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A Prospective Study of Intraarterial Infusion Chemotherapy in Advanced Wild-Type *BRAF* Melanoma Patients

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ABSTRACT

Background: Treatment strategies for advanced cutaneous melanoma (CM) patients, resistant or not treatable with novel target and immunotherapeutic drugs, remain a significant challenge, particularly for patients with unresectable stage IIIC/D disease localized to inferior limbs and pelvis, for whom specific outcomes are rarely considered.

Materials and Methods: This is a prospective study of multidisciplinary treatments, including locoregional melphalan chemotherapy, in 62 *BRAF* wild-type CM patients with locoregional metastases in the inferior limbs and pelvis, including inguinal regions. Patients were either in progression following or ineligible for, or not treatable with novel immunotherapy. For exclusively inferior limb-localised disease, patients received locoregional melphalan chemotherapy performed by hyperthermic isolated limb perfusion ($n = 19$) or isolated limb infusion ($n = 19$), and for synchronous lesions localised to inferior limbs and pelvis, received hypoxic pelvic and limb perfusion ($n = 24$). Additional multidisciplinary therapy included local, locoregional and systemic treatments and the primary endpoint was tumour response.

Abbreviations: WT, wild-type; BRAF, v-Raf murine sarcoma viral oncogene homolog B; Trk, tropomyosin receptor kinase.

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Results: The objective response rate following first cycle of locoregional chemotherapy was 37.1% at 3 mo and median progression-free survival was 4-mo, with 12.9% procedure-related complications, 30.6% low-grade haematological toxicity and 11.3% severe limb toxic tissue reactions. Multivariate logistic regression showed that the odds of response were significantly higher for patients ≤ 75 y of age and for patients with locoregional metastases exclusively located in the inferior limbs.

Conclusion: In this subgroup of CM patients with *BRAF* wild-type status, locoregional metastases localized to inferior limbs and pelvis, in progression following or ineligible for immunotherapy, melphalan locoregional chemotherapy demonstrated a safe and effective profile.

Trial Registration: ClinicalTrials.gov Identifier NCT01920516; date of trial registration: August 6, 2013.

Introduction

Cutaneous melanomas (CMs) are increasing in incidence and recur in approximately 10% of cases as locoregional metastases, including local recurrences, in-transit and satellites metastases, and regional lymph node metastases. Approximately 4% of locoregional metastases cumulatively localise to inferior limb, inguinal and pelvic regions, with synchronous inferior limb, inguinal region and/or pelvic involvement observed in approximately 2% of cases.[1-3]

Over the past 15 y, local, regional and systemic treatments for locoregional metastatic melanoma have evolved and high-volume specialist centres now provide local, regional, and systemic therapeutic options. However, these therapeutic options are not uniformly recommended by current international guidelines[1,4-10]; in particular, there are guideline differences in recommended local therapeutic procedures and both Japanese[10] and some European guidelines[7] do not recommend locoregional chemotherapy. Local treatments include surgical resection, electro-chemotherapy (ECT), ablative, topical and intralesional therapies.[11-13] Regional treatment options include regional radiation therapy[14] and locoregional chemotherapy by hyperthermic isolated limb perfusion (HILP) or isolated limb infusion (ILI) for locoregional melanoma metastases located in the limbs,[1] or hypoxic pelvic and limb perfusion (HPLP) for cases of synchronous inferior limb and inguinal region and/or pelvic involvement.[15] Systemic treatments, currently proposed for locoregional metastases judged to be unresectable for technical or clinical reasons, include single or combinations of agents with local and/or regional treatments.[11] In patients with unresectable stage III and IV CM, 4-y overall survival (OS) rate of approximately 58% and median survival time of approximately 20 mo have been reported.[16] Patients with unresectable stage III melanoma, however, represent only 3% of cases evaluated in novel target and immunotherapy studies over the past 5 y, making it impossible to extrapolate accurate outcomes of stage IIIC/D patients with synchronous locoregional metastases in inferior limbs and pelvis.[16] Furthermore, target therapy has been reported to provide a significant improvement in overall median survival of only 50% in patients with $BRAF^{V600E}$ mutated CM,[17,18] and novel immunotherapies reported to be efficacious in only approximately 45% of patients with wild-type *BRAF* CM.[19-21] Moreover, patients presenting with concomitant autoimmune disorders, chronic viral infections, organ dysfunction, organ transplants, brain metastases or who are either pregnant, too old or too frail have been excluded from the majority of immune checkpoint inhibitor clinical trials, although a number of trials have addressed systemic therapies in patients with brain metastases and trials are ongoing in patients with organ transplants.[22]

The current plethora of therapeutic options for melanoma not only reflects the fact that not all treatments are available in every institution but also that no single therapeutic strategy fits all cases. The selection of therapy is based on lesion number, size and anatomic location, regional lymph node involvement and/or distant metastases, biomolecular aspects, concomitant disease and response to previous therapies.[11] According to the majority of current guidelines,[1] therefore, a multidisciplinary therapeutic approach to CM should include locoregional chemotherapy, particularly for patients with locoregional inferior limb and pelvic metastases not responsive or eligible for novel target and immune therapies, who traditionally have bad therapeutic outcomes.[23,24]

Here, we address this issue in a prospective, real-life, open-label, multicentre study of a selected group of stage IIIC and IIID wild-type *BRAF* CM patients with locoregional inferior limb, pelvic and/or inguinal metastases, in progression following or not eligible for novel immune therapies. This patient population was submitted for multidisciplinary treatments, including locoregional melphalan chemotherapy.

Material and Methods

Patients

From 2012-2020, 62 CM patients (median age = 67.5 y, interquartile range = 58-75) with locoregional limb and pelvic

or inguinal metastases were prospectively enrolled as predefined subset of a larger trial database of CM patients treated with melphalan locoregional chemotherapy (ClinicalTrials. gov Identifier NCT01920516). Inclusion criteria for this subset were: (1) locoregional metastases (local recurrences, in-transit and satellite metastases, and regional lymph node metastases) located in inferior limbs, or in limbs and pelvis/including inguinal region (synchronous metastases); (2) *BRAF* wild-type status, and (3) progression following novel immunotherapy or ineligibility for clinical or non-clinical reasons, including: the absence of National Health System approval, administrative problems or for economic reasons. Patients with acral melanomas or upper limbs lesions were excluded from this study. None of the patients had nodal disease only. The patients included in this study were not involved in previous studies[3,24,25] and will not be included in future NCT01920516 reports. In accordance with Hospital Review Board regulations and the Helsinki Declaration, written consent was obtained from all patients and the study was approved by the relevant ethics committees (protocol numbers 0015956/2013 and 10/CE/2018).

Locoregional melphalan chemotherapy

Based on previous pharmacokinetic and clinical studies,[25-30] melphalan was used for locoregional chemotherapy at a dose of 35 mg/m^2. The selection of locoregional chemotherapeutic procedure was made by a multidisciplinary board, as follows: (1) HILP for patients $<$ 76 y old with $<$ 2 ECOG performance status and locoregional limb metastases; (2) ILI for patients $>$ 75 y old and/or with $\geq$ 2 ECOG performance status and locoregional limb metastases, and (3) HPLP for patients with locoregional limb and pelvic metastases. The HILP, ILI, and HPLP procedures have been previously described[15,25,30] and all procedures employed an extra-corporeal circulation machine. HILP was performed with oxygenation, high flow rates (150-1000 ml/min) and circuit hyperthermia to maintain tissue normothermia or mild tissue hyperthermia (39°C). ILI and HPLP were performed under hypoxic conditions with low flow-rates (50-150 ml/min) and mild circuit hyperthermia to maintain tissue normothermia, with the option of chemofiltration. Both HILP and HPLP procedures require specialized surgical skill, the HPLP procedure can also be performed percutaneously, whereas the ILI procedure requires an interventional radiologist. A percutaneous approach was chosen to minimize invasiveness and was contraindicated if: (1) iliac access was necessary in relation to fibrosis of the femoral vessel area; (2) lymphadenectomy was required, or (3) if the diameter of the common femoral artery was $\leq$ 7 mm, making vessel dissection risky.[28]

Further multidisciplinary treatments

Based on multidisciplinary board recommendations, following the first locoregional chemotherapy cycle, 28 patients received best supportive care for symptoms and 34 patients received treatments with curative intents, including surgery in 15 patients (6 of whom were submitted to ileo-inguinal lymph node dissections), surgery and diathermy-fulguration in six patients, ECT in one patient, locoregional chemotherapy procedures in 25 patients, systemic chemotherapy with temozolomide in one patient, immunotherapy with interleukin-2 inone patient and pembrolizumab in two patients. The two patients who received pembrolizumab were previously considered untreatable with this drug, due to prior absence of National Health System approval. At progression, all patients received best supportive care exclusively. Timing of locoregional chemotherapy repetitions (6/7-wk intervals) was based on previous studies, reporting disease-relapse in the presence of residual disease and initiation with progression by 8 wk in aggressive disease states.[15] Locoregional chemotherapy was not repeated, if: (1) locoregional metastases had progressed; (2) simultaneous distant relapses had occurred; (3) the general condition of the patient had worsened, or (4) if the patient refused treatment or withdrew consent. Bi-monthly surveillance included: clinical evaluation, photographic comparison, computed tomography (CT), magnetic resonance imaging (MRI) and/or positron emission tomography (PET).

Endpoints

The primary endpoint of this study was tumour response and secondary endpoints were adverse events, progression-free survival and OS.

Performance status, tumour response, and adverse events criteria

Patients' performance status was defined according to ECOG.[31] Response was determined approximately 45 d after the first melphalan locoregional chemotherapy procedure. The follow-up modalities and timing were not previously defined in the protocol of the NCT01920516 trial. Timing of response evaluation was based on previous studies, reporting disease-relapse in the presence of residual disease and initiation with progression by 8 wk in aggressive disease states.[15] Responses of deep mass and lymph node metastases were assessed by CT, MRI and PET, and classified using Response Evaluation Criteria in Solid Tumours (version 1.1), as either complete responses (CR), partial responses (PR), stable disease (SD) or progressive disease (PD).[32] Responses of superficial lesions were assessed by physical examination and photographic comparison. The objective response rate (ORR) reflects the percentage of patients exhibiting a minimum 30% reduction in tumour volume (PR) or the complete disappearance of tumour after treatment (CR). Adverse events were evaluated using CTCAE software (v4.03) (National Cancer Institute, Bethesda, Massachusetts, USA) and toxic reactions in infused/perfused tissues were evaluated according to the Wieberdink toxicity score.[33]

Statistical analysis

Data are summarized as medians and interquartile range (IQR). Univariate associations between response patterns and clinical variables were calculated using χ^2 tests, setting the I type error at 5%. In accordance with variables in this study, odds ratios responses were estimated, 95% confidence interval calculated using the Woolf method and multivariate anal-

ysis of responses addressed by multivariate logistic regression. Independent variable selection followed a 4-step process: (1) univariate test statistical significance; (2) a Cramer value (V) approaching 1; (3) a variance inflation analysis of previously selected variables (namely, variables were excluded if the variance inflation index was > 1.5), and (4) a model set according to optimal (minimum) Bayesian information criteria. Model specification was assessed by a link test for the single-equation model devised. Progression-free survival and OS were analysed by Kaplan Meyer survival curve and log-rank tests. Variables were categorized at the outset and unadjusted hazard ratios were estimated. Progression and mortality risk assessments were addressed using a multivariate Cox regression model, adjusted for selected confounders according to the aforementioned selection steps. Model fitting was checked by log-likelihood ratio test and statistical analyses were computed using STATA software (version 14) (Stata Corp, College Station, Texas, USA).

Results

Descriptive characteristics of the 62 patients included in this study are presented in Table 1. With respect to histologic characteristics, all metastatic tissues were characterized by the presence of epithelioid cells and the absence of spindle cells and tumour-infiltrating lymphocytes. With respect to previous immunotherapeutic treatments for locoregional metastases, three patients were in progression following ipilimumab therapy, pembrolizumab therapy was interrupted in one patient due to pneumonitis and 58 patients were deemed ineligible for novel immunotherapy by a multidisciplinary board (Supplementary Material Table).

Table 1 – Characteristics of 62 stage IIIC and IIID, *BRAF* wild type CM patients with locoregional limb and pelvic and/or inguinal metastases.

	N	%
Gender		
- Female	42	67.7
- Male	20	32.3
ECOG performance status		
- 0	18	29.0
- 1	27	43.6
- 2	16	25.8
- 3	1	1.6
- 4	0	0
Primary site of melanoma		
- Lower extremity	58	93.6
- Gluteal region	1	1.6
- Anterior abdominal wall	2	3.2
- Back	1	1.6
Location of the locoregional metastases		
- Inferior limbs plus pelvis including inguinal region	24	38.7
- Inferior limbs	38	61.3
Stage[26]		
- IIIC	47	75.8
- IIID	15	24.2
Burden[27]		
- Low burden (< 10 nodules; or no lesion > 3 cm)	33	53.2
- High burden (≥ 10 nodules; or one lesion > 3 cm)	29	46.8
Metastatic cells producing melanin		
- Yes	34	54.8
- No	28	45.2
Mitotic rate of the metastatic cells		
- ≥ 1 mitosis per mm^2	22	35.5
- < 1 mitosis per mm^2	40	64.5
Previous therapies of the locoregional metastases		
- No previous treatment	24	38.7
- Dacarbazine-based systemic chemotherapy	9	14.5
- Interferon alpha and/or interleukin-2	15	24.2
- Palliative excision	32	51.6
- Ipilimumab	3	4.8
- Pembrolizumab	1	1.6
- Electrochemotherapy	6	9.7

ECOG = Eastern Cooperative Oncology Group.

Primary endpoint

In this 62 WT-*BRAF* CM patient-cohort, the ORR was 37.1% following first cycle of locoregional chemotherapy and was comprised of 15 CRs (24.2%) and 8 PRs (12.9%). Responses (CR plus PR) were significantly higher in patients ≤ 75 y old, patients with ECOG performance status < 2, patients with locoregional metastases exclusively located in inferior limbs, patients with stage IIIC disease and patients with tumours with < 1 mitosis per mm^2 (Table 2). With respect to therapeutic procedure, HILP, ILI and HPLP elicited response rates of 89.5% (13 CR, 4 PR, 2 SD), 15.8% (1 CR, 2 PR, 15 SD, 1 PD) and 12.5% (1 CR, 2 PR, 21 SD), respectively, and responses were significantly higher in patients submitted for HILP (Table 2). Based on multivariate logistic regression, the odds of response were significantly higher for patients ≤ 75 y of age and for patients with locoregional metastases exclusively located in the inferior limbs (Table 3).

Secondary endpoints

Adverse events

Procedure-related complications occurred in 12.9% of patients and haematological toxicity occurred in 30.6% of patients and were not statistically different between procedures. Limb toxic tissue reactions were detected in 61.3% of patients (19 HILP patients and 19 ILI patients) and severe reactions (Wieberdink grade 3 or 4) were detected in 11.3% of patients. Although autonomous mobility was delayed by the HILP procedure, all patients were able to walk independently upon hospital discharge. One patient, however, developed postoperative foot drop (Table 4).

Progression-free survival

Post therapeutic local or loco-regional progression characterised 48 patients (90.6%) and distant site progression char-

Table 2 – Responses (CR plus PR) in relation to patient and/or tumour variables and therapeutic procedures.

Variables	N. of responses (% within groups)	P value of Pearson's χ^2 test	Univariate OR (95% CI)
Age			
≤ 75	21/47 (44.7%)	0.029	5.3 (1.1-25.9)
> 75	2/15 (13.3%)		
Gender			
Male	5/20 (25.0%)	0.174	0.4 (0.1-1.5)
Female)	18/42 (42.9%)		
ECOG performance status			
< 2	20/45 (44.4%)	0.050	3.7 (0.94-14.8)
≥ 2	3/17 (17.7%)		
Location of the locoregional metastases			
Inferior limbs plus pelvis	3/24 (12.5%)	0.001	0.1 (0.0-0.5)
Inferior limbs	20/38 (52.6%)		
Stage			
IIIC	23/47 (48.9%)	0.005	12.3 (1.5-101.4)
IIID	1/15 (6.7%)		
Burden			
Low	11/33 (33.3%)	0.513	0.7 (0.25-1.9)
High	12/29 (41.4%)		
Metastatic cells producing melanin			
No	8/28 (28.6%)	0.207	0.5 (0.17-1.5)
Yes	15/34 (44.1%)		
Mitotic rate of the metastatic cells			
< 1	20/40 (50.0%)	0.005	6.3 (1.62-24.8)
≥ 1	3/22 (13.6%)		
Previous therapies of the locoregional metastases			
No	12/24 (50.0%)	0.095	2.4 (0.85-7.1)
Yes	11/38 (29.0%)		
Type of locoregional chemotherapy			
- HILP versus ILI			
– HILP	17/19 (89.5%)	0.0001	45.3 (6.7-307.7)
– ILI	3/19 (15.8%)		
- HILP versus HPLP			
– HILP	17/19 (89.5%)	0.0001	59.5 (8.9-397.8)
– HPLP	3/24 (12.5%)		
- ILI versus HPLP			
– ILI	3/19 (15.8%)	0.757	1.3 (0.2-7.4)
– HPLP	3/24 (12.5%)		

CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; HILP = hyperthermic isolated limb perfusion; HPLP = hypoxic pelvic and limb perfusion; ILI = isolated limb infusion; OR = odds ratio.

acterised five patients (9.4%). The median time of progression-free survival was 4 mo (IQR = 3-6). Univariate analysis indicated that progression-free survival was significantly prolonged in patients with ECOG performance status < 2, lesions located exclusively in inferior limbs, stage IIIC disease, in patients with < 1 mitosis per mm^2 in metastatic tissues, and in patients submitted for the HILP locoregional chemotherapy procedure (Table 5; Part A). However, multivariate statistical analysis based upon location of the locoregional metastases, stage and mitotic rate of the metastatic cells, indicated that none of these three variables significantly affected progression-free survival in this advanced CM subgroup.

Overall survival

The median OS time following multidisciplinary treatments, including locoregional chemotherapy, was 20 mo (IQR = 14-30 mo) and 1-, 3- and 5-y survival rates were 85.5%, 22.6% and 8.1%, respectively. Univariate analysis indicated that OS was significantly prolonged in patients with ECOG perfor-

Table 3 – Logistic regression of selected patient/tumour variables on response to locoregional chemotherapy.

Variables	OR	95% CI	P value
Age			
≤ 75 *versus* > 75	8.74	1.46-52.27	0.017
ECOG performance status			
< 2 *versus* ≥ 2	2.01	0.35-11.57	0.432
Location of the locoregional metastases			
Inferior limbs plus pelvis *versus* Inferior limbs	0.18	0.03-0.97	0.046
Stage			
IIIC *versus* IIID	5.99	0.59-60.86	0.130
Mitotic rate of the metastatic cells			
< 1 *versus* ≥ 1	2.49	0.48-12.86	0.274

CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; OR = odds ratio.

Table 4 – Procedure-related complications and toxicities in 62 CM patients subjected to first cycle of melphalan locoregional chemotherapy.

Part A: Procedure-related complications	Grade 1-2	Grade ≥ 3	All patients (62) number (%)	HILP (19/62) number (%)	ILI (19/62) number (%)	HPLP (24/62) number (%)
Persistent leakage of fluid from the incision	+	-	2 (3.2)	1 (5.3)	0 (0)	1 (4.2)
Seroma	+	-	1 (1.6)	0 (0)	0 (0)	1 (4.2)
Wound infection	+	-	1 (1.6)	0 (0)	0 (0)	1 (4.2)
Scrotum swelling	+	-	1 (1.6)	0 (0)	0 (0)	1 (4.2)
Pelvic pain	+	-	1 (1.6)	0 (0)	0 (0)	1 (4.2)
Inguinal hematoma	+	-	2 (3.2)	0 (0)	1 (5.3)	1 (4.2)
Part B: Procedure-related toxicities						
Anaemia	+	-	4 (6.4)	3 (15.8)	1 (5.3)	0 (0)
Neutropenia	+	-	8 (12.9)	3 (15.8)	3 (15.8)	2 (8.3)
Thrombocytopaenia	+	-	7 (11.3)	3 (15.8)	3 (15.8)	1 (4.2)
Hypotension	+	-	1 (1.6)	1 (5.3)	0 (0)	0 (0)
Alopecia	+	-	1 (1.6)	0 (0)	0 (0)	1 (4.2)
Nausea and vomiting	+	-	5 (8.1)	1 (5.3)	3 (15.8)	1 (4.2)
Dyspeptic symptoms	+	-	1 (1.6)	1 (5.3)	0 (0)	0 (0)
Acidosis	+	-	1 (1.6)	1 (5.3)	0 (0)	0 (0)
Wieberdink toxic tissue reactions score	+	-	31 (50.0)	15 (79.0)	16 (84.2)	0 (0)
	-	+	7 (11.3)	4 (21.1)	3 (15.8)	0 (0)
Motor and sensory deficit	+	-	2 (3.2)	2 (10.6)	0 (0)	0 (0)
Foot drop	+	-	1 (1.6)	1 (5.3)	0 (0)	0 (0)

HILP = hyperthermic isolated limb perfusion; HPLP = hypoxic pelvic and limb perfusion; ILI = isolated limb infusion.

mance status < 2, lesions located exclusively in inferior limbs, stage IIIC disease, in patients exhibiting < 1 mitosis per mm^2 in metastatic tissues, and in patients submitted for the HILP locoregional chemotherapy procedure. However, multivariate analysis based upon location of the locoregional metastases, stage and mitotic rate of the metastatic cells, identified stage IIID as the only statistically significant variable affecting OS in this advanced CM subgroup (Table 5; Part B).

Discussion

Improvement in the treatment of CM patients with locoregional metastases in inferior limbs and in pelvic or inguinal regions, is an important objective of translational and clinical research, and is of particular relevance to wild-type *BRAF* CM patients, in progression following or ineligible for novel immunotherapy. The most relevant aspect of this study is

Table 5 – Univariate and multivariate analysis of variables affecting progression-free survival (Part A) and overall survival (Part B) in 62 CM patients.

Part A

Variables	PFS (mo)	Univariate analysis			Multivariate analysis		
	Median (IQR)	HR	95% CI	*P* value	HR	95% CI	*P* value
Age							
- ≤75	4 (3-12)						
- >75	3 (3-5)	0.63	0.34-1.17	0.15			
Gender							
- Female	4 (3-20)						
- Male	4 (3-5.5)	1.45	0.80-2.60	0.21			
ECOG performance status							
- <2	5 (4-12)						
- ≥2	3 (3-4)	0.34	0.18-0.63	0.001			
Location of the locoregional metastases							
- Inferior limbs plus pelvis	3 (3-4)	2.38	1.34-4.22	0.003	1.77	0.90-3.48	0.09
- Inferior limbs	5 (4-20)						
Stage							
- IIIC	4 (3-12)						
- IIID	4 (3-4)	0.44	(0.23-0.84)	0.01	0.64	0.32-1.29	0.21
Burden							
- Low	4 (3-6)						
- High	4 (3-12)	0.96	0.55-1.66	0.88			
Metastatic cells producing melanin							
- No	4 (3-5.5)	0.88	0.51-1.53	0.66			
- Yes	4.5 (3-12)						
Mitotic rate of the metastatic cells							
- <1	5 (3-16)						
- ≥1	3.5 (3-4)	2.09	1.18-3.70	0.01	1.43	0.73-2.78	0.29
Previous therapies of the locoregional metastases							
- No	5 (3.5-12)						
- Yes	4 (3-5)	1.62	0.90-2.91	0.10			
Type of locoregional chemotherapy							
- HILP	12 (6-41)	Baseline					
- ILI	4 (3-5)	3.57	1.64-7.69	0.001			
- HPLP	3 (3-4)	4.64	2.16-9.97	0.001			

Part B							
	Overall survival (mo)	**Univariate analysis**			**Multivariate analysis**		
Variables	Median (IQR)	HR	95% CI	*P* value	HR	95% CI	*P* value
Age							
- ≤75	21 (13-41)						
- >75	18 (14-21)	1.57	0.84-2.94	0.15			
Gender							
- Female	20.5 (14-41)						
- Male	19.5 (13.5-24)	1.36	0.76-2.43	0.29			

(*continued on next page*)

Table 5 – (*continued*)

Part B

Variables	Overall survival (mo)	Univariate analysis			Multivariate analysis		
	Median (IQR)	HR	95% CI	*P* value	HR	95% CI	*P* value
ECOG performance status							
- <2	24 (19-44)						
- ≥2	13 (9-14)	16.13	6.46-40.28	0.0001			
Location of the locoregional metastases							
- Inferior limbs plus pelvis	18.5 (10-21.5)	1.95	1.12-3.41	0.02	1.54	0.81-2.96	0.19
- Inferior limbs	22.5 (14-41)						
Stage							
- IIIC	22 (13-44)	2.66	(1.38-5.11)	0.003	2.15	1.05-4.39	0.04
- IIID	16 (14-20)						
Burden							
- Low	21 (13-28)						
- High	20 (14-30)	1.05	0.61-1.83	0.84			
Metastatic cells producing melanin							
- No	19 (13.5-23)	1.33	0.76-2.33	0.31			
- Yes	23 (14-47)						
Mitotic rate of the metastatic cells							
- <1	21 (14-40)						
- ≥1	19 (13-24)	1.73	0.98-3.03	0.05	1.12	0.57-2.21	0.74
Previous therapies of the locoregional metastases							
- No	24 (15.5-34.5)						
- Yes	18.5 (13-28)	1.62	0.91-2.91	0.10			
Type of locoregional chemotherapy							
- HILP	39 (24-48)	Baseline					
- ILI	17 (14-21)	4.28	1.94-9.45	0.0001			
- HPLP	18.5 (10-21.5)	3.94	1.88-8.29	0.0001			
Further therapies of the locoregional metastases							
- one cycle of locoregional chemotherapy and best supportive care	24 (14-40)						
- multidisciplinary treatment including locoregional chemotherapy	19.5 (13-24)	1.60	0.93-2.77	0.09			

CI = confidence interval; ECOG = Eastern Cooperative Oncology Group; HILP = hyperthermic isolated limb perfusion; HPLP = hypoxic pelvic and limb perfusion; HR = hazard ratio; ILI = isolated limb infusion; IQR = interquartile range; PFS = progression-free survival.

that it considers a subgroup of stage IIIC/D CM patients that, over the past 5 y, has only represented approximately 3% of reported cases in clinical trials of novel therapy drugs.[16] This subgroup is characterized by frequent bad outcomes when not treatable or not responsive to novel target and immunotherapeutic agents[17-21] and clearly requires a better therapeutic strategy, including those evaluated in the present report.

For this specific subgroup of advanced CM patients, our study supports the international guidelines recommending a multidisciplinary treatment including locoregional chemotherapy, by demonstrating a 37.1% ORR following the first cycle of locoregional chemotherapy, associated with 12.9% procedure-related complications, 30.6% low-grade haematological toxicity and 11.3% severe limb toxic tissue reactions. Furthermore, multivariate logistic regression revealed that responses were significantly higher in patients of ≤ 75 y of age and in patients with locoregional metastases located exclusively to inferior limbs. An additional important message from this study, is that patients eligible for maximally aggressive regional therapies had higher responses and enjoyed long-term benefits. Moreover, locoregional chemotherapy followed by multidisciplinary treatments elicited a median OS time of 20 mo (IQR = 14-30 mo), which is similar to the reported OS time for *BRAF*-mutated CM patients treated with novel target or immunotherapeutic agents,[16] though, a direct

comparison cannot accurately be made considering the high number of stage IV CM patients included in systemic therapeutic studies.

The choice of locoregional chemotherapeutic procedure depends initially upon the localisation of locoregional metastases. For metastatic disease localised to inferior limbs, pelvic and inguinal regions, HPLP is the only procedure that can deliver drugs to all metastases within the perfused compartment, considering that HILP and ILI only partially reach inguinal lesions and cannot deliver drugs to deep pelvic lesions.[34] In contrast, either HILP or ILI are appropriate procedures for metastatic lesions localised exclusively to inferior limbs. An important message arising from this study is that in this stage IIIC/D WT-BRAF CM cohort, consisting of patients with synchronous limb and pelvic locoregional metastases in progression after or ineligible for novel target and immunotherapy, HPLP elicited a 12.5% ORR following the first cycle of locoregional chemotherapy and a median OS time of 18.5 mo (IQR = 10-21.5), if followed by other multidisciplinary treatments. For this particular subset, the clinical benefits of systemic chemotherapy, interferon or interleukin-based immunotherapy are unsatisfactory, and remain to be defined for other therapies, such as local injectables, ECT or RT.[11] With respect to the choice between HILP and ILI, although phase III prospective randomized trials comparing these two procedures have not yet been initiated, there is general consensus that HILP is more effective than ILI but is more complex and associates with more adverse events.[1] It is for these reasons that our multidisciplinary board recommended the HILP procedure for the treatment of locoregional limb metastases in patients < 76 y old, with an ECOG performance status < 2, and the ILI procedure for the treatment of patients > 75 y old and/or ECOG performance status ≥ 2. This procedure selection bias was considered and weighted in both the logistic regression model used to evaluate the effect of patient/tumour variables on response and the multivariate Cox regression model used to evaluate PFS and OS. In our study, the 98.5% ORR and 39 mo median OS time associated to HILP, confirm the literature,[1] whereas the 15.8% ORR elicited by ILI is considerably lower than that (64.1%) reported in a previous multicentre study.[35] This difference may relate to the fact that patients in our study were significantly older (78 y, IQR = 70-84) than those in the latter study,[35] exhibited a worse ECOG performance status, did not include stage IIIB disease, had been unsuccessfully pretreated for locoregional metastases in 42% of cases, and were submitted for a different therapeutic regimen. Therefore, ILI should not be refused for frail >75-y-old CM patients, if one considers that HILP is too aggressive, the only parameter assessed during best supportive care is symptom improvement and the clinical benefit of alternative local therapies remains to be elucidated.[11]

In this non randomized study, OS was pre-defined as a secondary endpoint. This decision was based upon the relatively small number of patients enrolled, the high number of patients with stage IIID disease, the high percentage (61.3%) of patients previously treated for locoregional metastases, the high percentage of patients (30.6%) considered ineligible for novel immunotherapeutic drugs, and the heterogeneity of multidisciplinary treatments. Multivariate survival analysis, performed considering the criteria adopted for the choice of locoregional chemotherapy procedure and collinearity of variables, highlighted an increased risk of death in patients with stage IIID disease.

With regard to future perspectives, the 20-mo median OS time observed in the subgroup evaluated in this study could be improved by enhancing the efficacy of locoregional chemotherapy and by combining locoregional chemotherapy with other local, regional or systemic therapies. In this respect, although melphalan was the only therapeutic agent used in our study for reasons of sample homogeneity, several studies have reported interesting results for melphalan combined with other drugs.[36-40] Moreover, the use of fresh tissues biopsies, liquid biopsies and purified circulating tumour cells in chemosensitivity and tumour gene expression assays can be used to develop more personalized locoregional or systemic treatment strategies, as recently reported.[41] With respect to locoregional chemotherapy and other therapy combinations, the use of ablative techniques, ECT, topical and intralesional therapy is under current investigation[7,11-14] and novel immunotherapies are increasing due to a broadening of eligibility criteria, with several clinical trials now including patients previously considered ineligible.[22] Furthermore, it has been reported that ILI with melphalan followed by systemic administration of ipilimumab provided an 85% ORR associated to increased T-cell infiltration in locoregional metastases of 26 melanoma patients, suggesting that locoregional chemotherapy administered as immune stimulant therapy could potentiate immune-responses.[42] Finally, novel agnostic therapeutic agents, such as larotrectinib and entrectinib, that inhibit mutation and deletion-activated Trk oncogenes, elicit remarkable durable responses in a wide range of advanced stage Trk-fusion oncogene-driven cancers, including melanoma.[43]

Conclusion

Despite limitations of a prospective non-randomized trial, small sample size, high-degree of patient selection and locoregional chemotherapy treatment heterogeneity, this study demonstrates that locoregional chemotherapy is capable of eliciting responses that may be durable in a specific subgroup of advanced CM patients, not treatable with novel immunotherapeutic agents, and characterized by *BRAF* wild-type status, stage IIIC/D, and locoregional metastases localised in the inferior limbs and pelvis. This study also provides more precise clinical and biological reasons that should persuade surgeons, skilled in intraarterial chemotherapy approaches, not to abandon locoregional procedures, and supports the recent call[44] for larger prospective controlled, multicentre, interventional trials to evaluate locoregional chemotherapeutic efficacy in combination with other therapies.

Author Contributions

Study concept and design (SG, OZ, GF); acquisition of data (KL, MC, DS); analysis and interpretation of data (FM, SG, OZ, GF, KL, MC, DS); drafting of the manuscript (SG, ARM, KL, DS); critical revision of the manuscript for important intellectual content (ARF, DS, KL, GF, SG).

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Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jss.2021.05.054.

REFERENCES

1. Wright FC, Kellett S, Look Hong NJ, et al. Locoregional management of in-transit metastasis in melanoma: an Ontario Health (Cancer Care Ontario) clinical practice guideline. *Curr Oncol*. 2020;27:e318–e325.
2. Trout AT, Rabinowitz RS, Platt JF, Elsayes KM. Melanoma metastases in the abdomen and pelvis: frequency and patterns of spread. *World J Radiol*. 2013;5:25–32.
3. Guadagni S, Fiorentini G, Clementi M, et al. Melphalan hypoxic perfusion with hemofiltration for melanoma locoregional metastases in the pelvis. *J Surg Res*. 2017;215:114–124.
4. Coit DG, Thompson JA, Albertini MR, et al. Cutaneous melanoma, Version 2.2019. *J Natl Compr Canc Netw*. 2019;17:367–402.
5. Perone JA, Farrow N, Tyler DS, Beasley GM. Contemporary approaches to in-transit melanoma. *J Oncol Pract*. 2018;14:292–300.
6. Michielin O, van Akkooi A, Lorigan P, et al. ESMO consensus conference recommendations on the management of locoregional melanoma: under the auspices of the ESMO Guidelines Committee. *Ann Oncol*. 2020;31:1449–1461.
7. Garbe C, Amaral T, Peris K, et al. On behalf of the European Dermatology Forum (EDF), the European Association of Dermato-Oncology (EADO), and the European Organization for Research and Treatment of Cancer (EORTC). European consensus-based interdisciplinary guideline for melanoma. Part 2: treatment - update 2019. *Eur J Cancer*. 2020;126:159–177.
8. Cancer Council Australia Melanoma Guidelines Working Party. Clinical practice guidelines for the diagnosis and management of melanoma. Sydney: Cancer Council Australia. [Version URL: Available at: https://wiki.cancer.org.au/australiawiki/index.php?oldid=186442]. Available from: https://wiki.cancer.org.au/australia/Guidelines:Melanoma.
9. National Health Commission of the People's Republic of ChinaChinese guidelines for diagnosis and treatment of melanoma 2018. *Chin J Cancer Res*. 2019;31:578–585.
10. Nakamura Y, Asai J, Igaki H, et al. Japanese Dermatological Association Guidelines: outlines of guidelines for cutaneous melanoma 2019. *J. Dermatol.*. 2020;47:89–103.
11. Read RL, Thompson JF. Managing in-transit melanoma metastases in the new era of effective systemic therapies for melanoma. *Exp Rev Clin Pharmacol*. 2019;12:1107–1119.
12. Lardone RD, Chan AA, Lee AF, et al. Mycobacterium bovis Bacillus Calmette–Guérin alters melanoma microenvironment favoring antitumor T cell responses and improving M2 macrophage function. *Front Immunol*. 2017;8:965.
13. Caracò C, Marone U, Simeone E, et al. Electrochemotherapy in melanoma patients: a single institution experience. *Mel Manag*. 2015;2:127–132.
14. Hong A, Fogarty G. Role of radiation therapy in cutaneous melanoma. *Cancer J*. 2012;18:203–207.
15. Guadagni S, Santinami M, Patuzzo R, et al. Hypoxic pelvic and limb perfusion with melphalan and mitomycin C for recurrent limb melanoma: a pilot study. *Melanoma Res*. 2003;13:51–58.
16. Tie EN, Lai-Kwon JE, Gyorki DE. Systemic therapies for unresectable locoregional melanoma: a significant area of need. *Mel Manag*. 2019;6. doi:10.2217/mmt-2019-0010.
17. Khushalani NI, Sondak VK. Are we there yet? Prolonged MAPK inhibition in BRAF V600-mutant melanoma. *Lancet Oncol*. 2016;17:1178–1179.
18. Chan MM, Haydu LE, Menzies AM, et al. The nature and management of metastatic melanoma after progression on BRAF inhibitors: Effects of extended BRAF inhibition. *Cancer*. 2014;120:3142–3153.
19. Topollian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol*. 2014;32:1020–1030.
20. Ribas A, Hamid O, Daud A, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA*. 2016;315:1600–1609.
21. Daud A, Nandoskar P. Pembrolizumab for melanoma-safety profile and future trends. *Expert Opin Drug Saf*. 2016;15:727–729.
22. Johnson DB, Sullivan RJ, Menzies AM. Immune checkpoint inhibitors in challenging populations. *Cancer*. 2017;123:1904–1911.
23. Guadagni S, Fiorentini G, Clementi M, et al. Does locoregional chemotherapy still matter in the treatment of advanced pelvic melanoma? *Int J Mol Sci*. 2017;18:2382.
24. Guadagni S, Fiorentini G, Clementi M, et al. MGMT methylation correlates with melphalan pelvic perfusion survival in stage III melanoma patients: a pilot study. *Mel Res*. 2017;27:439–447.
25. Cecchini S, Sarti D, Ricci S, et al. Isolated limb infusion chemotherapy with or without hemofiltration for recurrent limb melanoma. *World J Clin Oncol*. 2015;6:57–63.
26. Gershenwald JE, Scolyer RA. Melanoma staging: American Joint Committee on Cancer (AJCC) 8th edition and beyond. *Ann Surg Oncol.*. 2018;25:2105–2110.
27. Steinman J, Ariyan C, Rafferty B, Brady MS. Factors associated with response, survival, and limb salvage in patients undergoing isolated limb infusion. *J Surg Oncol*. 2014;109:405–409.
28. Guadagni S, Palumbo G, Fiorentini G, Clementi M, Marsili L. Surgical versus percutaneous isolated pelvic perfusion (IPP) for advanced melanoma: comparison in terms of melphalan pharmacokinetic pelvic bio-availability. *BMC Res Notes*. 2017;10:411.
29. Guadagni S, Kanavos E, Schietroma M, Fiorentini G, Amicucci G. Selected hypoxic stop-flow perfusions: indication and limits. *Tumori*. 2006;92:402–406.
30. Lasithiotakis K, Economou G, Gogas H, et al. Hyperthermic isolated limb perfusion for recurrent melanomas and soft tissue sarcomas: feasibility and reproducibility in a multi-institutional Hellenic collaborative study. *Oncol Rep*. 2010;23:1077–1083.

31. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of The Eastern Cooperative Oncology Group. *Am J Clin Oncol*. 1982;5:649–655.
32. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response valuation criteria in solid tumors: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45:228–247.
33. Wieberdink J, Benckhuysen C, Braat RP, van Slooten EA, Olthuis GA. Dosimetry in isolation perfusion of the limbs by assessment of perfused tissue volume and grading of toxic tissue reactions. *Eur J Cancer Clin Oncol*. 1982;18: 905–910.
34. Guadagni S, Russo F, Rossi CR, et al. Deliberate hypoxic pelvic and limb chemoperfusion in the treatment of recurrent melanoma. *Am J Surg*. 2002;183:28–36.
35. Miura JT, Kroon HM, Beasley GM, et al. Long-term oncologic outcomes after isolated limb infusion for locoregionally metastatic melanoma: an international multicenter analysis. *Ann Surg Oncol*. 2019;26:2486–2494.
36. Nooijen PT, Manusama ER, Eggermont AM, et al. Synergistic effects of TNF-alpha and melphalan in an isolated limb perfusion model of rat sarcoma: A histopathological, immunohistochemical and electron microscopical study. *Br J Cancer*. 1996;74:1908–1915.
37. Beasley GM, Miura J, Zager JS, Tyler DS, Thompson JF, Kroon HM. Isolated limb infusion for melanoma. In: Balch CM, Thompson JF, Gershenwald JE, Atkins MB, Kirkwood JM, McArthur G, et al., editors. Cutaneous Melanoma. 6th edition. Switzerland: Springer Nature AG; 2020; pp. 827-50.
38. Beasley GM, Riboh JC, Augustine CK, et al. Prospective multicenter phase II trial of systemic ADH-1 in combination with melphalan via isolated limb infusion in patients with advanced extremity melanoma. *J Clin Oncol*. 2011;29:1210–1215.
39. Vo KT, Matthay KK, DuBois SG. Targeted antiangiogenic agents in combination with cytotoxic chemotherapy in preclinical and clinical studies in sarcoma. *Clin Sarcoma Res*. 2016;6:9.
40. Padussis JC, Steerman SN, Tyler DS, Mosca PJ. Pharmacokinetics & drug resistance of melphalan in regional chemotherapy: ILP versus ILI. *Int J Hyperthermia*. 2008;24:239–249.
41. Guadagni S, Fiorentini G, Papasotiriou I, et al. Circulating tumour cell liquid biopsy in selecting therapy for recurrent cutaneous melanoma with locoregional pelvic metastases: a pilot study. *BMC Res Notes*. 2020;13:176.
42. Ariyan CE, Brady MS, Siegelbaum RH, et al. Robust antitumor responses result from local chemotherapy and CTLA-4 blockade. *Cancer Immunol Res*. 2018;6:189–200.
43. Cappabianca L, Guadagni S, Maccarone R, et al. A pilot study of alternative TrkAIII splicing in Merkel cell carcinoma: A potential oncogenic mechanism and novel therapeutic target. *J Exp Clin Cancer Res*. 2019;38:424.
44. Bagge RO, Ny Lars, Ascierto PA, et al. The efficacy of immunotherapy for in-transit metastases of melanoma: an analysis of randomized controlled trials. *Mel Res*. 2021;31:181–185.