European Journal of Surgical Oncology 45 (2019) 832–837

Contents lists available at ScienceDirect

European Journal of Surgical Oncology

journal homepage: www.ejso.com

Evaluation of the efficacy and toxicity of upper extremity isolated limb infusion chemotherapy for melanoma: An Australian multi-center study

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ARTICLE INFO

Article history:
Accepted 20 February 2019
Available online 27 February 2019

Keywords:
Isolated limb infusion
Melanoma
Upper extremity
Melphalan
Actinomycin D
Efficacy
Toxicity

ABSTRACT

Background: Isolated limb infusion (ILI) is a minimally invasive treatment for patients with locally advanced extremity melanoma. Most studies combine results of upper-limb ILI (UL-ILI) and lower-limb ILI (LL-ILI), leaving UL-ILIs relatively underreported as LL-ILIs comprise the vast majority in these reports. However, differences between the two procedures may be clinically important. The aim of this study was to evaluate the efficacy and toxicity of UL-ILI in an Australian multi-center setting.

Patients and methods: 316 ILI procedures for melanoma performed between 1992 and 2008 in five Australian institutions were analyzed. In all institutions melphalan (±actinomycin D) was circulated in the isolated limb for 20–30 min.

Results: Baseline patient characteristics for UL-ILI ($n = 27$) and LL-ILI ($n = 289$) were similar, except that more men underwent UL-ILI (66% vs. 38%; $p = 0.007$) and disease in LL-ILI was mostly located on the distal limb ($p = 0.02$). Median tourniquet times were shorter for UL-ILI (38 vs. 48 min; $p = 0.04$) and UL-ILI patients experienced less limb toxicity (Grade III/IV in 24% vs. 31%; $p = 0.01$). Complete response (CR) rates were similar: 33% after LL-ILI ($p = 0.70$), 30% after UL-ILI, while overall response (OR) rates were higher after LL-ILI: (76%) than UL-ILI (59%; $p = 0.05$). No difference in survival was seen.

Conclusions: UL-ILI is safe to perform and effective, resulting in low limb toxicity. CR rates were similar to those for LL-ILI, but OR rates were lower for UL-ILI. It may be possible to improve OR rates achieved by UL-ILI by optimizing perioperative factors, while maintaining low toxicity.

Introduction

When bulky or numerous local melanoma recurrences or in-transit melanoma metastases develop in a limb, simple treatment modalities such as excision, cryotherapy, injection with Rose Bengal (PV-10) or talimogene laherparepvec (T-VEC) may be ineffective [1]. In these patients, isolated limb infusion (ILI) is a simple, safe and effective technique for delivering high-dose regional chemotherapy. Over the last decades, ILI has become the preferred treatment option for these patients at melanoma treatment centers around the world. Multicenter studies from Australia and the USA have reported complete response (CR) rates of 31–33%, partial response (PR) rates of 33–42% and a systematic review of 576 patients treated by ILI reported a CR rate of 33% and a PR rate of 40% [2–4]. Furthermore, all ILI studies using melphalan have reported mild to moderate limb toxicity without any catastrophic procedure-related complications such as amputation or mortality.

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https://doi.org/10.1016/j.ejso.2019.02.026
0748-7983/

Compared to the traditional, more invasive isolated limb perfusion (ILP) procedure, ILI is as effective but less morbid due to its minimally-invasive nature [4–6].

ILI is routinely performed for both upper-limb and lower-limb in-transit melanoma, with reported percentages of upper-limb ILI (UL-ILI) ranging from 7 to 25% of all ILIs performed [2,3,7,8]. The reason why UL-ILI is less frequently performed may lie in the fact that, despite a similar incidence of upper and lower limb melanomas, there is a lower risk of patients with upper-limb melanomas developing in-transit metastases [9,10]. A further explanation might be that UL-ILI is technically more challenging to perform the lower limb ILI (LL-ILI), resulting in the treating surgeon considering other treatment options [7].

Most studies have reported combined results, leaving UL-ILIs relatively under-reported. However, because of physiological and procedural differences, UL-ILI and LL-ILI may actually not be equivalent procedures and should therefore be studied separately. Due to the small number of UL-ILIs performed, reports focusing specifically on upper-limb procedures are limited. Only two smaller studies, both from the USA, have reported outcomes of UL-ILI, however, both included multiple tumor types and repeat procedures [7,8]. Therefore, the aim of the present study was to investigate safety and efficacy of the procedure in patients undergoing a first UL-ILI for irresectable melanoma confined to a limb at five Australian tertiary referral centers, and to compare the results with those of LL-ILI procedures performed at the same institutions.

Patients and Methods

The five Australian institutions contributing data were: Melanoma Institute Australia/Royal Prince Alfred Hospital (MIA/RPAH) in Sydney; the Royal Adelaide Hospital (RAH) in Adelaide; the Alfred Hospital (AH) and the Peter MacCallum Cancer Centre (PMCC), both in Melbourne; and the Princess Alexandra Hospital (PAH) in Brisbane. Patients were treated between 1992 and 2010. Follow-up information and survival data were obtained from hospital outpatient clinic records, supplemented when necessary by communicating with patients' family physicians and by consulting state cancer registries.

Patients with an advanced primary melanoma or with unresectable in-transit metastases of the limb, without or with involvement of lymph nodes (AJCC Stage IIIb, IIIc), who had been treated by ILI were included in the study [11]. A small number of patients with distant metastatic disease (stage IV) and concurrent symptomatic limb disease who had been treated by ILI with palliative intent were also included. For patients with metastatic groin or axillary disease requiring a regional lymph node dissection as well as an ILI, this was undertaken under the same anesthetic after completion of the ILI procedure. The current study includes results following the initial ILI procedure, with repeat procedures excluded. Informed consent was obtained from all patients and the study was approved by the relevant institutional human research ethics committees. The data for all patients were prospectively collected, combined and analyzed.

All five centers followed the same ILI protocol. The cytotoxic combination of melphalan and actinomycin D was used in four centers (MIA/RPAH, RAH, AH, PMCC); PAH used melphalan only. Based on preoperative measurements marked on the skin, the volume of limb tissue that had been isolated was estimated. The standard dose of melphalan was 7.5 mg/L for LL-ILI and 10 mg/L for UL-ILI, with a maximum total dose of 100 mg for LL-ILI and 50 mg for UL-ILI. The standard dose of actinomycin-D, if used, was 75 mg/L for LL-ILI and 100 mg/L for UL-ILI. Due to the smaller intravascular space, the infusate volume for UL-ILI was reduced to 300 ml, whereas 400 ml was used for LL-ILI. Further technical aspects of ILI have been described in detail previously [12,13]. A schematic overview of the procedure is shown in Fig. 1 [14].

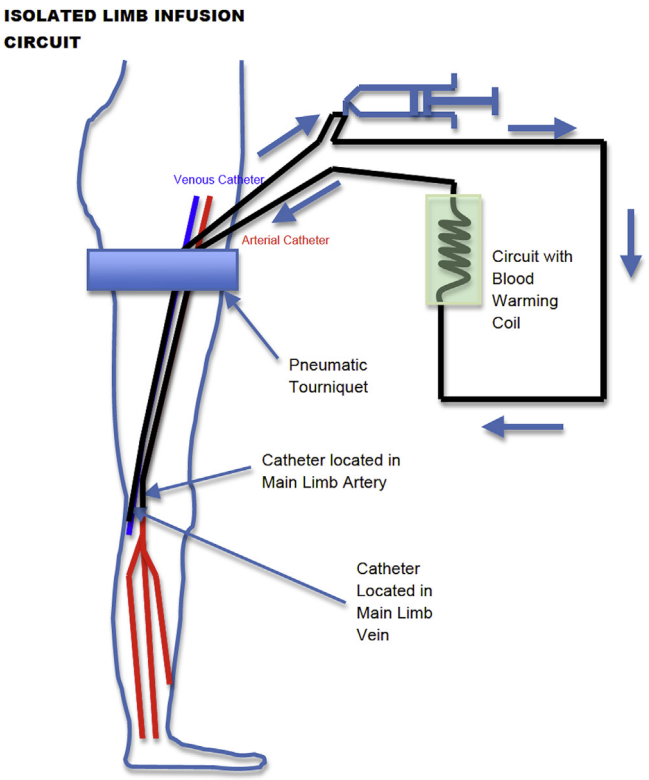

Fig. 1. Schematic illustration of the circuit used for isolated infusion of a lower-limb (adapted from Giles et al.) [14].

Postoperatively, limb toxicity was assessed using the Wieberdink [15] scale and serum creatine phosphokinase (CK) was measured daily [16]. Clinical responses were defined according to the World Health Organization (WHO) criteria for reporting results of cancer treatment [17], with a complete response (CR) being the disappearance of all measurable disease, determined by two observations 4 or more weeks apart, and a partial response (PR) as a $\geq$50% decrease in total tumor size determined by two observations 4 or more weeks apart without the appearance of new lesions or progression of disease.

Possible prognostic factors were tested for their influence on toxicity, response rates, and survival. The chi-squared and Fisher's exact tests were used for comparison of frequency distributions and the Mann–Whitney U test for nonparametric variables. Continuous variables were assessed using the analysis of variance (ANOVA) test for repeated measures. Survival curves were plotted using the Kaplan-Meier method [18]. A significant difference was assumed for a probability value of <0.05. Statistical calculations were performed using GraphPad Prism software (GraphPad software Inc., San Diego, CA, USA) and SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Twenty-seven patients (9%) underwent an UL-ILI and 289 patients (91%) a LL-ILI. The median age of UL-ILI patients was 76 years (range 57–88 years) and that of LL-ILI patients 73 years (range 28–100 years; $p = 0.07$). The UL-ILI group contained significantly more male patients compared to the LL-ILI group (66% vs. 38% respectively; $p = 0.007$). With regard to disease distribution in the

affected limb, in LL-ILI patients most melanoma metastases were located distally on the leg (between knee and ankle) (63%), whereas in UL-ILI patients most disease occurred more proximally, on the proximal arm (between elbow and shoulder) (44%; $p = 0.02$). Stage of disease and burden of disease, recorded as number and size of the lesions on the limb, were similar for the two groups (Table 1).

Intra- and post-operative data are documented in Table 2. Median infused limb volumes were substantially lower in the UL-ILI group (median 2.0 vs. 6.0 liters; $p < 0.001$). Consequently, the total melphalan dose was also significantly lower in this group (median 20 vs. 45 mg; $p < 0.001$). According to the ILI protocol, melphalan dosage (milligrams per liter of infused tissue) was higher in UL-ILI compared to LL-ILI; median 11.6 and 7.5 mg/L, respectively ($p < 0.001$). Drug circulation times were similar for the two groups (21 vs. 23 min for UL-ILI and LL-ILI respectively; $p = 0.47$), but tourniquet times were significantly shorter in the UL-ILI group (38 vs. 48 min respectively; $p = 0.04$).

The patients in the UL-ILI group experienced significantly lower Wieberdink toxicity grades without any grade IV toxicity compared to the LL-ILI group where 8 patients (3%) developed grade IV toxicity ($p = 0.01$). Grade V toxicity (necessitating amputation) did not occur in either group. Post-procedure median peak CK levels were 308 IU/L in the UL-ILI group, which was not significantly different from the median peak of 518 IU/L in the LL-ILI group ($p = 0.09$). The median length of hospital stay was 7 days for the UL-ILI group, and was similar (8 days) in the LL-ILI group. There was no mortality associated with the ILI procedure in either group.

A CR was seen in 8 (30%) of the UL-ILI patients and in 96 (33%) of the LL-ILI patients ($p = 0.70$), while a PR was seen in 8 (30%) and 125 (43%), respectively ($p = 0.17$). Overall responses (CR and PR combined: OR) were significantly different between groups, with responses in 16 (59%) after UL-ILI and 221 (76%) after LL-ILI ($p = 0.05$; Table 3). Stable disease (SD) was recorded in 8 (30%) after UL-ILI and 49 (17%) after LL-ILI, while progressive disease occurred in 3 (11%) and 19 (7%) patients, respectively.

Median follow-up was 25 months. No significant difference was seen in survival times, with a median survival of 36 months for the UL-ILI group and 39 months for the LL-ILI group ($p = 0.59$; Fig. 2).

Discussion

In this Australian multi-center ILI study including first procedures for melanoma only, we found that the incidence of UL-ILI procedures was low and comprised only 9% of all ILIs performed. UL-ILI was safe to perform and resulted in low regional toxicity. Although CR rates were similar compared to LL-ILI, OR rates were significantly lower.

The low frequency with which UL-ILIs were performed is similar to the frequencies reported in previously published ILI studies, in which percentages ranged from 7 to 25%. Similar percentages have been reported for ILP studies [2,3,7,8,19,20]. The 9% of UL-ILIs out of all ILIs performed in the current study fits within that range. Despite the similar incidence of primary melanoma on the upper and lower limbs, the lower rate of UL-ILI is probably due mainly to the significantly lower risk of patients with upper-limb melanomas developing in-transit metastases [9]. Perhaps also contributing to the lower frequency of UL-ILI compared with LL-ILI is the fact that patients with lower limb melanomas are of younger age, making it more likely that they are willing to undergo an ILI procedure compared to older patients with in-transit disease in the upper limb [10]. Another possible explanation could be the technically more challenging nature of UL-ILI compared to LL-ILI, making surgeons more likely to consider other treatment options.

In our study, UL-ILIs were performed more frequently in men (66% of all UL-ILIs), whereas LL-ILIs were more frequently performed in women (62% of all LL-ILIs; $p = 0.007$). Similar patterns between sexes for upper- and lower-limb procedures have been described in previous ILI and ILP studies, reflecting the higher incidence of upper-limb melanoma in male patients and the higher incidence of lower-limb melanoma in females [10]. Secondly, the disease was more evenly distributed proximally and distally in the upper limb, whereas in the lower limb most disease was concentrated distally (between knee and ankle). Variations of previous sun-exposure may play a role in the differences observed between sexes and the different patterns of disease distribution on the upper and lower limbs, but evidence supporting this hypothesis is scant.

In the current study of 316 procedures, there was no significant

Table 1
Patient characteristics.

Variable	Upper-limb	Lower-limb	*p*-value
No. of ILIs	27 (9%)	289 (91%)	–
Median age, y (range)	76 (57–88)	73 (28–100)	0.07
Sex (male/female)	18/9 (66%)	111/178 (38%)	**0.007**
Location on the limb[a]			**0.02**
Foot/Hand	4 (16%)	14 (5%)	
Lower leg/Forearm	10 (40%)	183 (63%)	
Thigh/Upper arm	11 (44%)	92 (32%)	
Stage of disease at time of ILI [11]			0.53
I Primary melanoma	2 (7%)	8 (3%)	
IIIb In-transit metastases	15 (56%)	167 (58%)	
IIIc In-transit metastases with nodal involvement	8 (30%)	79 (27%)	
IV Distant metastases	2 (7%)	35 (12%)	
Number of lesions, median (range)	3 (1–18)	5 (1–100)	0.56
Size of lesions, mm, median (range)[b]	9 (2–48)	6 (1–160)	0.52
Clark level of primary melanoma[c]			0.62
I	0 (0%)	1 (0%)	
II	2 (10%)	7 (3%)	
III	2 (10%)	31 (15%)	
IV	12 (57%)	130 (63%)	
V	5 (23%)	39 (19%)	
Breslow thickness of primary melanoma (mm; median (range)[d]	3.7 (0.9–8.5)	2.7 (0.15–18)	0.13

ILI, isolated limb infusion; mm, millimeter.
[a] Location on limb for 314 patients.
[b] Size of lesions for 300 patients.
[c] Clark levels for 229 patients.
[d] Breslow thickness for 238 patients.

Table 2
Intra- and postoperative data.

Variable (medians)	Upper-limb	Lower-limb	*p*-value
Limb Volume (L, range)	2.0 (0.8–3.1)	6.0 (1.6–13.5)	**<0.001**
Melphalan dose (mg, range)	20 (10–45)	45 (12.5–100)	**<0.001**
Infused melphalan (mg/L, range)	11.6 (7.2–20.0)	7.5 (4.0–14.0)	**<0.001**
Drug circulation time (min, range)	21 (14–36)	23 (3–43)	0.47
Tourniquet time (minutes, range)	38 (23–77)	48 (22–98)	**0.04**
Overall subcutaneous temperature (°C, range)[a]	36.2 (30.7–37.8)	37.0 (27.2–41.4)	0.33
Overall intramuscular temperature (°C, range)	36.6 (31.2–38.2)	37.2 (29.1–40.5)	0.31
Maximum subcutaneous temperature (°C, range)	37.3 (32.8–39.7)	37.9 (28.5–41.4)	0.80
Maximum intramuscular temperature (°C, range)	37.5 (34.1–39.0)	38.0 (32.6–41.1)	0.63
Wieberdink toxicity[b]			**0.01**
I no visible effect	8 (32%)	30 (10%)	
II slight erythema/oedema	11 (44%)	172 (59%)	
III considerable erythema/oedema with blistering	6 (24%)	80 (28%)	
IV extensive epidermolysis/obvious damage to deep tissues with threatened or actual compartment syndrome	0 (0%)	8 (3%)	
V severe tissue damage necessitating amputation			
Peak serum CK postoperatively, IU/L (median, range)[c]	308 (64–3705)	518 (25–56540)	0.09
Days elevated serum CK (median, range)	4 (1–6)	5 (1–16)	0.44
Number of days admitted (median, range)	7 (2–11)	8 (2–50)	0.27

[a] Temperatures for 281 patients.
[b] toxicity for 315 patients.
[c] CK (creatine phosphokinase) for 249 patients.

Table 3
Response rates.

Response	Upper-limb	Lower-limb	*p*-value
Complete response, no. of patients (%)	8 (30%)	96 (33%)	0.70
Partial response, no. of patients (%)	8 (30%)	125 (43%)	0.17
Overall response, no of patients (%)	16 (59%)	221 (76%)	**0.05**

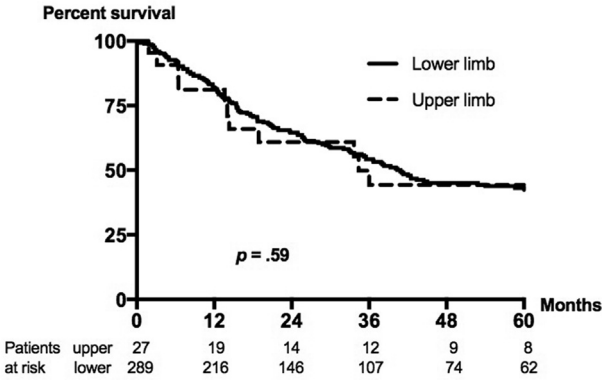

Fig. 2. Overall survival (in months) following upper-limb isolated limb infusion (dotted line) compared to lower-limb isolated limb infusion (solid line; $p = 0.59$).

difference in the CR rate (30% UL-ILI vs. 33% LL-ILI), but the OR rate was significantly lower after UL-ILI (59% vs. 76%; p = 0.05). The CR rates were similar to those reported by Beasley et al. when comparing UL-ILI (n = 36) to LL-ILI (n = 167) for melanoma (28% vs. 32%, respectively), but they found no significant difference in OR rates (50% vs. 53%, respectively) [7]. Similarly, no differences in response rates between UL-ILI (n = 12) and LL-ILI (n = 37) for melanoma were seen in the study conducted by Wong et al. [8].

The main purpose of an ILI is to achieve a CR or PR for locoregional disease control, but a favourable response to ILI is also associated with increased survival [2,21]. Therefore, efforts to increase response rates to UL-ILI are important. One possible way to do this would be to improve drug delivery to tumor cells during ILI to optimize drug exposure. In this regard, UL-ILI is more challenging to perform as it is more difficult to achieve satisfactory flow in the upper limb due to the smaller diameter of the brachial artery and vein compared to the femoral artery and vein, and the substantially lower intravascular volume of the upper limb. Also, the tips of the necessarily small calibre catheters are further from the access site compared with LL-ILI, which increases resistance [7]. In an effort to overcome this, Vyas et al. placed shorter venous catheters below the tourniquet on the ipsilateral side to improve blood flow [22]. In a similar fashion, at MIA/RPAH the venous catheter has occasionally been inserted antegrade via the wrist in UL-ILI cases, when venous access via the groin proved impossible, achieving satisfactory venous flow in these patients. With regard to the flow-rates, however, the ILI procedure was based on the “stop-flow” method of delivering cytotoxic drugs to tumors, as described by Aigner et al. [23], with the flow being simply intended to maintain or increase limb temperatures and to ensure thorough mixing of the cytotoxic drugs within the intravascular compartment, rather than improving drug delivery itself.

Another important consideration is that melphalan uptake into tumor cells is enhanced under hypoxic and acidotic conditions [21,24,25]. Therefore, the shorter tourniquet time in UL-ILI procedures in our study may have contributed to the lower OR rates. In the future, deliberately extending tourniquet times to increase the duration of limb (and tumor) hypoxia may be advantageous. If this is done, measurement of the hypoxia marker EF5 (2-[2 nitro-1H-imidazol-1-yl]-N-[2,2,3,3,3-pentafluoropropyl]) may be useful in order to determine how hypoxia and acidosis affect responses to ILI [26].

On the other hand, the shorter tourniquet time in UL-ILI may have played an important role in significantly reducing the limb toxicity experienced after UL-ILI when compared to LL-ILI. It is possible that the treating surgeon was inclined to limit the tourniquet time in the hope of minimizing limb toxicity, bearing in mind that this report also includes the learning curve of the five treating institutions, with an average of only five UL-ILI procedures performed per center. In our study, no UL-ILI patient required a fasciotomy (grade IV toxicity), while this was performed in 3% of the LL-ILI patients. These toxicity rates and shorter tourniquet times after UL-ILI are in accordance with previous ILI studies [7,8]. Another possible reason for the lower limb toxicity grades may be the fact that UL-ILI patients have lower limb tissue volumes, with a lower fat-to-muscle ratio. Since there is greater melphalan uptake

in muscle than in subcutaneous fat and skin, the muscles of patients with smaller limb volumes are exposed to a relatively lower total dose of melphalan than in patients with larger limb volumes [27,28]. With the intention of overcoming this, UL-ILI patients are routinely administered a higher melphalan dosage of 10.0 mg/L compared to LL-ILI (7.5 mg/L). In view of the lower OR rates and milder toxicity, it could be argued that the melphalan dosage per litre of tissue for UL-ILI should be increased even further, perhaps to 12.5 mg/L.

Despite the low limb toxicity after UL-ILI, the median hospital stay was seven days in this study. This may have been unnecessarily cautious, but as most patients were elderly and many lived in remote areas, treating surgeons usually chose to keep them in hospital until it was clear that limb toxicity would not be a problem rather than discharging them early and have them return several times in the first postoperative week for limb evaluation.

The results of the current study of UL-ILI should be interpreted with caution due to the low number of patients undergoing the procedure. However, it is important to be aware of the results as the procedural and physiological differences suggest that UL-ILI and LL-ILI are actually different procedures and should therefore be studied separately.

Only one small ILP report focusing on upper limb procedures specifically has been published previously and two larger ILP studies have performed a comparative analysis of upper-limb and lower-limb procedures, with no differences in outcomes [19,20,29]. Although response rates after ILP were higher overall compared to ILI (albeit in unmatched patient groups), there was no impact on survival, while limb toxicity was more severe.

In recent years, a number of effective systemic therapies for metastatic melanoma have been introduced, but to date none has been shown to be as safe or effective as ILI, which often produces durable responses, especially where there is bulky disease or when numerous lesions are present [5,30,31]. However, since UL-ILI is technically more challenging to perform, clinicians may in the near future administer systemic agents earlier to patients with unresectable upper-limb melanoma, with ILI as a fall-back option if the systemic therapy is unsuccessful. Also, the potential exists to improve ILI response rates, especially after UL-ILI since the OR rate in the current study was significantly lower than after LL-ILI, by combining or sequencing the procedure with new systemic therapies [32–34]. For example, a recently published phase II trial showed promising results of treatment by CTLA-4 blockade in combination with melphalan ILI, resulting in a CR rate of 62% [35]. Furthermore, systemic ipilimumab before or after ILI is currently being investigated (NCT01323517, NCT02115243) and trials utilizing anti-PD-1 therapy in association with ILI are being developed.

In conclusion, this Australian multicenter study shows that UL-ILI for locally advanced melanoma is safe and is associated with low limb toxicity. Although CR rates were similar, OR rates were lower than those achieved by LL-ILI. Given the differences observed, further studies are needed to analyse UL-ILI and LL-ILI separately in an attempt to optimize their therapeutic potential while keeping limb toxicity at an acceptably low level.

Conflicts of interest

None.

Funding

JF Thompson is supported by the Melanoma Foundation of the University of Sydney and by a NHMRC Programme Grant.

References

[1] Testori A, Faries MB, Thompson JF, et al. Local and intralesional therapy of in-transit melanoma metastases. J Surg Oncol 2011;104:391–6.
[2] Kroon HM, Coventry BJ, Giles MH, et al. Australian multi-center study of isolated limb infusion for melanoma. Ann Surg Oncol 2016;23:1096–103.
[3] Beasley GM, Caudle A, Petersen RP, et al. A multi-institutional experience of isolated limb infusion: defining response and toxicity in the US. J Am Coll Surg 2009;208:706–15.
[4] Kroon HM, Huismans AM, Kam PCA, et al. Isolated limb infusion with melphalan and actinomycin D for melanoma: a systematic review. J Surg Oncol 2014;109:348–51.
[5] Grünhagen DJ, Kroon HM, Verhoef C. Perfusion and infusion for melanoma in-transit metastases in the era of effective systemic therapy. Am Soc Clin Oncol Educ Book 2015;35:e528–34.
[6] Dossett LA, Ben-Shabat I, Olofsson Bagge R, Zager JS. Clinical response and regional toxicity following isolated limb infusion compared with isolated limb perfusion for in-transit melanoma. Ann Surg Oncol 2016;23:2330–5.
[7] Beasley GM, Sharma K, Wong J, et al. A multi-institution experience comparing the clinical and physiologic differences between upper extremity and lower extremity melphalan-based isolated limb infusion. Cancer 2012;118:6136–43.
[8] Wong J, Chen YA, Fisher KJ, Zager JS. Isolated limb infusion in a series of over 100 infusions: a single-center experience. Ann Surg Oncol 2013;20:1121–7.
[9] Egger ME, Tabler BL, Dunki-Jacobs EM, et al. Clinicopathologic and survival differences between upper and lower extremity melanomas. Am Surg 2012;78:779–87.
[10] Buettner PG, MacLennan R. Geographical variation of incidence of cutaneous melanoma in Queensland. Aust J Rural Health 2008;16:269–77.
[11] Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol 2009;27:6199–206.
[12] Thompson JF, Kam PCA, de Wilt JHW, et al. Isolated limb infusion for melanoma. In: Thompson JF, Morton DL, Kroon BBR, editors. Textbook of melanoma. London: Martin Dunitz; 2004. p. 429–37.
[13] Kroon HM, Huismans AM, Kam PCA, et al. Isolated limb infusion: technical aspects. J Surg Oncol 2014;109:352–6.
[14] Giles MH, Coventry BJ. Isolated limb infusion chemotherapy for melanoma: an overview of early experience at the Adelaide Melanoma Unit. Cancer Manag Res 2013;5:243–9.
[15] Wieberdink J, Benckhuysen C, Braat RP, et al. 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;18:905–10.
[16] Lai DTM, Ingvar C, Thompson JF. The value of monitoring serum creatine phosphokinase values following hyperthermic isolated limb perfusion for melanoma. Reg Cancer Treat 1993;6:36–9.
[17] World Health Organization. WHO handbook for reporting results of cancer treatments. WHO Offset Publication No. 48. Geneva: World Health Organization; 1979.
[18] Kaplan L, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1985;53:457–81.
[19] Deroose JP, Eggermont AM, van Geel AN, de Wilt JH, Burger JW, Verhoef C. 20 years experience of TNF-based isolated limb perfusion for in-transit melanoma metastases: TNF dose matters. Ann Surg Oncol 2012;19:627–35.
[20] Rossi CR, Pasquali S, Mocellin S, et al. Long-term results of melphalan based isolated limb perfusion with or without low-dose TNF for in-transit melanoma metastases. Ann Surg Oncol 2010;17:3000–7.
[21] Kroon HM, Moncrieff M, Kam PC, et al. Outcomes following isolated limb infusion for melanoma: a 14-year experience. Ann Surg Oncol 2008;15:3003–13.
[22] Vyas A, Avritscher R, Ensor J, Ross M, Wallace MJ. Isolated limb infusion with cytotoxic agents: a simplified approach for venous access. Cancer 2010;116:459–64.
[23] Aigner KR, Kaevel K. Pelvic stop flow infusion (PSI) and hypoxic pelvic perfusion (HPP) with mitomycin and melphalan for recurrent rectal cancer. Reg Cancer Treat 1994;1:6–11.
[24] Siemann DW, Chapman M, Beikirch A. Effects of oxygenation and pH on tumor cell response to alkylating chemotherapy. Int J Radiat Oncol Biol Phys 1991;20:287–9.
[25] Skarsgard LD, Skwarchuk MW, Vinczan A, et al. The cytotoxicity of melphalan and its relationship to pH, hypoxia and drug uptake. Anticancer Res 1995;15:219–23.
[26] Speicher PJ, Beasley GM, Jiang B, et al. Hypoxia in melanoma: using optical spectroscopy and EF5 to assess tumor oxygenation before and during regional chemotherapy for melanoma. Ann Surg Oncol 2014;21:1435–40.
[27] Kroon HM, Moncrieff M, Kam PC, Thompson JF. Factors predictive of acute regional toxicity after isolated limb infusion with melphalan and actinomycin-D in melanoma patients. Ann Surg Oncol 2009;16:1184–92.
[28] Klaase JM, Kroon BBR, Beijnen JH, van Slooten GW, van Dongen JA. Melphalan tissue concentrations in patients treated with regional isolated perfusion for melanoma of the lower limb. Br J Canc 1994;70:151–3.
[29] Würl P, Eichfeld U, Pauer HD, Gläser A, Rose U, Dralle H. Technique and results of regional hyperthermic cytostatic arm perfusion for malignant melanoma. Surg Today 1997;27:719–25.
[30] Muilenburg DJ, Beasley GM, Thompson ZJ, et al. Burden of disease predicts

response to isolated limb infusion with melphalan and actinomycin d in melanoma. Ann Surg Oncol 2015;22:482–8.
[31] Puzanov I, Skitzki J. New does not always mean better: isolated limb perfusion still has a role in the management of in-transit melanoma metastases. Oncology 2016;15:30. pii: 220161.
[32] Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med 2015;372:30–9.
[33] 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;29:1210–5.
[34] Beasley GM, Coleman AP, Raymond A, et al. A phase I multi-institutional study of systemic sorafenib in conjunction with regional melphalan for in-transit melanoma of the extremity. Ann Surg Oncol 2012;19:3896–905.
[35] Ariyan CE, Brady MS, Siegelbaum R, et al. Robust antitumor responses result from local chemotherapy and CTLA-4 blockade. Cancer Immunol Res 2018;6:189–200.