				
Female	62 (71%)	41 (67%)	103 (70%)	0.598
Male	25 (29%)	20 (33%)	45 (30%)	
<b>Age</b>				
<65 years	55 (56%)	26 (37%)	81 (49%)	<b>0.030</b>
≥65 years	44 (44%)	42 (63%)	86 (51%)	
<b>Location primary</b>				
Arm	3 (3%)	2 (3%)	5 (3%)	0.737
Leg	47 (54%)	40 (67%)	87 (59%)	
Foot	29 (34%)	16 (26%)	45 (31%)	
back	4 (5%)	2 (3%)	6 (4%)	
unknown primary.	3 (3%)	1 (1%)	4 (3%)	
missing	1	-	1	
<b>Breslow</b>				
median in mm (range)	2.89 (0.6-15.0)	3.00 (0.7-11.0)	2.97 (0.6-15.0)	0.579
missing	25 (29%)	12 (20%)	37 (25%)	
<b>Primary to IT-mets</b>				
≤1 year	30 (36%)	31 (52%)	61 (43%)	0.052
>1 year	53 (64%)	28 (48%)	81 (57%)	
missing	4	2	6	
<b>Time between IT-mets and ILP</b>				
≤6 months	41 (42%)	46 (69%)	87 (53%)	<b>0.001</b>
>6 months	57 (58%)	21 (31%)	78 (47%)	
missing	1	1	2	
<b>Location</b>				
arm	4 (4%)	3 (4%)	7 (4%)	0.906
leg	95 (96%)	65 (96%)	160 (96%)	
<b>Number of lesions</b>				
<10	41 (41%)	37 (54%)	78 (47%)	0.098
≥10	58 (59%)	31 (46%)	89 (53%)	
<b>Size largest</b>				
<40 mm	53 (53%)	49 (72%)	102 (61%)	<b>0.016</b>
≥40 mm	46 (47%)	19 (28%)	65 (39%)	
<b>AJCC stage</b>				
III-B	46 (47%)	35 (52%)	81 (48%)	0.706
III-C	39 (39%)	26 (38%)	65 (39%)	
IV	14 (14%)	7 (10%)	21 (13%)	
<b>Prior treatment</b>				
none	59 (60%)	56 (82%)	115 (69%)	<b>0.019</b>
ILP	17 (17%)	8 (12%)	25 (15%)	
RTx	3 (3%)	2 (3%)	5 (3%)	
CTx	9 (9%)	-	9 (5%)	
immuno	4 (4%)	1 (2%)	5 (3%)	
combination	7 (7%)	1 (2%)	8 (5%)	

ILP= isolated limb perfusion; RTx= radiotherapy; CTx = chemotherapy; immuno= immunotherapy

(13%). All demographic, disease presentation and ILP characteristics were summarized in table 1. Most remarkable evolutions on characteristics over time were a shift towards older

patients ( $p=0.030$ ), shorter period between diagnosis and TM-ILP ( $p=0.001$ ) and smaller lesions ( $p=0.016$ ).

### Treatment

Patients underwent ILP via the axillary ( $n=7$ , 4%), iliac ( $n=85$ , 51%) and femoral ( $n=75$ , 45%) approach. A significant shift from an iliacal approach to a femoral approach was observed in the later years, ( $p=0.003$ , table 2). Hospital length of stay decreased for every perfusion type (table 2).

	High dose (1991-2004)			Low dose (2004-2009)			Total (1991-2009)			p
<b>Type of ILP</b>										<b>0.003</b>
Axillary	4 (4%)			3 (4%)			7 (4%)			
Iliacal	61 (62%)			24 (35%)			85 (51%)			
Femoral	34 (34%)			41 (60%)			75 (45%)			
	Ax	Il	Fem	Ax	Il	Fem	Ax	Il	Fem	
<b>Dose (mg)</b>										
median melphalan	46	110	60	40	98	60	42	110	60	
<b>Hospitalization</b>										
median days	14	11	10	5	8	6	10	10	8	

Ax = axillary; Il = Iliacal; fem = femoral; ILP = isolated limb perfusion; TNF- $\alpha$  = tumor necrosis factor- $\alpha$

### Response rate and limb function

An overall response rate of 89% ( $n=148$ ) was observed. In 102 cases (61%) a CR was recorded, 46 patients (28%) had a PR and 19 (11%) had NC. Patients treated with a high dose TM-ILP had a CR rate of 70% compared to a CR rate of 49% for those treated with a low dose TM-ILP ( $p=0.006$ ). A CR was significantly more often observed in patients with stage III-B disease (77%) compared to patients with stage III-C or IV disease, 49% vs 38% respectively (III-B vs III-C,  $p=0.002$ ; III-B vs IV,  $p=0.003$ ; III-C vs. IV,  $p=0.45$ ). In multivariate analysis TNF- $\alpha$  dose, stage of disease and age remained significant prognostic factors for CR (table 3).

Limb function was assessed in all 148 patients which resulted in perfect function in 118 cases (80%), loss of function without the necessitate of using crutches in 15 cases (10%), 4 cases (3%) of severe limb function loss necessitating crutches. In 2 patients (1.5%) an amputation was necessary because of post ILP locoregional toxicity (Wieberdink grad V). In 8 patients

**Table 3: Analysis of prognostic factors for CR, local progression, systemic disease and OS**

Variable	CR		Local progression		Systemic disease		Overall survival	
	Univariate OR (p)	Multivariate OR (p)	Univariate HR (p)	Multivariate HR (p)	Univariate HR (p)	Multivariate HR (p)	Univariate HR (p)	Multivariate HR (p)
Sex female* vs male	0.56 (0.088)	-	1.01 (0.951)	-	<b>2.48 (&lt;0.001)</b>	<b>1.83 (0.022)</b>	<b>1.97 (&lt;0.001)</b>	-
Age <65 vs ≥65 years	<b>0.51 (0.039)</b>	<b>0.49 (0.047)</b>	1.36 (0.147)	-	1.27 (0.296)	-	<b>1.71 (0.004)</b>	<b>1.52 (0.031)</b>
BMI ≤30* vs >30	1.16 (0.725)	-	1.04 (0.886)	-	1.06 (0.851)	-	1.22 (0.403)	-
Location of primary limb* v acra vs else	0.82 (0.562)	-	0.88 (0.561)	-	1.50 (0.094)	-	<b>1.68 (0.009)</b>	-
Breslow thickness (in mm.)	0.71 (0.590)	-	0.73 (0.534)	-	2.79 (0.053)	-	<b>2.30 (0.030)</b>	-
Interval prim vs IT-mets ≤1* vs >1 year	0.93 (0.347)	-	1.04 (0.344)	-	1.08 (0.189)	-	<b>1.12 (0.003)</b>	-
Location of lesions total* vs lower vs upper	0.96 (0.893)	-	0.80 (0.298)	-	1.47 (0.111)	-	0.90 (0.548)	-
Number of lesion <10* vs ≥10 lesions	1.11 (0.770)	-	1.06 (0.809)	-	1.37 (0.327)	-	1.24 (0.330)	-
Size of largest lesion <4* vs ≥4 cm.	0.57 (0.266)	-	0.94 (0.846)	-	0.94 (0.826)	-	1.37 (0.285)	-
AJCC Stage of disease III-B* vs III-C vs IV	0.87 (0.666)	-	<b>1.77 (0.002)</b>	<b>1.84 (0.005)</b>	0.92 (0.697)	-	0.86 (0.418)	-
Prior ILP no* vs yes	0.75 (0.380)	-	0.79 (0.288)	-	<b>2.01 (0.002)</b>	<b>1.76 (0.023)</b>	<b>2.19 (&lt;0.001)</b>	<b>2.16 (&lt;0.001)</b>
Interval IT-mets vs ILP ≤6* vs >6 months	<b>0.30 (0.001)</b>	<b>0.23 (&lt;0.001)</b>	1.47 (0.075)	-	<b>2.74 (&lt;0.001)</b>	<b>1.95 (0.010)</b>	<b>2.32 (&lt;0.001)</b>	<b>1.64 (0.020)</b>
Period of ILP '91-'04* vs '04-'09 (high* vs low)	<b>0.19 (0.001)</b>	<b>0.13 (&lt;0.001)</b>	0.71 (0.430)	-	<b>2.74 (&lt;0.001)</b>	<b>1.95 (0.010)</b>	<b>4.46 (&lt;0.001)</b>	<b>2.58 (0.002)</b>
CR achieved no* vs yes	1.35 (0.463)	-	1.05 (0.855)	-	0.87 (0.628)	-	0.72 (0.156)	-
CR achieved no* vs yes	1.72 (0.094)	-	1.02 (0.925)	-	<b>0.62 (0.041)</b>	-	<b>0.57 (0.003)</b>	-
CR achieved no* vs yes	<b>2.44 (0.006)</b>	<b>2.57 (0.010)</b>	0.70 (0.084)	-	1.36 (0.236)	-	0.81 (0.280)	-
CR achieved no* vs yes	N/A	N/A	<b>0.34 (&lt;0.001)</b>	<b>0.32 (&lt;0.001)</b>	<b>0.46 (0.001)</b>	<b>0.53 (0.018)</b>	<b>0.29 (&lt;0.001)</b>	<b>0.35 (&lt;0.001)</b>

\*Reference group; CR = complete response; OS = overall survival; ILP = isolated limb perfusion; TNF-α = tumor necrosis factor-α; NA = not applicable

(6%) an amputation was necessary because of uncontrollable ulcerating locoregional tumor recurrences (n=8), In one patient an amputation was necessary for arthrosclerosis despite a CR. In case of amputation median time span between first ILP and amputation was 17 months (mean 19, range 2-32).

### Local progression

Local progression after ILP occurred in 56% of cases (n=93) after a median time of 13 months. Although not significant, a trend towards better local control could be observed in the high dose TM-ILP group. Median time to local progression (TLP) was 16 months after high dosed TM-ILPs while those treated after TNF-α dose reduction showed a median TLP of 11 months (p=0.076, figure 2-c). Patients with a CR after ILP had a significantly longer median TLP of 19 months whereas a PR or NC resulted in a median TLP of 6 months (p<0.001). Patients treated for ≥10 lesions had a shorter TLP compared to those with <10 lesions. (9 months vs. 24 months, resp, p=0.002). CR after ILP and amount of lesions remained significant prognostic factor for local progression in multivariate analysis (table 3).

### Systemic disease

Patients treated with curative intent (stage III-B and III-C, n=146) developed systemic disease (stage IV) in 79 cases (54%) with a median time to systemic progression (TSP) of 26 months. Patients with a CR had a median TSP of 39 months whereas patients with PR or NC showed a

median TSP of 11 months ( $p < 0.001$ ). Female sex ( $p < 0.001$ ), the size of the largest lesion ( $p = 0.002$ ) and stage of disease ( $p < 0.001$ ) were baseline factors reaching significance in univariate cox regression analysis. Sex, size, stage of disease and response to ILP remained significant prognostic factors for TSP in multivariate analysis. The dosage of TNF- $\alpha$  was not of influence on TSP ( $p = 0.236$ ). Once patients developed systemic disease median survival time was 7 months.

### *Survival*

The overall actuarial 3-year, 5-year and 10-year survival rate after ILP was 40% ( $\pm 4\%$ ), 26% ( $\pm 4\%$ ) and 13% ( $\pm 3\%$ ) respectively, median OS was 24 months. CR after perfusion resulted in a prolonged median OS of 44 months while patients with PR or NC had a median survival of 11 months (figure 2-a,  $p < 0.001$ ). Stratified for stage of disease, 5-year survival was 42% for stage III-B disease, 15% for stage III-C disease and 0% for stage IV disease (figure 2-b,  $p = 0.001$ ). In univariate regression analysis female sex ( $p < 0.001$ ), age ( $p = 0.004$ ), a primary on the limb ( $p = 0.009$ ), Breslow thickness ( $p = 0.003$ ), small size of IT-mets ( $p < 0.001$ ) and long interval between diagnosis of IT-mets and perfusion ( $p = 0.003$ ) appeared to be other favorable prognostic factors correlated with prolonged survival. In multivariate analysis age, small size, lower stage of disease and complete response after ILP remained, significant prognostic factors for prolonged survival. Analogously to time to systemic progression, dose of TNF- $\alpha$  was not associated with OS ( $p = 0.272$ , figure 2-d). All hazard ratios are summarized in table 3.

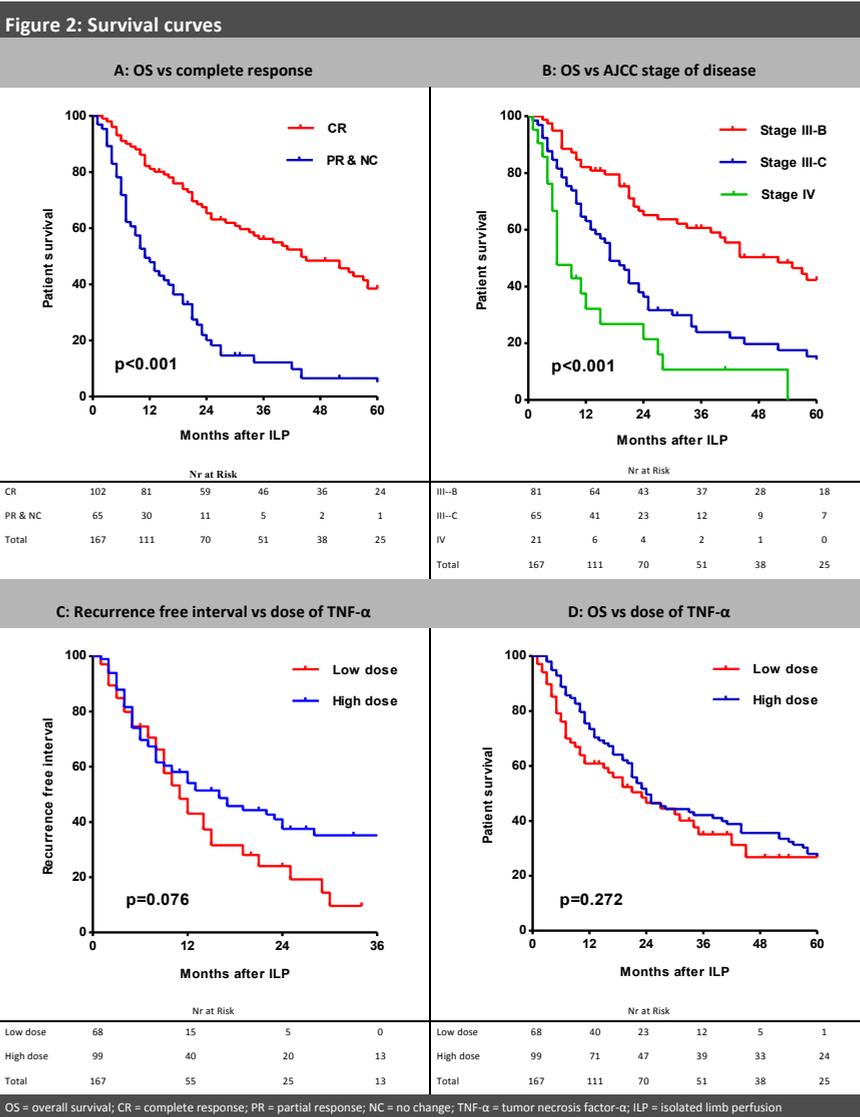
### *Body mass index*

Patients with a BMI  $> 30$  had a CR rate of 63% ( $n = 19$ ), which is similar to the CR rate of 60% for those with a BMI of  $\leq 30$  ( $p = 0.78$ ). Median TLP was 13 months for patients with a BMI  $\leq 30$  while patients with a BMI  $> 30$  had a median TLP of 12 months ( $p = 0.82$ ). In univariate analysis, BMI as prognostic baseline factor did not reach significance for clinical outcome, nor for TLP, TSP or OS.

### *Leakage and toxicity*

Local toxicity was not observed (Wieberdink I) in 31 cases (18%), slight (Wieberdink II) in 93 cases (56%), considerable (Wieberdink III) in 38 cases (23%) and severe (Wieberdink IV) in 3 cases (2%). Amputation due to perfusion reaction was necessitated for 2 patients (1%), one after 2 months, the other after 6 months. The dose of TNF- $\alpha$  could not be identified as significant predictor for local toxicity ( $p = 0.524$ ).

There was no or minor leakage ( $\leq 10\%$ ) in 160 ILPs (96%), median leakage was 0% (mean 1.34; range 0-25). Leakage was  $> 10\%$  in 7 patients of which one patient with 12% systemic



leakage had a myocardial infarction 2 days after ILP, after referral to a cardiac department this patient could be stabilized, had no further complications and could be discharged from hospital after 8 days. Two other patients experienced transient hypotension treated with vasopressors. One patient had a grade IV leucopenia that lasted for 1 day, which did not need any intervention. Three patients did not experience any inconvenience of the >10% systemic leakage.

## Discussion

With an overall response (OR) rate of 89% and a CR rate of 61% the present study demonstrates that TM-ILP is a successful treatment modality in obtaining local control of the limb in patients with melanoma in-transit metastases. Local and systemic toxicity is limited which emphasizes the safety of this procedure. The reduction of the dose of TNF- $\alpha$  was associated with a lower CR rate.

The introduction of TNF- $\alpha$  ushered in a new era for ILP in Europe. The present study reported on the evolution observed over these two decades. The most remarkable change was the dose reduction of TNF- $\alpha$  based on several previous studies describing comparable response rates with reduced local toxicity.<sup>10, 11, 13, 14</sup> In the present series, a CR was more often observed in the period of high dose TM-ILPs. In multivariate analysis this difference remained significant.

The lowering of the dose of TNF- $\alpha$  not only led to inferior clinical response, but to an inferior local control as well. This was emphasized by the fact that there were no cases of maintained local control after three years in the low dose TNF- $\alpha$  group (figure 2-c). There was no significant correlation between the dose of TNF- $\alpha$  and systemic progression or OS. These findings fit in the concept of a locoregional treatment having locoregional benefit only. In our opinion CR after TM-ILP occurs in patients with the more favorable biology,<sup>16</sup> which allows a similar effect after low dose perfusions. Patients with more unfavorable biology might experience more often a CR and prolonged local control after high dose perfusion compared to low dose perfusion. However, systemic development and overall survival are dictated by the biology of the tumor, which explains that despite lower response rates and inferior local control low dose perfusions show similar TSP and OS. This is illustrated in figure 2-c and 2-d.

The dose reduction of TNF- $\alpha$  in ILP for melanoma patients was mainly based on data in sarcoma patients. Our group published in 2005<sup>14</sup> a mixed series of sarcoma and melanoma patients with only 16 melanoma patients that received low dose TNF- $\alpha$ . Rossi et al.<sup>13</sup> described a series of 20 low dose perfusions in melanoma patients. The low numbers of patients might explain why these studies did not find the correlation between dose of TNF- $\alpha$  and CR-rate and local control. Our series is one of the largest in the world with a mature follow up and therefore the outcome might be different compared to our previous smaller series.

There is no consensus in the literature about the benefit of using TNF- $\alpha$  in ILP for IT-mets in melanoma patients. Cornett et al.<sup>30</sup> performed the only randomized controlled trial so far in which they report an increased local en systemic toxicity without any beneficial effect in clinical response (CR rate 26% for TM-ILP vs CR rate 25% for M-ILP). This study was subject of

several criticisms,<sup>31</sup> so their conclusions should be read with caution. First of all, they reported complete response rate after 3 months, which is an uncommon endpoint since a substantial proportion of patients reach CR between 3 and 6 months. Secondly, there was very little data provided concerning differences between patients and tumor characteristics between both arms. Thirdly, the true indication for TNF- $\alpha$  based ILP, bulky disease was not analyzed.

Alexander et al.<sup>20</sup> reported recently the long term follow up results of a mixed TM-ILP and M-ILP series. They did not identify a significant correlation between the addition of TNF- $\alpha$  to M-ILP and infield progression, which might be explained by the lower number of patients included in this study. The reported CR rate of 69% is slightly higher compared to ours in a more favorable patient population (68% stage III-A disease in their group versus 48% in the present study). Rossi et al.<sup>32</sup> reported a CR rate of 60% for TM-ILP and 42% for M-ILP, which was a significant difference ( $p=0.05$ ). With the correlation between CR rate and local control on one hand and the dose of TNF- $\alpha$  on the other, the present study emphasizes the important role of TNF- $\alpha$  in ILP for melanoma patients.

Certainly in bulky disease TNF- $\alpha$  is of additional value. Melphalan uptake is very low in large tumors, which can be improved by a three to six fold with the use of TNF- $\alpha$ .<sup>33</sup> Consequently, we consider TM-ILP indicated for patients with bulky disease and those resistant for M-ILP. When disease load is limited melphalan only based ILP might be effective in achieving local control.<sup>34, 35</sup> In case of small lesions restricted to the distal parts of the limb isolated limb infusion with melphalan can be of value.<sup>36</sup> Literature suggests that reduction of duration of TM-ILP has no influence on clinical response nor on local control.<sup>37</sup> However, these results are achieved in soft tissue sarcoma patients and should be investigated in an IT-mets melanoma study population.

A variety of treatment modalities for IT-mets have been used with various successes. If lesions are limited in number and size simple surgical excision is the preferred treatment modality. Smaller lesions too numerous for excision were treated with carbon dioxide laser therapy, intralesional injections and electro chemotherapy but all with poor clinical response rates.<sup>38-44</sup> After decades of failing identifying effective systemic therapy, there are promising results achieved with PLX4032 and ipilimumab in patients with stage III and IV disease. PLX4032 (vemurafenib) provides a rather limited PFS of only 5.5 months in irresectable stage III-IV disease and ipilimumab a response rate of only about 10%, so the role of ILP remains established whilst the of these new drugs in the treatment for IT-mets is still unclear.<sup>45-47</sup>

TNF- $\alpha$  increases the efficacy of ILP. We demonstrated that high doses of TNF- $\alpha$  are correlated with higher CR rates and superior local control in patients with high tumor burden and those having failed previous therapy. Since the main objective of TM-ILP in melanoma

patients is obtaining local control, rather than improving survival, high dose TNF- $\alpha$  perfusions seems preferable to low dose TNF- $\alpha$  perfusions.

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# Chapter 8

## Repeated isolated limb perfusion in melanoma patients with recurrent in-transit metastases

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## Abstract

### *Objective*

In-transit metastases of melanoma (IT-Mets) occur in 5-8% of all melanoma patients. In case of extensive loco-regional disease tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion (TM-ILP) had proven to obtain excellent local control. Herein we reported on repeat TM-ILP for loco-regional recurrence after ILP.

### *Methods*

Between 1991-2013, 37 consecutive repeat TM-ILPs were analyzed in 32 different patients. Three patients underwent a third TM-ILP

### *Main results*

During a median follow up of 20 months after repeat TM-ILP, overall response rate was 86%. Complete response (CR) was recorded after 24 TM-ILPs (65%). CR after first TM-ILP was a strong predictor for successful repeat TM-ILP in terms of clinical response and local recurrence. Local toxicity was mild (70% Wieberdink I-II). Local recurrence rate was 59%. Five year overall survival was 35%

### *Principal conclusions*

Repeat TM-ILP is a safe treatment modality in melanoma patients with recurrent IT-Mets. Those with a complete response after first TM-ILP benefit the most of repeat TM-ILP.

## Introduction

Cutaneous melanoma accounts for the vast majority of all deaths of skin cancer. Due to intermittent and excessive sun exposure, incidence is raising rapidly in western world.<sup>1</sup> Five to 8 percent of melanoma patients will develop in-transit metastases (IT-mets). IT-mets are known as an unfavorable prognostic factor and especially when accompanied by lymph node metastases associated with poor survival.<sup>2</sup> A variety of treatment modalities have been described with various success. When limited in number and small in size, surgical resection is the golden standard. In case of numerous or bulky disease radiotherapy, intralesional injections, laser therapy and systemic therapy might be considered.<sup>3, 4</sup> In locally advanced disease these treatment modalities often fail. Historically there has been a limited role for systemic treatment for IT-mets. However, the introduction of BRAF inhibitors, ipilimumab and the anti-PD1 antibodies nivolumab and pembrolizumab has ushered in a new era.<sup>5-8</sup> The effect of these promising medical treatment modalities in stage III-C melanoma patients with IT-mets is now subject of several studies. Results have to be awaited.

Tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion (TM-ILP) has proven to obtain excellent local control with complete response rates up to 70% in melanoma patients with extensive and bulky IT-Mets.<sup>9-11</sup> The true indication of TNF- $\alpha$  in ILP is bulky disease. After failure of M-ILP, TNF- $\alpha$  might improve response rates if repeat ILP is indicated.<sup>12</sup> IT-mets often precede overt systemic disease. Our long-term series contained 15 out of 105 patients with sustained control in the limb without evidence of distant metastases over a period of at least five years.<sup>13</sup> This suggests that in selected patients disease may be confined to the limb. In this particular group repeated limb perfusions might be indicated in case of locoregional recurrence.

This study aimed to report on repeated ILP in melanoma patients of the limb and compare the outcome results and local toxicity with those treated with a first ILP.

## Methods

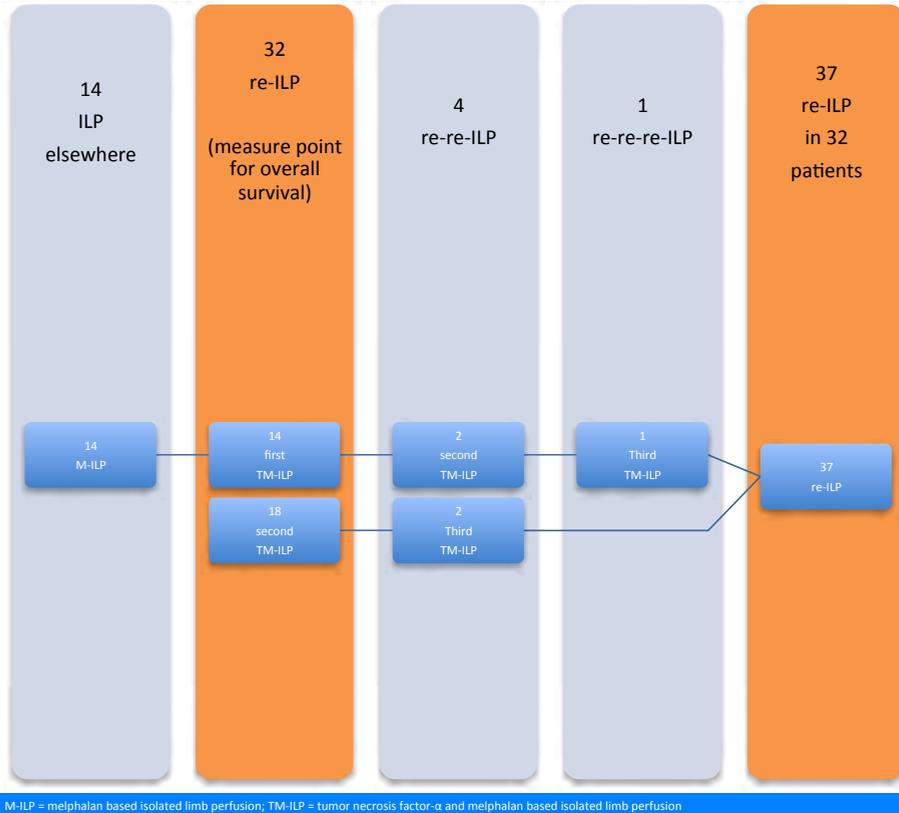
### *Patients*

Between 1991 and 2013, 185 consecutive TM-ILPs in melanoma patients were performed. Thirty-seven procedures were repeated TM-ILPs in 32 different patients. Fourteen patients were treated with M-ILP elsewhere before they were referred to our center, in 18 patients, repeat ILP was preceded by a TM-ILP. In 5 patients, >2 ILPs were performed (figure 1).

*Treatment*

ILP technique has been described extensively before.<sup>14, 15</sup> Briefly, the procedure is performed with patients under general anesthesia. After heparinization, the targeted blood circuit is isolated by clamping and cannulation of the major artery and vein and connected to an oxygenated extracorporeal circuit. A tourniquet compresses collateral vessels to prevent leakage. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored throughout the procedure. TNF- $\alpha$  -alpha and melphalan are the active compounds used and after 60-90 minutes of perfusion, the limb is rinsed and the original circulation restored.

Figure 1



*Response and toxicity*

Clinical response was obtained 2-4 weeks and 8 weeks after ILP. Afterwards follow up was three monthly in the first 2 years after ILP and at longer intervals hereafter. Response rates

were defined according to WHO criteria.<sup>16</sup> Overall response was defined a complete or a partial response. Toxicity after ILP was classified following Wieberdink et al.<sup>17</sup>

*Statistics*

IBM SPSS Statistics version 21.0 was used for statistic analysis. Chi-square and log rank scores were used. Overall survival was measured from second ILP (figure 1).

**Results**

*Patients and outcome*

Median age at time of repeat TM-ILP (n=37) was 63 years (IQR 52-76 years). Patients and disease characteristics are summarized in table 1. Overall response (OR) rate was 86% (n=32). A complete response (CR) was recorded after 24 procedures (65%). Compared to patients in our database that underwent a first ILP, no significant differences in clinical outcome were noted (CR-rate 61%; OR-rate 89%).<sup>11</sup> Median follow after repeat ILP was 20 months (IQR 8-44 months).



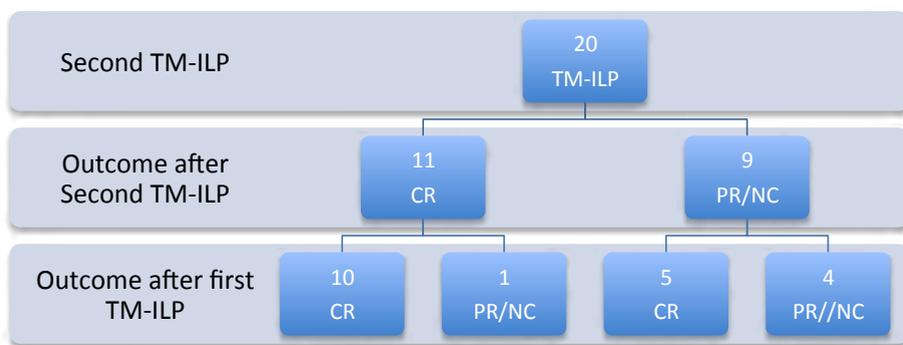
Table 1: patients, tumor and treatment characteristics	
<b>Gender</b>	
Female	25 (78%)
Male	7 (22%)
<b>Age</b>	
< 65 years	20 (54%)
≥ 65 years	17 (46%)
<b>Location</b>	
Arm	-
Leg	37 (100%)
<b>Breslow of the primary</b>	
Median thickness	2,70 mm
Data missing	13
<b>Number of IT-Mets</b>	
< 10 IT-Mets	9 (24%)
≥ 10 IT-Mets	28 (76%)
<b>Size of largest IT-Mets</b>	
< 40 mm	24 (65%)
≥ 40 mm	13 (35%)
<b>AJCC stage of disease</b>	
III-B	16 (43%)
III-C	16 (43%)
IV	5 (14%)
<b>Type of TM-ILP</b>	
Iliacal	20 (54%)
Femoral	17 (36%)

IT-Mets = in-transit metastases; AJCC = American Joint Committee on Cancer; TM-ILP tumor necrosis factor-α and melphalan based isolated limb perfusion

We performed a subanalysis in patients treated twice in our center since in these cases adequate data of the first procedure were available. Out of 20 second TM-ILP, 11 ended up in a CR (55%). Just one of these patients did not have a CR after the first TM-ILP. The procedures resulting in a PR or no change (n=9), were preceded by a CR in first TM-ILP in 5 cases (figure 2).

There was no significant correlation between interval of primary and second TM-ILPs and CR rate. Four out of 10 patients (40%) treated again within one year had a CR whereas CR rate was 50 % (5 out of 10) for those treated again with an interval over 1 year. In this subgroup analysis, median hospital length of stay was 9 days after first TM-ILP compared to 8 days after second TM-ILP.

Figure 2: Outcome after second TM-ILP related to first TM-ILP



TM-ILP = tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion; CR = complete response; PR = partial response; NC = no change

### Local control

Local recurrence rate was 59% (n=22 out of 37). Estimated local recurrence rate after 1 year and 2 years was 34% and 70%, respectively. Median time to local recurrence was 13 months. Patients treated with a single TM-ILP in our historical control had a local recurrence rate of 56% with a median time to recurrence of 13 months.<sup>11</sup>

After 15 repeat TM-ILPs the leg remained free of disease. Eleven patients died of systemic disease during follow up. Four patients were free of disease at the end of follow up. One patient underwent 2 M-ILPs elsewhere and was treated once with a TM-ILP resulting in a

long lasting CR until end of follow up after 126 months. The second patient was treated with a first TM-ILP 15 years after resection of a primary melanoma of the leg and 10 years after appearance of first IT-mets, resulting in a CR lasting for 15 months. Four years afterwards a second TM-ILP was performed and at the end of follow up (31 months) there was no evidence of disease. The third patient suffered of Papillon-Lefevre syndrome, which completely responded after first TM-ILP. After 2 years a local recurrence occurred but unfortunately the patient showed no response on the second TM-ILP. Finally this patient underwent a resection of the plantar fascia in order to resect all disease with severe impairment of pedal function. The last patient was known to have peripheral arterial occlusive disease (PAOD) in the medical history. A TM-ILP was performed for numerous IT-Mets of the lower leg resulting in a partial response. Since disease was progressive after 10 months there was decided to perform a repeat TM-ILP resulting in a complete response. Unfortunately this patient developed peripheral arterial occlusive disease (PAOD) grade IV resulting in an amputation 9 months after TM-ILP.

Seven out of the 15 patients with sustained local control during follow up were treated with a first TM-ILP in our center, hence previous response rates were available. Six out of these seven patients had a complete response after first TM-ILP.

Three patients underwent a third TM-ILP (figure 1). Two patients had stage IV melanoma, hence treated in palliative setting. The third patient received a third TM-ILP with curative intent for extensive local regional disease (IT-Mets > 20) resulting in a CR just lasting for 4 months. Afterwards local recurrences were observed in a milder pattern, always treated with local resection. After a follow up of 207 months after first TM-ILP and 149 months after third TM-ILP disease was still confined to the limb.

### *Survival*

Three and five-years survival after repeated ILP was 56% and 35% respectively. Estimated median overall survival was 45 months (IQR: 21-80).

### *Local toxicity*

Local toxicity was absent or mild (W-I & W-II) after 26 repeat TM-ILPs (70%). A W-III reaction was observed after 10 repeat TM-ILPs (27%). An amputation due to perfusion reaction was required after 1 TM-ILP (W-V, 2,7%). Compared to those treated with a first TM-ILP in our database, no significant differences in perfusion reaction were observed. ( $p=0,216$ ; W-I 22%, W-II 54%; W-III 21%, W-IV 2% and W-V 1%).

Subanalysis in the 20 patients that underwent a second TM-ILP in our center revealed no significant difference in local toxicity when comparing the first with the second TM-ILP

( $p=0.288$ ). Toxicity was mild or absent (W-I & W-II) after 80% of first procedures compared to 65% of second TM-ILPs.

## Discussion

This study shows that tumor necrosis factor- $\alpha$  and melphalan based isolated limb perfusion (TM-ILP) is a safe and effective option in repeated setting in case of loco-regional recurrence of in-transit metastases of melanoma.

Overall response rate was 86%, complete response rate was 65%. These rates did not differ significantly compared to those treated for the first time with TM-ILP.<sup>18</sup> Main goal of TM-ILP is to obtain local control in the affected limb. Local recurrence rate in this study was 59% and comparable with local control after a first TM-ILP.<sup>11, 18</sup> Two patients (5%) remained free of recurrence (follow up 31 and 126 months after last TM-ILP). Both patients were diagnosed with melanoma more than a decade before the last TM-ILP. This suggests a mild biology of disease and particularly this patient category can benefit of a repeat TM-ILP since it is most likely disease will remain confined to the limb.

In line with this hypothesis all but one of patients (6 out of 7) with local control until end of follow up after second TM-ILP performed in our center had a complete response after first TM-ILP. Complete response after TM-ILP is a well-known favorable prognostic factor reflecting a more indolent biology of tumor.<sup>19</sup> On the other hand there was just one complete response recorded after a repeat TM-ILP preceded by a first TM-ILP with a partial response. All other complete responses in this series were local recurrence after a complete response on first TM-ILP. These findings suggest that predominantly the patients with a complete response after first TM-ILP and a late loco-regional recurrence benefit most from a repeat TM-ILP. After a partial response or no change a successful repeat TM-ILP is uncommon. Response data after M-ILP performed in other centers were incomplete so correlation between outcome after M-ILP and repeat TM-ILP could not be examined appropriately. TM-ILP after failure (no response) of M-ILP is known to be successful with improved response rates.<sup>12, 20, 21</sup> The indication for a repeat TM-ILP after previous TM-ILP seems to be more dictated by the response of the first procedure.

Late onset of local recurrence did not correspond with higher complete response rates after repeat TM-ILP. Overall one might state that late onset is favorable for overall survival but does not guarantee another complete response after TM-ILP.

Five-year survival rate was 35%, which is considerably higher, compared to patients who are treated once.<sup>22, 23</sup> Other reports on repeat TM-ILP showed comparable overall survival.<sup>20, 21</sup> This remarkably better overall survival is likely to be a reflection of the selection of patients offered a repeat TM-ILP. Rapid onset of loco-regional disease is an indicator of an aggressive

biology of disease and therefore these patients are often refrained from repeat TM-ILP. Repeat TM-ILP is reserved for those with late onset of disease which still is confined to the limb, hence, patients with relatively favorable prognosis.

Overall repeat TM-ILP is a safe treatment modality in patients with loco-regional recurrence of melanoma. In selecting patients for a repeat TM-ILP, response on first TM-ILP and stage of disease should be taken into account.

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# Chapter 6

**Isolated limb perfusion for melanoma  
in-transit metastases: developments  
in recent years and the role of tumor  
necrosis factor- $\alpha$**

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## Abstract

### *Purpose of review*

The treatment of in transit metastasis of melanoma remains challenging and is essentially dictated by the biological behavior of melanoma. When lesions are large or numerous, isolated limb perfusion (ILP) is an attractive treatment modality. In this review an overview of literature on treatment options of melanoma in transit metastases will be discussed.

### *Recent findings*

Most recent studies report on TNF- $\alpha$  and melphalan based ILP (TM-ILP) series or mixed series of TM-ILP and melphalan only based ILP (M-ILP). After TM-ILP complete response rates varied from 59 to 90% while for M-ILP CR rates of 41-82% were reported. The only randomized trial comparing TM-ILP and M-ILP revealed no clear benefit of TNF- $\alpha$  at 3 months, but improved outcome at 6 months and in patients with bulky disease. Reports on Isolated limb infusion (ILI) with melphalan and actinomycin D indicate lower response rates, but similar local control rates as M-ILP at lower cost.

### *Summary*

ILP is an attractive treatment option in melanoma patients with multiple in-transit metastases. In our opinion TM-ILP is superior to M-ILP as it achieves higher response rates, especially in patients with bulky disease. When lesions are small and in the distal two-thirds of the leg only, ILI is a valuable alternative.

## Introduction

Melanoma incidence is rising fast in the western world at an average of 2.7% over the last two decades.<sup>1, 2</sup> The prognosis of melanoma patients varies widely and depends upon the stage of disease.<sup>3</sup> In about 5%-8% of melanoma patients in transit metastasis (IT-Mets) develop. IT-Mets are cutaneous or subcutaneous deposits of melanoma trapped within the lymphatics between the primary tumor and the regional lymph node basin.<sup>4</sup> The development of IT-Mets is not a mechanistic event that is the result of surgical interventions such as sentinel node biopsies or elective lymph node dissections, but depends on tumor biology and mainly occurs in high risk patients.<sup>5</sup> According to the latest American Joint Committee on Cancer (AJCC) system IT-Mets are categorized as IIIB (without metastatic node) or IIIC (with metastatic node).<sup>3</sup> They often precede the manifestation of systemic disease, and 5-year survival rates for patients with IT-mets of 32-69% are reported.<sup>3, 6-8</sup>

## Treatment

The management of IT-Mets remains challenging as it is essentially dictated by the biological behavior of melanoma.<sup>4</sup> A variety of treatment modalities have been used with varying results.

### *Surgical excision*

When IT-mets are limited in number and size a simple excision is the preferred treatment. Radical surgery is necessary but there is no need for wide excision margins as IT-Mets appear sharply circumscribed. Once IT-mets become irresectable an amputation does not improve survival and is hardly ever indicated.<sup>9</sup>

### *Carbon dioxide laser therapy*

The carbon dioxide laser can be of value when lesions are small but too numerous for surgical excision. Efficacy and wound healing depends on size, but most wounds heal by 6 weeks.<sup>10</sup> With local control during first year of 63% after a mean of 6 laser ablations results are encouraging.<sup>11</sup> Utility is restricted however as for lesions >1 cm in diameter laser therapy becomes less effective.

### *Intralesional therapy*

Regional immunotherapy with intralesional injections are studied over the last decades. The first introduced Intralesional therapy was based on Bacille Calmette-guérin (BCG),<sup>12</sup> followed by the use of cytokines as interferon (IFN),<sup>13</sup> and interleukine-2 (IL-2).<sup>14, 15</sup> Despite

measurable autoimmune response, clinical response rates are disappointing. Moreover multiple therapeutic sessions, not free of local toxicity, are needed and therefore these treatment modalities are mostly abandoned.

More recently intralesional technique with electrochemotherapy (ECT) was introduced. Due to electroporation cell permeability increases and allows the increased uptake of cytostatic agents like bleomycin or cisplatin.<sup>16, 17</sup> Results on the duration of effects and regional control remain to be firmly established for ECT.

### *Radiotherapy*

Melanoma cells were assumed relatively resistant to radiation, nevertheless radiotherapy can be of value when lesions are superficial, limited of size and number and in a small area. With local control rates of up to 80% radiotherapy has proven to be effective.<sup>18</sup> Although metastatic disease finally develops some studies showed prolonged disease free survival (DFS) and overall survival (OS).<sup>19</sup> At no cost of additional local toxicity the addition of hyperthermia to radiation resulted in significant prolonged DFS.<sup>20</sup>

### *Systemic therapies*

After decades of failing to identify effective systemic treatments in melanoma we have clearly entered a new era.<sup>21</sup> Both in terms of mutation-based targeted therapies, such as BRAF and C-kit mutations in melanoma, as well as in terms of novel ways of modulating the immune system new systemic therapies in melanoma are being developed with surprising results.<sup>22</sup> Flaherty et al.<sup>23</sup> reported of the use of a BRAF inhibitor in 32 metastatic melanoma patients leading to a complete response rate of 6% and partial response rate of 75% with a median progression free of 7 months. Hodi et al.<sup>24</sup> reported a statistically significant and clinically relevant impact on overall survival after ipilimumab-based immunotherapy in a randomized phase III trial in unresectable stage III and IV patients, in second line. With many other candidates in clinical development the landscape of melanoma treatments will change drastically and, this will also be true for patients with multiple in-transit metastases.

### **Isolated limb perfusion**

The technique of isolated limb perfusion (ILP) was introduced in 1958 by Creech et al.<sup>25</sup> at Tulane University in New Orleans, that allows tumors in extremities to be exposed to concentrations of chemotherapy up to 25 times higher than can be achieved with systemic administration.<sup>25</sup>

### *The technique*

After heparinization, the targeted blood circuit is isolated by clamping and cannulation of the major artery and vein and connected to an oxygenated extracorporeal circuit. By means of ligation of the collateral vessels and application of a tourniquet proximally to the perfusion level isolation is achieved and leakage is prevented. Using a precordial scintillation probe to detect technetium labeled albumen, leakage is monitored throughout the procedure.<sup>26</sup>

Once isolation is secured and the absence of leakage is demonstrated drugs can be introduced to the system. Melphalan is the most commonly used chemotherapeutic agent, at a dose of 10mg/L for a leg and 13 mg/L for an arm, because of its favorable local toxicity and its efficacy.<sup>27, 28</sup> Depending on the site of the tumors the ILP is executed via the axillary, brachial, iliacal or femoral artery. After 60-90 minutes of perfusions a wash out procedure using 1L (arm) to 4L (iliacal) of a physiological saline solution and 6% dextran (Macrodex Pharmacia, Uppsala, Sweden) is performed.

Clinical response is usually slow and it may often take 3-6 months before a CR is reached. Locoregional toxicity after ILP is usually classified following Wieberdink et al:<sup>29</sup> (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.

### *Hyperthermia*

Adequate temperature of perfused tissue prevents vasoconstriction in the cutis and the subcutis which especially is of importance when superficial lesions are treated.<sup>30</sup> Hyperthermia has been shown to improve cytotoxicity of chemotherapeutics in vitro especially at temperatures of >41°C.<sup>31, 32</sup> A big drawback is the increased damage to normal tissues with hyperthermia, reason for it to be used less these days.<sup>33, 34</sup> As a compromise between response rates and local toxicity perfusion is performed under mild hyperthermic conditions (39-40°C) with reported OR rates of 85-99%.<sup>35-37</sup> (table 1).

### *Perfusion agents*

In addition to melphalan several other chemotherapeutic drugs are used in order to improve response rates. In a single agents setting nitrogen mustard, dacarbazine cisplatin and carbopaltin are studied with various success. Never the less none of them appeared superior to melphalan.<sup>27, 28</sup> Several combinations of those cytostatics have been evaluated but none showed additional benefit. Nowadays the only used combination of cytostatics is melphalan

with dactinomycin in a hyperthermic setting (40-41°C) resulting in comparable results to melphalan alone based perfusion in a patient population with less favorable characteristics, like bulky disease and palpable lymph nodes or systemic disease.<sup>34, 38, 39, 52</sup> (table 1).

**Table 1: overview of literature in isolated limb perfusion**

Author	Ref	Year	Melphalan				
			Temp	n	CR	PR	OR
Klaase	35	1994	39-40	120	64	25	89
Lingam	36	1996	39-40	103	76	23	99
Aloia	37	2005	39-40	58	57	31	88
Bryant	38	1995	40-41	85	40	42	85
Thompson	39	1997	40-41	111	73	13	86
Di Filippo	33	1998	41,5-43	119	46	40	86

Author	Ref	Year	Melphalan +/- TNF- $\alpha$				
			Temp	n	CR	PR	OR
Noorda	40	2004	39-40	90 (TM-ILP)	59		
				40 (M-ILP)	45		
Cornett	41	2006	39-40	58 (TM-ILP)	26	43	69
				58 (M-ILP)	25	39	64
Alexander	7	2010	39-40	43 (TM-ILP) and 47 (M-ILP)	69*	26*	95*
Rossi	42	2010	39-40	58 (TM-ILP)	61	29	90
				53 (M-ILP)	42	49	91

Author	Ref	Year	Melphalan + TNF- $\alpha$				
			Temp	n	CR	PR	OR
lienard	43	1992	39-40	19	89	11	100
Lejeune	44	1993	39-40	44	90	10	100
Vaglioni	45	1994	39-40	11	64	0	64
Eggermont	46	1995	39-40	58	88	12	100
Lienard	47	1999	39-40	64	73	22	95
Rossi	48	2004	39-40	20	70	25	95
Grünhagen	8	2004	39-40	100	69	26	95
Hayes	49	2007	39-40	25	44	40	88
Di Filippo	50	2009	39-40	113	63	25	88
Lasithiotakis	51	2010	39-40	14	62	38	100

Ref = reference; Temp = temperature; CR = complete response; PR = partial response; OR = overall response; TNF- $\alpha$  = tumor necrosis factor-

### Isolated limb infusion

A simplification of the ILP procedure was developed by Thompson et al,<sup>53</sup> isolated limb infusion (ILI). Small catheters are inserted percutaneously into to the artery and vein of the

affected limb via the major vessels in the contralateral groin. Since the catheter tips are placed just proximal of the knee in the arteria poplitea, ILI is only effective for lesions at the distal two thirds whereas ILP via the iliacal approach can treat IT-mets all the way up to the groin. When a pneumatic tourniquet is applied a hyperthermic, hypoxic isolated infusion can be performed with melphalan (5-10 mg/L) and actinomycin D (50-100 µg/L). After 30 minutes the limb is flushed with saline, the tourniquet deflated and the catheters removed. Several studies reported response rates somewhat lower than after ILP (especially the CR rate) but with comparable local control rates after ILP with melphalan alone<sup>54-57</sup> but at lower cost and local toxicity.<sup>56,57</sup>

Overall ILI is a minimally invasive procedure with comparable results to M-ILP and is a valuable alternative for M-ILP for patients with IT-mets in the distal parts of the limb.

#### *Adjuvant perfusion*

With the encouraging results of melphalan based ILP (M-ILP) for in transit melanoma patients together with the premise that melanoma IT-mets commonly arise from primarily clinically undetectable lymphogenic tumor deposits present at the time of primary excision of the tumor, one could hypothesize that high-risk melanoma patients could benefit for adjuvant M-ILP after surgical excision. In a large multicenter trial published in 1998 with 832 patients Koops et al.<sup>58</sup> reported of a small benefit on locoregional recurrence with reduction of development of IT-mets and regional lymph node metastasis but no benefit on overall survival. Since ILP can be accompanied by comorbidity and costs the authors conclude that adjuvant M-ILP after surgical excision is not recommended.

In a randomized trial surgical excision followed by M-ILP was compared to excision alone in a patient group (n=69) with resectable IT-mets. Since in these patients IT-mets already occurred one could expect more benefit of adjuvant M-ILP. Despite significant better locoregional control there was no benefit of overall survival and the authors concluded that there is no indication for M-ILP in case of resectable IT-mets.<sup>59</sup>

#### *Double perfusion schedules*

Similar to schedules in systemic fashion one could assume that more frequent application of chemotherapeutic drugs raises efficiency. The idea of double perfusion schemes is based on the idea that residual tumor cells after the first perfusion may be eliminated after a second since they might be more exposed to the drugs in a partial damaged tumor. In normothermic fashion patients were offered a reduced second dose of melphalan after a time interval of 3-4 weeks which led to higher response rates but had no influence on local, regional lymphnode and distance recurrence rate.<sup>60</sup> More recently, a double perfusion schedule existing of a first perfusion with true hyperthermia (42-43°C) without chemotherapeutic

drugs follow by a second normothermic M-ILP one week later showed high complete response rates (CR = 65%). This, probably due to the synergistic effect of hyperthermia and melphalan whereas local toxicity was acceptable which might be realized by the concept of fractionated application of the melphalan.

Based on the same principle as in ILP double ILI schedules were topic in literature with no benefit on clinical response, local or systemic progression and survival,<sup>61</sup> however a second ILI appeared to be of value after local recurrence.<sup>62</sup>

### **Tumor necrosis factor- $\alpha$**

Probably the most successful modification to melphalan based ILP was the introduction of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by Lienard and Lejeune.<sup>43</sup> The role of this cytokine is a dual one; (I) High dose TNF- $\alpha$  has a cytotoxic effect which plays a role in the anti tumor activity,<sup>63</sup> (II) even more important is the effect of TNF- $\alpha$  on the tumor vasculature leading to rapid change in tumor morphology characterized by hemorrhagic necrosis.<sup>64</sup>

#### *TNF- $\alpha$ in combination with melphalan*

In our laboratory it was shown, that in rats with advanced large limb tumors, TNF- $\alpha$  achieved a factor 3 to 6 improvement of drugs uptake of the tumor in comparison to an ILP with melphalan or doxorubicine alone.<sup>65, 66</sup> We also showed that true hyperthermia (42-43 C) in combination with TNF- $\alpha$  and melphalan was very toxic to normal tissues.<sup>67</sup> An enhancing effect of hypoxia in M-ILP was not demonstrated in our findings of TM-ILP.<sup>67</sup>

#### *TNF- $\alpha$ based ILP*

The first study on the addition of TNF- $\alpha$  to M-ILP was by Lienard et al.<sup>43</sup> This study reported of an OR of 100% in 19 patients (table 1). Subsequently more studies reported very high response rates.<sup>7, 8, 40, 44-51, 68</sup> These results seems better compared to M-ILP. Whereas a large meta-analysis on M-ILP showed a CR-rate of 54%,<sup>69</sup> all listed studies in table 1 about TM-ILP show higher CR rates.

Certainly in bulky disease, TNF- $\alpha$  is of additional value. Melphalan uptake is very low in large tumors and TNF- $\alpha$  improves drug uptake significantly so they respond like large soft tissue sarcomas (STS). ILP was largely abandoned for STS till the introduction of TNF- $\alpha$ , now very large tumors reacted well to ILP,<sup>70</sup> which has led to a successful multi center trial and the approval of TNF- $\alpha$  for irresectable extremity STS in Europe.<sup>71</sup>

Yet, the results of a randomized control trial in 2006 published by Cornett et al.<sup>41</sup> were disappointing. They reported of increased local and systemic toxicity while no beneficial effect of TNF- $\alpha$  was observed. (CR rate 26% for TM-ILP vs. 25% for M-ILP). The methods and

conclusions of this publication were subject of several criticisms;<sup>72</sup> (I) Clinical response was assessed after 3 months, which is an uncommon endpoint since most CRs are reached between 3 to 6 months after ILP, and thus a low CR rate was reported. (II) The true indication, bulky disease was not analyzable. Therefore conclusions on this trial should be read with caution.

In a non-randomized mixed retrospective series of TM-ILP and M-ILP Rossi et al.<sup>42\*\*</sup> were able to demonstrate a significant difference between both perfusions types, a CR rate of 61% was achieved after TM-ILP while patients treated with M-ILP showed a CR-rate of 42% (p=0.05). Overall TM-ILP has been demonstrated to be a safe treatment modality with limited local and systemic toxicity, which can be repeated with retained activity.<sup>73-76</sup>

### *Prognosis after perfusion*

Unfortunately IT-mets of the melanoma are known to have a worse prognosis. Main prognostic factors for survival after a relapse of the first melanoma are: the usual characteristics of the primary (thickness, ulceration), stage of disease and type of recurrence (local, in transit, regional lymph node and distant).<sup>6</sup> It is interesting that several studies report of a very strong correlation between a CR after TM-ILP and prolonged survival.<sup>8, 42</sup> Apparently TM-ILP selects those patients with a CR that have a more favorable biology of the tumor. This is emphasized by the fact that: CR after ILP is correlated with time to local and systemic progression as well.<sup>8, 42</sup>

## **Conclusion**

Management of IT-mets in melanoma patients remains challenging as relapse and survival are mainly dictated by the biological behavior of the tumor. When simple surgical excision fails TM-ILP is an excellent treatment modality for IT-mets, as it achieves high complete response rates with limited local and systemic toxicity. We consider TM-ILP indicated for M-ILP resistant patients and in all patients with bulky tumors. When lesions are small and when disease is restricted to the distal parts of the limb, ILI is a cost effective good alternative.

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# PART III

Summary, conclusions and appendices



two decades of tumor  
necrosis factor- $\alpha$   
and melphalan based  
isolated limb perfusion

de meeste woorden  
begrijp ik niet...



Discussion and future perspectives

# Chapter 10

With the introduction of isolated limb perfusion (ILP) in 1957 an effective and safe treatment modality in order to obtain local control in melanoma patients with in-transit metastases (IT-mets) became available.<sup>1</sup> However, melphalan based ILP (M-ILP) failed to treat bulky IT-mets as uptake of melphalan was inhomogeneous in large lesions. Comprehensibly initial results of M-ILP in extremity soft tissue sarcoma (STS) patients were disappointing. The addition of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) to M-ILP changed this situation dramatically.<sup>2</sup> Now extensive bulky melanoma IT-mets reacted very well with overall response rates up to 100%,<sup>2, 3</sup> whereas STS showed overall response rates around 80%.<sup>4, 5</sup>

### **TNF- $\alpha$ based ILP in sarcoma patients**

#### *General discussion*

The most common indication in our center to perform a TNF- $\alpha$  and melphalan based ILP (TM-ILP) was locally advanced extremity STS, more than half (n=303, 57%) of all 535 performed TM-ILPs was in STS patients. With the emerging evidence that limb amputation does not prolong survival compared to radical resection with adjuvant radiotherapy (RTx)<sup>6, 7</sup> there has been growing interest in limb saving treatment options. In case of locally advanced extremity STS a radical resection might end up in anatomical or functional amputation. TM-ILP is an attractive neoadjuvant treatment option reducing tumor size in order to make a radical resection possible. Currently, TM-ILP followed by an oncological resection and adjuvant RTx is an established limb saving strategy in Europe.

However, the role of adjuvant RTx is still a matter of debate. The adjuvance of RTx after resection of an extremity STS is at cost of morbidity. Hoven-Gonderie et al.<sup>8</sup> reported that two-thirds of all patients experienced serious late toxic problems after combined treatment for STS. Major problems with wound healing en continuous wound infections (8%-14%) are described in literature.<sup>9, 10</sup> Vascular damage (4-14%) is a common long-term complication of radiotherapy.<sup>10-12</sup> The beneficial effect of irradiation after limb saving surgery for STS was first demonstrated by Rosenberg et al.<sup>6</sup> This favorable effect of adjuvant RTx on local control was re-established in series with patients treated with TM-ILP before oncological resection.<sup>13, 14</sup> Despite reporting a significant decrease in STS local recurrence after irradiation therapy, Yang et al. concluded that in selected patients with low risk for recurrence irradiation should not be considered because of important life time risk for complications.<sup>15</sup> **Chapter 3** described a selection of patients with no evidence of local recurrence regardless the adjuvance of RTx. This group consists of patients with primary unifocal disease whom underwent a R0 resection with a strong pathological response on TM-ILP (>50% necrosis).

The reduction of TNF- $\alpha$  dose, which was the major topic in **Chapter 5**, was not of influence on clinical outcome or local recurrence. However, pathological response was worse after low dose TM-ILP. The goal of ILP in unifocal disease is to enable a radical resection of a previously irresectable sarcoma. In our series, dose reduction of TNF- $\alpha$  did not influence the proportion of R0 resections. Consequently, reduced pathological response rate in the low-dose era, has no clinical impact. However, if adjuvant RTx after resection is undesirable (e.g. in peri-articular areas) maximum pathological response may be pursued and in these cases, high-dose ILP can be considered.

Our long-term results, as described in **Chapter 1**, confirms that TM-ILP is an efficient and safe treatment modality in order to render locally advanced irresectable extremity STS into resectable disease without the need for extensive mutilating surgery.

### *Future perspectives*

In last 2 decades, both retrospective and prospective studies have proven that TM-ILP is an important neoadjuvant therapy with pathological complete response rates up to 30%. These rates have not been reached by other conventional treatment modalities. However, a superior effect in terms of local control has never been demonstrated. As mentioned in an editorial by Bonvalot and Gronchi<sup>16</sup> future studies should not be expected for several reasons. First, because of the rarity of the disease, too few patients are available to show an expected improvement of 90% in the rate of local control. Second, the cost of this type of study for the community—and for most of the patients who would not experience recurrence anyway—with no expected effect on survival is prohibitive. Third, new tailored histotype drugs, such as trabectedine, commonly used in the neoadjuvant setting of myxoid liposarcoma, are already achieving a 30% complete pathological response.<sup>17</sup>

## **TNF- $\alpha$ based ILP in melanoma patients**

### *General discussion*

Treatment of melanoma IT-mets remains challenging. Survival of patients suffering of melanoma IT-mets is poor and mainly dictated by the aggressive biology of the primary tumor. Therefore minimal invasive treatment modalities as simple surgical resection or intralesional injections are preferable. In case of numerous or bulky lesions these options might fail and ILP can be of value.

The value of TNF- $\alpha$  in addition to M-ILP has been subject of several discussions. So far, only 1 trial by Cornett et al.<sup>18</sup> has been published. Results of this study were outright disappointing. No beneficial effect of TNF- $\alpha$  was observed. (CR rate 26% for TM-ILP vs. 25% for M-ILP) and

an increased local toxicity after the use of TNF- $\alpha$  was reported. However, the methods and conclusions of this publication were subject of several criticisms;<sup>19</sup> (I) Clinical response was assessed after 3 months, which is an uncommon endpoint since most CRs are reached between 3 to 6 months after ILP, and thus a low CR rate was reported. (II) The true indication, bulky disease was not analyzable. Therefore conclusions on this trial should be read with caution. In a non-randomized retrospective series Rossi et al<sup>20</sup> were able to demonstrate a significant benefit of the addition of TNF- $\alpha$  to M-ILP ( $p=0.05$ ).

The findings in this last study fit more in the observations made in this thesis. In **chapter 7** a significant worse response was observed after the lowering of dose of TNF- $\alpha$  suggesting that in our series the use of TNF- $\alpha$  is of benefit. The lowering of the dose of TNF- $\alpha$  not only led to inferior clinical response, but to an inferior local control as well. This was emphasized by the fact that there were no cases of maintained local control after three years in the low dose TNF- $\alpha$  group. No significant correlation between the dose of TNF- $\alpha$  and systemic progression or overall survival was observed. These findings fit in the concept of a locoregional treatment having locoregional benefit only. In our opinion CR after TM-ILP occurs in patients with the more favorable biology,<sup>21</sup> which allows a similar effect after low dose perfusions. Patients with more unfavorable biology might experience more often a CR and prolonged local control after high dose perfusion compared to low dose perfusion. However, systemic development and overall survival are dictated by the biology of the tumor.

The superior local control after higher dosing of TNF- $\alpha$  in TM-ILP is further emphasized in the long-term results described in this thesis. In the low dose group no cases of long lasting local control could be observed. **Chapter 6**, showed that long-term local control with a minimal follow up of 5 years was achieved in 14% of patients. Local control was defined as no evidence of recurrence in the field of perfusion or in case of recurrence one re-intervention (re-ILP or surgical excision)

Overall the addition of TNF- $\alpha$  increases the efficacy of ILP. High doses of TNF- $\alpha$  are correlated with higher CR rates and superior local control. If the biology of tumor permits long-term overall survival high dose TM-ILP might obtain long-term local control. Since the main objective of TM-ILP in melanoma patients is obtaining local control, rather than improving survival, high dose TNF- $\alpha$  perfusions seems preferable to low dose TNF- $\alpha$  perfusions.

### *Future perspectives*

The poor survival after perfusion give rise to the question whether a local therapy such as ILP should still be used on a stand-alone basis. A parallel between IT-mets and deep pelvic nodes can be seen. Both reflect extensive disease and it has been shown that the extent of surgery to the deep pelvic and obturator nodes does not improve the outcome of these

patients. In other words: the prognosis of these patients is dictated by the biology of the disease rather than by the extent of surgery.<sup>22</sup> The same mechanism is likely to be applicable to the IT-mets situation.

After decades of failing to identify effective systemic treatments in melanoma we have clearly entered a new era.<sup>23</sup> Both in terms of mutation-based targeted therapies, such as BRAF, MEK and C-kit mutations in melanoma, as well as in terms of novel ways of modulating the immune system new systemic therapies in melanoma are being developed with surprising results.<sup>24, 25</sup> Patients with stage IV melanoma experience response rates of around 50% and in a subset of patients long-term benefit can be obtained.<sup>25</sup> Although the majority of patients who entered the trials of these new drugs had indeed stage IV disease, most protocols allowed patients with irresectable stage III disease to enroll as well. The patient with IT-mets eligible for perfusion is by definition an irresectable stage III patient and therefore trial results can be extrapolated to this subset of patients, albeit with caution and in the awareness that response to ILP is dependent on stage of disease. It is however unlikely that systemic therapy will replace ILP in patients with extensive IT-mets. The response rates of the new agents are impressive compared to standard chemotherapy, but are far below those that can be achieved with an ILP. BRAF inhibitors have impressive and rapid response rates in BRAF mutant patients of about 60% (OR). Drawbacks are numerous however: the median duration of response is only around 7 months after which resistance seems almost inevitable, there is a wide interpatient variability in response to treatment and toxicity associated with BRAF inhibition cannot be neglected<sup>25</sup>. A more durable response can be obtained with anti-CTLA4 antibodies, but only a minority of patients will respond. Patients with multiple irresectable IT-mets will remain irresectable after a PR, so that the aim of therapy should ideally be CR. This thesis reported of a CR-rate of 70% after high dose TM-ILP. This order of CR –rates are still unmet by any systemic therapy. The superior local control rate of a perfusion combined with the mild toxicity compared to systemic treatment make that a perfusion remains the optimal treatment option in these patients. Table 1 presents an overview of overall and complete response rates of current treatment modalities for melanoma IT-mets.

The combination of both treatment modalities might satisfy two main objectives, local control and overall survival. Potentially combining systemic and local therapies might effectuate synergistic effects leading to improved outcome in terms of local control and survival. By example, chemoresistance can possibly be overcome by increasing the cytotoxic efficacy and thereby the responses.<sup>26</sup> In a multi-center phase II study combining melphalan isolated limb infusion (ILI) and systemic ADH-1, a cyclic pentapeptide that disrupts N-cadherin adhesion complexes, an overall response rate of 60% was achieved without increasing toxicity, compared with an overall response rate of 40% achieved previously with

melphalan alone at the same institutions.<sup>27</sup> Improved responses were also seen when melphalan ILI was performed after systemic bevacizumab, a monoclonal antibody against VEGF causing increased delivery of melphalan to the tumor cells, in a pre-clinical melanoma model.<sup>28</sup> A clinical trial administering bevacizumab in combination with melphalan ILI is eagerly awaited. Currently the use of systemic the anti-CTLA-4 antibody ipilimumab, before or after melphalan ILI is being investigated in phase I and II trials.

Potentially more attractive is the combination of TM-ILP for rapid response, followed by systemic therapy for survival benefit. A phase II trial protocol is presently open for recruitment combining ILP and systemic ipilimumab.

In conclusion TM-ILP is here to stay. The procedures provide rapid responses that are still superior to the responses achieved by systemic therapy, even in the latest trials. The responses are obtained at the cost of only very mild local, and virtually absent systemic toxicity. Patients with extensive IT-mets do need local treatment for disease control, but as irresectable stage III disease is a reflection of poor tumor biology, survival remains relatively poor. We firmly believe that this provides a massive opportunity for systemic agents. The results of the combination trials are awaited with great expectations.

**Table 1: Resonse rates of current treatment modalities**

	CR (%)	ORR (%)	MTTP
DTIC	1%	10-20%	1.5
High dose IL-2	1%	10-20%	2
BRAF-inh	6%	50-70%	7
MEK-inh	1%	20-30%	5
BRAF- & MAK-inh	10%	60-75%	11
Anti-CTLA-4	0%	10-20%	5
Anti-PD-1	5%	20-40%	5
Anti-CTLA-4 & Anti-PD-1	22%	50-60%	Unknown
TM-ILP	60-80%	85-95%	13

CR = complete response; ORR = overall response rate; MTTP = median time to progression

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Nederlandse samenvatting

# Chapter 11

Met de introductie van geïsoleerde ledemaat perfusie (GLP) in 1957 kwam er een effectieve en veilige behandeling om lokale controle te verkrijgen bij patiënten met in transit melanoom metastasen (IT-mets).<sup>1</sup> GLP met melphalan (M-GLP) faalde echter bij grote IT-mets vanwege een inhomogene uptake van melphalan in de laesie. Logischerwijs vielen de eerste resultaten van M-GLP voor patiënten met een extremiteten weke delen sarcoom (WDS) tegen. De toevoeging van tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was een enorme stap voorwaarts voor M-GLP.<sup>2</sup> Grote IT-mets reageerden nu wel erg goed met response rates tot 100%.<sup>2,3</sup> In patiënten met sarcomen werden response rates van 80% gerapporteerd.<sup>4,5</sup>

### Geïsoleerde ledemaat perfusie met TNF- $\alpha$ voor sarcoom patiënten

Meer dan de helft van alle uitgevoerde GLP met TNF- $\alpha$  en melphalan (TM-GLP) was vanwege een WDS. Met het overtuigende bewijs dat een amputatie van het aangedane ledemaat niet is geassocieerd met een betere overleving, vergeleken met een radicale resectie gevolgd door adjuvante radiotherapie (RTx)<sup>6, 7</sup>, nam de interesse in ledemaat sparende behandelingen toe. Patiënten met een locally advanced WDS van een extremitet eindigden vaak met een amputatie of ondergingen mutilerende chirurgie. TM-GLP is een aantrekkelijke behandeling om reductie van het tumorvolume te bewerkstelligen om vervolgens een oncologische resectie te kunnen verrichten zonder de noodzaak tot mutilerende chirurgie. Tegenwoordig is TM-GLP gevolgd door een oncologische resectie en adjuvante RTx een gebruikelijke behandeling in Europa voor locally advanced WDS.

De rol van adjuvante RTx blijft echter onderwerp van discussie. RTx na TM-GLP en resectie gaat gepaard met aanzienlijke morbiditeit. Hoven-Gonderie et al.<sup>8</sup> rapporteerden ernstige bijwerkingen bij twee-derde van alle patiënten die een gecombineerde behandeling vanwege WDS ondergingen. Vooral wondgenezingsstoornissen en vasculaire schade worden beschreven in de literatuur. De meerwaarde van adjuvante RTx na resectie van een WDS in termen van lokale controle werd voor het eerst beschreven door Rosenberg et al.<sup>6</sup> Vele studies bevestigden dit resultaat. Ondanks de verbeterde lokale controle concludeerde Yang et al.<sup>9</sup> dat patiënten met een laag risico op een recidief geen RTx moeten krijgen vanwege de mogelijke ernstige bijwerkingen. **Hoofdstuk 3** beschrijft een groep WDS patiënten die geen lokaal recidief hebben gekregen na TM-GLP gevolgd door resectie. Dit zijn patiënten met primaire ziekte die een R0 resectie ondergingen en een goede pathologische respons (>50% necrose) in het resectie preparaat hadden. Deze groep bevat zowel patiënten die zijn behandeld met adjuvante RTx als patiënten die geen RTx kregen.

De reductie van de TNF- $\alpha$  dosis, het belangrijkste onderwerp in **Hoofdstuk 5**, had geen invloed op de klinische respons of lokale controle. De pathologische respons was echter wel slechter na TM-GLP met een lage dosis TNF- $\alpha$ . Het doel van GLP in unifocale WDS van de

extremiteten is om irresectabele tumoren resectabel te maken. De reductie van TNF- $\alpha$  had geen invloed op het percentage R0 resecties. De slechtere pathologische response had dus geen klinische consequenties. Als adjuvante RTx ongewenst is (bv: gewrichten in het bestralingsveld) wordt maximale pathologische respons echter wel nagestreefd en zou een hogere dosering TNF- $\alpha$  overwogen kunnen worden.

De lange termijn resultaten zijn beschreven in **Hoofdstuk 1** en bevestigen dat TM-GLP een efficiënte en veilige behandeling is om locally advanced irresectabele WDS neo-adjuvant te behandelen en zodoende te verkleinen om een oncologische resectie mogelijk te maken zonder mutilerende chirurgie.

### **Geïsoleerde ledemaat perfusie met TNF- $\alpha$ voor melanoom patiënten**

Behandeling van IT-mets van het melanoom is uitdagend. De overleving is slecht en wordt vooral bepaald door de vaak agressieve biologie van de primaire tumor. Daarom verdienen minimaal invasieve behandelingen zoals lokale resectie of lokale injecties de voorkeur. Als er teveel of te grote metastasen zijn en deze minimaal invasieve opties falen, kan TM-GLP van grote waarde zijn.

De toevoeging van TNF- $\alpha$  bij M-GLP is al geruime tijd onderwerp van discussie. Tot nu toe is er slechts één trial, van Cornett et al,<sup>10</sup> gepubliceerd. De resultaten van deze studie zijn ronduit teleurstellend. Er werd geen voordelig effect waargenomen van TNF- $\alpha$ . (Complete respons rate 26% voor TM-GLP vs 25% voor M-GLP). Wel werd er een toename van toxiciteit waargenomen in de TNF- $\alpha$  groep. Er is echter veel kritiek op de methodologie van deze studie.<sup>11</sup> (I) De klinische respons werd gemeten na 3 maanden. Dit is een ongebruikelijk eindpunt, een complete respons wordt vaak pas waargenomen na 3 tot 6 maanden na TM-GLP. (II) De belangrijkste indicatie, grote metastasen, werd niet beschreven in de studie. Derhalve moeten de resultaten met enige voorzichtigheid en reserve worden geïnterpreteerd. In een niet gerandomiseerde studie van Rossi et al.<sup>12</sup> werd wel een voordelig effect van de toevoeging van TNF- $\alpha$  aan M-GLP waargenomen ( $p=0.05$ ).

De bevindingen van deze laatste studie passen beter bij de uitkomsten van dit proefschrift. **In hoofdstuk 7** werd er een significant slechtere respons beschreven na reductie van de dosis van TNF- $\alpha$  in TM-GLP. Deze bevindingen suggereren wel degelijk een voordelig effect van de toevoeging van TNF- $\alpha$  aan M-GLP. Niet alleen de klinische respons was slechter, ook werd er een slechtere lokale controle waargenomen. Het feit dat geen langdurige lokale controle werd waargenomen in de lage doseringsgroep na 3 jaar onderstreept dit. De dosis van TNF- $\alpha$  was niet gecorreleerd met systemische progressie of overleving. Dit past in het concept dat locoregionale behandeling enkel een locoregionaal effect heeft en geen invloed heeft op overleving. Een complete respons na TM-GLP komt voor in patiënten met een

gunstige biologie van de tumor.<sup>13</sup> Dit effect wordt ook bereikt na een laag gedoseerde TM-GLP. Patiënten met een slechtere biologie hebben mogelijk vaker een complete respons en verlengde lokale controle na een hoog gedoseerde TM-GLP. Echter systemische progressie en overleving worden bepaald door de biologie van de primaire tumor.

De superieure lokale controle wordt onderstreept in de lange termijn resultaten beschreven in dit proefschrift. In de laag gedoseerde TNF- $\alpha$  groep werden geen patiënten met langdurige lokale controle waargenomen. **Hoofdstuk 6**, beschreef dat er op de lange termijn met een minimale follow up van 5 jaar een lokale controle van 14% werd bereikt. Lokale controle werd gedefinieerd als geen tekenen van recidief in het veld van perfusie of een recidief dat met een enkele interventie behandeld kon worden.

Alles bij elkaar leidt de toevoeging van TNF- $\alpha$  tot meer effectiviteit van GLP. Hoge dosis TNF- $\alpha$  leidt tot hogere complete respons rates en betere lokale controle. Omdat het doel van GLP locoregionale controle is lijkt hoog gedoseerde TM-GLP de voorkeur te genieten boven laag gedoseerde TM-GLP.

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# Chapter 12

## Appendices

- Curriculum •
- List of publications •
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## Curriculum

Jan Piet Deroose werd geboren op 24 juli 1979 als oudste van twee broers. Hij groeide op in Ekeren (België). Op dertienjarige leeftijd vertrok het gezin naar Nederland. In 1997 rondde hij zijn eindexamen af aan het Rijnlands Lyceum in Oegstgeest. Zijn ouders en broer gingen terug naar België, maar Jan besloot om in Nederland te blijven om economie te gaan studeren aan de Erasmus Universiteit Rotterdam. Na 4 jaar studeren en een jaar in het bestuur van het Rotterdamse Studenten Corps, wilde hij toch niets liever dan arts worden. In 2003 is hij begonnen aan de studie geneeskunde aan de Erasmus Universiteit. In dit eerste studiejaar wist hij zijn studie economie ook nog met goed gevolg af te ronden.

Tijdens zijn studie werkte hij in het medisch studententeam 'Les Forgerons' in het Ikazia ziekenhuis. Hier werd zijn interesse voor de heelkunde gewekt. Na zijn afstuderen in 2009 heeft hij een half jaar in het Ikazia ziekenhuis gewerkt als ANIOS heelkunde. In december 2009 begon hij in de Daniel den Hoed Kliniek aan het promotieonderzoek dat heeft geresulteerd in dit proefschrift onder supervisie van prof dr. C. Verhoef en prof dr. A.M.M. Eggermont.

Op 1 juli 2011 is hij gestart met de opleiding tot chirurg in het Amphia ziekenhuis en het Erasmus Medisch Centrum (opleiders dr. L. van der Laan en dr. B.P.L. Wijnhoven). Jan woont in Rotterdam samen met Irene Goverse en zijn twee dochters Julie en Floor.



## List of publications

*Radiotherapy for soft tissue sarcomas after isolated limb perfusion and surgical resection: essential for local control in all patients?*

**Deroose JP**, Burger JWA, van Geel AN, den Bakker MA, de Jong JS, Eggermont AM, Verhoef C  
*Ann Surg Oncol.* 2011 Feb;18(2):321-7

*Isolated limb perfusion for melanoma in-transit metastases: developments in recent years and the role of tumor necrosis factor alpha*

**Deroose JP**, Eggermont AMM, van Geel AN, Verhoef C  
*Curr Opin Oncol.* 2011 Mar;23(2):183-8

*Long term follow-up of TNF $\alpha$  based isolated limb perfusion in melanoma patients*

**Deroose JP**, Grünhagen DJ, van Geel AN, de Wilt JHW, Eggermont AMM, Verhoef C  
*Br J Surg.* 2011 Nov;98(11):1573-80

*Long-term results of TNF $\alpha$  and melphalan based isolated limb perfusion in locally advanced extremity sarcomas*

**Deroose JP**, van Geel AN, Burger JWA, den Bakker MA, Eggermont AMM, Verhoef C  
*J Clin Oncol.* 2011 Oct 20;29(30):4036-44

*Local and intralesional therapy of in-transit melanoma metastases*

Testori A, Faries MB, Thompson JF, Pennacchioli E, **Deroose JP**, van Geel AN, Verhoef C, Verrecchia F, Soteldo J.  
*J Surg Oncol.* 2011 Sep;104(4):391-6

*20 Years experience of TNF based isolated limb perfusion for in-transit melanoma patients: TNF dose matters*

**Deroose JP**, Eggermont AMM, van Geel AN, de Wilt JHW, Burger JWA, Verhoef C  
*Ann Surg Oncol.* 2012 Feb;19(2):627-35

*Isolated limb perfusion with TNF $\alpha$  and melphalan for the distal parts of the limb in soft tissue sarcoma patients*

**Deroose JP**, van Geel AN, Burger JWA, Eggermont AMM, Verhoef C  
*J Surg Oncol.* 2012 May;105(6):563-9

*Isolated limb perfusion by tumor necrosis factor alpha and melphalan in patients with advanced aggressive fibromatosis*

van Broekhoven DLM, **Deroose JP**, Bonvalot S, Gronchi A, Grünhagen DJ, Eggermont AMM, Verhoef C

*Br J Surg.* 2014 Dec; 101(13):1674-80

*Treatment modifications in TNF-based isolated limb perfusion in patients with advanced extremity soft tissue sarcomas*

**Deroose JP**, Grünhagen DJ, de Wilt JHW, Eggermont AMM, Verhoef C

*Eur J Cancer.* 2015 Feb; 51(3):367-73

*Repeated isolated limb perfusion in melanoma patients with recurrent in-transit metastases*

**Deroose JP**, Grünhagen DJ, Eggermont AMM, Verhoef C

*Melanoma Res.* 2015 Oct; 25(5):427-31

## Dankwoord

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