Hyperthermic Isolation Limb Perfusion with TNFα in the Treatment of In-transit Melanoma Metastasis

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Abstract. Background: Hyperthermic isolation limb perfusion (HILP) with tumor necrosis factor alpha (TNFα) and IFNγ was pioneered by Liénard and Lejeune in 1988. The TNFα was empirically employed at a dosage of 3-4 mg, that is ten times the systemic maximum tolerated dose (MTD). After eighteen years from its first clinical application, more than 300 patients have been treated. The aim of this study is to clarify two major arguments: the TNFα dose and eligibility criteria for patient selection. Patients and Methods: A phase I-II study has previously been conducted in 20 patients with in-transit melanoma metastases using a combination of melphalan and TNFα at dosages ranging from 0.5 to 3.3 mg. Twenty patients were treated and a complete pathological response of 70% was recorded, with no correlation between tumor response and TNFα. The dose of 1 mg of TNFα provided the best results regarding efficacy and toxicity. On the basis of this results a large phase II SITILO study was undertaken. Patients with stage IIIA – IIIB (presence of in transit metastases and/or regional node involvement) were considered eligible; a total of 113 patients were enrolled in the study. The disease was bulky (>10 nodules or fewer nodules with a diameter ≥3 cm) in 42.5% of the patients and unresectable in 33%. Forty patients were treated with a TNFα dosage >1 mg and 73 with 1 mg. All the patients were submitted to HILP via axillary and iliac vessels for tumor of upper and lower limb, respectively. TNFα was injected in the extracorporeal circuit at the pre-established dose, followed after 30 minutes by melphalan (13 and 10 mg/L of limb volume for upper and lower limbs, respectively). Results: A grade 1 and 2 limb toxicity was found in 52.9% and 30.1% of the patients, respectively, 5.5% of patients exhibited a grade 3 and 4, whereas grade 5 limb toxicity was not found. The complete and partial responses were 63% and 24.5%, respectively, with an objective response of 87.5%. We tried to correlate the typed tumor response (CR or not CR) and the TNFα dosage ≤1 mg or >1 mg, but no statistically significant difference was found between the two groups. The bulky disease was the only prognostic factor able to influence the tumor response. Conclusion: Only patients with bulky melanoma disease can benefit from HILP with TNFα at a low dose of 1 mg.

Hyperthermic isolation limb perfusion (HILP) has been employed in the treatment of advanced limb tumors, in-transit melanoma metastasis and non resectable limb soft tissue sarcoma. This technique permits the use of a combination of hyperthermia and high dosages of antineoplastic drug, given that the limb is temporarily isolated from the systemic circulation. In patients affected with in-transit melanoma metastasis the mean complete response rate was 54% (1).

Recently, the complete response rate was further improved up to 70% with the use of tumor necrosis factor alpha (TNFα) (2). Patient eligibility criteria for TNFα HILP and drug dosage are still under investigation.

A phase I-II study has been previously conducted, using a combination of melphalan and TNFα at dosages ranging from 0.5 to 3.3 mg. The results of the study demonstrated that 1 mg of TNFα is the best dose in terms of treatment efficacy and toxicity (3).

The aim of this paper is to report the SITILO (Italian Society of Integrated Locoregional Therapies) experience in 113 stage IIIA-IIIB patients.

Patients and Methods

A hundred and thirteen patients with histologically proven in-transit metastasis were subjected to HILP: 37 were males and 76 females, with a median age of 60 years (range 23-82 years). The nodules were located in the upper and lower limbs, in 14 and 99
patients, respectively. The disease was bulky (≥10 nodules or fewer nodules with a diameter ≥3 cm) in 42.5% of the patients and unresectable in 33%. The median follow-up was 27 months (range, 3-123 months). Forty patients were treated with a TNFα dosage >1 mg and 73 with 1 mg.

HILP technique. The technique used for HILP has been described previously (1). Only a few details that are strictly related to the treatment will, therefore, be reported.

The axillary and iliac vessels were cannulated for tumors of the upper and lower limbs, respectively. As soon as the tumor temperature reached 41.5ÆC, TNFα was injected in the extracorporal circuit at the pre-established dose, followed by melphalan after 30 min (13 and 10 mg/L of limb volume for upper and lower limbs, respectively). During regional perfusion, leakage was accurately monitored with technetium-99 labeled albumin (0.5 Bg/kg) (4). At the end of perfusion, the extracorporeal circuit was washed with saline solution and low molecular weight dextran to remove residual drug.

In patients undergoing iliac perfusion, an iliac lymph node dissection was performed; an axillary lymph node dissection was performed in patients undergoing an axillary HILP. In patients with palpable groin nodal disease, inguinal lymph node dissection was carried out at the time of maximum tumor response (generally within 2 months).

Results

The results of this large phase II study are described in terms of toxicity and efficacy, namely tumor response, loco-regional control and survival.

Toxicity. As far as the loco-regional toxicity is concerned, the Wieberdink’s staging system was employed (5). A grade 1 and 2 limb toxicity was recorded in 52.9% and 30.1% of the patients, respectively; 5.5% of patients exhibited a grade 3 and 4, whereas grade 5 limb toxicity was never recorded. The systemic toxicity was generally mild and characterized by a G2 hematological toxicity recorded in 13% of the patients. Only a G3 pulmonary toxicity was observed, and was treated in ICU with an intubation of 12 hours.

### Table I. TNFα limb perfusion for stage III melanoma: correlation between type of tumor and response.

<table>
<thead>
<tr>
<th>Type of Tumor</th>
<th>CR + PC (%)</th>
<th>NC + P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulky</td>
<td>74.1</td>
<td>25.9</td>
</tr>
<tr>
<td>No bulky</td>
<td>89.1</td>
<td>10.1</td>
</tr>
</tbody>
</table>

CR=complete response; PC=partial response; NC=no change; P=progression

An attempt was made to correlate the type of tumor response (CR or not CR) and the TNFα dosage ≤1 mg or >1 mg, but no statistically significant difference was found between the two groups.

### Table II. Influence of complete response on loco-regional control and survival

<table>
<thead>
<tr>
<th>Type of response</th>
<th>5-year loco-regional control (%)</th>
<th>5-year overall survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete response</td>
<td>41.2</td>
<td>66.9</td>
</tr>
</tbody>
</table>

### Table III. Hyperthermic Antiblastic Perfusion with L.PAM±TNFα: impact on CR. Rates in relation to low and high tumor burden.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Schedule</th>
<th>Complete response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraker (7)</td>
<td>L.PAM-TNFα-IFN-γ</td>
<td>low tumor burden 87</td>
</tr>
<tr>
<td></td>
<td>L.PAM</td>
<td>high tumor burden 58</td>
</tr>
<tr>
<td>Di Filipo (3)</td>
<td>L.PAM+TNFα</td>
<td>low tumor burden 80</td>
</tr>
<tr>
<td></td>
<td>L.PAM</td>
<td>high tumor burden 67</td>
</tr>
</tbody>
</table>

Tumor response. The complete and partial tumor responses were 63% and 24.5%, respectively, with an objective response of 87.5%; stable disease was observed in only 12.5% of the patients.

An attempt was made to correlate the type of tumor response (CR or not CR) and the TNFα dosage ≤1 mg or >1 mg, but no statistically significant difference was found between the two groups.

In patients treated with a tumor temperature >41°C a complete response (CR) rate of 70% was found, which was greater compared to that of 62% observed in patients treated with a tumor temperature ≤41°C, but the difference was not statistically significant.

The bulky disease was the only prognostic factor which influenced the tumor response. As a matter of fact, in bulky disease patients the overall response (OR) rate was 74.1% and compared to 89.1% in no bulky disease patients, the difference was considered statistically significant (p=0.05) (Table I).

The overall 5-year loco-regional control was 42.7%; 41.2% and 26.9% for complete and not complete responders, respectively. The 5-year overall survival was 49%. CR rates were also better in terms of overall survival with a 5-year overall survival of 66.9% and 27.1% in CR and not CR, respectively, with a statistically significant difference (p=0.0001) (Table II).
Discussion

After its first clinical application in 1988, HILP with TNFα has been employed in more than 300 patients affected with in-transit melanoma metastasis. The mean tumor CR rate is 70%, superior to that of 54% CR rate obtained with L Phenil-Alanin-Mustard (L. PAM) alone.

At the present time, there is still uncertainty whether the TNFα-L.PAM regimen is superior to L.PAM alone. Two randomized studies have been conducted to answer this question (6-7). In both studies no great differences have been recorded between the two regimes, even though in the Fraker et al. experience, in a subset of patients with bulky "sarcoma-like" melanoma in-transit metastases (i.e., more than 10 lesions or lesions larger than 5 cm), the CR rate was 58% in the group of patients treated with the TNFα-L.PAM combination as opposed to 19% when only L.PAM was employed.

These results are comparable to our own (Table III), clearly indicate which patients really benefit from TNFα-HILP. Most probably the greater efficacy of TNFα against bulky tumors is based on the selective action mechanism of this cytokine on tumor vasculature that leads to a specific hemorrhagic necrosis. Recently, this hypothesis has been confirmed by results obtained in a series of 20 patients, all affected with bulky disease and treated with TNFα-L.PAM regimen. Complete response was 70%, PR 25% with an OR of 95% (8). These findings accords with results with TNFα plus melphalan in soft tissue sarcoma (9).

TNFα dosage is another crucial issue. The dose of 3-4 mg was empirically established and not determined on the basis of a dose-escalating study, which may be unnecessary. Results from a phase I study previously conducted with a starting dose of 0.5 mg, which is very close to the systemic MTD, showed no correlation between tumor response and TNFα dose. A complete response rate of 70% was achieved with a low TNFα dose (14 out of 20 patients were treated with a dose ranging between 0.5 and 1.6 mg). Therefore 1 mg might represent a good clinical compromise between treatment efficacy and toxicity, using a temperature ≥41.5°C (3). This was also demonstrated in a larger patient population.

In an experimental animal model using soft-tissue sarcoma bearing rats treated by HILP several parameters related to the treatment efficacy have been evaluated. A combination of 50 μg TNFα and 40 μg melphalan demonstrated synergistic activity leading to an OR rate of 71%. A temperature of 38-39°C or 42-43°C resulted in a higher response rate, even though at 42-43°C local toxicity decreased limb functionality dramatically. Synergy between TNFα and melphalan was lost at a dose of TNF below 10 μg in 5 ml perfusate. The author concludes that for an optimal HILP, a minimum perfusion time of 30 min and minimum temperature of 38°C is mandatory. Moreover, the dose of TNFα could be lowered to 10 μg per 5 ml perfusate, which might permit the use of TNFα in a less leakage-free or less inert perfusion settings (10). This dosage is five times less than the standard dose and corresponds to the 1 mg used in our clinical setting.

Several possible mechanisms for the synergistic antitumor effect of TNFα and melphalan have been proposed. De Wilt et al. have demonstrated that the major action mechanism of TNFα is a selective six-fold increase in melphalan concentration in the tumor tissue, whereas the melphalan concentration in healthy tissue is very low (11).

The high response rate with 1 mg could be related to the fact that this dosage is sufficient to obtain in vivo saturation of TNFα receptors, as described in vitro (12). There are two reasons why low TNFα dose (1 mg) is better than high doses (3-4 mg): i) despite accurate leakage monitoring, some systemic toxicity was recorded; therefore, the use of low TNFα dose further reduces the possibility of systemic toxicity. ii) TNFα is commercially available at a cost of €9200 at full dosage (3-4 mg); hence using 1 mg the cost is four-fold reduced.

In conclusion, the combination of TNFα and L.PAM is worthwhile in clinical practice for the treatment of limb melanoma in-transit metastases with bulky disease, after failure to respond to other treatments. Experimental and clinical results strongly indicate that 1 mg of TNFα is sufficient to achieve very satisfactory results.

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References


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