Hyperthermic Isolated Limb Perfusion With Tumor Necrosis Factor α, Interferon Gamma, and Melphalan for Locally Advanced Nonmelanoma Skin Tumors of the Extremities

A Multicenter Study

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Background: Hyperthermic isolated limb perfusion (HILP) with tumor necrosis factor α (TNF-α), interferon gamma, and melphalan has proved to be useful in the treatment of recurrent malignant melanoma and of locally advanced soft tissue sarcomas of the extremities.

Objective: To determine whether this modality is also effective in the treatment of locally advanced nonmelanoma skin tumors of the extremities.

Patients and Methods: Fifteen patients with locally advanced primary, recurrent, or metastatic skin tumors of the extremities (12 with squamous cell carcinoma and 3 with Merkel cell carcinoma) underwent HILP with TNF-α, interferon gamma, and melphalan. Six tumors were localized in the upper extremity (40%), and 9 in the lower extremity (60%). Treatment-related complications, limb salvage rate, local recurrence, and regional and distant metastases were scored during a median follow-up of 20 months.

Results: After HILP, 9 patients (60%) showed a complete response (with histopathological confirmation). Four patients (27%) showed a partial response (with histopathological confirmation in 1 patient), and 2 patients (13%) showed no change (with histopathological confirmation in 1 patient and with clinical evidence in 1 patient). Two patients (13%) showed treatment-related complications. The limb salvage was achieved in 12 patients (80%), and the local recurrences developed in 4 patients (27%). During follow-up, regional lymph node metastases were observed in 2 patients (13%) and distant metastases in 2 patients (13%).

Conclusion: Based on our results, HILP with TNF-α, interferon gamma, and melphalan should be considered as a limb-saving treatment modality in patients with locally advanced nonmelanoma skin tumors of the extremities who would otherwise be candidates for ablative surgery.


After Creech et al1 introduced the technique of hyperthermic isolated limb perfusion (HILP) in 1957, it has most commonly been used in patients with recurrent malignant melanoma of the extremities, satellitosis, and in-transit metastases.1-4 The standard cytotoxic drug in HILP for melanoma is melphalan.5 In HILP for soft tissue sarcoma (STS) of the extremities, melphalan and various other cytotoxic agents did not improve treatment results, compared with previously described combined-treatment modalities of surgery and radiotherapy.5-7 After the addition of high-dose tumor necrosis factor α (TNF-α), the results improved dramatically. Meanwhile, HILP with TNF-α and melphalan has proved to be a promising limb-saving treatment modality for patients with locally advanced STS of the extremities.8-10 The treatment of locally advanced carcinomas of the skin, other than malignant melanoma, also can be a major problem for the surgical oncologist. It may be impossible to resect the tumor curatively without losing or causing severe functional impairment of the extremity. At present, there is no standard therapy for this group of patients. The promising results of HILP with TNF-α, interferon gamma, and melphalan, under circumstances of mild (39°C-40°C) hyperthermia, in patients with melanoma and STS prompted us to investigate this treatment modality in patients with locally advanced nonmelanoma skin tumors of the extremities who otherwise cannot undergo curative resection. We evaluated the efficacy of HILP with TNF-α, interferon gamma, and melphalan and the treatment-related complications in patients with locally advanced

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PATIENTS AND METHODS

Fifteen patients (11 male and 4 female; median age, 71 years; range, 31-78 years) underwent HILP with TNF-α, interferon gamma, and melphalan for primary, recurrent, or metastatic squamous and Merkel cell carcinoma localized in the extremities. Patients were treated at 1 of the 4 institutes involved in this multicenter study (University Hospital Groningen, Groningen, University Hospital–Daniel den Hoed Cancer Centre Rotterdam, Rotterdam, Netherlands Cancer Institute Antoni van Leeuwenhoek ziekenhuis, Amsterdam, the Netherlands; and the Centre Pluridisciplinaire d’Onco logie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland). In 6 patients (40%), the tumor was localized in the upper extremity; in 9 patients (60%), in the lower extremity. Tumor size varied from 3 × 3 to 15 × 12 cm (median, 52 cm²). Owing to the size and/or the location, curative resection of these tumors was impossible without causing severe mutilation of the extremity or impairment of function. Two patients (13%) had local recurrence after surgical resection and external beam radiotherapy (patients 6 and 9), 2 patients (13%) had metastatic lesions in the soft tissues of the extremity (patient 3), 1 patient (7%) was treated for a primary tumor and a metastasis in the same extremity (patient 3), and 1 patient (7%) was treated for tumor recurrence (after surgery) and a metastasis of that tumor in the same extremity (patient 10). Characteristics of patients and tumors are provided in the Table.

Before HILP, an incisional biopsy was performed to obtain the definitive histopathological diagnosis. Twelve patients were treated for squamous cell carcinoma, and 3 patients were treated for Merkel cell carcinoma. Besides incisional biopsy, preoperative work-up consisted of magnetic resonance imaging or computed tomographic scan (patients treated in Lausanne), selective angiography of the affected extremity, chest x-ray and computed tomographic scan (patient 6), and 1 patient (7%) was treated for tumor recurrence after surgical resection and external beam radiotherapy (patients 6 and 9), 2 patients (13%) had metastatic lesions in the soft tissues of the extremity (patient 3), and 1 patient (7%) was treated for tumor recurrence (after surgery) and a metastasis of that tumor in the same extremity (patient 10). Characteristics of patients and tumors are provided in the Table.

Before HILP, an incisional biopsy was performed to obtain the definitive histopathological diagnosis. Twelve patients were treated for squamous cell carcinoma, and 3 patients were treated for Merkel cell carcinoma. Besides incisional biopsy, preoperative work-up consisted of magnetic resonance imaging or computed tomographic scan (patients treated in Lausanne), selective angiography of the affected extremity, chest x-ray and computed tomographic scan of the lungs. After informed consent was obtained, the patients were treated in a phase 2 study consisting of HILP with TNF-α, interferon gamma, and melphalan.

The perfusion technique has previously been described extensively.11,12 Before HILP, the patients received 0.2 mg recombinant interferon gamma (Boehringer Ingelheim, Ingelheim/Rhein, Germany) subcutaneously once a day for 2 days. Isolation of the blood circulation of the limb was achieved by clamping the major artery and vein after heparinization. After inserting the cannulae, the patient was connected to a heart-lung machine. Collateral vessels were ligated, and a tourniquet was applied to compress the remaining minor vessels. Leakage of the drugs from the perfusion circuit to the systemic circulation was measured using human serum albumin with radioactive iodine and technetium.13,14 The extremities were perfused for 90 minutes under mild hyperthermic conditions (39°C-40°C) with 0.2 mg of interferon gamma, 3 (arm) to 4 (leg) mg of TNF-α (Boehringer Ingelheim), and 10 (limb volume, leg) to 13 (limb volume, arm) mg/L of melphalan (L-phenylalanine mustard; Glaxo-Wellcome, London, England). After perfusion, patients were admitted to the intensive care unit to monitor the clinical toxic effects of HILP.

Six to eight weeks after perfusion, the patients underwent restaging with magnetic resonance imaging or computed tomographic scan and angiography. Tumor resection, if possible, was performed 6 to 8 weeks after perfusion. The histopathological response to HILP was standardized, based on results of the histopathological examination and scored with the following criteria: complete histopathological response, indicated by no viable tumor cells; partial histopathological response, indicated by more than 50% necrosis; and no change, indicated by less than 50% necrosis.5 Treatment results are summarized in the Table.

All patients participated in an intensive physical therapy rehabilitation program to improve functional morbidity. During follow-up, we scored treatment-related complications, limb salvage rate, local recurrence, local progression, regional recurrence, and distant metastases. Survival curves were calculated according to the Kaplan-Meier method.

**Table**: Patient, Treatment, and Follow-up Characteristics

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Tumor Localization (Size, cm)</th>
<th>Tumor Histological Characteristics</th>
<th>Synchronous Metastases at Time of HILP</th>
<th>Artery Used for HILP</th>
<th>Resection</th>
<th>Histopathological Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/44</td>
<td>Foot (3 × 3)</td>
<td>Primary squamous cell</td>
<td>No</td>
<td>Popliteal</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>2/M/72</td>
<td>Elbow region (6 × 8)</td>
<td>Primary squamous cell</td>
<td>No</td>
<td>Brachial</td>
<td>Yes</td>
<td>Partial</td>
</tr>
<tr>
<td>3/M/74</td>
<td>Hand and wrist (15 × 12); forearm (0.5 × 0.5)</td>
<td>Primary squamous cell; metastatic squamous cell</td>
<td>No</td>
<td>Axillary</td>
<td>No</td>
<td>No change</td>
</tr>
<tr>
<td>4/M/58</td>
<td>Hand (13 × 11)</td>
<td>Recurrent squamous cell</td>
<td>No</td>
<td>Axillary</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>5/M/61</td>
<td>Lower leg (7 × 5)</td>
<td>Recurrent squamous cell</td>
<td>No</td>
<td>Iliac</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>6/F/71</td>
<td>Lower leg (10 × 4)</td>
<td>Recurrent squamous cell</td>
<td>No</td>
<td>Iliac</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>7/M/62</td>
<td>Lower leg (15 × 12)</td>
<td>Recurrent squamous cell</td>
<td>No</td>
<td>Femoral</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>8/M/78</td>
<td>Lower leg (15 × 15)</td>
<td>Recurrent squamous cell</td>
<td>No</td>
<td>Femoral</td>
<td>No</td>
<td>Partial</td>
</tr>
<tr>
<td>9/M/58</td>
<td>Lower leg (8 × 10)</td>
<td>Recurrent squamous cell</td>
<td>No</td>
<td>Femoral</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>10/F/77</td>
<td>Forearm (7 × 3); upper arm (5 × 3)</td>
<td>Recurrent squamous cell; metastatic squamous cell</td>
<td>No</td>
<td>Axillary</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>11/F/71</td>
<td>Foot (8 × 7)</td>
<td>Metastatic squamous cell</td>
<td>Inguinal lymph nodes</td>
<td>Femoral</td>
<td>No</td>
<td>Partial</td>
</tr>
<tr>
<td>12/M/73</td>
<td>Lower leg (5 × 5)</td>
<td>Metastatic squamous cell</td>
<td>Lung</td>
<td>Popliteal</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>13/M/31</td>
<td>Hand (5 × 2)</td>
<td>Primary Merkel cell</td>
<td>No</td>
<td>Axillary lymph nodes</td>
<td>Yes</td>
<td>No change</td>
</tr>
<tr>
<td>14/F/78</td>
<td>Lower leg (multiple)</td>
<td>Recurrent Merkel cell</td>
<td>No</td>
<td>Femoral</td>
<td>Yes</td>
<td>Complete</td>
</tr>
<tr>
<td>15/M/73</td>
<td>Elbow (9 × 7)</td>
<td>Recurrent Merkel cell</td>
<td>No</td>
<td>Axillary lymph nodes</td>
<td>Yes</td>
<td>No change</td>
</tr>
</tbody>
</table>

*HILP indicates hyperthermic isolated limb perfusion; NED, no evidence of disease; MOF, multiple organ failure; DRT, death related to treatment; AWD, alive with disease; DND, death not related to malignant lesion; and DOD, death due to disease.
squamous cell carcinoma or Merkel cell carcinoma localized in the extremities.

### Results

The median follow-up was 20 months (range, 1-65 months). Resection of the residual tumor mass following HILP was performed in 11 of the 15 patients. In these patients, the histopathological response of the tumor to HILP with TNF-α, interferon gamma, and melphalan was evaluated microscopically, whereas in the other 4 patients, the response was assessed clinically. Reasons for not performing tumor resection were death due to other causes before biopsy or resection (patient 11) and deterioration of the general condition of the patient due to metastatic disease (patient 15). In patients 3 and 8, a complete resection of the tumor after HILP was not possible, so an amputation was performed after 2 and 5 months, respectively. After HILP, the tumors showed complete histopathological response in 8 patients with squamous cell carcinoma and 1 patient with Merkel cell carcinoma (60%, with confirmation in all), partial response in 3 patients with squamous cell carcinoma and 1 patient with Merkel cell carcinoma (27%, with histopathological confirmation in 1), and no change in 2 patients (13%, squamous cell carcinoma without histopathological confirmation in 1 patient, and Merkel cell carcinoma with histopathological confirmation in 1). Figure 1 demonstrates the effect of HILP in patient 4, who was treated for an unresectable squamous cell carcinoma, localized on the hand.

Limb salvage could be achieved in 12 patients (80%; 9 with squamous cell carcinoma and 3 with Merkel cell carcinoma). Reasons for ablative surgery were deep infection of the tumor bed shortly after perfusion in 1 patient (patient 5) and local progression during follow-up in 2 patients (patients 3 and 8). Complications occurred in 2 patients (13%). After HILP, a distributive shock with multiple organ failure developed in patient 5, followed by a deep infection of the tumor bed that necessitated amputation. He died of septic shock in the postoperative period. A superficial wound infection developed in patient 11. Altogether, treatment-related mortality was 7% (1 patient).

During follow-up, local recurrence was observed in 2 patients (patients 10 and 13), 6 and 8 months after HILP, respectively, after a complete histopathological response of a squamous cell carcinoma in 1 patient and after no histopathological change of a Merkel cell carcinoma in the other. In the latter patient (patient 13), amputation was not performed due to the development of fulminant disseminated disease. In 2 patients in whom resection of the residual tumor mass was not performed, local progressive disease developed 2 and 5 months after HILP (patients 3 and 8, respectively). Thus, a total of 4 patients (27%; 3 patients with squamous cell carcinoma and 1 patient with Merkel cell carcinoma) suffered local recurrence or local progressive disease.

Regional lymph node metastases developed during follow-up in 2 patients (13%), 1 patient treated for squamous cell carcinoma and 1 patient treated for Merkel cell carcinoma (patients 3 and 14, respectively). In 2 patients treated for Merkel cell carcinoma (13%), distant metastases developed during follow-up (patients 13 and 15). Both patients died during follow-up due to metastatic disease, 16 and 7 months, respectively, after HILP. One patient with multiple metastases from a squamous cell carcinoma at the time of HILP died 8 months following HILP, due to progressive metastatic disease (patient 12). The 2 other deaths (13%) during follow-up were due to suicide (patient 11) and sepsis as described in patient 5. The overall survival and the local and regional recurrence in patients are presented in Figure 2.

### Comment

Maximum locoregional control with minimum treatment-related morbidity was the primary goal of our study. The incidence of local recurrence and (functional) morbidity after HILP could therefore be used to measure these criteria.12 As a considerable number of patients with locally advanced malignant neoplasms of the extremities will die of distant metastases, limb-sparing treatment modalities now are considered preferable for a significant number of musculoskeletal tumors.15,16 In this respect, HILP with TNF-α, interferon gamma, and melphalan has meant a breakthrough in the limb-saving treatment of locally advanced STS of the extremities.8,9 Treatment with TNF-α causes early and selective changes in tumor-associated endothelial cells of human tumors, which can lead to necrosis of the tumor.17-19 Disappearance of all tumor-associated vessels has been described within 7 to 14 days after HILP with TNF-α, interferon gamma, and melphalan, whereas other vessels remained unchanged.20,21 Even with palliative intent, HILP with TNF-α, interferon gamma, and melphalan can be an effective treatment modality to control local disease, alleviate pain, and avoid ablative surgery.8,10,22-24

All patients treated in our study had locally advanced disease or local recurrence after previous treat-
ment and were considered candidates for ablative surgery. In the selection of a treatment modality for squamous cell carcinoma, factors such as size and localization of the lesion, depth of infiltration, age of the patient, and desired cosmetic result all play a role. Tumor resection with adequate margins and radiotherapy are the most frequently used therapies, whereas radiotherapy or chemotherapy may be used for local or distant metastases.\(^2^5\) In the past, isolated limb perfusion with methotrexate has been described as a limb-saving treatment modality in 2 patients with locally advanced squamous cell carcinoma of the lower extremities. However, both perfusions were performed under true hyperthermia (\(>41^\circ\mathrm{C}\)), which may lead to serious local toxic side effects.\(^2^6\)

On the basis of our results, we can conclude that the limb salvage rate and the local control rate in patients treated with HILP consisting of TNF-\(\alpha\), interferon gamma, and melphalan followed by tumor resection for squamous cell carcinoma correspond with those in patients treated with the same modality for locally advanced STS of the extremities.\(^9\)

In our study, 1 patient had major treatment-related complications (patient 5), leading to the death of this patient. Preoperatively, this patient had severe lymphedema. Lymphedema may lead to retention of TNF-\(\alpha\) and thereby to distributive shock and multiple organ failure. Therefore, lymphedema must be an exclusion criteria in the TNF-\(\alpha\)-based protocols.

Merkel cell carcinomas are known to have a high incidence of locoregional recurrences, regional lymph node involvement, and distant metastases, resulting in a very poor prognosis after surgical treatment.\(^2^7\) In the 3
patients we treated with this condition, however, limb salvage was achieved. We conclude that in patients with locally advanced nonmelanoma skin tumors of the extremities who cannot be treated curatively with conventional treatment modalities, HILP with TNF-α, interferon gamma, and melphalan is a promising limb-saving treatment modality mostly without major treatment-related morbidity.

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REFERENCES