Hyperthermic isolated limb perfusion for recurrent melanomas and soft tissue sarcomas: Feasibility and reproducibility in a multi-institutional Hellenic collaborative study

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Abstract. Hyperthermic isolated limb perfusion with TNF-α and melphalan (TM-HILP) is a complicated surgical procedure. Herein, we present the experience of the Hellenic collaborating centers with TM-HILP for inoperable in-transit melanoma and soft tissue sarcoma (STS) of the extremities to examine safety and feasibility of collaborating as a multi-institutional group for future research studies. From 2001 to 2009, twenty patients (median age 63.5 years) underwent TM-HILP for locally advanced in-transit melanoma (n=14) or unresectable STS (n=6). All patients underwent a 90-min isolated limb perfusion with melphalan (10 mg/l limb volume) and TNF-α (1-2 mg) under mild hyperthermia (39-40˚C). No major intra-operative complications occurred and all patients completed the procedure successfully. One patient developed postoperative ischemic necrosis of the limb necessitating amputation. All melanoma patients showed a response to TM-HILP with 7 (62%) of them experiencing complete response. All STS patients attained complete response after excision of residual tumor. The median disease specific and limb-relapse-free survival was 15 and 12 months, respectively. TM-HILP can be safely applied even in low volume tertiary hospitals provided that technology to minimize intraoperative systemic leakage is available. Future prospective studies can be performed reproducibly by this multi-institutional collaborative group.

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Introduction

Soft tissue sarcoma (STS) accounts for 1% of all malignant tumors in adults. Approximately 50% of STSs are located in a lower or upper extremity and due to their asymptomatic presentation they can become quite large by the time they are diagnosed (1). Tumor size, histogenetic type, grade, surgical margin status and presence of metastases are the main prognostic factors. Most patients with extremity STS present with clinically localized disease (2,3). Lymph node metastasis is evident in <3% and distant metastasis is present in 10% at the time of primary staging (4). Prognosis of patients with distant metastasis is poor and does not depend on the local control of disease. In the absence of metastasis, the mainstay treatment of STS is radical excision followed by radiotherapy and/or chemotherapy but this therapy is not feasible in a significant number of patients presenting with large (>5 cm), deep seated, high grade sarcomas (5).

Cutaneous melanoma (CM) accounts for 5% of malignant tumors and it is localized on the limbs in approximately one third of the patients (1). Its main prognostic factors are tumor thickness, histological ulceration and presence of metastasis (6,7). Most of the patients with CM present without clinical evidence of metastasis at the time of primary staging but one fourth of them experiences tumor progression. Nearly half of all first recurrences occur in the regional lymph nodes, 30% in distant sites and 20% are satellite or in-transit metastases (8). Satellite and in-transit metastasis represent intralymphatic spread around a primary melanoma and between the primary melanoma and the regional lymph nodes, respectively. Satellite and in-transit disease is worrisome because it is associated with synchronous and metachronous distant disease, it is frequently

very difficult to treat and its persistent recurrence is associated with significant morbidity. Simple surgical excision, laser or cryoablation, intralesional injection of biologically active agents (BCG, IL-2, and interferon), radiation therapy electroporation or combinations of the above are currently available treatment options but they are limited by the extent of the disease and their efficacy (9).

For patients with unresectable STS of the extremities or bulky in-transit melanoma who are considered candidates for amputation, as well as for patients with distant metastases and symptomatic, disabling local disease, TM-HILP is an excellent palliative treatment offering significant symptom relief and high limb salvage rates (10). Hyperthermic isolated limb perfusion with TNF-α and melphalan (HILP-TM) is a surgical procedure of delivering high doses of chemotherapeutic agents in the tumor-bearing area without producing systemic effects. The combination of high dose melphalan and TNF-α with hyperthermia in the form of TM-HILP has been associated with high rates of responses and limb salvage mainly in patients with locally advanced soft tissue sarcomas (STS) and cutaneous melanomas (CM). Herein we review our results of HILP-TM in patients with inoperable locally advanced soft tissue sarcomas and in-transit melanomas to examine safety and feasibility of collaborating as a multi-institutional group for future research studies.

Patients and methods

Between March 2001 and August 2009, 20 patients, 14 females and 6 males, were treated in the frame of the Hellenic Collaborating Centres, with the indication of locally advanced soft tissue sarcoma (6 patients) or recurrent in transit melanoma metastases (14 patients). Median age was 63.5 years (range 30-80). All patients with soft tissue sarcoma had unresectable tumors for which amputation or debilitating surgery was the only alternative. Patients with recurrent in transit melanoma metastases had large and/or multiple inoperative tumors. All patients had histological confirmation of diagnosis. Demographic data, presentation of disease, prior treatment history, treatment details and outcome were retrieved from prospectively maintained database of the Department of Surgery. In total, 14 in-transit melanomas and 6 soft tissue sarcomas are presented. Patient characteristics and demographics, histopathological diagnoses and treatment details are shown in Table I. All patients were initially treated elsewhere and referred to us after recurrence or after failure to control recurrence by chemotherapy or surgical treatment. Two STS patients had clinical evidence of visceral metastasis at the time of HILP.

Routine superficial and/or deep inguinal lymph node dissection was performed in all patients with melanoma. Depending on the localization (above or below knee) and size of the tumor and previous operations, HILP was carried out at the level of external iliac or common femoral vessels. Generally in case of disease remaining localised for a period of months below knee we performed femoral perfusion. External iliac vessels were approached through an oblique iliac incision, splitting of the abdominal wall muscle to the retroperitoneal space and after appropriate exposure and dissection, inferior epigastric and deep circumflex arteries as well as the obturator vein were ligated to optimise isolation of the lower limb. Careful search and ligation of all vessels behind the inguinal ligament such as external circumflex ileum profunda, obturatoria and epigastric iliac veins was performed to minimise systemic leakage. Systemic heparinization (ACT 200-250 sec) was administered ~3 min before the occlusion of vessels with vascular tourniquets. Subsequently, external iliac or femoral venotomy and arteriotomy were performed to insert perfusion catheters. For iliac exposures, the catheters were advanced to the common femoral artery and vein, distal to the inguinal ligament and just before their bifurcation, thus allowing adequate room for the placement of an Esmarch elastic limb tourniquet which was stabilized around a Steinmann pin placed in the iliac crest. In femoral approach cases the catheters were placed at the level of the superficial femoral vessels through the common femoral vessels. Collateral circulation was obstructed by placing a pressure air cuff and inflating it to ~250 mm Hg. Similarly, for arm catheterization, the subclavian vessels were isolated and the catheters were positioned in the axillary vessels, distally enough to place an elastic arm tourniquet or pressure cuff. Catheters were fixed in respective vessels using vascular tourniquets.

Thereafter, arterial and venous perfusion catheters were connected to the extracorporeal circuit. The flow rate was adjusted to 35-40 ml/l of limb volume/min depending on the leakage. Perfusion pressure was maintained at ≥15 mm Hg below the systemic mean arterial pressure to achieve adequate tissue oxygenation. Perfusate consisted of ~250 ml Ringer-lactate, 1 U red blood cell concentrate, 10-30 ml sodium bicarbonate, depending on the pH of the perfusate and 0.5ml of 2500-5000 IU heparin/ml. The total volume of the perfusate was 700-800 ml and the hematocrit in the extracorporeal unit 25-30%. In order to maximize tumor reduction the whole procedure is usually performed under mild hyperthermia (38.5-40˚C) that may improve the clinical outcome by direct proteolysis of the tumor (11). Mild tissue hyperthermia was maintained by heated perfusion circuit and by inserting the limb between air-warming blankets covered by isothermal foil. Hyperthermia was monitored by 2 thermocouples inserted through skin to the subdermal tissues and muscles of femur and leg. After 20 min of leakage control and monitoring (described below) and after reaching limb temperature of at least 38.5˚C, 2 mg of TNF-α-1a (Tasonermin, Beromun®, Boehringer-Ingelheim) as bolus was injected into the extracorporeal circuit. After perfusion of the limb for 30 min another perfusion session followed with melphalan (10 mg/l limb volume) for 60 min. Drug concentrations in the perfusate may reach 15-25 times higher than conventional systemic administration. Finally the limb was flushed out with 4-6 l Haemacel (5% dextran-40.5% glucose), Esmarch tourniquet (or pressure cuff) was released, catheters were removed and cannulated vein and then artery were reconstructed. Following HILP, all patients were transferred to the intensive care unit for rigorous monitoring for at least one night without endotracheal intubation according to protocol.

Leakage control and monitoring. Continuous intraoperative external monitoring of perfusate leakage was performed for

Table I. Patient and HILP-TM treatment characteristics.

Patient	Age/ sex	Histological diagnosis	Anatomical site	No. of lesions	Related symptoms	Distant metastasis	Previous treatment	Level of isolation	Melphalan dose (mg)	Fasciotomy	LND results
1	80/F	Liposarcoma	Femur	1 (12 cm)	Pain, limb edema	No	XRT, chem	Iliac	100	Yes	NP
2	57/F	Leiomyosarcoma	Calf	1 (7 cm)	Pain	No	Chem	Femoral	80	No	NP
3	79/F	Leiomyosarcoma	Calf	1 (10 cm)	Pain	Lung	Chem	Femoral	90	No	NP
4	63/M	Histiocytoma, angiosarcoma	Arm	1 (10 cm)	Pain, limp, severe edema	No	Surg, chem	Subclavian	60	Yes	NP
5	30/M	Neurinosarcoma	Femur	1 (15 cm)	Pain, edema	Yes	Surg, chem	Femoral	100	Yes	NP
6	64/M	Histiocytoma	Forearm	1 (>10 cm)	Pain, edema	No	Surg, chem	Brachial	60	Yes	NP
7	57/F	Melanoma in transit	Calf	>10	Pain, bleeding, edema	No	None	Femoral	90	No	Positive
8	60/F	Melanoma in transit	Calf+femur	>20	-	No	None	Femoral	140	No	Positive
9	61/F	Melanoma in transit	Calf	>20	Pain	No	Chem, surg	Iliac	90	Yes	Negative
10	71/F	Melanoma in transit	Calf	>10	-	No	IFN, surg	Iliac	95	Yes	Positive
11	58/M	Melanoma in transit	Calf+femur	>100	Limp, limb edema	No	Surg	Iliac	130	No	Positive
12	75/F	Melanoma in transit	Calf+femur	>20	Limb	No	Surg, chem	Femoral	100	No	Negative
13	58/F	Melanoma in transit	Calf+femur	35	-	No	Surg, IFN	Iliac	100	No	Positive
14	68/F	Melanoma in transit	Calf+femur	>20	Edema on exertion	No	Surg	Iliac	100	No	NP
15	64/F	Melanoma in transit	Calf+femur	>10	Pain, bleeding, edema	No	Surg, chem	Iliac	90	Yes	NP
16	68/M	Melanoma in transit	Calf+femur	>50	Pain, bleeding edema	No	Surg, chem	Femoral	90	Yes	NP
17	69/F	Melanoma in transit	Big toe, femur		Pain, edema	No	Surg, chem	Iliac	90	Yes	Positive
18	30/F	Melanoma in transit	Calf+femur	>20	Pain, bleeding	No	Surg, chem	Femoral	90	Yes	Positive
19	76/M	Melanoma in transit	Calf+femur	>30	Pain, bleeding, edema	No	Surg, chem	Femoral	85	Yes	NP
20	31/F	Melanoma in transit	Calf	>10	Pain, bleeding, edema	No	Surg, chem, ILP	Popliteal	60	Yes	NP

HILP-TM, hyperthermic isolated limb perfusion with TNF-α and melphalan. F, female; XRT, radiotherapy; chem, chemotherapy; surg, surgical excision, LND, inguinal lymph node dissection; NP, not performed.

all patients using a collimated gamma Nal(TI) scintillator and Tc-99m labelled human serum albumin (HSA). The detector was connected to a rate-meter and a strip-chart recorder and all these devices were mounted on a in-house-made mechanically articulated mobile stand which allowed proper alignment and immobilisation of the detector close to the chest over the heart of the treated patient. Following surgical isolation, the lead-tube collimated detector wrapped in a sterile plastic bag was carefully positioned over patient's heart and appropriately aligned to exclude photons emitted from the perfusion unit. A dose o 1 MBq of Tc-99m labelled HSA was injected in the systemic circulation to determine baseline count rate over heart. Following stabilization of baseline count-rate, 10 MBq of Tc-99m labelled HSA were injected into the perfusion circuit. Doubling of baseline count-rate corresponds to 10% leakage. Leakage was determined for at least 15 min by monitoring the % difference of count-rate from baseline. If there was no leakage, the chemotherapy agent was introduced and the therapy was initiated. If there was considerable leakage (>0.5% for 15 min), surgical isolation was checked again and systemic pressure, flow rate of extracorporeal circulation system, and limb temperature were appropriately tuned to minimise or even eliminate leakage. Initiation of chemotherapy was permitted only if the leakage for a 20 min duration was <0.5%. The radiopharmaceutical Tc-99m HSA was prepared in the Nuclear Medicine Department of our Hospital 1 h prior to use.

Acute local toxicity of the HILP procedure was classified according to Wieberdink *et al* (12). Treatment response was evaluated 3 and 6 months after operation according to the response evaluation criteria in solid tumors (RECIST).

Results

All patients completed the surgical operation successfully. No major intra-operative complication necessitating re-operation occurred. In one patient femoral dissection was noticed after the removal of the catheters and was treated with patch reconstruction. No patient experienced systemic leakage exceeding 10%, necessitating interruption of the procedure. Total dose of melphalan in the perfusate ranged from 80 to 140 mg. Prophylactic fasciotomies were performed in 60% of the patients due to threatened compartment syndrome. Median postoperative stay was 15 days (range, 10-30 days).

Regional toxicity was minimum (grade I) in 11 (55%) of the patients. Grade II and III toxicity occurred in 7 and 2 patients, respectively. One melanoma patient experienced grade V toxicity and underwent amputation due to progressive necrosis of the limb. This patient was the second case of TM-HILP in the particular center, had undergone prophylactic fasciotomy and intraoperative systemic leakage was determined to <1%. This incident led to an extensive review of the details of the procedure in the particular patient according to our protocol which, however, did not reveal any preventable reason for this serious complication. No serious regional or systemic toxicity was noted in the following 7 patients operated in that centre. Systemic toxicity is presented in Table II. Observed side effects were mild (grade I and II) and included fever, thrombopenia, leucopenia, nausea and tachycardia without any clinical impact on patient outcome. Postoperative serum level of creatine kinase was increased significantly in 17 (85%) patients ranging from x1.8-x28 maximum upper normal value. Surgical postoperative complications (wound infection and dehiscence) were minimal in the majority of patients and were observed in patients undergoing simultaneous radical lymph node dissection. Two patients experienced limited skin flap necrosis and were successfully treated with surgical debridement; one of them experienced also seroma which was managed by drainage. No patient developed postoperative fascial compartment syndrome.

All patients, except from the one who underwent amputation, were able to walk unassisted when they were discharged. The patient who underwent amputation died 6 months later of disseminated melanoma. Clinical response to TM-HILP is outlined in Table II. Limb salvage was 95% in our series. All melanoma patients experienced a clinical response to treatment. Patients with STS attained complete response after excision of residual tumor. Histological examination of dissected lymph nodes in melanoma patients showed metastatic disease in 6 out of 14 melanoma patients. Median local relapse-free interval was 17 months for melanoma patients. Median disease specific survival was 15 months for both melanoma and sarcoma patients. All patients presenting with pain had significant relief of their symptom concurrent with their response to TM-HILP and no significant loss of limb function was noted as patients were able to return to their pre-treatment daily activities.

Discussion

In this retrospective study, we reported the experience and the outcome of TM-HILP in patients with unresectable in-transit melanoma or soft tissue sarcoma treated within the framework of the Hellenic Collaborative Centres. These are comprised of 6 tertiary hospitals localised in Greece and 1 in Cyprus. These collaborating centres are the only providers TM-HILP in these two countries since 2001 (13). The results of our small series show that this treatment modality is able to offer palliation and limb salvage without perioperative mortality in a selected group of patients with tumors refractory or not amenable to conventional surgery or chemotherapy who otherwise were considered candidates for amputation.

The overall response rate in the current study was 100% for melanoma excluding one patient who underwent immediate amputation due to ischemic necrosis of the limb. The complete response rate was 62%. These results are comparable with those reported in the literature with overall and complete response rates ranging from 95 to 100% and 70 to 90% respectively (10). Complete response rates differ significantly according to disease stage in patients with in transit melanoma. In the series of Gruenhagen *et al* (14), patients with coexisting lymph node involvement exhibited complete response rates of 64% that was significantly lower than patients without lymph node metastasis who experienced complete response rate of 82% (14). In our study, the majority of patients had lymph node metastases (54%) which might account for the fact that our CR rates are at the lower limit of the range reported in the literature. One third of our melanoma patients

Table II. Toxicity, clinical response and follow-up data of patients who underwent HILP-TM.

Patient	Systemic leakage %	Systemic toxicity	Local toxicity (Wieberdink[a])	Post HILP CK (U/l)	Days of post-operative hospital stay	Clinical response[b]	Complete excision of residual tumor	Follow-up (months)	Patient status at last follow-up	Locoregional relapse-free survival (months)
1	<0.5	PLTs, WBCs, K, rash	I	3472	17	SD	Yes	29	DF	29
2	<1.5	Nausea, tachycardia, fever	II	3726	12	PR	Yes	36	D	36
3	<0.5	Flutter, K	I	n.l.	13	CR	Yes	15	D	15
4	8%	PLT, WBC, nausea, tachycardia	II	3750	18	PR	Yes	96	DOD	60
5	8%	PLT, WBC, tachycardia	II	3700	30	SD	Yes	12	DOD	12
6	<0.5%	Nausea, rash, PLT, WBC	I	2100	15	PR	Yes	12	DOD	6
7	<0.5	Fever	I	n.l.	28	CR	-	6	D[a]	6
8	<0.5	None	I	300	12	CR	-	18	LR	17
9	<1.0	Fever	I	2400	17	CR	-	19	DM	19
10	<0.5	None	II	598	12	CR	-	10	D	8
11	<1.0	Bilirubinemia	II	3944	12	PR	-	7	DM	7
12	<0.5	None	III	n.l.	16	PR	-	6	DF	6
13	<1.0	Metabolic acidosis	I	250	12	CR	-	3	DF	3
14	<1.0	None	II	350	10	CR	-	1	DF	1
15	<1%	PLT, WBC, nausea	V	3950	30	-	-	6	DOD	-
16	<1%	Nausea, fever, PLT, WBC, rash	II	2800	18	PR	-	18	DOD	18
17	<1%	Fever, nausea, rash, WBC, PLT	II	3500	19	PR	-	8	DOD	8
18	<1%	Fever, rash, tachycardia	I	2100	15	PR	-	15	DOD	7
19	<1%	Nausea, tachycardia, PLT, WBC	I	2600	14	CR	-	18	DOD	10
20	<1%	Nausea, tachycardia, fever, PLT, WBC	I	2200	15	CR	-	8	DOD	8

HILP-TM, hyperthermic isolated limb perfusion with TNF-α and melfalan; CK (U/l), creatine kinase (units/liter); n.l., normal limits range (35-180 U/l); PR, partial response; ↓PLTs, thrombopenia; ↓WBCs, leucopenia; ↓K, hypokalemia; D, died of melanoma; DOD, died of disease; DM, alive distant metastasis present; LR, alive local relapse; D, died of other cause; DF, alive free of disease. [a]Ref. 16. [b]Assessed 3 and 6 months after operation according to RECIST criteria.

developed local relapse during follow-up and half of them presented with distant metastasis and died shortly after. Limb salvage, the primary objective of this treatment reached 92% in our series that is comparable with rates reported in the literature demonstrating high efficacy of TM-HILP in unresectable in-transit melanoma (14).

Overall response rates reported in the literature for unresectable soft tissue sarcomas are generally lower compared with melanomas and vary from 53 to 91% (15). Complete and partial response rates constitute ~20 and 50% of the total, respectively and limb salvage ranges between 58 and 89% in several single centre studies (15,16). Due to small number of patients meaningful comparison of our results with those reported in the literature is not feasible. However, limb salvage was 100% in our sample and all patients were able to undergo complete excision of their tumors.

Isolated limb perfusion with melphalan only has been effective in the case of small multiple in transit metastases and it is still widely used with this indication. This approach however has been ineffective in the case of bulky melanomas metastasis and has failed in the case of soft tissue sarcoma (17,18). The addition of TNF-α to isolated limb perfusion with cytotoxic agents increased dramatically the efficacy of the method as indicated by several multicenter trials (10). This was attributed to the vasculotoxic effect of TNF-α, leading to ischemic necrosis of the tumor within 16 h from the perfusion and increased uptake of cytotoxic drugs by the tumor (19,20). Another mechanism of the synergistic effect of TNF-α might be the increase of chemosensitivity of tumor cells to cytotoxic treatment. Recent experimental studies suggest that attenuation of integrin mediated PI3K signaling sensitizes neoplastic cells to cytotoxic treatment *in vitro* and *in vivo* (21). Moreover, reduced integrin activation has been implicated in the antivascular activity of TNF-α (22). It is possible that TNF-α mediated attenuation of PI3K signaling in melanoma cells renders it more sensitive to cytotoxic treatment but this remains to be investigated (23).

TM-HILP is considered a palliative treatment even though its impact to the overall survival of patients with in transit melanoma or STS is not yet clear (10). Therefore, it is essential that it is associated with minimum toxicity and essentially no mortality. Moreover, as in every palliative procedure it has to be characterized by quick recovery, symptom palliation and good functional result. In this series, as in several others, mortality associated with the procedure was zero. Main systemic adverse effect of the TM-HILP was mild leucopenia and thrombopenia, sinus tachycardia and postoperative fever. Systemic toxicity was mainly grade 1 and 2, transient; it did not require therapeutic intervention and did not affect treatment outcome or post-operative hospital stay. Grade 3 or 4 toxicity was not observed in any patient, even in patients with significant but acceptable systemic leakage. Minimal systemic toxicity is reported from several European centres and it is attributed to the strict and continuous monitoring of systemic leakage during the procedure (14,24-27). When leakage is prevented and a thorough wash out of the limb vasculature is performed at the end of the procedure mild systemic toxicity is observed on the day of surgery and it is similar to the toxicity after ILP with melphalan alone (28).

Regional toxicity was mild (grade I and II) in the majority of patients (90%) and comparable with results of larger patient series (29). This is critical, since the severity of acute regional toxicity is related to long-term morbidity and functionality of the limb. In patients experiencing grade III acute regional toxicity the rate of permanent long-term regional morbidity reaches 50% (muscle atrophy, tissue fibrosis, limb malfunction) (30,31). Moreover grade III reactions are not well accepted by patients and may prolong significantly hospital stay. Acute regional toxicity necessitating amputation is a devastating complication of the procedure given its palliative nature even though the patients undergoing HILP are candidates for amputation if no therapy is offered. The frequency of treatment related amputation is generally below 5% in the literature (14,24,25,32). Probably, due to the low frequency of the event no specific associated factors have been reported so far. The addition of TNF-α to HILP with melphalan was not related with any increase in grade 5 regional toxicity in a retrospective analysis of 294 melanoma patients (27).

Even though the general impression is that the functional morbidity in patients undergoing TNF-α based ILP is minimal, studies addressing this issue in the literature are scarce. In the study of Grunhagen *et al* (14) where limb function did not affect standard daily activities in 84 out of 87 patients undergoing TM-HILP for multiple in-transit melanoma metastases. On the other hand, Noorda *et al* (32) reported that 31% of the STS patients experienced significant extremity malfunction after TM-HILP. Higher malfunction rates in soft tissue sarcoma patients might be explained by lower complete response rates and the added morbidity of the subsequent surgical excision of the remaining tumor. Moreover, in view of the short survival of patients undergoing ILP, health related quality of life is a major outcome of the treatment. There is only one published study evaluating the quality of life of long-term melanoma survivors after ILP. The results of this study suggested that the quality of life of these patients is equivalent to that of matched controls of the general population, even though only 20% of the patients remained completely free of limitations and symptoms at the end of follow-up. Further studies are necessary, including patients with STS as well as studies assessing rehabilitation activities to reduce long-term morbidity of this therapeutic approach (33).

In conclusion, TM-HILP represents a safe and effective limb sparing palliative procedure for patients with inoperable soft tissue sarcomas and bulky in-transit melanomas which are considered candidates for amputation. TM-HILP can be safely performed even by low volume tertiary hospitals provided that technology to minimize intraoperative systemic leakage is available. The results of our study are comparable to those of large centers pointing that future prospective studies can be performed reproducibly by this multi-institutional collaborative group.

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