Isolated Limb Perfusion with Tumor Necrosis Factor-Alpha and Melphalan
An Alternative to Amputation for Locally Advanced Soft Tissue Sarcomas

Peter M. Schlag, Per-Ulf Tunn

SUMMARY
Introduction: Treatment for soft tissue sarcoma of the extremities has shifted in recent years from amputation to wide local excision combined with postoperative irradiation. In locally advanced soft tissue sarcoma of the limb, this limb-sparing approach is often not feasible. In these circumstances, isolated limb perfusion (ILP) with tumor necrosis factor-alpha (TNF-α) and melphalan, delivered under mild hyperthermic conditions, can achieve limb salvage in a significant percentage.

Methods: Selective literature review and presentation of the authors’ own results.

Results: The 5-year overall survival rate of patients with high malignant soft tissue sarcoma of the limb is about 50% to 60%. As it has become apparent that amputation for soft tissue sarcoma does not improve survival rates, interest in limb salvage approaches has increased. TNF-α and melphalan based ILP can provide long term limb salvage in about 80% of the patients with locally advanced and recurrent soft tissue sarcoma of the extremities. The overall response rate is higher than 70%. ILP represents the most effective neoadjuvant treatment approach of primary locally advanced and recurrent soft tissue sarcoma at present. In cases with widespread metastases, it can be used palliatively without subsequent tumor resection to avoid ablative surgery.

Discussion: ILP should strongly be considered indicated in locally advanced and recurrent soft tissue sarcoma of the extremities to avoid amputations and improve R0-resectability.

Key words: soft tissue sarcoma, isolated limb perfusion, surgery, limb salvage, prognosis, interdisciplinary therapy

The treatment of resectable soft tissue sarcomas of the limbs is primarily surgical and limb-sparing. In the non-metastasizing stage in adults, it is supplemented when appropriate by postoperative radiotherapy depending on the grading, tumor localization and the results of the histopathological examination. In locally advanced tumors of the limbs, ablative procedures with curative intent are still widely practiced. Since these mutilating approaches generally fail to influence the prognosis, multimodal solutions are called for before resorting to ablative procedures. Isolated limb perfusion is an innovative neoadjuvant therapy proposed in this context as a means of increasing the proportion of limb-sparing interventions without accepting a worsening of the prognosis and with the goal of improving the patient’s quality of life.

Soft tissue sarcomas are rare, with an incidence of 1 to 2 new cases per 100,000 population and year, and account for 1 to 2% of all malignant neoplasms of adulthood (1, 2). The very heterogeneous group of soft tissue sarcomas includes all malignant tumors of non-epithelial tissue (muscle, fatty tissue, connective tissue, vessels etc.). At about 60%, the limbs are the commonest sites affected, with the lower limb predominating at around 40%.

Because of the relative rareness of soft tissue sarcoma, specialized knowledge and experience of the various therapeutic options are limited. Local tumor therapy employing the usually available surgical resources can present problems despite a high level of surgical skill. Especially for patients with locally advanced soft tissue sarcomas of the limbs, mutilating resections or even amputations should be consigned to a second-line...
category due to the availability of neoadjuvant strategies. Besides local radiotherapy and systemic chemotherapy, with or without local hyperthermia, these neoadjuvant therapeutic options include isolated hyperthermic limb perfusion. Before proceeding to amputation, it is therefore recommended to seek a second opinion from an interdisciplinary sarcoma center.

After diagnosis and staging, local tumor therapy is the central element in the management of non-metastasizing soft tissue sarcoma in adults. The goal is long-term local tumor control and cure combined with limb salvage. The use of multimodal therapeutic concepts can provide a high level of local tumor control associated with preservation of function and limb. Depending on the site of involvement, the majority of soft tissue sarcomas can be treated by limb-sparing compartment oriented or wide local resection, when appropriate combined with radio / chemotherapy. About 10% of the tumors, however, are locally so advanced as to rule out primary curative, limb-sparing resection (R0 resection). Most of them are soft tissue sarcomas arising in extracompartmental locations immediately adjacent to or infiltrating vascular and neural structures or joints. In these cases, isolated limb perfusion (ILP) with tumor necrosis factor-alpha (TNF-α) (1 to 4 mg) and melphalan (10 to 13 mg/l limb volume) with mild hyperthermia (38 to 40 °C) has proved successful as neoadjuvant therapy to devitalize and reduce the size of the tumor. This treatment strategy allows a function and limb-sparing resection in about 80% of the patients directly threatened by amputation because of the localization and infiltration depth of the tumor. Both a primary tumor and a local tumor recurrence are indications for ILP. Even in the metastasizing stage of the disease with locally advanced soft tissue sarcoma of the limb, ILP is also indicated as a palliative procedure with the intention of sparing the limb. Curative amputation is only justified if an R0 resection of the tumor, i.e., removal of diseased tissue in histologically healthy tissue, is not possible with neoadjuvant therapy.

At present, ILP is used mainly for soft tissue sarcomas of adulthood. For childhood sarcomas, the indication is much rarer and is reserved particularly for cases of relapse (3). The principles of the method and the results achieved with this therapeutic concept are now presented on the basis of a selective literature review and our own therapeutic results.

**Principle of action of TNF-α and melphalan**

Tumor necrosis factor-alpha (TNF-α) is a potent cytotoxin with effective antitumoral activity (4). Systemic administration in therapeutically effective doses raises problems because of the

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**Figure 1:** Angiographic findings of a soft tissue sarcoma of the distal thigh / popliteal fossa before and after ILP with TNF-α and melphalan. The images show complete disappearance / obliteration of the tumor vessels 10 days after ILP.
associated high toxicity: the dose of 1 to 4 mg TNF-α used in ILP is 10 times higher than the systemic maximum tolerated dose (MTD) of 0.350 mg/m² body surface area (5).

TNF-α exerts its effect via direct and indirect cytotoxic/apoptotic mechanisms and ultimately leads to devitalization of the tumor. Besides the direct antiproliferative and cytotoxic effects on the tumor cells, TNF-α activates adhesion molecules on the surface of endothelial cells inside the tumor. The tumor specific action is presumably based on inhibition of integrin av beta 3, an adhesion molecule expressed by endothelial cells especially during angiogenesis and encountered to only a very limited extent in the dormant endothelial cells of healthy tissue. Inhibition of the av beta integrin function finally leads to apoptosis of vascular endothelial cells in the tumor. In a complex interaction with macrophages and leukocytes, vascular endothelial structures of the tumor are then destroyed, leading to tumor necrosis (5, 6).

The selective action of TNF-α on the vascular system of the tumor can be impressively demonstrated by, for example, comparing pre- and post-ILP angiographic findings (diagram 1). The tumor vascularization can be obliterated completely within 7 days after ILP with TNF-α.

In addition to the effects on the vascular system of a soft tissue sarcoma observed after administration of TNF-α, animal studies have also shown a 4 to 6 fold higher concentration of melphalan in the tumor tissue compared to administration of melphalan alone (7). Via this mechanism, TNF-α potentiates the cytotoxic effect of melphalan. The more pronounced the tumor vascularization, the stronger may be expected to be the synergistic effects of TNF-α and melphalan.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Extent of reaction</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>I</td>
<td>No subjective or objective reaction</td>
<td>26.4%</td>
</tr>
<tr>
<td>II</td>
<td>Slight reddening and/or edema</td>
<td>45.9%</td>
</tr>
<tr>
<td>III</td>
<td>Marked erythema and/or edema with vesiculation; slight impairment of motility</td>
<td>22.3%</td>
</tr>
<tr>
<td>IV</td>
<td>Extensive epidermolyses; damage to deep tissue with permanent loss of function: impending or clinically apparent compartment syndrome</td>
<td>5.4%</td>
</tr>
<tr>
<td>V</td>
<td>Damage necessitating amputation</td>
<td>1.6%</td>
</tr>
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</table>
Principles of isolated hyperthermic limb perfusion

Isolated hyperthermic limb perfusion (ILP) is a regional cytotoxic therapeutic procedure used in clinical medicine mainly for soft tissue sarcomas and for recurrent sarcomas of the limbs that are not amenable to primary curative resection. The aim of this procedure is to optimize or first make possible the resectability of the tumor.

ILP was first introduced into clinical practice in 1957 by Creech and Krementz for the management of locally advanced tumors in the limbs (8). Isolation of the limb circulation with an extracorporeal circulation unit (heart-lung machine) allows the administration of high dose, regionally effective cytotoxic agents and prevents serious systemic side effects. The additional warming of the tissue enhances the pharmacological action (9). In ILP, the affected limb is isolated from the patient’s systemic circulation by a surgical intervention. Depending on the localization of the tumor, an iliac, high femoral or adductor access, and in the upper limb a transpectoral, axillary or cubital access is distinguished. This requires exposure of the corresponding arteries and veins. After introducing the intravascular catheter the extracorporeal circulation is completed with a heart-lung machine. Smaller collateral vessels are temporarily closed by applying a tourniquet, thereby preventing transfer of the administered agents to the systemic circulation. To monitor for leakages, i.e. possibly insufficient separation of the limb and systemic circulation, continuous measurement of the leakage rate with intravascularly administered radioactive tracers (labeled autologous erythrocytes or human albumin) both in the systemic and limb circulation is particularly important. Administration of the antitumor medication into the limb circulation can only be commenced when the system is judged to be stable. The medications are administered intra-arterially, flow through the tumor-bearing limb and travel via the venous return flow back to the extracorporeal circuit. By means of an oxygenator and heat exchanger incorporated in the venous system, the pO₂ and the temperature of the limb circulation are maintained constant within a temperature range of 38 to a maximum 40°C (diagram). The total perfusion time is usually 90 minutes. The perfusate is then “washed out” of the treated limb, the intravascular catheters removed and, after venous and arterial suture, the intervention is concluded with wound closure. The median duration of the total procedure is 330 minutes.

ILP was first performed with melphalan as monotherapy. Melphalan has been used in a 20 fold higher dosage compared to the usual systemic administration while being appropriately tolerated. The response rates for malignant melanoma were 75 to 80% (complete remission in more than 50% of patients) (10, 11, 12). In the treatment of locally advanced soft tissue sarcoma of the limbs, however, melphalan alone has shown only very limited efficacy.

Figure 2:

a) Ulcerating soft tissue sarcoma of the right popliteal fossa before therapy.
b) Clinical result 6 weeks after ILP with TNF-α and melphalan with demonstration of partial remission.
c) Result after further tumor resection and plastic reconstruction of the soft tissue defect, 12 weeks after ILP.
Similar results have also been obtained with other cytostatics (such as adriamycin, cisplatin, actinomycin) as monotherapy, but were associated with more severe undesirable effects. Only when melphalan was combined with TNF-α were high response rates reported for the first time by Lejeune et al., resulting in 1992 in the breakthrough for ILP in the management of locally advanced soft tissue sarcomas of the limbs (13).

The convincing results of a multicenter study, in which our own clinic also participated (14) and in which 186 patients were enrolled, finally led in 1999 to the European approval of TNF-α for use in combination with melphalan in the management of locally advanced soft tissue sarcoma of the limbs. This was a prospective surveillance study. A randomized study was not ethically acceptable in this context, since only patients were enrolled for whom amputation of the affected limb had been recommended on the basis of conventional criteria. During the further course, with increasing clinical experience and complementary comparative surveillance studies, it was also demonstrated that a dose reduction of TNF-α from 4 to 1 mg produced the same results (15).

Because of the complexity of ILP with TNF-α and melphalan, this technique is reserved for a small number of clinics with sufficient experience and expertise. Within Europe, 35 centers have been trained to perform this very demanding and interdisciplinary therapy in cooperation with the Daniel den Hoed Cancer Center in Rotterdam, Netherlands. In Germany, ILP with TNF-α and melphalan is performed at appropriately trained centers in locations including Berlin, Bochum, Erlangen, Essen, Homburg/Saar and Mannheim.

**Postoperative management**

Because of the possibility of systemic intolerance reactions, postoperative intensive medical supervision of the patient is obligatory. The rate of serious adverse events, such as sepsis-like reactions, renal failure, liver failure, paralytic ileus or extremely severe toxic damage to the affected limb, which can lead to amputation, is below 3%. The general surgical complications potentially associated with ILP include infections, disorders of wound healing, thrombosis, embolism, injury to vessels or nerves, and the formation of lymphatic fistulas, usually associated with lymph dissection. Treatment related mortality is below 0.5% (16). The local and systemic side effects are therefore justifiable considering all the problems associated with the disease itself. Moderate systemic side effects of TNF-α such as fever and chills are relatively easy to manage. Forced diuresis is performed for shock prophylaxis or prevention of renal disease before, during and after ILP.

Most patients develop local cutaneous reactions following ILP, but in the majority of cases require no special therapy. The Wieberdink system (17) has become established as a means of classifying the toxic reactions. About half the patients exhibit local reactions and grade II edemas. Table 1 gives an overview of the incidence of local side effects in our own patients. The cutaneous reactions that have occurred were generally in remission within 14 days.

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**Table 2**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Patients (n)</th>
<th>Response rate (PR + CR) (%)</th>
<th>R0 resection (%)</th>
<th>Local recurrence rate (%)</th>
<th>Limb salvage (%)</th>
<th>Total survival (median) (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lev-Chelouche et al. (1999)</td>
<td>13</td>
<td>54 + 38</td>
<td>74</td>
<td>38</td>
<td>85</td>
<td>29</td>
</tr>
<tr>
<td>Lejeune et al. (2000)</td>
<td>22</td>
<td>64 + 18</td>
<td>n.d.</td>
<td>45</td>
<td>86</td>
<td>19</td>
</tr>
<tr>
<td>Olieman et al. (1998)</td>
<td>34</td>
<td>59 + 35</td>
<td>45</td>
<td>26</td>
<td>85</td>
<td>28</td>
</tr>
<tr>
<td>Grünhagen et al. (2006)</td>
<td>197</td>
<td>51 + 18</td>
<td>n.d.</td>
<td>n.d.</td>
<td>87</td>
<td>57</td>
</tr>
<tr>
<td>Bonvalot et al. (2005)</td>
<td>100</td>
<td>17 + 49</td>
<td>n.d.</td>
<td>n.d.</td>
<td>87</td>
<td>n.d.</td>
</tr>
<tr>
<td>Own results (2005)</td>
<td>125</td>
<td>53 + 19</td>
<td>91</td>
<td>18</td>
<td>81</td>
<td>63</td>
</tr>
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PR, partial remission; CR, complete remission; n.d., no data
days. The rate of serious local damage (Wieberdink grade IV: for example impending or clinically apparent compartment syndrome) was 5% (table 1).

After 4 to 8 weeks, restaging for evaluation of response to therapy is followed by resection of the tumor. The resection is planned on the basis of the post-ILP imaging results (MRI) and taking into account the pre-therapy tumor extent. Since these are predominantly extracompartmental tumor localizations, wide excisions are most commonly performed. Plastic reconstruction of the soft tissue defect, in some cases also under functional aspects, is required in more than 30% of the patients (diagram 2a-c). The resected material is examined histologically to determine the extent of tumor necrosis and hence the response rate, and to allow recommendations to be made regarding possible adjuvant therapy requirements.

Clinical results
ILP with TNF-α and melphalan has been shown in several clinical studies to be an effective induction therapy for locally advanced soft tissue sarcoma for which primary curative, limb-sparing R0 resection is not feasible (3, 9, 13, 14, 16, 22, 23). The overall response rate is 70 to 80% (complete remission: 20 to 30%, partial remission approximately 50%). In about 80% of patients the limb threatened by amputation could be salvaged (table 2). The indications for ILP are shown in table 3.

Between 1993 and 2005, 917 patients with soft tissue sarcomas underwent interdisciplinary treatment in our clinic. In 125 patients we performed ILP for locally advanced soft tissue sarcoma of the limbs. We treated 54% men and 46% women with a mean age of 51 years (11 to 76 years). The tumors of various histological types (synovial sarcoma 23%, malignant fibrotic histiocytoma 19%, liposarcoma 17%, malignant peripheral neural sheath tumor 7%, pleomorphic sarcoma 7%, leiomyosarcoma 5%, and other sarcoma types 23%) were predominantly localized on the lower limb (87%). The limb could be spared in 80% of the patients after ILP. The 5-year survival rate of these patients is 63%, the disease-free 5-year survival rate 46% and the local relapse-free 5-year survival rate 76%.

Conclusion
Mutilating resections and ablative procedures for the management of locally advanced soft tissue sarcoma of the limbs are avoidable in most cases. Amputations usually do not improve the prognosis and are generally associated with poor quality of life. ILP with TNF-α and melphalan is a very effective additive therapy for increasing the rate of limb-sparing R0 resections. Both our and international experience shows that limb amputations can be avoided in about 80% of cases.

Conflict of Interest Statement
Prof. Schlag received fees from Boehringer Ingelheim Research Funding in 2004-2006. Dr Tunn declares that he has no conflict of interest as defined by the guidelines of the International Committee of Medical Journal Editors.

Table 3
Indications for ILP in locally advanced soft tissue sarcoma of the limbs

<table>
<thead>
<tr>
<th>Adulthood</th>
<th>Childhood</th>
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<tr>
<td>Primary tumor: Extracompartmental or inter-compartmental malignant soft tissue tumor of the upper and lower limb (incl. joint, vessel and nerve contact) in which primary R0 resection is doubtful and possible only with a highly mutilating intervention (e.g. amputation).</td>
<td>Primary tumor: Progression during systemic induction chemotherapy (e.g. CWS protocol), alternative to amputation.</td>
</tr>
<tr>
<td>Local recurrence: Generally to be preferred, especially for locally advanced or multilocularly growing recurrent soft tissue sarcoma of the limbs with previous primary multimodal treatment (radiotherapy, chemotherapy).</td>
<td>Local recurrence: After prior multimodal therapy and impending amputation with curative or, if appropriate, palliative intent.</td>
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REFERENCES