Abstract. Background: Tumor necrosis factor-alpha (TNFα)-based hyperthermic isolated limb perfusion (HILP) is routinely carried out at most oncological institutions in the treatment of locally advanced soft tissue limb sarcoma (STS), employing high TNFα dosages. After a phase I-II study, the SITILO (Italian Society of Integrated Locoregional Therapies in Oncology) centers began to employ the lower dose of 1 mg of TNFα. The aim of this paper is to report on the results obtained in 75 patients with limb-threatening STS treated with a low TNFα dose and doxorubicin (Dx). Patients and Methods: HILP with TNFα (at a dosage of either ≤1 mg or >1 mg) and Dx was administered to 75 patients with limb-threatening STS: 37 males and 38 females; median age 50 years; tumor in the lower and upper limbs in 58 and 17 patients, respectively; primary and recurrent tumors in 45 and 30 patients, respectively. Most tumors (77%) were high grade. Tumor resection was carried out 6 to 8 weeks after HILP. Results: The grade of limb toxicity was mild to moderate in the vast majority of patients (76%). Grades IV and V were observed, but only when high muscle temperatures were recorded and high TNFα dosages were employed. Systemic toxicity was also mild to moderate and there were no postoperative deaths. Complete and partial tumor responses were 34% and 48%, respectively, with an overall response of 82%. Limb sparing surgery was carried out in 85.3% of patients. At a median follow-up of 28 months, 16 recurrences (21.3%) were recorded, with a 5-year locoregional disease-free survival of 63%. The 5-year disease-free survival and overall survival were 36.7% and 61.6%, respectively. Conclusion: HILP with 1 mg of TNFα is an effective neoadjuvant therapy resulting in a high rate of limb sparing in limb-threatening STS, with acceptable local reactions and negligible systemic toxicity.

Soft tissue sarcomas (STS) are a heterogeneous group of malignant tumors that originate in mesenchymal tissue. They account for approximately 1% of all malignancies. Approximately 60% of these tumors occur in the extremities, in which case they are often large at presentation and represent a local management problem (1). Surgical resection combined with high-dose adjuvant external radiotherapy is the treatment of choice (2-3). The local situation in the limb often requires extensive and mutilating surgery causing severe impairment in limb functionality. Unfortunately, in 10% of cases, amputation is inevitable, but demolitive surgery does not improve life expectancy as prognosis is mainly related to the occurrence of distant metastases (4).

Hyperthermic isolated limb perfusion (HILP) has been employed as a neoadjuvant treatment in order to obtain limb salvage in primary unresectable STS. HILP effectiveness is based on: (a) temporary limb isolation from the systemic circulation, as a means of achieving drug concentrations 15- to 20-fold greater than those achieved with systemic administration (2, 5, 6); and (b) hyperthermia, as a means of increasing the tumoricidal effect of antiblastic agents such as melphalan and doxorubicin (7-8). Moreover, the introduction of tumor necrosis factor-alpha (TNFα) in the HILP regimen by Lienard et al. further improved the therapeutic effectiveness of this neoadjuvant procedure, especially in advanced STS of extremities (9). The overall response rates ranged between 75% and 83%, and limb salvage was achieved in a similar percentage of patients (10-11).
In previous phase I-II studies, we identified the maximum tolerable dose (MTD) of TNFα in association with doxorubicin. Interestingly, the administration of 1 mg of TNFα resulted in tumor responses comparable to those obtained with higher dosages (3-4 mg). The aim of this paper is to report on the tumor response, locoregional control and survival observed in a large phase II HILP study enrolling 75 patients with limb-threatening STS, conducted by the SITILO (Italian Society of Integrated Locoregional Therapies in Oncology) centers employing a pre-selected range of TNFα dosages.

Patients and Methods

Patients. The characteristics of the 75 patients with advanced STS enrolled in this study are reported in Table 1. All the STS patients enrolled in this study were candidates for amputation due to extra-compartmental or multi-compartmental lesions, and/or tumors with gross bone or sciatic nerve infiltration, and/or multiple lesions in the same limb. Other eligibility criteria were the following: (a) age (18-75 years); (b) histological confirmation of diagnosis; (c) Eastern Cooperative Oncology Group Performance status up to 2; (d) fully informed consent. Patients were excluded if any of the following criteria were met: (a) severe cardiovascular, hepatic, or renal disease; (b) severe vascular disease of the involved limb; (c) tumor lesion(s) located in the proximal third of the thigh, too close to the inguinal ligament or gluteus. Accurate staging before HILP included physical examination, complete chemical profile, chest and abdomen computed tomography (CT), as well as CT or magnetic resonance imaging (MRI) of the involved limb.

HILP technique. The technique of isolation perfusion has been extensively described in previous papers (12-13). Briefly, the axillary or iliac vessels were cannulated and temperature was monitored by multiple muscle thermocouples inserted into the skin, thigh, leg muscles and the tumor. As soon as the tumor temperature reached 41°C, TNFα was injected into the extracorporeal circuit at the pre-established dose. After 30 minutes, doxorubicin was administered at a dose of 8.5 mg/l of limb volume, and the extracorporeal circulation continued for other 60 minutes. During regional perfusion, technetium 99 labelled-albumin was injected into the circuit and a scintillation probe was placed on the cardiac area in order to continuously monitor the systemic leakage. All the patients were admitted to the intensive care unit for at least 24 hours in order to carefully monitor acute systemic and local side-effects. Systemic toxicity was evaluated according to World Health Organization (WHO) criteria, whereas the Wieberdink classification was adopted to evaluate locoregional toxicity (14).

The tumor response to treatment was evaluated radiologically and pathologically. A CT scan or MRI was carried out before the perfusion and again after 20-30 days. Tumor response was defined as the percentage increase of the tumor necrosis rate before the perfusion (liquid component) applying the ellipsoid formula (diameter x diameter x diameter x 0.523). The pathological evaluation was both macroscopic (longitudinal and transversal) and microscopic (the decrease in percentage of viable nuclei in the tumor surrounding the necrotic area after HILP compared with the pre-treatment nuclear density). Depending on their response to treatment, patients were categorized as complete responders (CR: 100% of histological necrosis) partial responders (PR: necrosis between 50-99%), or no-change (NC; necrosis <50%).

The post-perfusional treatment, carried out 4-6 weeks after perfusion, consisted of marginal resection, wide resection, or amputation. External beam radiotherapy was delivered only in cases of intralesional or marginal resection. After completion of the treatment, patients were followed up by physical examination, chest and upper abdomen CT, and CT/MRI of the treated limb every 4 months for the first 2 years after surgery, and then every 6 months for 3 more years.

Statistical methods. Descriptive statistics were used to summarize pertinent study information. The association between variables was tested by the Pearson Chi-Square test or Fisher’s exact test. Overall survival (OS) and disease-free survival (DFS) were calculated by the Kaplan-Meier product-limit method from the date of the perfusion until relapse of disease or death from any cause or disease. If a patient had not relapsed/died, survival or relapse was censored at the time of the last visit. The log-rank test was used to assess differences between subgroups. Significance was defined at the p<0.05 level (15). The SPSS version 13.0 (SPSS, Milan, Italy) statistical program was used for analysis.

Results

The results of the study are reported in terms of toxicity and treatment efficacy, namely tumor response, percentage of limb sparing, locoregional control and survival.

Toxicity. The grade of limb reaction was mild to moderate (I-II-III) in 57 out of 75 patients (76%). A grade IV reaction

Table 1. Soft tissue limb sarcoma treated with TNFα-based HILP: patient characteristics.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age (years)</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
<th>Site</th>
<th>Lower limb</th>
<th>Upper limb</th>
<th>Size (cm)</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Type of tumor</th>
<th>Primary</th>
<th>Recurrent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Median</td>
<td>50</td>
<td>18</td>
<td>85</td>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>Site</td>
<td>Lower limb</td>
<td>Upper limb</td>
<td>Size (cm)</td>
<td>Median</td>
<td>Min</td>
<td>Max</td>
<td>Type of tumor</td>
<td>Primary</td>
<td>Recurrent</td>
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<td>Size (cm)</td>
<td>Median</td>
<td>Min</td>
<td>Max</td>
<td>Type of tumor</td>
<td>Primary</td>
<td>Recurrent</td>
</tr>
</tbody>
</table>
was recorded in 16 out of 75 patients (21%), whereas a grade V reaction was observed only in 3% of the patients. Systemic toxicity was generally mild to moderate, e.g. haematological grade 2 (11 patients), and hepatic grade 1-3 (10 patients). Grade 3 pulmonary toxicity was observed in 1 patient. Grade 4 renal toxicity was also observed in 1 patient only, due to rhabdomyolysis in the postoperative period (grade V limb reaction), but the patient fully recovered with medical therapy (Table II).

Treatment efficacy. The tumor response was assessed in all patients submitted to surgery after HILP. CR and PR rates were 34% and 48% respectively, with an overall response (OR) of 82%. Stable disease (SD) and progressive disease (PD) were recorded in 13% and 5% of the patients, respectively (Table III). We looked for correlations between the CR rate on the one hand and the TNFα dose (>1 mg vs. ≤1 mg) or tumor temperature (≥41°C vs. <41°C) on the other, but only the latter reached statistical significance, in that patients treated with the higher tumor temperature achieved a CR rate of 56.7% as opposed to 17.6% obtained in patients treated with the lower tumor temperature (p=0.001; Figure 1).

Conservative surgery was carried out in 85.3% of the patients, whereas amputation was necessary only in 14.7% of the patients. This is remarkable, since all 75 patients were candidates for amputation before the neoadjuvant treatment (Table III). Sixteen out of 75 patients (21.3%) relapsed locoregionally. The 5-year locoregional DFS was 63% . This is also remarkable, as most of the patients had large, deep-seated, multi-compartmental and high-grade tumors (Figure 2). The 5-year DFS and OS were 36.7% and 61.6% , respectively (Figures 3-4).

Discussion

The rationale for using doxorubicin during hyperthermic perfusion is based on the following clinical observations: (a) doxorubicin is the most active cytotoxic agent against STS, with objective response rates ranging between 15% and 35% (CR 6%); (b) a dose–response relationship has been observed, although this has not been formally proven in randomized studies; (c) in vivo studies have demonstrated that the simultaneous application of heat (41.5°C) and doxorubicin increases the antitumor effect of the latter (16); (d) 90% to 97% of doxorubicin is bound to the tumor tissue after hyperthermic perfusion; (e) the tissue concentrations of doxorubicin obtained with hyperthermic perfusion are 5, 25 and 45 times greater than those obtained with normothermic perfusion and with intra-arterial and systemic infusions, respectively.

The rationale supporting a trimodality association of doxorubicin, hyperthermia and TNFα appears to be even stronger. In vitro studies by Alexander et al. demonstrated that the association of TNFα with doxorubicin displays the greatest efficacy as compared to a number of other TNFα/drug combinations (17). More recently, the efficacy of HILP association regimens with doxorubicin and TNFα was demonstrated in animal models (the BN175 fibrosarcoma and ROS-1 osteosarcoma), in which synergistic antitumor effects were observed that resulted in tumor regression in 54% to 100% of the cases, depending on the tumor. In both tumor models, the association with TNFα enhanced (1.8- and 3.1-fold in ROS-1 and BN175, respectively) doxorubicin accumulation in the tumor tissue as compared to the administration of doxorubicin alone. This

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**Table II. Locoregional and systemic toxicity.**

<table>
<thead>
<tr>
<th>Grade of limb reaction</th>
<th>No. patients</th>
<th>Systemic toxicity (grade, no. patients)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Hematological</td>
</tr>
<tr>
<td>I</td>
<td>27</td>
<td>G2 11</td>
</tr>
<tr>
<td>II</td>
<td>17</td>
<td>G2 5</td>
</tr>
<tr>
<td>III</td>
<td>13</td>
<td>G3 3</td>
</tr>
<tr>
<td>IV</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

**Table III. Locoregional clinical outcome.**

<table>
<thead>
<tr>
<th>Tumor response %</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR, PR, SD, PD</td>
<td>Conservative %</td>
</tr>
<tr>
<td>34, 48, 13, 5</td>
<td>85.3</td>
</tr>
</tbody>
</table>

CR, Complete response; PR, partial response; SD, stable disease; PD, progressive disease. 1 Wide excision or compartmental resection.
increase in local drug concentration may explain the synergistic antitumor response of combination HILP treatments (18).

Most likely, hyperthermia synergizes not only with doxorubicin but also with TNFα, thereby exerting a two-tiered influence on the overall biological and clinical effects of the two drugs. This view is strongly supported by in vitro and in vivo observations documenting a direct correlation between TNFα efficacy and temperature (19).

On these premises, we conducted a phase I/II study aimed at evaluating the maximum tolerable dose of TNFα in association with doxorubicin and hyperthermia (20), and report herein on a larger cohort of 75 patients. In agreement with our previous study, we found that at high temperature (>41˚C), the TNFα-hyperthermia association results in a greater than additive effect. Patients treated with this tumor temperature achieved a CR rate of 56.7% as opposed to the poor 17.6% obtained in patients treated with the lower tumor temperature of <41˚C (p=0.001).

Some authors have argued that hyperthermia results in unacceptable toxicity in the perfused limb. However, our previous phase I/II study provided initial evidence that limb toxicity occurs above certain TNFα dosages and temperatures. Herein, we confirm that severe (grades IV-V) limb reactions were exclusively observed when the mean muscle temperature was above the critical threshold of 41.5˚C, and the TNFα dose was >2.2 mg. Therefore, the present study conclusively demonstrates that it is mandatory to pilot the HILP temperature in order to remain within the 40.8˚C - 41.5˚C range and that the TNFα dose of 1 mg must not be exceeded since, within these parameters, limb reactions were mostly grade II and occasionally grade III.

The results of the present phase II study strongly support the observations made in our previous phase I/II study in other respects too. These studies altogether demonstrate that there is no significant correlation between the TNFα dosage and the...
type of tumor response. Thus, it is our firm opinion that 1 mg of TNFα is the best dosage in the HILP setting. This statement is in agreement with in vivo experiments in which the TNFα dosage was able to provoke a synergistic effect with doxorubicin, and melphalan at dosages equivalent to 1 mg (21). Likewise, a recent randomized study in which TNFα was administered at different dosages has pointed out that the tumor response is not correlated with the TNFα dosage (22).

In conclusion, hyperthermic perfusion with doxorubicin and TNFα has been proven herein to be an excellent neoadjuvant therapy for unresectable soft tissue limb sarcoma because it permits limb-sparing surgery in a high percentage of the cases with satisfactory functional results.

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References


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