Prognostic Factors Influencing Tumor Response, Locoregional Control and Survival, in Melanoma Patients with Multiple Limb In-transit Metastases Treated with TNFα-based Isolated Limb Perfusion

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Abstract. Background: In isolated limb perfusion (ILP) with tumor necrosis factor-alpha (TNFα) and interferon (IFN)-γ, pioneered by Lienard and Lejenne in 1988, TNFα was empirically employed at a dosage (3-4 mg) ten times higher than the systemic maximum tolerable dose (MTD). We previously conducted a phase II/III study 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. The dose of 1 mg of TNFα was identified as optimal in terms of both efficacy and toxicity. The aim of the present study was to describe our experience with 113 stage IIIA/IIIB melanoma patients treated with a TNFα-based ILP and identify prognostic factors for response, locoregional control and survival. Patients and Methods: Patients at stage IIIA-IIIB (presence of in-transit metastases and/or regional node involvement) were considered eligible. The disease was bulky (≥10 nodules ≥3 cm 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 of >1 mg and 73 with 1 mg. Patients with tumors in the upper and lower limbs were submitted to ILP via axillary and iliac vessels, respectively. TNFα was injected in the arterial line of an extracorporeal circuit at the pre-established dose, followed by melphalan (13 and 10 mg/l of limb volume for the upper and lower limbs, respectively) 30 minutes later. Results: Complete responses (CR) and partial responses (PR) were 63% and 24.5%, respectively, with an objective response (OR) of 87.5%. No change (NC) was observed in only 12.5% of the patients. Upon multivariate analysis, only bulky disease maintained its independent value for tumor response with an odds ratio of 4.07 and a p-value of 0.02. The 5-year locoregional disease-free survival was 42.7%. Upon multivariate analysis, the only prognostic factors were stage, age and bulky disease. The 5-year overall survival was 49%. Multivariate analysis showed that only sex, stage and CR maintained their independent values. Conclusion: TNFα-based ILP was proven to be an effective treatment for melanoma patients with in-transit metastases. The TNFα dosage of 1 mg was as effective as 3-4 mg, with lower toxicity and cost. We propose that TNFα and melphalan-based ILP should be employed for bulky tumors or after failure of melphalan-based ILP.

The prognosis of melanoma patients varies widely and largely depends on disease stage (1). In-transit melanoma metastases (IT-mets) occur in 5-8% high-risk melanoma patients. IT-mets are cutaneous or subcutaneous melanoma nodules between the primary site and the regional lymph node basin. The presence of IT-mets causes considerable discomfort, especially when multiple and ulcerated nodules spread along the limb. The treatment of IT-mets is a major challenge because of their high recurrence rates following surgery and radiotherapy, as well as systemic chemotherapy. Isolated limb perfusion (ILP), developed by Creech and Kremetz has been proven to be the most effective regional treatment (2). The temporary exclusion of the affected limb from the systemic circulation permits the delivery of antineoplastic drugs at dosages of more than 20 times higher than those administered systemically. Moreover, hyperthermia enhances the efficacy of antineoplastic drugs. In fact, melphalan-based ILP for melanoma IT-mets can achieve...
Patients and Methods

Patients. One hundred and thirteen patients with histologically proven IT-mets were subjected to ILP; 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. According to the MD Anderson staging system (6), 58% and 42% of the patients presented at stages IIIA and IIIB, respectively. The disease was bulky (≥10 nodules ≤3 cm or fewer nodules with a diameter >3 cm) in 42.5% and unrespectable in 33% of the patients. The median follow-up was 27 months (range: 3-123 months). Forty patients were treated with TNFα at a dosage of >1 mg and 73 at a dosage of 1 mg.

ILP technique. Because the technique used for ILP has been described previously (7), only a few relevant details will be given here. Following cannulation of either the axillary or iliac vessels and upon establishment of the desired tumor temperature (at least 41°C), TNFα (Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany) was injected into the arterial line of the extracorporeal circuit at the pre-established dose, followed by melphalan (L-phenylalanine mustard [L-PAM]; Alkeran, Welcome, London, UK) after 30 minutes, at dosages of 13 and 10 mg/l of limb volume for the upper and lower limbs, respectively. During regional perfusion, leakage was accurately monitored with technetium-99-labelled albumin (0.5 MBq/kg) (8). At the end of perfusion, the extracorporeal circuit was washed with saline solution and low molecular weight dextran to remove residual drug.

Iliac or axillary (as appropriate) lymph node dissections were performed in all the patients. 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).

Evaluation of response and toxicity. Limb toxicity was classified according to the Weberdink staging system (9) as follows: (I) no reaction; (II) slight erythema or edema; (III) considerable erythema or edema with some blistering; slightly disturbed motility permissible; (IV) extensive epidermolysis or damage to deep tissue, causing definitive functional disturbance and either threatening or causing a manifest compartmental syndrome; and (V) reaction that may necessitate amputation. Response evaluation was carried out 2 to 4 and 8 weeks after ILP. Response rates were reported according to the WHO criteria (10).

Statistical evaluation. 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 (11). The hazard risk (HR) and the confidence limits (CI) were estimated for each variable using the Cox univariate model and adopting the most suitable prognostic category as reference group (12). A multivariate Cox proportional hazard model was also developed using stepwise regression (forward selection) with predictive variables which were significant upon univariate analysis. Enter limit and remove limit were p<0.10 and p=0.15, respectively. Logistic regression analysis was used to assess the impact of different variables on the response. The cut-off p-values to enter in or to be removed from the model were set to 0.10 and 0.15. Results are reported as odds ratio (OR) with 95% CI. The SPSS version 13.0 statistical program (SPSS, Milan, Italy) was used for analysis.

Results

The results of this phase II study are described in terms of toxicity and tumor response, locoregional control and survival. A series of prognostic factors related both to the treatment parameters (tumor temperature, TNFα dose, ILP duration) and the clinical characteristics (sex, age, stage of disease, number of lesions) were evaluated for each of the aforementioned endpoints.

Toxicity. Limb toxicity grades I and II were recorded in 56% and 34% of the patients, respectively; 5% of patients exhibited grades III or IV, whereas grade V limb toxicity was never recorded. The systemic toxicity was generally mild and characterized by a grade 2 hematological toxicity recorded in 13% of the patients. Only one grade 3 pulmonary toxicity was observed, which was treated in ICU with an intubation of 12 hours.

Tumor response. The CR and partial tumor responses (PR) were 63% and 24.5%, respectively, with an OR of 87.5%. Only 12.5% of the patients displayed no response. We assessed whether any correlation existed between the type of tumor response, e.g. CR or PR vs. no change (NC) or progression (P), and the TNFα dosage ≤1 mg or >1 mg, but no statistically significant differences were found. Bulky disease was the only prognostic factor that significantly influenced tumor response. Specifically, in patients with bulky disease, the objective response rate was 74.1% as compared to 89.1% in patients with non-bulky disease (p=0.05).
Multivariate analysis (Table I) confirmed that, among the 8 prognostic factors taken into consideration, only bulky disease maintained its independent value (OR=4.07, p=0.02).

Locoregional control. The 5-year locoregional control was 42.7%. Upon multivariate analysis, the only prognostic factors for locoregional control were disease stage, age and bulky disease (Table I).

Survival. The 5-year DFS and OS for the entire patient population were 24.3% and 49%, respectively. Multivariate analysis identified age (HR=3.16, p-value=0.008) and stage (HR=2.13, p-value=0.06) as prognostic factors for disease-free survival. Upon multivariate analysis, only sex, stage of disease and CR maintained their independent prognostic values for OS. Responders with CR did better with a 5-year OS of 66.9% as compared with non-CR whose 5-year overall survival was 27.1% (p=0.0001).

In patients at stages IIIA and IIIB, the 5-year OS rates were 68.6% and 28.0%, respectively, the difference being statistically significant (p=0.03).

Discussion

Since its first clinical application in 1988, combination ILP with L-PAM and TNFα (TM-ILP) has been employed in more than 400 patients carrying IT-mets. The mean tumor CR rate of the TM-ILP treatment was shown to be 70%, i.e. superior to the 54% CR rate obtained with L.PAM alone (M-ILP). Because of this relatively small difference, it was readily evident that it is of paramount importance to identify those patients who may really benefit from treatment with TNFα. Previous studies on this topic were conducted by several groups, including our own, but concerned limited numbers of melanoma patients (13-16; Table II). In agreement with these studies, but taking into account a conspicuous retrospective case collection totalling 113 homogeneously treated patients, we report herein (Table I) that bulky tumor is the only relevant parameter selected for tumor response by multivariate analysis, with an OR of 4.07 (1.29-12.8) and a p-value of 0.01. An impressive CR rate of 67% of TM-ILP, as compared to the very poor 28% CR rate associated with the M-ILP regimen, clearly illustrates that TNFα finds elective application in treatment of bulky tumors, whereas it does not provide any significant advantage in treatment of non-bulky tumors (Table II). The importance of TNFα in bulky disease is in line with recent results in a smaller series of 20 patients treated with the TM-ILP regimen. CR in this series was 70%, PR was 25%, with an overall response of 95% (17). Similar clinical indications were obtained in the treatment of soft tissue sarcomas (18).

The high rates of CR in patients with bulky disease is an observation of considerable clinical relevance, since CR has been proven to also influence locoregional control in other series (13-15). For instance, in the Grunhagen et al. series (14), local progression occurred at a median time of 16 months in 55% of patients undergoing ILP. Median time to local progression (TTLP) of patients after CR was 22 months versus 6 months after PR or NC (p<0.001, significant upon both univariate analysis and multivariate analysis). These results show that control of bulky disease is central to the achievement of both CR and locoregional control, and this provides a strong rationale to optimise TNFα treatment in this subset of patients.

In our experience, age and disease stage (IIIA vs. IIIB) were proven (Table I) to be strong predictors of locoregional DFS, as shown in Figure 1. In this regard, one could argue that stage may influence locoregional control by enhancing extravasation of melanoma cells. It is conceivable that
melanoma cells spreading along lymphatic channels from the primary tumor to the regional lymph nodal basin might pass through the lymphatic channel wall and be harboured in the skin and/or subcutaneous tissue. This process is most likely facilitated at stage IIIAB in which regional nodes are loaded by tumor cells. As to 5-year OS rates, they were also influenced by the disease stage (68.6% and 28.0% for stages IIIA and IIIAB, respectively; Figure 2), but tumor response (CR vs. not CR) was the strongest predictor of OS, with an HR of 5.77 (2.07-16.09) and \( p \)-value of 0.001. This is illustrated by Figure 3 in which the 5-year overall survival rates of stage IIIA patients were stratified according to CR vs. non-CR, providing figures of 82.8% and 20.8%, respectively \( (p=0.0004) \).

In summary, a patient-related factor (disease stage) and, more so, a treatment-related factor (tumor response) are major determinants in the outcome of ILP-treated melanoma patients, and the latter defines a major, achievable therapeutic goal. We recommend that patients with bulky tumors be treated with a TM-ILP regimen because the CR rates are considerably higher than those achieved with M-ILP alone \( (13, 16, 19) \) and CR drastically impacts on all clinical parameters, including survival. Having clearly identified prognostically relevant parameters, all efforts have to be made in order to achieve a complete tumor response.

In this regard, a few considerations should be added about the hyperthermic schedule. In patients treated with a tumor temperature of \( \geq 41^\circ \text{C} \), a CR rate of 70% was found, which was greater than that of 62% observed in patients treated with a tumor temperature of \( \approx 41^\circ \text{C} \), although the difference was not statistically significant. Pain for gain, we suggest that a tumor temperature of \( \geq 41^\circ \text{C} \) should be employed in
association with TNFα and melphalan because it may conceivably increase the effectiveness of the two drugs without incurring major adverse effects.

High TNFα dosages did not influence the tumor response in our complete series of 113 stage IIIA–IIIB patients. This was previously demonstrated in a phase I/II study in melanoma patients, in which CR rates of 70% were obtained with a TNFα dose ranging from 0.5 to 1.6 mg (5). Since then, we have used 1 mg of TNFα that might represent a good compromise between treatment efficacy and toxicity. The same results were also obtained in experimental animal models (20). The authors conclude that for an optimal ILP, a minimum perfusion time of 30 min and a minimum temperature of 38°C is mandatory. Moreover, the dose of TNFα could be lowered to 10 mg or 5 ml perfusate, which might permit the use of TNFα in a less leakage-free or less inert perfusion setting. This dose is five times lower than the standard dose and corresponds to the dose of 1 mg used in our clinical setting. TNFα is commercially available at a cost of $9,200.00 at full dosage (3–4 mg); hence using 1 mg reduces the cost four-fold.

The complex interplay among TNFα dose, bulky disease, CR and disease stage illustrates a few biological concepts that are worth discussing because of their relevance to future strategies to optimize the in vivo use of TM-ILT. Most likely, the elective targeting of bulky tumors by TNFα has its biological basis in the selective mechanism of action of this cytokine against well-vascularized tumors and newly formed vessels, ultimately leading to a specific hemorrhagic necrosis. On the contrary, micrometastatic disease is less sensitive to this cytokine, as indicated by two observations: (a) adjuvant ILP does not seem to completely eradicate micrometastases (21), and (b) after CR to ILP, recurrences tend to localize in new sites within the perfusional field (22).

In conclusion, the combination of TNFα and melphalan is suggested in the clinical practice for the treatment of limb melanoma IT-mets with bulky disease, or after failure of melphalan ILP.

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