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TNF-Based Isolated Limb Perfusion Followed by Consolidation Biotherapy with Systemic Low-dose Interferon Alpha 2b in Patients with In-transit Melanoma Metastases: A Pilot Trial

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Background: Tumor necrosis factor (TNF)-based isolated limb perfusion (ILP) yields high tumor response rates in patients with in-transit melanoma metastases. However, most patients will ultimately experience disease recurrence. The aim of this pilot study was to test the hypothesis that systemic low-dose interferon α-2b (LDI) might consolidate the therapeutic effect of ILP.

Methods: A total of 12 patients with in-transit melanoma metastases not amenable to surgical excision were given LDI subcutaneously (3 million IU/day, 7 days/week for 12 months) after TNF-based ILP (TNF 1 mg + melphalan (L-PAM) 10 mg/L) (group A). The clinical outcome of these patients was historically compared with that of 19 patients with similar anthropometric and disease characteristics who underwent TNF-based ILP alone (group B).

Results: In group A, LDI was well tolerated, only grade 2 systemic toxicity being recorded in 50% of patients. The progression-free survival analysis showed a statistically significant advantage for group A patients as compared with group B (median time to progression: 26 and 17 months, respectively; log-rank test *P*-value: 0.037). This survival benefit was confirmed at multivariate analysis, where treatment was the only prognostic factor retained by the prediction model. The analysis of the risk of disease progression over time suggested that this survival benefit appears to vanish after LDI discontinuation, which further strengthens the hypothesis that LDI might consolidate the therapeutic effect of TNF-based ILP.

Conclusions: These preliminary findings support the conduction of larger trials to formally assess the ability of LDI to improve the clinical outcome of melanoma patients with in-transit metastases undergoing TNF-based ILP.

Key Words: Interferon-alpha (IFNα)—Tumor necrosis factor (TNF)—Isolated limb perfusion—Melanoma.

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In-transit metastases occur in 5–10% of all patients with cutaneous melanoma, the lower limb being the main location (70% of cases).[1] Surgical resection is indicated when lesions are small and limited in

TABLE 1. *Study eligibility criteria*

Inclusion criteria	*Exclusion criteria*
■ Age >18 and <75 years	■ Pregnancy
■ Histologically confirmed diagnosis of in-transit melanoma metastases	■ Autoimmune disease
■ Isolated limb perfusion (with TNF and L-PAM) within the past 2 months	■ Distant metastasis (e.g., lung, brain, bone, liver, and subcutaneous metastasis outside the extremity treated with isolated limb perfusion)
■ ECOG[a] status 0–2	■ Systemic chemotherapy within the past 2 months
■ Normal liver and renal function	■ Steroid therapy (ongoing)
■ Fully informed consent	

[a] Eastern cooperative oncology group

number, while amputation should be considered only if the limb function is severely impaired or if the hygienic conditions are poor. When the extent of the disease contraindicates surgical excision, isolated limb perfusion (ILP) should be taken into consideration, as amputation does not provide any advantage in terms of survival and systemic chemotherapy has shown limited effect.[2] Isolated limb perfusion is a well-established locoregional procedure to deliver high-dose anticancer agents to an extremity with multiple in-transit lesions.[3] Melphalan (L-PAM) has been used as the referral drug since its first clinical application with a reported complete response rate of around 50%; by contrast, other chemotherapeutic agents (e.g., dacarbazine, actinomycin-D, cisplatin) did not show any therapeutic advantage in terms of either tumor response rate or duration.[4,5]

Since the early 1990s, tumor necrosis factor (TNF) was used in ILP in combination with L-PAM, with high complete response rates (up to 70–80%) in several published series.[2,6]

Despite the encouraging results obtained with ILP in terms of local disease control, disease recurrence remains a major issue in the therapeutic management of these patients. In fact, even after complete tumor response, most patients will develop disease progression either locally or systemically or both.[2–4] Therefore, any effort to consolidate the anticancer effect of ILP is justified.

In this respect, the present pilot study tested the hypothesis that the systemic administration of low-dose interferon alpha 2b (LDI) might increase the duration of progression-free survival of patients who underwent TNF-based ILP. The rationale to administer this new therapeutic regimen to melanoma patients with in-transit metastases was based on two main considerations:

1. The main anticancer mechanism of TNF is believed to be the selective disruption of the tumor vasculature, which ultimately leads to an ischemic insult to malignant cells.[7] In particular, investigators have demonstrated that TNF inhibits the activity of integrin-alpha-V-beta-3, which is overexpressed by proliferating endothelial cells (such as those of growing tumor masses);[8] the antagonization of this cell surface molecule has been shown to induce apoptosis of angiogenic blood vessels[9] and thus might be responsible for the tumor selective activity of TNF.
2. The anticancer activity of interferon-alpha (IFNα, the only agent approved for the adjuvant treatment of melanoma) has been largely documented in different models; however, the molecular mechanisms underlying the IFNα antitumor activity are largely unknown.[10] At low doses, IFNα appears to inhibit tumor angiogenesis[11,12] by directly inhibiting endothelial cell proliferation and by downregulating the expression of pro-angiogenetic factors (e.g., VEGF, b-FGF, IL-8, and matrix metalloproteinases).[13,14]Consequently, we hypothesized that LDI might exert an anti-angiogenic effect complementary to that of TNF; in particular, LDI might hamper the blood vessel formation necessary for the growth of tumor remnants following TNF-based ILP.

The present study introduces the results obtained in a pilot trial of TNF-based ILP followed by consolidation biotherapy with systemic LDI.

PATIENTS AND METHODS

From April 2002 to April 2005, 12 patients with lower limb in-transit melanoma metastases not amenable to surgical excision were enrolled in this study. Eligibility criteria are listed in Table 1.

The patients underwent ILP with TNF (1 mg; Beromun, Boehringer-Ingelheim Italia, Milan, Italy) and L-PAM (10 mg per liter of limb volume; Alkeran, Glaxo-Wellcome Italia, Verona, Italy), as we de-

TABLE 2. *Patients and tumor characteristics in study group A (isolated limb perfusion followed by consolidation biotherapy) and group B (isolated limb perfusion alone)*

	Group A	Group B	P-value[a]
Patients	12	19	–
Mean age (range)	63 (41–76)	61 (38–73)	0.74
Sex			
Male (%)	4 (33)	8 (42)	0.71
Female (%)	8 (67)	11 (58)	
Mean thickness (range)[b]	2.5 (1–10)	2.3 (1–6)	0.84
TNM stage[c]			1.00
IIIB (%)	10 (83)	15 (78)	
IIIC (%)	2 (17)	4 (22)	
ECOG PS[d]			0.48
0–1 (%)	8 (67)	10 (53)	
2 (%)	4 (33)	9 (47)	

[a] Student's *t*-test or Fisher's exact test *P*-values, as appropriate.
[b] Primary tumor thickness expressed in millimeters.
[c] In-transit metastases with (IIIC) or without (IIIB) regional lymph node metastasis at the time of isolated limb perfusion.
[d] Performance status (PS) assessed 2 months after isolated limb perfusion.

scribed in detail elsewhere,[15] within 2 months from TNF-based ILP. These patients (group A) were also given subcutaneous low-dose IFNα (LDI, 3 million IU/day, 7 days/week; Intron-A, Shering Corporation, Kenilworth, New Jersey, USA). By study design, LDI was administered for 12 months and was stopped earlier only in case of disease progression or drug intolerance.

The clinical information about 19 patients with characteristics (i.e., sex, age, and tumor stage) similar to group A but treated with TNF-based ILP alone was extracted from our database (historical controls, group B). Patients and tumor characteristics are listed in Table 2.

Patients' Follow-up and Clinical Outcome Evaluation

Following ILP, patients were followed-up with clinical examination every 2 months for the first 6 months and thereafter every 3 months until disease progression.

Considering both study groups ($n = 31$), the median follow-up was 18 months (range: 4–48 months).

Tumor response to ILP was assessed 2 months after the locoregional treatment and was classified as follows: (1) complete response: disappearance of all in-transit metastases; (2) partial response: at least 50% decrease in the sum of the product of the diameters of measured lesions without any simultaneous increase in size of the remaining nodules or the appearance of any new lesions; (3) no change: decrease in tumor size $< 50\%$ or increase of tumor size $< 25\%$; (4) progressive disease: increase of $> 25\%$ in the size of any measured lesion, appearance of a new tumor, or both.

TABLE 3. *Toxicity grade assessment of the perfused limb according to the Wieberdink's scale*

Grade	Description
I	No evidence of limb damage
II	Slight or mild skin erythema or edema
III	Considerable skin erythema or edema with some blistering; slightly disturbed motility possible
IV	Extensive epidermolysis or evident damage to the deep tissues causing definite functional disturbances; threatening or clinically evident compartmental syndrome
V	Toxicity that necessitates limb amputation

Local (limb) toxicity (to ILP) and systemic toxicity (to IFNα) were classified according to the Wieberdink's criteria (Table 3)[16] and the World Health Organization (WHO) criteria, respectively.

The evaluation of local tumor progression response was mainly clinical. Ultrasonography and fine needle aspiration were performed in case of suspected lesions, upon clinician's judgment.

Chest X-ray, liver ultrasound scan, and computed tomography scan were performed every 4 months to detect distant metastases.

For survival analysis purposes, progression-free survival (PFS) which was considered as the interval between ILP and disease progression.

Statistical Analysis

Survival estimates were calculated by means of the Kaplan–Meier method and survival curves were compared by means of the log-rank test.

The Cox proportional hazards model was used to calculate the hazard ratio (HR) associated with treatment as well as to identify independent prognostic factors (stepwise variable selection mode) among the following: age, sex, tumor response after ILP, Breslow thickness (primary tumor), regional lymph node involvement (at the time of ILP), and treatment (ILP + LDI versus ILP alone). Considering the median PFS of group B as the reference time to progression (17 months, see below), the sample size of this pilot study allowed to detect a minimum survival difference of 30, 20, and 10 months with a statistical power of 80%, 60%, and 28%, respectively.

Probability values lower than 0.05 were considered significant.

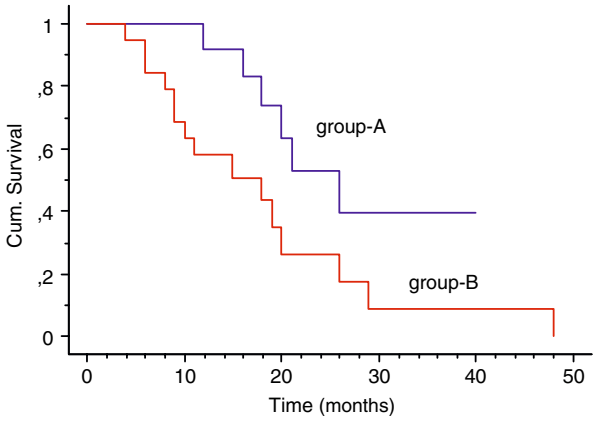

FIG. 1. Progression-free survival of 12 patients with in-transit melanoma metastases who underwent TNF-based isolated limb perfusion (ILP) and consolidation biotherapy with systemic low-dose IFN-alpha (group A) compared with 19 patients who underwent ILP alone (group B). Log-rank test, $P = 0.037$.

All statistical analyses were performed by using the StatView statistical package (SAS Institute, USA).

RESULTS

Toxicity

In group A, the mean drug leakage (as measured by the scintigraphic method described in detail elsewhere)[17] from the perfusate to the systemic circulation during ILP was 4.5% (range: 1–8%), with no significant difference between the two study groups (group B mean leakage: 4%).

As regards ILP-related side effects, in group A, 10 out of 12 patients (83%) showed grade 2 local toxicity, a rate similar to that observed in group B (15/19, 79%; Fisher's exact test $P = 0.70$).

In group A, LDI was well tolerated. In particular, systemic toxicity was represented by grade 2 hematological toxicity ($n = 4$, 33%), grade 2 fatigue ($n = 5$, 42%), and grade 2 fever ($n = 6$, 50%), the latter being always responsive to common antipyretic drugs. Overall, the toxicity associated with LDI occurred in a significant proportion of patients (50%) but it was minimal or mild and easily controlled.

Clinical Outcome

Considering group A ($n = 12$), the median follow-up was 20.5 months (range: 12–40 months). After TNF-based ILP, complete tumor response was observed in six out of 12 patients (50%). After a median follow-up of 17 months, four of these patients are alive with no evidence of disease, one is alive with disease and another died of disease. Among the six cases with partial tumor response (median follow-up: 22.5 months), four patients died of disease, one is alive with disease, and one died in a car accident.

Considering group B ($n = 19$), the median follow-up was 12 months (range: 4–48 months). Ten patients (53%) showed complete response after TNF-based ILP. After a median follow-up of 12 months, five of these patients are alive with no evidence of disease, two are alive with disease and three died of disease. Among partial responders ($n = 9$), after a median follow-up of 12.5 months, one patient is alive with disease and eight died of disease.

Progression-free Survival

According to the Kaplan–Meier survival estimates, the median PFS for group A and group B patients was 26 months and 17 months, respectively (Fig. 1).

The log-rank test-based comparison of the two survival curves ($P = 0.037$) and the Cox model (HR: 0.38, 95% confidence interval: 0.14–0.98; $P = 0.049$) showed that this difference represented a significant advantage for patients who were administered consolidation biotherapy after ILP (group A) as compared with those who underwent ILP alone (group B).

At multivariate analysis, the only independent prognostic factor was treatment, with a risk reduction of 62% in favor of group A.

By analyzing the trend of the cumulative risk for disease progression plotted against the time on a natural logarithm scale (Fig. 2), it can be noted that the curves tend to draw closer after a period of approximately 12–16 months. Taking into consideration the mean duration of IFNα treatment (10 months), one might hypothesize that in group A the risk of disease progression approaches that observed in group B after LDI discontinuation, which would indirectly further supports the protective activity of consolidation biotherapy.

DISCUSSION

Isolated limb perfusion (ILP) is nowadays considered a well-established treatment for in-transit melanoma metastases not amenable to surgical excision.[2–5] Following systemic chemotherapy, tumor response rates are very low; on the other hand, limb amputation is held by most authors to be an over-

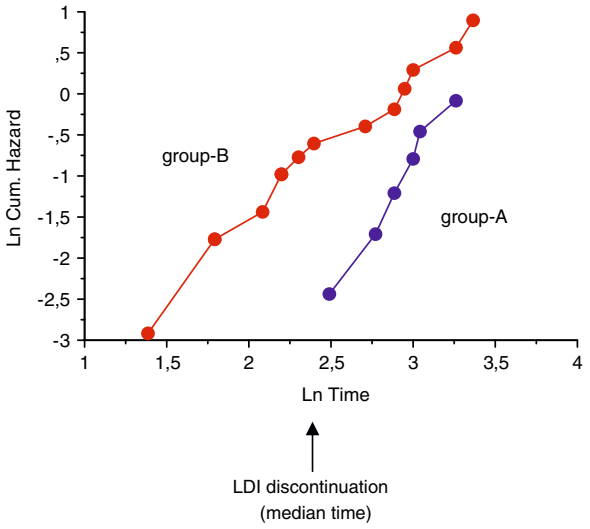

FIG. 2. Cumulative risk of disease recurrence in patients with in-transit melanoma metastases who underwent TNF-based isolated limb perfusion (ILP) and consolidation biotherapy with systemic low-dose IFN-alpha (LDI, group A) and in patients who underwent ILP alone (group B). As compared with group B, the risk of disease progression is shifted to the right for group A patients (interpretation: protective effect of treatment → delayed disease progression). Moreover, in group A the risk appears to approach that observed in group B over time (the slope of group A is steeper than that of group B), which may be interpreted as the loss of LDI protective effect after its discontinuation.

treatment that does not allow any advantage in terms of survival.

L-PAM is the referral drug for ILP, both because of its acceptable tolerability and the high rate of complete responses observed following L-PAM-based ILP. Combined with L-PAM, TNF appears to increase tumor response rates, particularly in patients with "bulky" tumor. This remarkable antitumor effect is due to multiple factors, such as: (1) the greater sensitivity of tumor to the cytotoxic effects of heat as compared with normal tissues, (2) the synergism between hyperthermia (≥41.0°C) and both L-PAM and TNF, and (3) the TNF-mediated disruption of the tumor microvasculature that adds to the direct cytotoxic effect of L-PAM against malignant cells. Moving from these premises, we started to treat melanoma patients with locally advanced in-transit metastases of the extremities with TNF-based ILP.[15]

However, despite the aforementioned favorable results, TNF activity has been recently questioned in a randomized controlled trial;[18] furthermore, following ILP, disease progression occurs in the majority of patients (approximately 50% of patients with complete tumor response and virtually all cases showing partial tumor response), which ultimately jeopardizes the therapeutic effort of a surgically demanding procedure such as ILP. Finally, stage IIIB–IIIC melanoma patients show a 5-year disease specific mortality of around 60–70%, which is due to the systemic progression of the disease.[19] According to these considerations, it is clear that treatments complementary to ILP must be actively investigated in order to improve the clinical outcome of these patients.

To the best of our knowledge, no clinical experience has been so far reported in the literature on the application of any complementary treatment to ILP.

With the present study, we intended to explore the hypothesis that the use of LDI might improve the clinical outcome of patients with in-transit melanoma metastases who undergo TNF-based ILP. This pilot study was thus based on the comparison of the clinical outcome observed in a small group of patients undergoing ILP plus LDI (group A) with that of a historical control group of patients with similar anthropometric and disease characteristics (group B). Like the pretreatment features, also the tumor response rates to ILP were similar: in fact, a complete tumor response was observed in 50% and 53% of patients from group A and group B, respectively (Fisher's exact test $P = 0.79$). This observation supports the substantial reliability of the comparison of the prospectively enrolled patients (group A, experimental therapy) with those retrospectively considered (group B, historical control).

As regards tumor recurrence among patients with tumor complete response, two out of six (33%) patients developed a local relapse in group A and 5 out of 10 (50%) developed a local relapse in group B (Fisher's exact test, $P = 0.49$). Because these figures are too small to draw any conclusion and because the effect of consolidation biotherapy is more likely to delay tumor progression rather than to reduce the rate of disease relapse (which over time will occur in all cases, unless cure is obtained), we relied upon survival analysis to unravel the potential therapeutic effect of LDI in this explorative trial.

Progression-free survival analysis showed a significant advantage (median PFS difference: 9 months) for patients who underwent the sequential treatment (ILP followed by consolidation biotherapy) (Fig. 1), with a 62% reduction of the risk of disease progression. Interestingly, treatment was the only variable retained by the Cox proportional hazards model, ruling out the potential bias of other well-known

prognostic factors, such as primary tumor thickness, lymph node status, and tumor response to ILP.

Moreover, by analyzing the trend of the cumulative risk of disease progression over time, we could observe that this risk tends to decrease after 12–16 months (Fig. 2), which might be due to the fact that the protective effect of the LDI vanishes after its discontinuation (which occurred on average after 10 months). Besides indirectly strengthening our hypothesis, this finding suggests that future trials of consolidation biotherapy should be designed to administer LDI until disease progression (instead of stopping it after a fixed time of 12 months as provided in the present pilot study).

Taken together, our findings support the hypothesis that consolidation biotherapy with systemic LDI might increase the duration of PFS after TNF-based ILP in patients with in-transit melanoma metastasis, with no significant patient discomfort in terms of treatment related toxicity.

The results of this pilot study has prompted us to start a formal phase II trial of TNF-based ILP followed by long-term LDI in order to validate the observed survival benefit in a larger series of patients.

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