Isolated Limb Perfusion in Primary and Recurrent Melanoma: Indications and Results

DANIELLE LIÉNARD, MD,1* ALEXANDER M. EGGERMONT, MD, PhD,2 BIN B.R. KROON, MD, PhD,3 HEIMEN SCHRAFFORDT KOOPS, MD, PhD,4 AND FERDINAND J. LEJEUNE, MD, PhD1
1Centre Pluridisciplinaire d’Oncologie, CHUV, Lausanne, Switzerland
2Dr. Daniel den Hoed Cancer Centre, Rotterdam, The Netherlands
3The Netherlands Cancer Institute, Amsterdam, The Netherlands
4Groningen University Hospital, Groningen, The Netherlands

In advanced melanoma of the limbs with in-transit metastasis, melphalan with isolated limb perfusion (M-ILP) produces around 50% complete remissions (CR). The combination of melphalan with tumour necrosis factor-alpha (TNFα) and interferon-gamma (IFNγ) in isolated limb perfusion (TIM-ILP) gives around 80% CR. A prospective randomised phase II study compared 32 patients who received TIM-ILP with 32 patients who received TM-ILP (without IFNγ). The overall remission rate (ORR) and the CR rate were superior with TIM-ILP as compared to TM-ILP, 100% vs. 91% and 78% vs. 69% respectively, but the differences are not significant. Given the efficacy of M-ILP on in-transit metastasis, the procedure was tested as an adjunct to surgery in high-risk (Breslow ≥1.5 mm) primary melanoma of the limbs. Through the combined effort of the melanoma groups of the European Organization for Research and Treatment of Cancer (EORTC), the World Health Organization (WHO), and the North American Perfusion Group, 832 evaluable patients from 16 centres were entered in a phase III study. Median followup is 6.4 years. There was a trend for a longer disease-free interval after M-ILP. The difference is significant if the patients without elective lymph node dissection (ELND) are separately analysed, with a high significance in the 1.5 to 3 mm thickness subgroup. The occurrence of in-transit metastases was reduced from 6.6% to 3.3% by M-ILP. There was, however, no benefit of M-ILP in terms of survival. Prophylactic M-ILP cannot be recommended as a standard adjunct to surgery in high-risk primary limb melanoma. TIM-ILP or TM-ILP is a regional therapy with a very high regional response rate on melanoma in-transit metastasis. Semin. Surg. Oncol. 14:202–209, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: melanoma; tumor necrosis factor; regional perfusion; melphalan; recombinant interferon-gamma; neoplasm metastasis; induced hyperthermia; phase II clinical trials; phase III clinical trials; remission induction; survival rate

INTRODUCTION

Among high-risk melanomas (thicker than 1.5 mm according to Breslow) located on the limbs, approximately 10% will develop loco-regional metastases between the site of the primary tumour and the regional lymph nodes. Called in-transit metastases, their growth can rapidly induce pain, bleeding, and problems with quality of life. If we know systemic chemotherapy can provide a response rate of only about 20%, it is easy to understand why a regional therapy—isolated limb perfusion (ILP)—has been developed during the last 40 years. ILP can be separated into two categories: therapeutic ILP, used to treat established melanoma metastases that are not amenable to surgery; and prophylactic or adjuvant ILP, used to eliminate micrometastatic disease which is not removed by primary surgery in high-risk primary melanoma.

The ILP technique is described in detail in many publications [1–3]. Briefly, ILP involves isolation of the diseased limb, its subsequent connection to a heart-lung machine, and administration of high-dose chemotherapy or immuno-chemotherapy. The rationale is to improve the

*Correspondence to: Dr. Danielle Liénard, Centre Pluridisciplinaire d’Oncologie, CHUV, Level 06, Rue du Bugnon 46, CH-1011 Lausanne, Switzerland. Tel: +41-21-314.01.63; Fax: +41-21-314.01.67. E-mail: Danielle.Lienard@chuv.hospvd.ch
tumour response rate by increasing the drug concentration in the limb, while abolishing or reducing systemic toxicity, depending upon the efficiency of the isolation. Dose limitation is then dependent only on regional toxicity. General anesthesia is used. The isolation perfusion system consists of a heart-lung machine circuit that provides the circulation of about 2 L total of perfusate (blood, crystalloids and macromolecules), under hyperthermic conditions. The perfusate is heated and oxygenated by a membrane or bubble oxygenator. Cutaneous tissues of the perfused limb are given extra heat from a heating blanket. At the end of ILP, the limb is washed to remove residual active drugs from the vascular space and the tissues. A key point of the technique is continuous monitoring for possible leakage from the limb to the body. The method for leakage monitoring [4–6] is based on continuous counting, with a probe in the precordial region, while a tracing dose of labelled albumin is injected in the extracorporeal circuit. Monitoring is the only way to check the quality of the isolation and avoid severe systemic toxicity.

**THERAPEUTIC ILP**

*Experimental Data*

For treatment of melanoma in-transit metastases of the limbs, ILP with the alkylating agent melphalan, has been the “gold standard”. It has resulted in 50% complete remissions (CR)—as compared to less than 1% when injected intravenously—and an overall response rate of approximately 75% [1]. Systemic toxicity consists only of hematologic disturbances (white blood cells and platelets).

Tumour necrosis factor-alpha (TNFα) was discovered as a serum factor in mice treated with Bacillus Calmette Guerin (BCG) and endotoxin, producing hemorrhagic and coagulative necrosis of tumours in recipient mice [7]. In fact, TNFα is the first cytokine able to produce very fast and effective necrosis of tumours in a manner more efficient than chemotherapy itself. In 1985, the human TNFα gene [8] was cloned and expressed in *Escherichia coli*, followed in the same year by the murine TNFα gene [9,10]. It is commonly accepted that human TNFα structure is a non-glycosilated trimer of 157 amino acids with several cystein bounds [11].

The recombinant TNFα was made available for clinical trials but unfortunately, it was then found to be involved in septic shock in human. Not surprisingly, phase I and II studies in humans were hampered by high levels of toxicity and the studies seldom showed anti-tumour effects. The efficacious dose of TNFα in mice, either in syngeneic tumours or in nude mice carrying human xenografts, is around 50 µg/kg [12,13]. The maximum tolerated dose (MTD) in humans is 350 µg/m² or 5 µg/kg [14,15]. This dose—10 times lower in humans—produces only infrequent (partial) responses, sometimes accompanied by severe side effects. It is worth emphasizing that most phase I and II studies were designed in a way similar to that for chemotherapeutic agents, that is, with no special protocol for preventing the so-called “septic shock-like syndrome”. In fact, TNFα produces a general vasoplegia leading to a drop of the vascular resistance. Therefore, it is not surprising that following intravenous—or even intra-tumoural TNFα—most reports indicate severe hypotension. Recently, it has been proposed that the side effects induced by TNFα do not completely mimicking those of septic shock. Indeed, the latter requires other components such as endotoxin that was shown to have a very strong synergism in toxicity, presumably because of difference in cytokine cascade. The TNFα-induced vasoplegia can be easily controlled by fluid loading and if necessary by vasoactive amines.

TNFα not only can induce vasoplegia on normal vessels, it also can cause specific anti-tumour effects on tumour vessels. Cancer growth depends on angiogenesis, which is promoted by angiogenic factors secreted by tumour cells. Old’s group discovered that TNFα was acting through a selective destruction of tumour microvasculature [16]. In contrast to this activity, TNFα is moderately cytotoxic to tumour cells, and only around 30% of the cell lines tested so far show some sign of cytotoxicity. From the various publications on experimental models using either syngeneic tumours or human tumour xenografts, it appears that TNFα alone is rarely able to induce tumour regression of long duration.

Chemotherapeutic agents, such as alkylating agents or 5-flououracil (5FU) or antracyclines were synergistic with TNFα in human tumour xenografts such as melanoma, colon, ovarian and gastric cancers [17]. In an experimental isolated perfusion model on rat sarcoma, a synergistic effect of the combination of melphalan with TNFα was demonstrated [18]. IFNγ, which has a very poor anti-tumour effect, was shown to act synergistically with TNFα in sarcoma and melanoma models [13,17]. Moreover, IFNγ was demonstrated to upregulate TNFα receptors [19]. However, the synergy of TNFα and IFNγ is translated in higher systemic toxicity [15]. Clearly, the experimental models indicate that if TNFα has the unique property of destroying the tumour associated vasculature, it should be used in combination with other agents to achieve optimal tumour response.

**ILP STUDIES ON IN-TRANSIT MELANOMA METASTASES**

*Phase I Study*

Six patients were perfused with TNFα only, receiving a dose of 2 mg (one patient), 3 mg (one patient) and 4 mg (4 patients), respectively, with tissue temperatures ranging around 39°C. This intent of this pilot study was to discover whether the side effects observed in the systemic setting could be abrogated. Partial response (PR) of less
than 1 month was seen in two patients, while no response was noted in three patients. One patient had a CR of 7-months duration and then progressed. Three patients have been re-perfused with the triple drug (TIM–ILP), which resulted in 2 CRs and 1 PR. We concluded that ILP with TNFα alone has inadequate activity to warrant further investigation [20].

Pilot and Phase II Studies

Considering the above-mentioned data that shows the synergistic effects of TNFα, IFNγ and alkylating agents, we decided to combine melphalan with these two cytokines under mild hyperthermic conditions because hyperthermia has been shown to potentiate the activity of both TNFα and melphalan [21,22]. All patients were subjected to a thorough examination, which included a brain and an thoraco-abdominal CT. When previous ILP had been performed, or an old patient was involved, a selective angiogram was made to verify the integrity of the vessels, and, in case of bulky melanoma metastases, the tumour vascularization. Complete hematological work-up and coagulation screening were performed, together with an assessment of kidney and liver functions. The patients, who had given informed consent, received 0.2 mg IFNγ subcutaneously in the evening of days 2 and 1, preceded by the administration of 500 mg of acetaminophen; during the ILP, the injected doses of IFNγ was also 0.2 mg.

During this protocol, an arterial and a pulmonary (Swan-Ganz®) catheter were routinely used as well as infusion with dopamine 3 µg/kg/minute before and during TNFα administration [23]. In a pilot study, conducted in Brussels and Lausanne from 1989 to 1993, the 90-minute triple combination TIM–ILP protocol (TNFα 3-4 mg, IFNγ 0.2 mg, melphalan [10 mg, lower limb], 13 mg [upper limb]/l perfused tissue) provided 90% CR [23,24]. In a multicentre (Lausanne, Rotterdam, Groningen, Amsterdam) phase II study, 53 patients with in-transit melanoma metastases received the same protocol. Thirty-four of these patients had Stage IIIA (in-transit metastases only), 15 had Stage IIIAB (in-transit metastases plus lymph node metastases), and 4 had, in addition, distant metastases (Stage IV). Most of them had more than five in-transit metastases; half of them had been previously treated by regional chemotherapy.

The objective response rate was 100%, including 91% CR, based on clinical measurements and on sonography in the case of deep lesions. The responses of these patients were compared to standard ILP with melphalan alone (M-ILP). For that purpose, a database was built of 103 patients with comparable Stage III regionally recurrent melanoma who were treated between 1980 and 1988 by the same four teams [25]. M-ILP produced the predicted 52% CR rate, while the combined TIM treatment gave a CR percentage of 91% (Table I). In systemic chemotherapy, it is frequently found that when combination therapy is successfully increases the response rate, the duration of the response diminishes. To address this finding, the duration of CR for M-ILP and TIM treatments was compared. The median duration has not yet been reached, but the means are 3.9 years for the 103 melphalan alone treated patients and 3.6 years for the 53 TIM–ILP patients, a difference that is not significant (logrank test: \( P = 0.35 \)). These results confirm that the quality of this high CR rate, obtained with the triple combination, is equal to that of the 52% CR rate obtained by ILP with melphalan alone.

Randomised Phase II Trial

Although experimental models demonstrated a synergy between TNFα and IFNγ (see above), it was necessary to verify whether ILP without IFNγ is still efficient. A multicentre randomised phase II study was conducted from January 1992 to June 1994 (unpublished observations). The study compared TIM–ILP to perfusion with the same schedule but without IFNγ (TM–ILP) [26].

Thirty-two patients received the triple combination and 32 patients received the double combination (67% of patients had Stage IIIA melanoma and 33% had Stage IIIAB melanoma). The two groups are well balanced for stage, Breslow thickness, location, body weight and surface, and age. Overall response rate (ORR) in the TIM–ILP group again reached 100%, with a slightly lower CR of 78.1% as

<table>
<thead>
<tr>
<th>Study</th>
<th>Institutions</th>
<th>No. of Patients</th>
<th>TIM(a)</th>
<th>TM(b)</th>
<th>M(c)</th>
<th>Author/Year/Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase II</td>
<td>Single institution</td>
<td>29</td>
<td>90%</td>
<td>10%</td>
<td></td>
<td>Liénard et al. 1992 [23]</td>
</tr>
<tr>
<td>Phase II</td>
<td>Multicentric</td>
<td>53</td>
<td>90%</td>
<td>10%</td>
<td></td>
<td>Liénard et al. 1994 [27]</td>
</tr>
<tr>
<td>Randomised phase II</td>
<td>Multicentric</td>
<td>64</td>
<td>78.1%</td>
<td>21.9%</td>
<td>68.8%</td>
<td>21.9%</td>
</tr>
<tr>
<td>Randomised phase III</td>
<td>Multicentric</td>
<td>43</td>
<td>80%</td>
<td>1%</td>
<td>61%</td>
<td></td>
</tr>
<tr>
<td>Historical control</td>
<td>Multicentric</td>
<td>103</td>
<td>52.4%</td>
<td>25.2%</td>
<td></td>
<td>Lejeune 1995 [25]</td>
</tr>
</tbody>
</table>

ILP = Isolated limb perfusion; CR = complete response; PR = partial response.

\(a\)TIM: Tumour necrosis factor-alpha 3-4 mg + Interferon-gamma 0.2 mg + Melphalan 10–13 mg/L.

\(b\)TM: Tumour necrosis factor-alpha 3-4 mg + Interferon-gamma 0.2 mg + Melphalan 10–13 mg/L.

\(c\)M: Melphalan 10–13 mg/L.
compared to previous study. In the TM-ILP group, there was an ORR of 95.3% with 69% CR. This was a trend without statistical significance (Table I). Duration of response, disease-free interval and survival are currently under evaluation.

Toxicity of ILP with TNFα

It is well established that systemic toxicity from ILP with any drug is the result of leakage. When we analysed the pharmacokinetics of TNFα, however, it was obvious that even in the absence of any detectable leakage (and after intense wash-out at the end of ILP to remove the intravascular residual TNFα) there was always a release of TNFα into the systemic circulation after restoration of the physiological circulation. This can be explained by the fact that even in case of no leakage, TNFα is slowly released from the perfused tissues with a hyperdynamic state followed by vasoplegia (a drop in systemic vascular resistance) and grade 1 or 2 hypotension. These effects are easily controlled by fluid loading and, depending on local policy, by administration of vasoactive amines. From the randomised phase II trial, it appeared that the addition of IFNγ local nor systemic toxicity. IFNγ trial, it appeared that the addition of IFNγ into the systemic circulation after restoration of the physiological circulation. This can be explained by the fact that even in case of no leakage, TNFα is slowly released from the perfused tissues with a hyperdynamic state followed by vasoplegia (a drop in systemic vascular resistance) and grade 1 or 2 hypotension. These effects are easily controlled by fluid loading and, depending on local policy, by administration of vasoactive amines. From the randomised phase II trial, it appeared that the addition of IFNγ did not increase local nor systemic toxicity. IFNγ-induced only some typical, flu-like symptoms in the pre-operative period [26].

In one centre, high leakage values were observed during the pilot study using a high pump flow. TNFα levels as high as 100 to 300 ng/ml were detected in the systemic blood with transient hyperdynamic shock symptoms including hypotension, acute pulmonary edema, and in some patients, transient liver failure. In some cases, mixed hyperdynamic and cardiogenic shock was observed. Two cases of multi-organ failure (MOF) clearly related to consistent, high TNFα leakage have been observed. These symptoms and MOF were reversible and no toxic death occurred [28]. These data clearly suggest that TNFα is not the main mediator of septic shock, as claimed previously. Today, high leakage values are exceptional due to improvements in the ILP technique, especially low pump flow (with a maximum 40 to 45 ml/l of perfused tissue) and continuous leakage monitoring by isotopes. It is clear that pump flow represents a very critical parameter in leakage control. Minute changes such as 50 ml/min—around 10% of the total flow—can modify the delicate balance between the two pressure compartments, i.e., the isolated limb and the rest of the body. It seems that the surgical devascularization and the isolating tourniquet sometimes have no or little influence on the opening of deep shunts, probably situated in the periarterial area—the coxo-femoral joint—and in the pelvis. A leakage of 10% represents around 400 µg of TNFα, nearly the MTD (see above). Slowing down the pump flow and performing an extensive wash-out at the end of the perfusion, drastically reduced systemic leakage of TNFα during ILP and TNFα release after revascularization [29].

Antivascular Effects of ILP with TNFα

Tumour regression after ILP with TNFα can be dramatic. An early softening of the tumour is the most common clinical finding. In-transit melanoma metastases, especially when exophytic and bulky, show necrosis within a few days as do some superficial soft tissue sarcomas. Usually, shrinkage happens more frequently in superficial tumours.

These clinical events are clearly due to specific damage to the tumour vessels by TNFα [24,30]. This is best demonstrated by angiography: the tumour vessels selectively disappear after 1–2 weeks, but all vessels in the normal tissues are spared. Echography combined with Doppler can evaluate not only the regression of (deep) tumours, but also the disappearance of intra-tumoural blood flow [Jean-Yves Neuwley, MD; Department of Radiology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; personal communication, 1997].

The early changes as seen by nuclear magnetic resonance (NMR) and by positron emission tomography (PET) indicate a specific hit of the blood supply to the tumour, with dramatic reduction of the metabolism. A semi-quantitative assessment of the tumour necrosis can be done [31,32]. Serial biopsies taken at different times after ILP with TNFα allowed analysis of the morphological and immunohistochemical changes and correlation of them with the clinical and imaging findings. It was found that the early events include an up-regulation of adhesion molecules on the endothelial cells (ECs), especially E-Selectin and VCAM-1 [33]. As early as 4 hours after ILP, ECs became swollen and died. The role of neutrophils in tumour necrosis was indirectly suggested by the inhibition of the antitumour effect of ILP with TNFα by irradiation in a rat model [18]. It was also shown that thrombocytes lined up on the vessel walls and penetrated the tumours [18]. In contrast to experimental animal data, necrosis in human tumours was more of the coagulative rather than hemorrhagic type [33]. When compared to these findings, analysis of cases treated by M-ILP did not show anything other than slight perivascular infiltration and late atypical necrosis [34].

Thus, TM-ILP appears to act through a dual targeting system: TNFα hits the tumour ECs and melphalan is mainly toxic to tumour cells. This combination leads to a fast and efficient necrosis of the tumours. The unique property of TNFα is the sparing of normal vessels—with one exception: fresh scar tissues can also become necrotic after ILP with TNFα. A current working hypothesis is that ECs in tumour and scar tissues are proliferating, which renders them highly sensitive to signals leading to activation and death. One of the effects of endothelium activation and damage is the increase of vascular permeability, resulting in a drop of the intra-tumoural pressure [35].
Future of TNFα in the Clinical Setting

A valid comparison of TIM/TM-ILP with M-ILP can be obtained only from a randomised prospective phase III study. The current National Cancer Institute (Bethesda) study [36] recently was subjected to interim analysis which suggested a superiority of TIM in terms of CR, especially in high tumour bulk cases (67% CR vs. 14% CR). This rather specific efficacy on high tumour burden in melanoma was recently confirmed in an Italian phase I/II study [37] (Table II). In 1997, a phase III study began which aims to accrue 284 patients from 11 centres in Europe to compare TM-ILP to perfusion with M-ILP (study co-ordinator, D. Liénard).

Although most of the side effects initially observed are successfully avoided today, high-dose TIM/TM-ILP remains a potentially hazardous procedure. Pharmacokinetic data show a 90-minute plateau with 2 to 6 µg/ml TNFα in the perfusate [38]. This would indicate that all receptors are saturated, and that there is an excess of the cytokine. Lower TNFα dosages were tried by three groups with apparently no loss of overall activity, although the series were small [37,39,40]. Starting from 4 mg TNFα, Fraker performed an escalation study but he did not find any improvement in response [41]. Future work in this respect should concentrate on de-escalation of TNFα dosage. In addition, the synergy of IFNγ with TNFα has not been well explored. Higher dosage of IFNγ should be tried.

TIM/TM-ILP is a major surgical procedure. It is therefore not surprising that only 8 to 9% of the patients received more than one perfusion. Nevertheless, ILP with TNFα should be considered an induction therapy, because a single treatment cycle is rarely efficacious in oncology. In melanoma, the lack of efficient systemic treatment hampers the design of maintenance therapy. Since ILP with TNFα seems to hit the tumour vasculature selectively, some of us currently are addressing the use of an antivascular strategy, including gene transfer, in experimental settings.

ADJUVANT ILP

Although the therapeutic efficacy of ILP is well established for the treatment of in-transit metastases, its value as an adjuvant treatment after resection of a high-risk primary melanoma was not well defined until recently [42,43]. Survival benefit has been reported from non-randomised and historical series of adjuvant M-ILP. In the literature, only one randomised phase III study of adjuvant M-ILP has been reported previously [44,45]. In this small single centre trial, only 17 and 18 patients with primary melanoma were entered into the wide-excision-only (WE) and the WE + M-ILP arms, respectively. The remaining randomised patients had Stage II and Stage III disease, with satellites or in-transit metastases being removed before perfusion. Although M-ILP was reported to result in a significant reduction in relapse rate and an increase in survival, there was an exceedingly high rate of early recurrences (39%) in the control group. In contrast, all patients but one (52/53) survived 5 years after M-ILP, even though 66% of the patients had Stage II and Stage III disease, a result unparalleled in the literature. Between the 1984 report [44,45] and the 1988 update [46], the difference in survival increased. Taken together, it can be surmised that due to the low number of cases, there was a bias expansion in that study.

Prospective Randomised Study in High-Risk Primary Melanoma

In an attempt to resolve the controversy and overcome the shortcomings of retrospective evaluation, a collaborative randomised prospective study began in 1984. The EORTC Melanoma Group, the WHO Melanoma Program, and the North American Perfusion Group examined the influence of M-ILP on tumour recurrence and survival in high-risk primary melanoma patients. Patients with primary melanoma of the limbs (≥1.5 mm, Breslow thickness) were randomised in this study to be treated either by

---

**TABLE II. Tumor Response after TIM-, TM-, and M-ILP in Different Studies**

<table>
<thead>
<tr>
<th>Author/Ref/Year</th>
<th>Protocol</th>
<th>Tumor Necrosis Factor-alpha</th>
<th>Complete Remission Rates (%)</th>
<th>All</th>
<th>Low tumor burden</th>
<th>High tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraker [36] 1997</td>
<td>TIM</td>
<td>3–4 mg</td>
<td>81</td>
<td>87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>67&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>—</td>
<td>65</td>
<td>81</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Di Filippo et al. [37]1997</td>
<td>TM</td>
<td>0.5–3.3 mg</td>
<td>70</td>
<td>80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>75&lt;sup&gt;d&lt;/sup&gt;–75&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>


<sup>a</sup> = <3 cm and <10 nodules.
<sup>b</sup> = any ≥3 cm or ≥10 nodules.
<sup>c</sup> = 1 nodule.
<sup>d</sup> = 2–5 nodules.
<sup>e</sup> = ≥5 nodules.
<sup>f</sup> = historical.
WE-only or by WE + M-ILP. Elective lymph node dissection (ELND) of the appropriate groin or axilla was optional, but it was specified that the same policy had to be followed consistently by each participating centre throughout the period of the trial. Between 1984 and 1994, a total of 832 evaluable patients from 16 centres entered the study: 412 were randomised to WE-only, with margins of 3 cm, and 420 to WE + M-ILP (10 mg melphalan/l perfused tissue for lower limb and 13 mg/l for upper limb [47] during 60 minutes and with mild hyperthermia.

Randomisation used the following stratification criteria: centre, gender, anatomical location of the melanoma (upper vs. lower limb), Breslow thickness (1.5 to 2.99 mm vs. 3.0 to 3.99 mm vs. ≥4.0 mm), ulceration (present or absent), and previous biopsy (yes or no). At the time of statistical analysis, the median follow up was 6.4 years (range 1–11 years). The main prognostic factors were well balanced in the two treatment groups: median age was 50 years, 68% of patients were female, 80% of melanomas were located on a lower limb, and 50% of melanomas had a Breslow thickness ≥3 mm. The only important imbalance between the two groups concerned ELND, which was performed in 47% of the WE + M-ILP group vs. 38% of the WE-only group.

RESULTS

The impact of M-ILP on disease-free interval (DFI) was transient: within the first 2 to 3 years after surgery, a high effect was observed, but almost no long-term effect was apparent (62% of patients evaluable at 8 years in both treatment arms were disease-free). Similar results were obtained after taking into consideration the treatment imbalance between ELND and no ELND (P = 0.027 Wilcoxon). The beneficial impact of M-ILP was clearly on the occurrence—as the first site of progression—of in-transit metastases, which were reduced from 6.6% to 3.3%, and of metastases in regional lymph nodes, which were reduced from 16.7% to 12.6% (Table III). In the more favourable prognostic group (Breslow thickness 1.5-2.99 mm), M-ILP significantly improved DFI (from 62% to 75% at 6 years) when no ELND was performed. On the other hand, the rate of distant metastases was higher in the M-ILP arm (12.9% vs. 9.7%), whereas the incidence of local recurrences and second primary tumours (generally melanoma) were equally low (around 3%) in the two treatment groups.

There was no benefit from M-ILP in terms of survival (Fig. 1). Treatment comparison stratified by whether or not ELND was performed gave similar results: P = 0.82 (logrank) and P = 0.75 (Wilcoxon). Subgroup analysis (in particular, the no-ELND patients and/or those with melanomas <3 mm) did not show any significant survival difference between the two treatment arms. However, caution must be exercised in drawing conclusions at this stage because in some of these subgroups, the number of deaths was small. It should be noted that the median survival time has not yet been reached. The estimated overall survival is 75% at 6 years and 65% at 10 years.

Toxicity

As expected, limb toxicity was higher in the M-ILP arm. Skin toxicity is typical after M-ILP and, although 20% of patients still had grade 2 reaction (mild erythema and edema) one month after perfusion, it was always reversible. Pain and nerve injury, especially of the perineal nerve, was mostly due to M-ILP, while the combination of M-ILP with ELND increased the severity of limb toxicity. Arterial and/or venous thrombosis occurred in 1% to 2.6% of patients and was most common in the group treated by WE + ELND + M-ILP. Due to toxicity, amputation was performed in two patients. The addition of M-ILP increased treatment costs and prolonged hospitalisation time by 70%. Long-term side effects were also higher after M-ILP, and the combination of M-ILP with ELND increased this frequency.

<p>| TABLE III. First Progression Site by Randomization of Treatment (WE vs. WE + M-ILP), Elective Lymph Node Dissection, and Breslow Thickness |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Local Recurrence (%) of patients | In-transit Metastases (%) of patients | Regional Lymph Nodes (%) of patients | Distant Metastases (%) of patients |</p>
<table>
<thead>
<tr>
<th>WE</th>
<th>WE + M-ILP</th>
<th>WE</th>
<th>WE + M-ILP</th>
<th>WE</th>
<th>WE + M-ILP</th>
<th>WE</th>
<th>WE + M-ILP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>2.9</td>
<td>2.9</td>
<td>6.6</td>
<td>3.3</td>
<td>16.7</td>
<td>12.6</td>
<td>9.7</td>
</tr>
<tr>
<td>No ELND</td>
<td>3.1</td>
<td>3.1</td>
<td>6.6</td>
<td>2.2</td>
<td>25.0</td>
<td>19.7</td>
<td>7.4</td>
</tr>
<tr>
<td>Breslow &lt; 3 mm</td>
<td>2.4</td>
<td>2.3</td>
<td>4.8</td>
<td>1.6</td>
<td>21.4</td>
<td>14.8</td>
<td>7.1</td>
</tr>
<tr>
<td>Breslow ≥ 3 mm</td>
<td>3.8</td>
<td>4.2</td>
<td>8.5</td>
<td>3.2</td>
<td>28.5</td>
<td>26.3</td>
<td>7.7</td>
</tr>
<tr>
<td>ELND</td>
<td>2.6</td>
<td>2.5</td>
<td>6.4</td>
<td>4.6</td>
<td>3.2</td>
<td>4.6</td>
<td>13.5</td>
</tr>
<tr>
<td>Breslow &lt; 3 mm</td>
<td>3.2</td>
<td>4.3</td>
<td>6.5</td>
<td>3.2</td>
<td>4.3</td>
<td>1.1</td>
<td>6.5</td>
</tr>
<tr>
<td>Breslow ≥ 3 mm</td>
<td>1.6</td>
<td>1.0</td>
<td>6.3</td>
<td>5.8</td>
<td>1.6</td>
<td>7.8</td>
<td>23.8</td>
</tr>
</tbody>
</table>

European Organization for Research and Treatment of Cancer (EORTC)/World Health Organization (WHO)/North American Perfusion Group prospective randomised adjuvant perfusion trial of patients with high-risk primary melanoma (≥1.5 mm).

ELND = elective lymph node dissection; WE = wide excision; M = melphalan; ILP = isolated limb perfusion.
CONCLUSIONS

The results of this prospective randomised study demonstrate a limited but definite benefit of M-ILP in terms of loco-regional disease control, but show no impact on survival. Moreover, the addition of M-ILP to standard WE induces toxicity, increases treatment costs, prolongs hospital stay, and can be associated with long-term morbidity. For all these reasons, we conclude from this randomised trial that prophylactic M-ILP cannot be recommended as an adjunct to standard surgery in high-risk primary limb melanoma.

ACKNOWLEDGEMENTS

The authors thank Boehringer Ingelheim, GmbH, Ingelheim/Rhein, Germany, for providing TNFα and IFNγ.

REFERENCES


Fig. 1. Survival by treatment group. EORTC/WHO/North American Perfusion Group prospective randomised adjuvant perfusion trial of patients with high-risk primary melanoma (Breslow thickness ≥ 1.5 mm).