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Isolated Limb Perfusion for In-Transit Melanoma Metastases: Melphalan or TNF-Melphalan Perfusion?

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Indications for treatment of melanoma in-transit metastases (ITMs) confined to the limb with isolated limb perfusion (ILP) are not well defined. This study reports the Groningen regional therapeutic perfusion experience with melphalan (M-ILP) and TNF-melphalan (TM-ILP) for ITMs, and reviews of the melanoma TNF-melphalan ILP literature. Between 1991 and 2012, 60 patients were treated with ILP. Patients with "small" ITMs received M-ILP (10–13 mg melphalan/L limb volume) and patients with "bulky" disease TM-ILP (1–4 mg TNF); 19 M-ILPs and 41 TM-ILPs were performed, 26 Stage IIIB, 31 Stage IIIB and 1 stage IV disease. Overall response after 57 ILPs was 90%; CR 27 (45%), PR 27 (45%), no response 3 (5%); after 9 M-ILPs CR 6 (32%) and 41 TM-ILPs CR 21 (51%, $P = 0.124$). For younger patients (<65 years) CR was 69% and for elderly patients 29% ($P = 0.003$). For low volume disease (<5 ITMs) CR was 75% and for high volume disease (≥5 ITMs) 41% ($P = 0.038$). After median follow-up of 15 months (range, 1–144) there was local recurrence or disease progression in 36 patients (60%). Positive lymph node status was associated with local progression, absence of CR and Stage IIIC disease; these were independent prognostic factors for progression to systemic disease. M-ILP is an effective regional treatment for melanoma ITMs, whereas for bulky disease TM-ILP should be the first choice. In-field progression-free survival after ILP is determined by the biological behavior of the ITMs and the patient's immune system.
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KEY WORDS: melanoma; perfusion; in-transit metastases; regional chemotherapy; melphalan; TNFα

INTRODUCTION

Although most types of cancers are declining in incidence, melanoma incidence has increased steadily worldwide over the last 20 years. In the Netherlands the incidence rate increased over the last decade from 17.3 per 100,000 in 2002 to 30.4 per 100,000 in 2011. The increased incidence has been especially in the elderly [1]. The current melanoma incidence in the USA is 21.1 per 100,000, while the highest incidence rate of 49.8 per 100,000 is in Australia [2,3].

The first presentation of melanoma is as localized disease in 84% of patients, in regional lymph nodes in 9%, as metastatic disease in 4% and unknown stage in 4%, with relative 5 year survival rates of 98.3%, 62.4%, 16%, and 76.5%, respectively [1,2]. Of all primary melanomas 50% are located on the limbs (30% lower limb and 20% upper limb), 36% on the trunk and 14% in the head and neck region [1].

The treatment for primary melanoma, regional and/or distant disease is well defined in the various national melanoma management guidelines with respect to surgical margins, sentinel lymph node biopsy (SLNB), radiation therapy, immunotherapy, chemotherapy, drug targeting therapy and follow-up [4]. Melanoma is currently one of the most survivable cancers, although the behavior of individual melanomas is unpredictable. Important prognostic factors for disease-free survival are Breslow thickness, presence or absence of ulceration, mitotic rate, gender, and body site [5].

According to the incubator hypothesis the lymphatic route is the principal method of spread of melanomas from their original site to the lymph node field where the metastatic melanoma cells may survive and grow slowly or remain latent before, in some patients, spreading to distant sites [6]. The risk of developing in-transit metastases (ITMs) is higher if the pathology shows lymphatic invasion in the primary tumor [7]. Another risk factor is a high tumor mitotic rate (TMR) in the primary tumor [8]. Ultimately, 3–5% of melanoma patients will develop local recurrence or ITMs, 5–13% regional disease and 3–10% distant disease [9]. The median time to the development of ITMs is 13–16 months after initial adequate local excision of the melanoma [9]. Adjuvant isolated limb perfusion (ILP) with melphalan (M-ILP) did not prevent local and/or regional ITMs, or influence disease-free or overall survival in melanoma confined to a limb [10]. The risks of local recurrence or ITMs after wide local excision in the EORTC perfusion trial (18832) were 2.9% and 6.6%, respectively [10].

Melanomas that recur locally may be curable by wide local excision and those that have spread to regional lymph nodes may be curable with therapeutic lymph node dissection [11]. Although melanomas that have spread to distant sites are rarely curable, a small proportion of patients can be cured by surgical resection of all metastatic disease, with 5-year survival rates of up to 39% [12,13].

Dacarbazine (DTIC), approved since 1970, was until recently the most effective drug for unresectable disease, with response rates of 10–20% but no significant improvement in survival. Recently, two different treatment approaches, immunotherapy with a monoclonal antibody against cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4 (ipilimumab)) and molecular/drug-targeted therapy with a BRAF and/or a MEK inhibitor produced improvement in progression-free survival (PFS) and overall survival (OS) in melanoma patients with Stage IV disease [14–16]. Current studies are exploring the safety and effectiveness of the anti programmed cell death 1 (PD-1) receptor (anti-PD-1) in the treatment of advanced melanoma [17].

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In-Transit Metastases

The treatment of ITMs is less straightforward. ITMs are tumor emboli trapped in the lymphatics between a primary tumor and the regional lymph node basin. It has been suggested that elective lymph node dissection, sentinel lymph node biopsy or completion lymph node dissection increase the risk of development of ITMs. However, the risk of ITMs depends on the tumor biology and not to the surgical approach to the regional nodes [18]. Although ITMs can occur in any part of the body, the majority are diagnosed in the lower limb. It has been suggested that the higher incidence of ITMs in the lower limb may be caused by gravity and delayed lymphatic drainage.

The current management options for ITMs are local treatment, regional treatment or systemic treatment (Fig. 1). Isolated limb infusion (ILI) or isolated limb perfusion (ILP) with melphalan, M-ILI and M-ILP, are effective locoregional treatments for ITMs [19,20]. To further improve the outcome of ILP other drugs such as dacarbazine (DTIC), melphalan in combination with actinomycin C, adriamycin, mitomycin-C, thiothepa, cisplatin, carboplatin have been used to treat ITMs, but their effectiveness was limited or the local toxicity too high and they have therefore been abandoned in the perfusion setting [21,22].

Tumor Necrosis Factor Alpha

Twenty-five years ago Lejeune [23] explored the use of high-dose tumor necrosis factor-alpha (TNFα), interferon-gamma (IFN-) and melphalan in the ILP treatment (TM-ILP) of ITMs. After a small multicenter pilot study it was concluded a few years later that TM-ILP was the treatment of choice for in-transit melanoma metastases [24]. In a multi-center study ILP with TNFα (Beromun®) and melphalan was successfully investigated as a limb-saving treatment for locally advanced sarcomas [25,26]. The European Medicine Agency (EMA) approved Beromun® for irresectable sarcomas and "bulky melanoma," so called "melanosarcomas," in 1999 [27].

This article reports the Groningen ILP experience with therapeutic perfusions with M-ILP and TM-ILP for melanoma ITMs, and reviews the current status of TM-ILPs as reported in the literature.

PATIENTS AND METHODS

Patients

Between 1991 and 2012, 60 patients with ITMs were treated with ILP in Groningen, median age 65 (range 33–84) years, 14 males (23%) and 46 females (77%). Patients with "small" (low volume) ITMs were treated with melphalan ILP (M-ILP), whereas patients with "bulky" (high volume) disease were treated with TNFα and melphalan ILP (TM-ILP). There were 19 M-ILPs (32%) and 41 TM-ILPs (68%) performed. Patient, tumor/disease characteristics and AJCC stage of disease are summarized in Table I.

Perfusion Treatment

Lower limb ILP was performed at three different levels (iliac, inguinal (femoral), and popliteal), and upper limb ILP at two levels (axillary or brachial), depending on the anatomical location of the ITMs (Fig. 2). Isolation of the blood supply of the limb was achieved by clamping and cannulating the major artery and vein after heparinization of the patient, connection to the oxygenated extracorporeal circuit, ligation of collateral vessels, and application of a tourniquet at the root of the limb to occlude superficial veins.

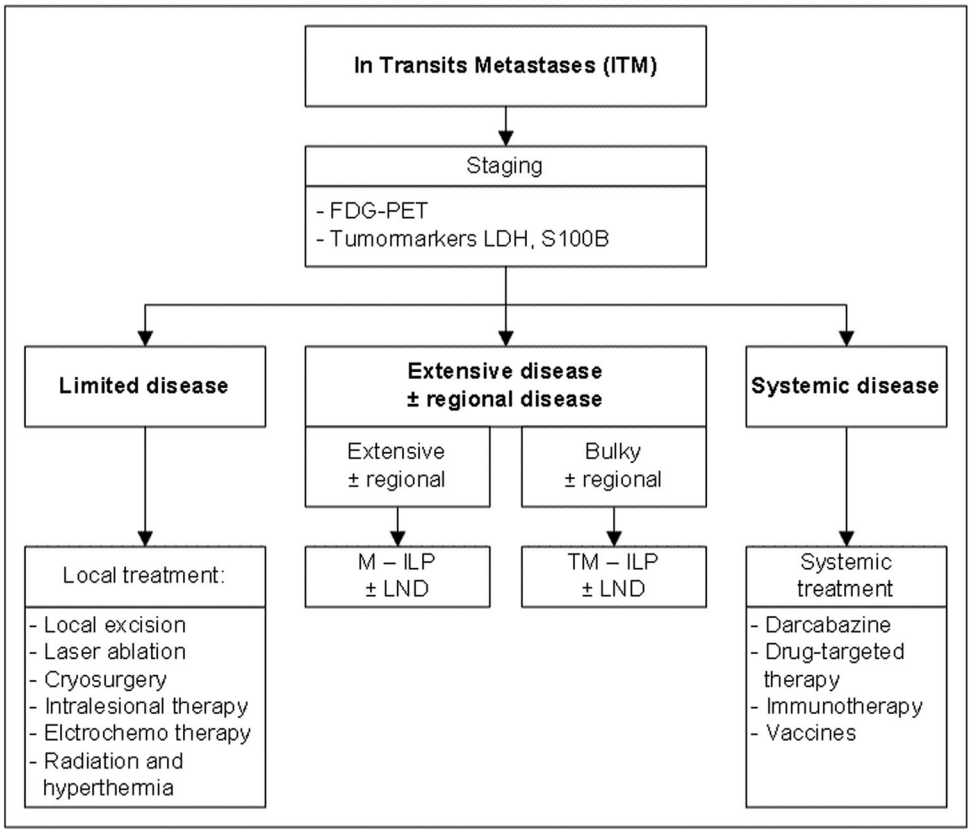

Fig. 1. Treatment options in-transit metastases (ITMs).

TABLE I. Patient and Tumor Characteristics

	Melphalan (n = 19) (No, %)	Melphalan + TNF-α (n = 41) (No, %)	Total (n = 60) (No, %)	*P*-value
Gender				
Male	5 (26%)	9 (22%)	14 (23%)	0.479
Female	14 (74%)	32 (78%)	46 (77%)	
Age				
Median in years (range)	62 (33–80)	65 (39–84)	65 (33–84)	0.556
<65 years	10 (53%)	18 (44%)	28 (47%)	0.586
≥65 years	9 (47%)	23 (56%)	32 (53%)	
Location PT				
Arm	1 (5%)	2 (5%)	3 (5%)	0.998
Leg	17 (90%)	36 (88%)	53 (88%)	
Unknown primary	1 (5%)	2 (5%)	3 (5%)	
Other	0 (0%)	1 (2%)	1 (2%)	
Breslow thickness				
Median in mm (range)	2.70 (0.70–16.0)	2.60 (0.80–10.5)	2.60 (0.70–16.0)	0.903
Ulceration				
Present	6 (32%)	14 (34%)	20 (33%)	0.412
Absent	10 (53%)	15 (37%)	25 (42%)	
Unknown	3 (15%)	12 (30%)	15 (25%)	
PT histology				
SSM	7 (37%)	11 (27%)	18 (30%)	0.629
Nodular	6 (32%)	10 (24%)	16 (27%)	
Acral lentiginous	1 (5%)	2 (5%)	3 (5%)	
Other/unknown	5 (26%)	18 (44%)	23 (38%)	
Interval PT and ITMs[a]				
<12 months	9 (50%)	17 (45%)	26 (46%)	0.466
≥12 months	9 (50%)	21 (55%)	30 (54%)	
Interval ITMs and ILP[b]				
<3 months	7 (37%)	18 (45%)	25 (42%)	0.380
≥3 months	12 (63%)	22 (55%)	34 (58%)	
Location ITMs[b]				
Arm	1 (5%)	2 (5%)	3 (5%)	0.653
Leg	18 (95%)	38 (95%)	56 (95%)	
Number of ITMs				
Median (range)	19 (4–35)	12 (1–40)	15 (1–40)	0.148
AJCC stage				
IIIB	10 (53%)	16 (39%)	26 (43%)	0.591
IIIC	8 (42%)	23 (56%)	31 (52%)	
IV	1 (5%)	2 (5%)	3 (5%)	

PT, primary tumor; SSM, superficial spreading melanoma; ITMs, in-transit metastases; ILP, isolated limb perfusion; AJCC stage, American Joint Committee on Cancer.
[a]Analysis on 56 patients: three missing because of unknown primary tumor. In one patient stage IV without ITMs indicated ILP.
[b]Analysis on 59 patients: in one patient stage IV without IT-metastases indicated ILP.

The pressure-regulated perfusion was performed under mild hyperthermia (38.5–40°C) with an extracorporeal circuit flow rate of approximately 500 ml/min [28]. The extremity was wrapped in a heating blanket to maintain optimal temperature. Temperature was monitored with thermistors. Leakage from the limb to the systemic circulation was monitored with radio-labeled 131-I human serum albumin using a precordial scintillation probe [29] (Fig. 3).

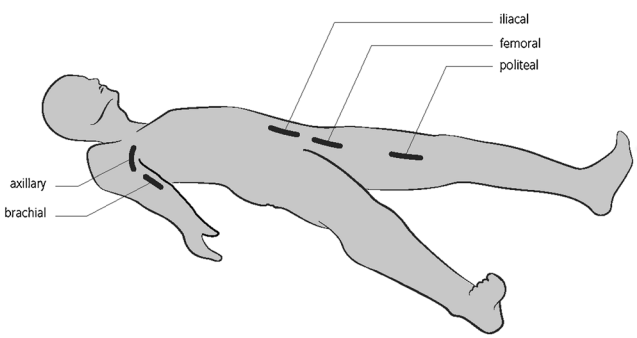

Fig. 2. Perfusions levels.

The perfusion time for M-ILP was 60 min, for TM-ILP 15 min with TNFα, and 45 min with mephalan. The dosage of melphalan was based on limb volume, 10 mg/L lower limb volume and 13 mg/L upper limb volume (Alkeran®, Burroughs Wellcome LTD., London, UK) [30]. The dosage of TNFα (Boehringer Ingelheim International GMbH, Ingelheim am Rhein, Germany) was 1–2 mg for the lower extremity (iliac/femoral ILP 2 mg, popliteal ILP 1 mg) and 1 mg for the upper extremity. The perfusion was stopped after 60 min and the extremity washed out with 3–6 L saline and filled, if indicated, with one unit red blood cell concentrate. Catheters were removed, vessels repaired, and heparin neutralized with protamine sulphate. The operative and technical details of the ILP procedure have recently been updated and described in detail [29,31].

A prophylactic, closed fasciotomy of the anterior compartment of the lower leg, or ventral and dorsal compartments of the forearm was performed through a 1 cm longitudinal incision of the skin in all patients to prevent a compartment syndrome.

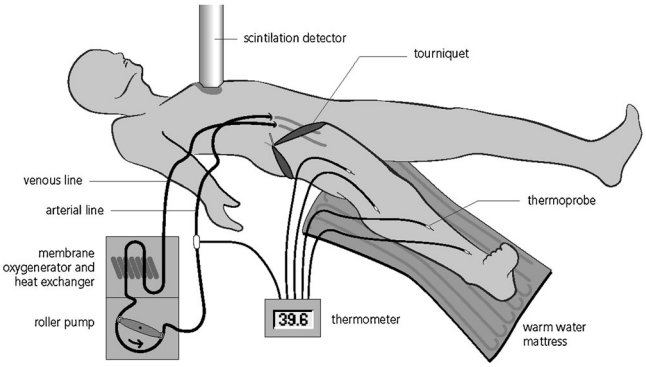

Fig. 3. Isolated regional perfusion.

Response Rates and Toxicty

Response rates were defined according to WHO toxicity criteria [32]. The treatment toxicity was recorded according the Wieberdink toxicity criteria [33] (Table II). Responses were assessed at 3 months after ILP and afterwards at 3-monthly intervals for the first year, 4-monthly intervals for the second year and at 6-monthly intervals thereafter.

Statistical Evaluation

Overall survival (OS), time to local progression (TLP) and time to systemic progression (TSP) were defined as the time between the ILP and death, local progression, or systemic progression, respectively. Survival curves were constructed by the Kaplan–Meier method and differences were assessed using the log-rank test. The Chi square test was used to calculate if there was a significant difference ($P < 0.05$) in the categorical variables between the M-ILPs and TM-ILPs. For continuous variables the Mann–Whitney *U*-test and the independent *t*-test were used, depending on the distribution of the variable. The Cox proportional hazards model was used to determine independent prognostic variables for TLP, TSP, and MSS. Prognostic variables for clinical response rate were determined by logistic regression analysis.

RESULTS

Treatment

In total 60 ILPs were performed, 19 with melphalan alone and 41 with melphalan plus TNFα; 57 (95%) of the ILPs were for lower limb disease, and 3 (5%) for upper limb disease. There were 21 (35%) iliac, 8 (13%) femoral, 28 (47%) popliteal, and 3 (5%) axillary perfusions. Iliac ILP was combined with deep groin dissection, femoral ILP with superficial groin dissection and axillary ILP with a level I–III axillary dissection. The median time between the diagnosis of ITM and ILP treatment was 4 (range, 0.3–66) months.

TABLE II. Wieberdink Toxicity Scale

Grade	Clinical characteristics
I	No subjective or objective evidence of reaction
II	Slight erythema or edema
III	Considerable erythema or edema with some blistering; slightly disturbed motility permissible
IV	Extensive epidermolysis or obvious damage to deep tissue causing definite functional disturbances; threatened or manifest compartmental syndrome
V	Reaction that necessitates amputation

Perfusions were performed at a median maximum temperature of 40.0°C (range, 38.6–41.9°C) with a median systemic leakage of 0.95% (range, 0–15%). A major leakage (>10%) was encountered in three patients (5%, Table III).

Some degree of acute regional toxicity was encountered in all patients after both M-ILPs and TM-ILPs: 38 Grade II (63%), 17 Grade III (28%), 4 Grade IV (7%) and 1 Grade V (2%) toxicity.

The median post-operative hospital stay after M-ILP was 9 (range, 4–34) days and after TM-ILPs 13 (range, 4–86) days (Table III); after axillary ILPs it was 14 (range, 5–15) days, after iliac ILPs 14 (range, 5–86) days, after femoral ILPs 23 (range, 9–66) days, and after popliteal ILPs 8 (range, 4–41) days.

Complications

There were two major perfusion-related complications requiring amputations; one after a severe technical perfusion pump-related complication with an air embolism and one after extensive tumor necrosis accompanied with severe infection of the limb, thus the initial limb salvage rate was 93%. There was no peri- or post-perfusion mortality.

Response Rates

The clinical response could not be assessed in three patients (5%), two who had amputation of the affected limb and one who was lost to follow up.

Three patients showed no response or progressive disease. The OR rate after 57 ILPs was 90%, for respectively 16 M-ILPs (84%) and 38 TM-ILPs (93%). There were 27 CRs (45%), 27 PRs (45%), and 3 patients showed no response or progressive disease (5%).

Eighteen M-ILPs resulted in 6 CRs (33%) and 39 TM-ILPs in 21 CRs (54%, $P = 0.124$) Patients less than 65 years of age had a CR rate of 69% after ILP, compared to 29% in the elderly patients (≥ 65 years, $P = 0.003$). CR was observed in 9 patients (75%) with low volume ITMs (1–5 ITMs), compared to 18 patients (41%) with high volume ITMs (>5 ITMs) ($P = 0.038$, Table IV).

Local Disease Control

Local recurrence or disease progression occurred in 36 patients (63%) after a median follow-up of 15 (range, 1–144) months; in 14 patients (78%) after M-ILP with a median progressive free interval of 14 months and in 22 patients (56%) after TM-ILP with a median progressive free interval of 16 months ($P = 0.466$).

Seventeen patients with a CR developed a local recurrence (63%) after a median follow-up of 19 months, whereas 19 patients with a PR (63%) developed progressive local disease after a median progressive free interval of 14 months ($P = 0.584$). This local disease progression necessitated amputation of the affected limb in two patients (3%), after 24 and 95 months, respectively.

Positive lymph node status was associated with local progression and was the only significant prognostic factor for local progression in multivariable analysis ($P = 0.036$, Table V).

Systemic Disease

Of the 57 patients who underwent ILP for ITMs with curative intent systemic disease developed in 33 patients (55%), after a median follow-up 40 (range 2–135) months, with no significant difference between the M-ILP and TM-ILP groups ($P = 0.613$). Of these 33 patients, 19 developed metastases in multiple locations (58%) and in 14 patients the metastases were limited to one organ (42%). The locations of distant metastases were lung (15), brain (7), bone (4), cutaneous (6), and intra-abdominal (9).

TABLE III. Perfusion Treatment Characteristics

	Melphalan	Melphalan + TNF-á	Total
Temperature			
Median °C (range)	39.8 (38.6–40.7)	40.0 (38.6–41.9)	40.0 (38.6–41.9)
Leakage			
Median % (range)	0.90 (0.00–12.00)	1.10 (0.00–15.00)	0.95 (0.00–15.00)
0–2% leakage	12 (71%)	25 (64%)	37 (66%)
2–10% leakage	3 (18%)	13 (33%)	16 (29%)
Major leakage	2 (12%)	1 (3%)	3 (5%)
Hospital stay			
Median days (range)	9 (4–34)	13 (5–86)	10 (4–86)

Eleven CR patients (41%) showed progression to systemic disease after a median follow up of 62 (range 2–67) months, and 21 PR patients (70%) after a median follow up of 17 (range 2–135) months ($P = 0.025$), also significant in a multivariable analysis ($P = 0.010$, Table V).

Patients with ulcerated primary melanomas developed systemic disease after a median time of 13 (range 2–62) months, compared to 67 (range 4–76) months for non-ulcerated primary melanomas ($P = 0.007$).

Patients with a short time interval (<12 months) between the primary tumor treatment and development of ITMs had a median TSP of 13 (range 2–62) months in contrast to patients with a time interval of >12 months who had a median TSP of 56 (range 2–135) months ($P = 0.035$). In patients with an interval of ≥18 months between primary tumor treatment and ILP a median TSP of 53 (range 2–13) months was observed, compared to 12 (range 2–62) months for patients with an interval <18 months ($P = 0.038$).

In multivariable analysis absence of CR and Stage IIIC disease were independent factors for progression to systemic disease (Table V).

Melanoma-Specific Survival

Fourteen M-ILP patients (74%) and 14 TM-ILP patients (34%) died of melanoma ($P = 0.006$). The median MSS for M-ILP/TM-ILP, Stage IIIB/C, treatment response, CR/PR + NR + PD and ulceration status are presented in the Figure 4A–D.

The overall 1-year, 3-year, and 5-year MSS rates after ILP were respectively 89%, 65%, and 39%. The median MSS of the complete cohort was 52 (range, 1–173) months; 51 months after M-ILP and 68 months after TM-ILP (Fig. 4A, $P = 0.196$). Median survival was 68 months for stage IIIB patients and 33 months for stage IIIC patients (Fig. 4B, $P = 0.003$). The median MSS after CR was 68 months and after PR/NR/PD 38 months (Fig. 4C, $P = 0.018$). The median MSS for ulcerated melanomas was 33 months and for non-ulcerated melanomas 83 months (Fig. 4D, $P = 0.021$).

The 5-year MSS for patients with a short time interval (<12 months) between primary melanoma treatment and development of ITMs was 15% and for patients with a longer time interval (≥ 12 months) it was 60% ($P = 0.077$).

In the univariate analysis absence of ulceration, AJCC stage IIIB, and CR were prognostic factors for prolonged MSS. In the multivariable analysis AJCC stage and clinical response rate were the two significant prognostic factors for survival (Table V).

DISCUSSION

The initial management of limited ITMs without signs of disseminated disease is local treatment, for example, surgical excision, cryosurgery, laser ablation, intralesional therapy, electrochemotherapy, and/or radiation ± hyperthermia (Fig. 1) [34]. If ITMs recur, local treatment is applied as long as possible. The disease free interval cannot be predicted and favorable immune responses are sporadically seen.

When the disease free interval after local treatment of ITMs in the limb is fast decreasing and/or the number of ITMs is rapidly increasing, i.e. where there is extensive or bulky disease, there may be an indication for regional therapy, for example, ILP or ILI. A drawback of ILP is the invasive and complex character of the procedure. ILI is as a minimally invasive alternative to ILP. There is no substantial difference in the DFS after ILP or ILI when melphalan is used [35]. Before initiating a regional therapy these IIIB/C patients are staged with FDG-PET and the tumor markers LDH and S100B [11]. There is no indication for a routine MRI of the brain in asymptomatic stage III melanoma patients [36]. Patients with disseminated disease are usually treated with systemic therapy, although there is sometimes an indication for a palliative ILP or ILI [37,38]. With M-ILP or M-ILI for ITMs remarkably effective regional control is achieved, but there is still a need for further therapeutic improvement [39]. TM-ILP has been shown to be an extremely effective limb salvage procedure for locally advanced sarcomas, but there is only a limited experience with TM-ILP for locally advanced ITMs [40].

TNFα in combination with melphalan can only be used in the regional setting, since TNFα plays a key role as a polypeptide mediator in the pathogenesis of septic shock [41]. TNFα is a vasoactive drug that causes destruction of the tumor vasculature in tumors that are highly vascularized and increases intratumoral vessel permeability, facilitating 3- to 6-fold higher drug uptake of melphalan in the tumor [42]. This is the

TABLE IV. Complete Response, Age and Number of ITMs

ILP	M-ILP (n = 6) (No, %)	TM-ILP (n = 21) (No, %)	Total (n = 27) (No, %)	*P*-value
Age				
<65 years	5 (56%)	13 (77%)	18 (69%)	0.003
≥65 years	1 (11%)	8 (36%)	9 (29%)	
Number of ITMs				
1–5 ITMs	1 (100%)	8 (73%)	9 (75%)	0.038
>5 ITMs	5 (29%)	13 (48%)	18 (41%)	

TABLE V. Analysis of Prognostic Variables for CR, Local Progression, Systemic Disease and Melanoma-Specific Survival

	Complete response		Local progression		Systemic disease		Melanoma-specific survival	
Variables	Univariable OR (*P*)	Multivariable OR (*P*)	Univariable HR (*P*)	Multivariable HR (*P*)	Univariable HR (*P*)	Multivariable HR (*P*)	Univariable HR (*P*)	Multivariable HR (*P*)
Gender								
Female[a] vs. male	1.00 (0.887)	—	1.28 (0.557)	—	1.25 (0.651)	—	1.80 (0.338)	—
Age								
<65 years[a] vs. ≥65 years	**0.18 (0.003)**	**0.17 (0.004)**	1.14 (0.708)	—	1.61 (0.202)	—	1.68 (0.191)	—
Location PT								
Leg[a] vs. arm	0.00 (0.999)	—	0.92 (0.909)	—	1.11 (0.883)	—	1.15 (0.431)	—
Breslow thickness (in mm)	0.83 (0.122)	—	1.03 (0.663)	—	1.08 (0.159)	—	1.04 (0.585)	—
Ulceration PT								
Absent[a] vs. Present	0.90 (0.864)	—	1.54 (0.284)	—	**3.35 (0.007)**	—	**3.06 (0.029)**	—
Interval PT and IT-metastases								
<12 months[a] vs. ≥12 months	2.37 (0.127)	—	0.74 (0.396)	—	**0.46 (0.035)**	—	0.45 (0.083)	—
Number of IT-metatases								
1–5[a] vs.>5 IT-metastases	**0.23 (0.046)**	**0.19 (0.039)**	1.30 (0.561)	—	2.29 (0.172)	—	2.81 (0.099)	—
Lymph node status								
Negative[a] vs. positive	1.86 (0.251)	—	**2.07 (0.036)**	**2.07 (0.036)**	1.78 (0.137)	—	2.09 (0.075)	—
AJCC stage of disease								
IIIB[a] vs. IIIC	2.18 (0.164)	—	1.36 (0.372)	—	**3.58 (0.003)**	**4.15 (0.002)**	**3.61 (0.005)**	**4.32 (0.003)**
Interval PT and ILP								
<18 months[a] vs. ≥18 months	1.82 (0.307)	—	0.73 (0.390)	—	**0.47 (0.038)**	—	0.49 (0.077)	—
Drug regimen								
Melp[a] vs. melp + TNF	2.33 (0.154)	—	0.73 (0.360)	—	0.83 (0.613)	—	0.62 (0.201)	—
TNF dose								
Low[a] vs. high	0.77 (0.688)	—	1.01 (0.974)	—	1.41 (0.465)	—	0.93 (0.954)	—
Clinical response								
No CR[a] vs. CR	NA	NA	0.72 (0.343)	—	0.18 (0.054)	**0.36 (0.010)**	**0.39 (0.023)**	**0.30 (0.006)**

CR, complete response; PT, primary tumor; IT-metastases, in-transit metastases; AJCC, American Joint Committee on Cancer; ILP, isolated limb perfusion; Melp, Melphalan; NA, not applicable. Bold these are the signifcant data, in the univariate and multivariate analyses.

[a]Reference group.

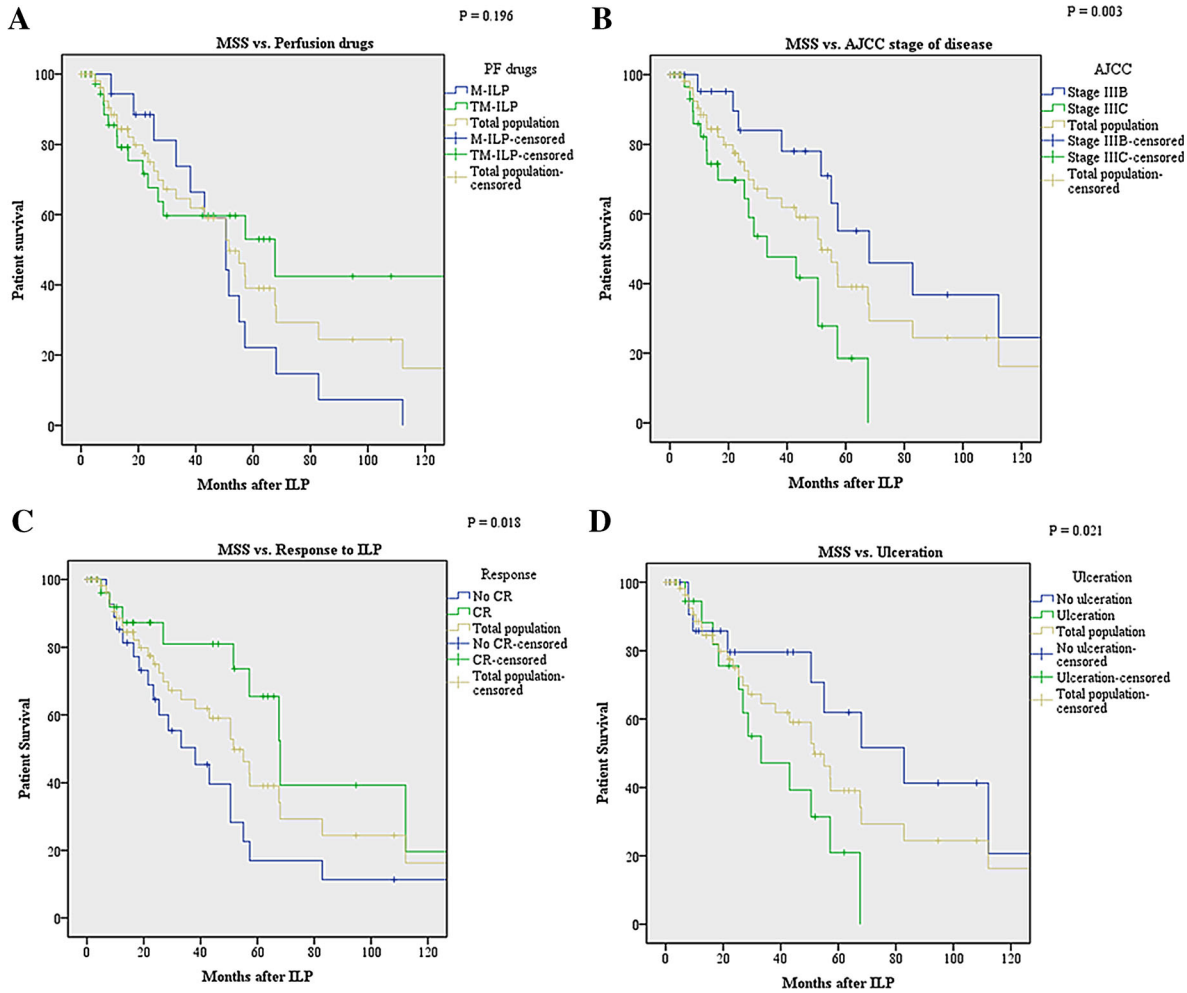

Fig. 4. Melanoma-specific survival: (**A**) MSS versus perfusion drugs; (**B**) MSS versus AJCC stage of disease; (**C**) MSS versus complete response; (**D**) MSS versus ulceration.

rationale for using M-ILP for "limited disease" and TM-ILP for the more vascularized "bulky disease." One of the disadvantages of TM-ILP is the systemic toxicity which is significantly correlated with high TNFα doses [43]. This was the major reason for the perfusion centers in Rotterdam and Groningen to lower the TNFα dose a decade ago. The reduced perfusion time with TNFα and melphalan and the better washout with an increased volume of saline resulted in decreased cardiovascular instability of the perfused patient during and after TM-ILP [44].

One clinical dose-finding study for TNFα in TM-ILP for melanoma was performed and two groups reported the use of low dose TNFα in ILP [45–49]. Escalating the TNF dose to 6 mg did not increase the complete response rate, but increased regional toxicity [45]. Grünhagen et al. [47] showed in a series of 100 TM-ILPs for melanoma ITMs that TNFα dose reduction did not alter the ILP outcome with respect to overall response or disease outcome The TNFα dose reduction reduced perfusion cost by 3,000–4,000 per ILP procedure. A recent update of this study showed however that larger doses of TNFα were significantly more effective in achieving CRs (70% vs. 49%, $P < 0.006$). Nevertheless, the high percentage of CRs achieved with high-dose TNFα did not translate into a prolongation of OS (16 vs. 11 months, $P = 0.076$) [49].

There are limited data about the use of TM-ILP for ITMs in melanoma patients, as shown in Table VI. There has been only one randomized trial, the ACSOG Z0020 trial, and four retrospective studies comparing M-ILP versus TM-ILP, plus the current series [46,55–58]. The initial results of the ACSOG Z0020 trial published in an abstract were more impressive than the final results [57,59]. The results of the retrospective non-randomized studies showed more CRs after TM-ILP overall, in contrast to Cornett who found no significant difference in the CR rate after 3 months [46,55–58]. However, the CR rate in the Cornett series was extremely low in comparison to the CR rates reported by others and summarized in Table VI.

There is no higher regional complication rate after TM-ILP compared to M-ILP [65]. Therefore double perfusions with TM-ILP can be performed. This is the main reason that most perfusion centers start to perfuse (1) at the most distant perfusion level which is possible and, if indicated, a second perfusion can be performed more proximally and (2) start with M-ILP, if possible, so that a second ILP with TNFα and melphalan can be performed if necessary for recurrent disease.

Although TNFα (Beromun®) is registered in Europe [27], it is not FDA-approved. The FDAs's attitude is mainly based on the preliminary results of the multi-institutional study and ACOSOG trial, which was closed early after an interim analysis showed no evidence of improved responses after 3 months. This decision was extensive criticized. However, a recent large single center study also failed to show improvement in regional in-field progression-free survival [63].

TABLE VI. Therapeutic TM-ILPs for In-Transit Metastases

Study	Year	Refs.	Design	Perfusion treatment	N	Objective	CR	CR
Lienard	1992	[50]	Retrospective	Melphalan and TNFα	19	100%	89%	
Lejeune	1993	[51]	Retrospective	Melphalan and TNFα	44	100%	90%	
Lienard	1994	[52]	Retrospective	Melphalan and TNFα	53	100%	90%	
Vaglini	1994	[53]	Retrospective	Melphalan and TNFα	22	77%	64%	
Eggermont	1995	[54]	Retrospective	Melphalan and TNFα	22	100%	88%	
Fraker	1996	[45]	Retrospective	Melphalan and TNFα	26	92%	76%	
Bartlett	1997	[55]	Retrospective	Melphalan and TNFα	58	94%	65%	
Lienard	1999	[56]	Retrospective	Melphalan ± TNFα	167	95%	73%	52% vs 73%
Fraker	2002	[57]	RCT	Melphalan ± TNFα	103	—	—	58% vs 72%
Noorda	2004	[58]	Retrospective	Melphalan ± TNFα	130	77%	55%	45% vs 59%
Grunhagen	2004	[47]	Retrospective	Melphalan and TNFα	100	95%	69%	
Rossi	2004	[48]	Retrospective	Melphalan and TNFα	20	95%	70%	
Cornett	2006	[59]	RCT	Melphalan ± TNFα	124	—	—	25% vs 26%
Hayes	2007	[60]	Retrospective	Melphalan and TNFα	27	77%	41%	
Rossi	2008	[61]	Retrospective	Melphalan and TNFα	31	—	2%	
Di Filippo	2009	[62]	Retrospective	Melphalan and TNFα	113	88%	63%	
Alexander	2010	[63]	Retrospective	Melphalan and TNFα	91	95%	69%	
Rossi	2010	[46]	Retrospective	Melphalan ± TNFα	112	90%	51%	40% vs 60%
Deroose	2011	[64]	Retrospective	Melphalan and TNFα	105	93%	68%	
Deroose	2012	[49]	Retrospective	Melphalan and TNFα	167	89%	61%	
Hoekstra[a]	2014		Retrospective	Melphalan ± TNFα	57	90%	45%	32% vs 51%

TM-ILPs, tumor necrosis factor alpha and melphalan isolated limb perfusion; CR, complete response; Ref, references.
[a]Current series.

In conclusion, the current study and the M-ILP and TM-ILP literature show clearly that the longer the time interval between primary tumor treatment and the development of ITMs and the smaller the tumor load the better the MSS and overall survival. TM-ILP and TM-ILI are effective regional treatments for ITMs of melanoma, without a substantial difference in outcome. The first ILP option is a M-ILP, whereas for bulky disease TM-ILP should be the first choice, since reponses for TM-ILP are overall better than M-ILP. The regional in-field progression-free survival after regional therapy is determined (1) by the biological behavior of the ITMs and (2) the patient's immune system.

For patients who are not candidates for regional M-ILP, TM-ILP or M-ILI therapy, novel promising local therapies are currently being tested in phase II and phase III trials. Such novel therapies include intralesional therapy with Rose Bengal for chemoablation of ITMs [66]. It will also be essential to study the effect of new systemic therapies, such as drug-targeted BRAF and/or MEK inhibitors as well as immune targeting therapy in ITM patients.

The introduction of TM-ILP 25 years ago for the treatment of ITMs was exciting. Today we have further exciting new treatments, for example, drug-targeting and immune targeting therapies, for advanced melanoma. Whether these systemic treatments will have the same or better local effects on melanoma ITMs will be studied in the coming years. Until then, regional therapy with TM-ILP will remain an effective local therapy for locally advanced melanoma confined to a limb, with acceptable morbidity and a high limb salvage rate.

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