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ORIGINAL ARTICLE – MELANOMAS

Long-Term Results of Melphalan-Based Isolated Limb Perfusion With or Without Low-Dose TNF for In-Transit Melanoma Metastases

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ABSTRACT

Background. The aims of the study were: (1) to determine toxicity, response rate, local-regional control, and survival in the entire population of the perfused patients; (2) to compare toxicity, response, and survival among patients who underwent melphalan-based perfusion with or without low-dose tumor necrosis factor (TNF); and (3) to identify factors that predict a complete response and survival.

Materials and Methods. A total of 53 patients with extensive in-transit metastases (47%) underwent perfusion with melphalan, and 59 (53%) also received low-dose TNF.

Results. No difference was observed between the 2 drug regimens for what concerns local toxicity ($P = 1.0$). The tumor complete response rate was higher in patients treated with TNF (60.3% versus 41.5%, $P = .036$), in particular in the case of locally advanced tumors (66.7% versus 30%, $P = .049$). The presence of lymph node metastases had a negative influence on the tumor response rate ($P = .003$). Median time to local progression and survival were 19.6 and 34.5 months, respectively. Long-term complete response was achieved in 68% of the patients with initial CR (39 of 57 patients). The tumor response after perfusion was the only prognostic factor for local control and survival ($P < .0001$ and $P = .002$, respectively).

Conclusions. In the case of locally advanced disease, the addition of low-dose TNF to melphalan-based isolated limb perfusion appears safe and particularly useful. The presence of lymph node metastases is associated with decreased response rates. A sustained complete response was obtained in about one-third of the patients.

The incidence of in-transit melanoma metastases depends on the initial stage of primary melanoma.[1] Patients who develop a limited number of small in-transit metastases can be treated with several approaches, such as surgical excision, intralesional injections, laser ablation, chemotherapy, radiotherapy, or electrochemotherapy.[1,2]

Isolated limb perfusion is indicated for patients with advanced in-transit metastases or in case of frequently recurrent limb disease.[1,3] It allows achieving regional concentrations of chemotherapeutic agents that are 15–25 times higher than after systemic administration. Melphalan has been identified as the standard drug for its local toxicity profile and efficacy; it allows achieving a complete response in around 50% of patients after ILP, with an overall response rate up to 80%.[4,5]

Extended melanoma in-transit lesions are difficult to treat using isolated limb perfusion. This is probably because of their poor and inhomogeneous vascularization and drug uptake. The association of tumor necrosis factor (TNF) α with melphalan seems to have an important therapeutic impact in this clinical setting, since it leads to the selective disruption of the tumor microvasculate, and consequently to an ischemic insult toward the melanoma cells.[6,7] Moreover, a higher melphalan concentration was observed in the tumors after TNF-based perfusion.[8] In several published series, a complete tumor response rate up to 60%–80% has been reported.[9–13] However, a higher local and systemic toxicity has been observed after isolated limb perfusion with this drug regimen, even though low-dose (0.5–1.0 mg) seems to prevent the occurrence of side effects.[14,15]

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Melphalan-based isolated limb perfusion is followed by a persistent complete tumor response in about one-third of patients, and the combination of TNF with melphalan seems not to influence the long-term tumor local control.[9,11,16–19]

The only published full-text phase III randomized controlled trial failed to demonstrate a significant difference in tumor response between patients submitted to melphalan-based perfusion with or without TNF.[20] However, these results have been argued, and consensus about the role of TNF in isolated limb perfusion for melanoma is still lacking.[21]

We performed the present study to determine toxicity, tumor response rate, local-regional control, and survival in the entire population of the perfused patients; to compare toxicity, tumor response, and survival among patients who had undergone melphalan-based perfusion with or without low-dose TNF and to identify factors that predict complete tumor response and survival.

PATIENTS AND METHODS

The medical records of patients with cutaneous melanoma who had signed an informed consent and who underwent isolated limb perfusion at our Institution from 1989 through 2007 were reviewed. TNF was introduced in our perfusion schedule in October 1994; therefore, patients underwent the procedure with melphalan only before that date. Between 1994 and 2002 we administered both melphalan and TNF in isolated perfusion of all our patients. Given the good results obtained through TNF-based isolated limb perfusion in the subgroup of patients with large amount of in-transit metastases (i.e., locally advanced disease, defined as more than 15 singular lesions or the diameter of the greatest nodule greater than 3 cm), between 2002 and 2007 TNF was administered to patients with locally advanced melanoma, while the others were treated with melphalan only.

Patients were considered eligible for the study if the following data were available: (1) histologically proven in-transit metastases of the limb; (2) absence of distant metastases at isolated limb perfusion; (3) isolated limb perfusion performed with melphalan and/or low dose TNF (0.5–1.0 mg); and (4) patients who were examined at regular follow-ups.

Only the first isolated limb perfusion was considered for patients who underwent more than 1 procedure.

The perfusion technique and the postoperative care is described in detail elsewhere.[22–24] In the presence of synchronous lymph-node metastases in the regional basin (groin or axilla), radical lymph node dissection was performed.

Statistical Analysis

The following parameters were taken into consideration in order to predict the tumor response: age (continuous variable), sex (male, female), site of the primary tumor (upper or lower limb), lymph node status before, or at the time of, the isolated limb perfusion (negative or positive), lesion diameter (<3 or ≥ 3 cm), number of lesions (<5 or 5–15 or >15), locally advanced disease, drug regimen (melphalan or melphalan plus TNF), maximum tumor temperature (continuous variable), and mean flow (continuous variable). Univariate and multivariate analyses were performed with logistic regression; in the latter, variables were considered in case the P value of univariate analysis was less or equal to .15.

The times to local progression and overall survival were calculated from the date of the isolated limb perfusion to local recurrence and the last follow-up or patient's death, respectively. For univariate survival analysis purposes, the Cox proportional hazards model were used to assess the association between clinical-pathological variables and time to local progression and overall survival. In order to identify independent prognostic factors, multivariate analysis was performed according to the Cox model using the stepwise mode for selection of significant variables by considering parameters with at least $P \leq .15$.

Results were considered significant with P values of less than .05. All analyses were performed using the SPSS Statistical Software (SPSS Inc., Chicago, IL, version 13.0).

RESULTS

Caseload

Considering the above inclusion criteria, 117 out of 144 melanoma patients with in-transit metastases of the extremities treated by isolated limb perfusion were considered for the present study. Out of this group, 5 patients were lost to follow-up and/or had incomplete information regarding tumor response to perfusion. Therefore, 112 patients were eligible for the present study, of whom 53 patients (47%) underwent melphalan-based isolated limb perfusion and 59 (53%) received TNF as well.

Patient and tumor characteristics and the technical aspects of isolated limb perfusion are listed in Table 1.

Postoperative Morbidity and Local Toxicity

No postoperative deaths occurred among the patients, nor were there cases with significant systemic toxicity. During the early postoperative days, all patients showed augmented plasmatic myoglobin levels, but none

TABLE 1 Patient, tumor, and isolated limb perfusion characteristics according to the drug regimen (melphalan and/or tumor necrosis factor [TNF]) in 112 TNM stage III melanoma patients with in-transit metastasis

Variables	Melphalan $n = 53$		Melphalan + TNF $n = 59$		Overall $n = 112$	
	No.	%	No.	%	No.	%
Mean age (y)	60.8		63.2		62.1	
Gender						
Male	12	22.6	17	28.8	29	25.9
Female	41	77.4	42	71.2	83	74.1
Site						
Upper limb	1	1.9	1	1.7	2	1.8
Lower limb	52	98.1	58	98.3	110	98.2
Lymph node status (3 missing)						
Negative	26	50.0	27	47.4	53	48.6
Positive	26	50.0	30	52.6	56	51.4
Lesion diameter (6 missing)						
<3 cm	43	82.7	41	75.9	84	79.2
≥3 cm	9	17.3	13	24.1	22	20.8
Number of lesions (7 missing)						
<5	37	71.2	28	52.8	65	61.9
5–15	11	21.2	12	22.6	23	21.9
>15	4	7.6	13	24.5	17	16.2
Locally advanced disease						
Absent	43	81.1	28	47.5	71	63.4
Present	10	18.9	31	52.5	41	36.6
Maximum temperature mean (°C)	41.2		40.8		41.0	
Mean flow (mL)	649		467		555	
Low toxicity						
Grade I	8	15.1	27	45.8	35	31.3
Grade II	41	77.4	28	47.5	69	61.6
Grade III	4	7.5	2	3.4	6	5.4
Grade IV	0	0	1	1.7	1	0.9
Grade V	0	0	1	1.7	1	0.9
Response						
Complete	22	41.5	35	60.3	57	51.4
Partial	26	49.1	17	29.3	43	38.7
No change	5	9.4	6	10.3	11	9.9

developed acute postoperative renal failure. The mean time for patients to revert to normal myoglobin values was 3 days (range, 2–9 days).

Local toxicity after isolated limb perfusion (Table 1) was mild (Wieberdink grade I–II) in 104 patients (93%). In 1 patient, fasciotomy was performed because of grade IV toxicity, and in another patient extensive rhabdomyolysis of a lower limb brought an amputation (Wieberdink grade V). Higher local toxicity (Wieberdink III–V) was more frequently observed among the patients treated with melphalan and TNF; however, the difference was not statistically significant between the two drug regimens ($P = 1.0$).

Response Rates

Tumor response rates are reported in Table 1. Complete response rate was higher among the patients who underwent isolated limb perfusion with TNF with respect to those who had undergone isolated limb perfusion with melphalan only (60.3% versus 41.5%; $P = .036$). Univariate and multivariate analysis of tumor response are detailed in Table 2. In the multivariate logistic regression model, only lymph node status independently predicted complete response after isolated limb perfusion ($P = .003$). Among those patients without metastases in the regional lymphatic basin ($n = 52$) a complete response

rate of 65.4% ($n = 34$) was observed, while 21 patients of 56 (37.5%) with lymph node metastases had a complete tumor response.

Among 40 patients with locally advanced in-transit metastases (i.e., more than 15 singular lesions or the diameter of the greatest nodule greater than 3 cm), 23 showed a complete response (57.5%). In 30 patients of this subgroup, isolated limb perfusion was performed with melphalan and TNF, and 20 (66.7%) had a complete response. Overall, this complete response rate is significantly higher ($P = .049$) than the one observed among patients treated with melphalan alone (3 of 10 patients, 30%).

Tumor Local Control

The median follow-up was 21 months (range, 3–194 months). Local progression occurred in 51 patients (46%), at a median time of 17.4 months (95% confidence interval [95% CI], 11.1–23.7 months). The 5-year local control was 38.8%. There was no significant difference in local control ($P = .26$) among the patients who had received melphalan alone (median time to local progression, 14.2 months; 95% CI, 12.0–16.4) or melphalan plus TNF (median time to local progression, 22.7 months; 95% CI, 5.1–40.3).

By considering the subset of complete responders after isolated perfusion, we observed a not significant time to local progression difference ($P = .14$) with (median time to local progression, not reached) or without (median time to local progression, 35.4 months; 95% CI, 16.9–53.9) the use of TNF. Median time to local progression was significantly longer ($P < .0001$) among patients who had a complete response (median time to local progression, not reached) versus those with a partial response and no change after perfusion (median time to local progression, 5.4 months; 95% CI, 3.5–7.3 months).

At a univariate and multivariate Cox regression analysis (Table 3), time to local progression was influenced only by the response to isolated limb perfusion ($P < .0001$).

Complete remission was maintained until death or last follow-up in 39 of 57 patients (68%) after complete response (median follow up of this subset: 25.7 months; range, 3–182). Within this group of patients, no significant predictors of long-term response duration were identified (data not shown).

Overall Survival

The 5-year overall survival rate was 28.5%; the median survival of the entire caseload was 34.8 months (95% CI, 25.3–44.3). There was no significant difference in survival ($P = .47$) among patients who received melphalan alone (median overall survival, 33.5 months; 95% CI, 23.2–43.8) or melphalan plus TNF (median overall survival, 34.8 months, 95% CI 9.5–60.1). Overall survival was significantly longer ($P = .0046$) in complete responders (median overall survival, 43.6 months; 95% CI, 2.2–85.0) than in patients with a partial response or no change after perfusion (median overall survival, 22.1 months; 95% CI, 7.9–36.3). Among complete responders patients, overall survival was not statistically different ($P = .09$) comparing those who received melphalan alone (median overall survival, 27.1 months; 95% CI, 12.3–41.9) or melphalan plus TNF (median overall survival, 95.5.8 months; 95% CI, 16.0–175.0).

At a univariate and multivariate Cox analysis, the survival was influenced only by tumor response ($P = .005$ and $P = .002$, respectively; Table 4).

TABLE 2 Univariate and multivariate analysis of clinical predictive factors for complete response achievement after isolated limb perfusion

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	*P*	OR	95% CI	*P*
Age (y)	1.01	0.98–1.05	.33			
Gender (female)	0.67	0.28–1.58	.36			
Lymph node status (negative)	0.31	0.14–0.69	.004	0.29	0.13–0.66	.003
Lesion diameter (≥3 cm)	1.33	0.50–3.49	.55			
Number of lesions						
5–15	0.51	0.17–1.51	.22			
>15	0.91	0.25–3.24	.88			
Locally advanced disease (present)	1.47	0.67–3.21	.33			
Drug regimen (melphalan + TNF)	2.14	1.004–4.57	.049	2.18	0.97–4.89	.059
Maximum temperature (°C)	1.39	0.82–2.38	.21			
Mean flow (mL)	1.00	0.99–1.00	.57			

TABLE 3 Univariate and multivariate analysis of clinical predictive factors for time to local progression after isolated limb perfusion

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (y)	0.99	0.97–1.02	.89			
Gender (female)	0.84	0.44–1.62	.61			
Lymph node status (negative)	0.57	0.32–1.007	.053			
Lesion diameter (≥3 cm)	0.89	0.45–1.76	.7			
Number of lesions						
5–15	0.82	0.37–1.84	.64			
>15	1.94	0.82–4.55	.12			
Locally advanced disease (present)	1.11	0.63–1.97	.7			
Drug regimen (melphalan + TNF)	1.37	0.78–2.39	.27			
Complete response to perfusion	0.17	0.09–0.31	<.0001	0.18	0.1–0.32	<.0001

TABLE 4 Univariate and multivariate analysis of clinical predictive factors for overall survival after isolated limb perfusion

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (y)	1.01	0.99–1.03	.29			
Gender (female)	1.60	0.94–2.72	.081			
Lymph node status (negative)	0.6	0.37–1.001	.05			
Lesion diameter (≥3 cm)	0.88	0.48–1.62	.68			
Number of lesions						
5–15	1.32	0.61–2.82	.47			
>15	1.35	0.57–3.20	.49			
Locally advanced disease (present)	1.19	0.72–1.98	.48			
Drug regimen (melphalan + TNF)	1.19	0.73–1.94	.47			
Complete response to perfusion	0.5	0.31–0.81	.005	0.46	0.28–0.76	.002

DISCUSSION

Isolated limb perfusion is nowadays a well-established approach for treating in-transit metastases from melanoma not amenable to excision and confined to a limb, since systemic chemotherapy can only provide a very low response rate and amputation is mostly considered an overtreatment that offers no advantage in terms of survival.[1,3] Melphalan is still considered the gold standard drug in terms of tumor response and tolerability, though the proposed association of TNF may have substantial effects on modulating patients' outcome.[6] However, the consensus about the role of this cytokine in isolated limb perfusion for melanoma patients is still lacking. In fact, the only published randomized trial comparing melphalan-based perfusion with or without TNF brought to a negative result (complete response rate of 26% in the experimental arm versus 25% when melphalan was administered alone).[20] Nevertheless, these findings have been criticized for many reasons: (1) the interval between isolated limb perfusion and tumor response evaluation (3 months) may be too short, since many cases of complete response are observed later than that; (2) there is a major overall discrepancy between the results of this study (complete response rate 25%–26%) and those reported by uncontrolled trials; (3) the absence of adequate stratification criteria for patients randomization (i.e., tumor burden and stage); (4) no long-term results regarding local tumor control were investigated; and (5) the statistical design of this trial was not based on an intention-to-treat analysis.[21]

Moreover, the optimal dosage of TNF (high, 3–4 mg versus low, 0.5–1 mg) in isolated limb perfusion is still under discussion since no prospective clinical trial addressing this issue has been so far conducted.[25]

For the reasons reported previously, the analysis of retrospective series, in particular those with a long-term follow-up, can still be of value in order to better standardize the isolated limb perfusion procedure and to ascertain possible clinical advantage derived from the use of TNF in association with melphalan.

Toxicity

Whether local toxicity is higher when TNF is associated to melphalan still remains a matter of discussion. A significantly higher regional toxicity after isolated limb perfusion with TNF was reported by Vrouenraets.[15] However, no significant difference in limb toxicity between the 2 drug regimens was observed in a retrospective series with high-dose TNF.[11]

In the present group of patients, low-dose TNF was employed, and local toxicity ranged from mild to moderate in the vast majority of cases. Although higher grades of local toxicity were experienced in some of our patients, no statistically significant difference was detected by comparing patients who received TNF with those treated with melphalan only.

Regarding the TNF dosage, a relationship between the amount of TNF administered via perfusion and the local toxicity was described by some authors.[26] These findings were not confirmed by an uncontrolled series, which reported similar local toxicity when TNF was used in high or low dose.[25]

In absence of randomized trials comparing clinical outcome after low- or high-dose TNF-based perfusion, these findings seem to suggest that the association of low-dose TNF does not significantly increase locoregional toxicity in melanoma patients undergoing isolated limb perfusion.

Short-Term Results

An overall complete response rate of 51% was observed in our patients. This is 42% when melphalan is perfused alone and 60% when TNF is associated with melphalan. The difference is particularly evident in patients with locally advanced tumor who experienced a complete response rate of 67% when TNF was used.

These findings are consistent with those reported in our previous experience on locally advanced melanoma and with those of other authors.[9–13,27]

The high response rate obtained after TNF-based isolated limb perfusion might be explained by the results from an in vitro study, in which the saturation of TNF receptors was obtained at a concentration level corresponding to a dose of 1 mg.[28] Moreover, in advanced limb sarcomas treated with low-dose TNF, we reported TNF perfusate concentrations 20-fold greater than those considered cytotoxic when in vitro, which remained steady during perfusion, supporting the hypothesis that 1 mg of cytokine is enough to saturate the entire uptake capacity of the limb.[29]

Complete tumor response is predicted by the use of TNF and the absence of regional lymph node involvement, which is the only independent predictor of complete response. This is in concordance with previously reported findings, thus suggesting that less aggressive disease might have a better chance of response after isolated limb perfusion.[10,16]

Long-Term Results

Considering that 60%–70% of our patients experienced a complete response, and local progression occurred in about half of these, an optimal local control was eventually achieved in about 1 patient of 3 who underwent isolated limb perfusion. Although patients treated with TNF had a longer median time to local progression when compared with those who received only melphalan, this difference did not reach statistical significance. The same results were reported in randomized trials and uncontrolled series that compared the efficacy of isolated limb perfusion with or without TNF (Table 5).[9,17,19,30] Overall, it seems that patients' long-term tumor local control is not influenced by TNF. Nevertheless, this observation is based on underpowered and heterogeneous studies, carried out on patients with different tumor burden. In fact, it might be speculated that an advantage in terms of tumor control with TNF might be observed in patients with locally advanced disease.

In the light of these considerations, only a randomized controlled trial with an adequate statistical power and patient stratification (i.e., based on tumor burden) will give a definitive answer on the impact of melphalan-based isolated perfusion with TNF in long-term tumor local control.

In the same context, a formal meta-analysis of the results so far obtained is not feasible. In fact, these studies did not report the minimum required information on time-to-event data, such as the number of events and the number of patients at risk at different time points, and hazard ratios, confidence interval and *P* values for both univariable and multivariable survival analyses.[31]

In the present study, the achievement of complete response was the only independent predictor for both tumor local control and survival. Waiting for the identification of reliable molecular prognostic factors, information such as tumor response to isolated limb perfusion might be of help when selecting the patients for adjuvant therapies, maximizing the likelihood of prolonged survival. For instance, the findings from our pilot study support the hypothesis that consolidation therapy with systemic low-dose interferon alpha might increase the duration of tumor local control after TNF-based isolated limb perfusion in patients with in-transit melanoma metastasis.[32]

CONCLUSIONS

In conclusion, patients who underwent isolated limb perfusion experienced a complete response in near 50% of

TABLE 5 Studies comparing the overall response rate, time to local progression, and overall survival after melphalan-based isolated limb perfusion with or without TNF for in-transit melanoma metastases of the limb

Authors, date	Study design	Median FU (mo)	TNF dosage (mg)	Patients	Complete response rate (%)	Time to local progression *P* value	Overall survival *P* value
Lienard, 1999[17]	RCT/Retrospective	NR	3–4	Melphalan: 103 TNF + melphalan[a]: 64	Melphalan: 52 TNF + melphalan: 73	NR	NR
Fraker, 2002[9]	RCT	NR	3–4	Melphalan: 51 TNF + melphalan[b]: 52	Melphalan: 58 TNF + melphalan: 72	NS	NS
Noorda, 2004[11]	Retrospective	21	3–4	Melphalan: 40 TNF + melphalan: 90	Melphalan: 45 TNF + Melphalan: 59	NS	NS
Alexander, 2009[19]	Retrospective	NR	3–4/6	Melphalan: 48 TNF + Melphalan[c]: 43	Melphalan: NR TNF + Melphalan: NR	NS	NS
Our series	Retrospective	21	0.5–1.0	Melphalan: 53 TNF + Melphalan: 59	Melphalan: 41 TNF + melphalan: 60	NS	NS

FU follow-up, *RCT* randomized-controlled trial, *NS* not significant, *NR* not reported

[a] A total of 31 patients also received interferon

[b] Patients received interferon along with TNF and melphalan

[c] 6 patients received 6 mg of TNF; 37 received also interferon

cases with a major regional toxicity in about 3%. Complete response was maintained in about one-third of patients who underwent isolated perfusion. Our results support the use of low-dose TNF in isolated limb perfusion mainly in melanoma patients with locally advanced tumor, on the basis of the high complete response rate (67%), which is superior to that achieved with melphalan alone especially in presence of locally advanced disease, and low local and systemic toxicity (3%). Given the decreasing response rate obtained in patients with lymphatic metastases, the lymph node status seems to be the best predictor of tumor response after perfusion. The best predictor of tumor local control and survival is the response to isolated limb perfusion.

Only a well-designed prospective randomized trial that compares the melphalan-based perfusions with or without TNF with sufficient statistical power, an appropriate time frame to assess tumor response, patient stratification (i.e., according to tumor burden), and investigation of tumor local control during follow-up, will give definitive evidence on TNF use in melanoma patients with unresectable limb in-transit metastases.

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