Original article

Annals of Oncology 16: 1061–1068, 2005
doi:10.1093/annonc/mdi229
Published online 1 June 2005

Limb salvage with isolated perfusion for soft tissue sarcoma: could less TNF-α be better?

S. Bonvalot[1]*, A. Laplanche[2], F. Lejeune[3], E. Stoeckle[4], C. Le Péchoux[5], D. Vanel[6], P. Terrier[7], J. Lumbroso[8], M. Ricard[8], G. Antoni[2], A. Cavalcanti[1], C. Robert[9], N. Lassau[6], J. Y. Blay[10] & A. Le Cesne[9]

Departments of [1]Surgery, [2] Public Health, [5] Radiotherapy, [6] Radiology, [7] Pathology, [8]Nuclear Medicine and [9]Medical Oncology, Institut Gustave Roussy, Villejuif, France; [3] Multidisciplinary Oncology Center, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; [4]Department of Surgery, Institut Bergonié, Bordeaux, France; [10]Department of Medical Oncology, Centre Léon Bérard, Lyon, France

Received 6 February 2005; revised 31 March 2005; accepted 7 April 2005

Background: The optimal dose of TNF-α delivered by isolated limb perfusion (ILP) in patients with locally advanced soft tissue sarcoma is still unknown.

Patients and methods: Randomised phase II trial comparing hyperthermic ILP (38–40°) with melphalan and one of the four assigned doses of TNF-α: 0.5 mg, 1 mg, 2 mg, and 3/4 mg upper/lower limb. The main end point was objective tumour response on MRI. Secondary end points were histological response, rate of amputation and toxicity. Resection of the remnant tumour was performed 2–3 months after ILP. The sample size was calculated assuming a linear increase of 10% in the objective response rates between each dose level group.

Results: One hundred patients (25 per arm) were included. Thirteen per cent of patients had a systemic leakage with a cardiac toxicity in six patients correlated with high doses of TNF-α. Objective tumour responses were: 68%, 56%, 72% and 64% in the 0.5 mg, 1 mg, 2 mg and 3 or 4 mg arms, respectively (NS). Sixteen per cent of patients were not operated, 71% had a conservative surgery and 13% were amputated with no difference between the groups. With a median follow-up of 24 months, the 2 year overall and disease-free survival rates (95% CI) were 82% (73% to 89%) and 49% (39% to 59%), respectively.

Conclusion: At the range of TNF-α doses tested, there was no dose effect detected for the objective tumour response, but systemic toxicity was significantly correlated with higher TNF-α doses. Efficacy and safety of low-dose TNF-α could greatly facilitate ILP procedures in the near future.

Key words: isolated limb perfusion, soft tissue sarcoma, TNF-α

Introduction

Isolated limb perfusion (ILP) was introduced by Creech in 1957 [1] for the treatment of patients with locoregionally recurrent melanoma, with approximately 50%–65% complete responses with melphalan alone [2]. In contrast, ILP with melphalan alone or other drugs has been used in the treatment of patients with extremity soft tissue sarcoma with limited success [3]. The addition of hyperthermia enhances cytotoxic effects of alkylating agents such as melphalan [4]. Tumour necrosis factor-alpha (TNF-α) was first described as an antitumour factor present in the serum of animals that had been primed with immunomodulators such as BCG, and then treated with endotoxin [5]. Response rates seen with TNF-α as a single systemic agent in different trials were very low, with dose-limiting toxicity being constitutional symptoms and hypotension [6]. Liénard et al. pioneered the administration via ILP of high-dose TNFα and γ–interferon with melphalan [7]. The initial doses of 4 mg rHuTNF-α in the lower extremity and 3 mg in the upper extremity were tested arbitrarily as 10 times the maximum tolerated dose in humans [8], and equivalent to the effective dose in rats, i.e. around 50 μg/kg [9]. Subsequently, this choice of dose has been continued unaltered because of the high response rate. In a multicenter European trial, ILP with high-dose TNF-α and melphalan resulted in a 76% response rate and 71% limb salvage in patients with limb-threatening soft-tissue sarcomas, leading to the approval of TNF-α in Europe [10].

Until now, no other available treatment seems to give comparable results when applied to limb-threatening soft tissue sarcomas. Mechanisms involved are an increase of melphalan

**Correspondence to:* Dr S. Bonvalot, Department of Surgery, Institut Gustave Roussy, 39 rue Camille, Desmoulins, 94805 Villejuif Cedex, France. Tel: +33-1-42-11-43-83; Fax: +33-1-42-11-52-56; E-mail: bonvalot@igr.fr

uptake in tumour tissue [11] and a selective destruction of tumour vessels with the addition of TNF-α [7, 12, 13]. However, the optimal dose of TNF-α delivered by isolated limb perfusion in patients with advanced soft tissue sarcoma (ASTS) is still unknown. In patients with melanoma, increasing the TNF-α dose to 6 mg did not increase the complete response rate but increased regional toxicity [14]. Pharmacokinetics data [15] showed plateau levels of micrograms of TNF-α in perfusates during the whole 90 min ILP, suggesting a saturation of the TNF-α receptors.

In a de-escalation study in rats, de Wilt demonstrated that the synergy between rHuTNF-α and melphalan was lost at a dose of TNF-α below 40 μg/kg [16]. Since rHuTNF-α in mice binds only to the p55 receptor and not to the p75, its activity is five to 10 times less than murine TNF-α (MuTNF) [16, 17]. This suggests that the dose of TNF-α currently used in clinical setting (approximately 50 μg/kg) might be reduced five to 10-fold while retaining the synergistic effects. Preclinical studies have showed that targeted delivery to tumour vessels of very low doses (picograms) of TNF-α enhances the penetration of doxorubicin in murine models [18] and that systemic administration of low-dose TNF-α increases the antitumour activity of a liposomal formulation of doxorubicin [19]. Clinical observations also favour a TNF-α dose decrease. Kinetics revealed that with 3 or 4 mg of rHuTNF-α, the perfusion system is supersaturated [20]. There is no significant difference in tumour response between patients with leakage over 2%, where the exposure of the perfused limb to TNF-α was 18.7% lower, and those without leakage [21].

Severe systemic toxicity and haemodynamic changes after ILP with TNF-α and melphalan, with or without interferon-γ, have been reported in several series, although reduced when leakage is adequately controlled [20]. The potential advantage of a lower dose of TNF-α includes a lower incidence of systemic adverse events leading to a more simple and safe procedure with a significantly lower cost. Subsequently, this approach could be expanded to a wider spectrum of malignancies and patients.

In order to challenge the use of the usual high dose of TNF-α (3 mg for the upper limb and 4 mg for the lower limb), we conducted a randomised phase II comparing four doses of TNF-α: 0.5 mg, 1 mg, 2 mg or the registered dose for ILP in humans. Our primary end point was radiological response, which is the main criterion to make the correct decision about limb salvage or amputation.

Patients and methods

Eligibility criteria

It was required that the patient had a locally limb advanced soft tissue sarcoma considered non-resectable by the referring surgeon of one of the three participating centres, i.e. that could only be treated by amputation or functionally mutilating surgery. The tumours were considered unresectable because of either multifocal disease or fixation/invasion to the neurovascular bundle and/or bone. The diagnosis was confirmed by histological re-examination of the primary tumour specimen by an expert pathologist. Sarcomas were classified as low (I), intermediate (II) or high grade (III), based upon histological examination of the primary according to the French cancer center's histological grading [22]. Primary and recurrent sarcomas were included. Patients with synchronous metastasis were not excluded.

Patients had to be older than 16 years, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and normal cardiac function. All patients provided written consent before randomisation. The protocol was approved by the Ethics Committees in the three participating institutions.

Randomisation

Eligible patients were randomly assigned to receive one of the four TNF-α doses: 0.5 mg, 1 mg, 2 mg or 3/4 mg for upper/lower limb. Randomisation was done by telephone or fax upon eligibility checklist, using a block allocation scheme.

ILP

Recombinant human TNF-α (TNF-α-1a, Beromun^{TM}) was provided by Boehringer Ingelheim GmbH, Ingelheim/Rhein, Germany. Melphalan (Alkeran) was provided by Glaxo-Wellcome (London, UK). Under general anaesthesia, the main artery and vein of the affected limb were clamped and canulated after heparinisation. Cannulae were then connected to a heart–lung machine and a pneumatic tourniquet was applied proximally to prevent leakage into the general circulation. ILP consisted of extracorporeal circulation with mild hyperthermia (tissue temperature 38–40°C) obtained with a heat-exchanger. When sarcoma did not affect feet or hands, they were wrapped tightly with an Esmarch rubber bandage immediately before injection of the drugs into the perfusion circuit to prevent sequelae [23]. The allocated dose of TNF-α according to randomisation was injected into the arterial line when the limb tissue temperature was greater than 38°C. Thirty minutes later, 10 mg/l (leg) or 13 mg/l (arm) of melphalan per limb volume was then added for the following 60 min, as performed in the European trial [10]. Limb volume was calculated using specifically dedicated software (Liénard D., unpublished). A 4–6 l washout of the limbs, using a mixture of Hartmann's solution and Macrodex, was performed at the end of the procedure. During the washout, the limb was extensively massaged.

Leakage from the isolated perfusion circuit to the systemic compartment was assessed with technetium 99m radiolabelled human serum albumin (Vasculocis, Cis Biointernational, Schering, Gif-sur-Yvette, France). After injection of a small activity (4 MBq) in the systemic circulation (calibration of the blood volume and efficiency of a precordial NaI(Tl) scintillation probe), 100 MBq of radiolabelled human serum albumin were injected into the isolated circuit. Any increase in the precordial counting rate (continuous curve recording) was interpreted as a leakage from the isolated circuit to the systemic circulation, and was quantified according to calibration parameters.

Post ILP surgery and adjuvant treatments

A delayed resection of the remnant tumour was usually planned 2–3 months after ILP. The aim was to perform en bloc resection, with free margins whenever possible. Major vessels or nerve trunk included in the tumour were resected en bloc to avoid tumour rupture. Vascular graft, muscular flaps and skin grafts were used when necessary. Transpositions of tendons were used to palliate nerve trunk resection.

Adjuvant radiotherapy was considered in patients who had no previous radiotherapy and when the margins were close to histologically viable tumour. Adjuvant chemotherapy was optional for non-metastatic patients, its indication relying mainly upon high histological grade and young age of the patient.

End points and clinical follow-up

The primary end point was objective tumour response [complete response (CR) or partial response (PR)], assessed on MRI performed 1 and 2 months after ILP, immediately prior to surgery. The MRI examinations included T1-weighted SE and fast SE T2-weighted fat-saturated sequences, as well as dynamic sequences (T1-weighted SE repeated six times every 40 s), displaying the maximum intensity slope in each pixel [2]). CR was defined by complete necrosis of the tumour or disappearance of all measurable disease. A partial response (PR) was defined as a regression of the tumour size greater than 50% in the product of the bi-dimensional measurements. Progressive disease (PD) was defined as a greater than 25% disease progression or the appearance of any new lesion [24].

Secondary end points were the ability to perform a conservative surgery, histological response, toxicity and survival. Histopathological response was defined as complete response (pCR) if no residual identifiable tumour cells were present, very good response between 1% and 10% of identifiable tumour cells, partial response between 11% and 50% of identifiable tumour cells, and no change if more than 50% identifiable tumour cells were present in the resection specimen. The R classification of UICC was used to classify the quality of resection [25]. Systemic toxicity and peripheral neuro-toxicity were graded according to the World Health Organisation (WHO) criteria scale [26]. Local toxicity was graded according to Wieberdink's scale [27]. Late toxicity was evaluated 6 months after ILP.

Statistical design

The sample size was calculated assuming a linear increase of 10% in the objective response (OR) rate between each dose level group, i.e. between 45% (0.5 mg group) and 75% (3 mg/4 mg group). With a type I error of 5% and a type II error of 25%, 25 patients per group were required assuming this hypothesis [28]. Results are expressed as percentages with 95% confidence intervals (CIs) or as medians and range. The Wilcoxon test for trend was used to compare the percentages between the four dose groups. Survival was calculated using the Kaplan and Meier method [29] with Rothman's 95% CIs [30]. Median time of follow-up was calculated with the Schemper method [31]. In the calculation of the local recurrence rate, deaths without recurrence were censored. All tests were two-sided.

Results

Patient's characteristics

One hundred consecutive patients were enrolled in three centres between June 2000 and July 2003 (83 patients in Gustave Roussy Institute, nine patients in Centre Hospitalier Universitaire Vaudois and eight patients in Bergonié Institute). Twenty-five patients were randomly assigned to each TNF-α dose group. Initial characteristics per group are presented in Table 1. Size, recurrences, grade and multifocality are well balanced in the four groups.

Histopathological subtypes are: liposarcoma ($n=23$), undifferentiated sarcoma ($n=20$), synovial sarcoma ($n=16$), epithelioid sarcoma ($n=8$), angiosarcoma ($n=7$), malignant peripheral nerve sheath tumours ($n=7$), fibroblastic sarcoma ($n=6$), muscular sarcoma ($n=5$), and miscellaneous ($n=8$).

The median tumour size was 100 mm (range 20–208) for lower limbs and 70 mm (range 10–125) for upper limbs. ILP was performed in a previously irradiated volume for 27 patients.

ILP and leakage

All but four patients received the allocated TNF-α dose. Three patients received a different dose in error: one patient of the 1 mg group received 2 mg, one patient of the 2 mg group received 1 mg, and one patient of the 3 mg/4 mg group with

Table 1. Patient characteristics

Group	0.5 mg ($n=25$)	1 mg ($n=25$)	2 mg ($n=25$)	3 mg/4 mg ($n=25$)
No. of males	13	15	15	9
Age, median (range)	48 (21–76)	52 (20–86)	59 (21–71)	51 (17–81)
Location				
Superior limb	12	10	6	13
Inferior limb	13	15	19	12
Multifocality	11	10	12	10
Size (mm): median (range)	70 (10–150)	80 (15–150)	116 (35–208)	80 (10–200)
Recurrence	19	15	13	16
1st	8	9	5	8
2nd	7	3	1	2
3rd	1	0	3	2
>3rd	3	3	4	4
Synchronous metastasis	4	3	3	2
Histological grade				
I	3	4	4	4
II	9	11	9	10
III	13	10	11	9

Table 2. ILP toxicity

Group	0.5 mg ($n=25$)	1 mg ($n=25$)	2 mg ($n=25$)	3 mg/4 mg ($n=24$)
Acute regional tissue reactions	12 (48%)	10 (40%)	8 (32%)	9 (38%)
Grade II	9	8	6	8
Grade III	3	2	1	1
Grade IV	0	0	1	0
Paraesthesiae (grade I)	6 (24%)	3 (12%)	1 (4%)	3 (13%)
Nerve palsy (grade III)	3 (12%)	4 (16%)	0 (0%)	3 (13%)
Stiffness	5 (20%)	4 (16%)	3 (12%)	6 (25%)
Hemodynamic grade II toxicity	0 (0%)	0 (0%)	1 (4%)	4 (16.6%)
Hemodynamic grade III toxicity	0 (0%)	0 (0%)	0 (0%)	1 (4%)

Acute regional tissue reactions according to Wieberdink et al. [26].
Hemodynamic and peripheral neurotoxicity according to WHO grading [25].

a lower limb tumour received 3 mg instead of 4 mg. One patient of the 3 mg/4 mg group did not have the perfusion because of massive nodal involvement discovered during the cannulation.

Median duration to reach the adequate tissue temperature was 20 min (range 5–109). Median superficial/deep tissue temperatures were 38.6/39.1°C and 39.6/39.7°C at the girdle of the limb and distally, respectively. Thirteen per cent of patients had a drug leakage after drug injection (median 3%, range 1%–12%). Reducing the perfusion flow rate corrected the leakage most of the time. High leakages were due to a bad venous return: for eight patients, reposition of the cannula of the femoral vein under the level of the tourniquet corrected this technical problem. Despite extensive washout, 17% patients had leakage (median 3%, range 1%–14%) after reconnection of the limb circulation.

Toxicity

There was no toxic or surgery related deaths. Grade II and III cardio-vascular toxicity (Table 2) was correlated with the TNF-α dose ($P<0.01$). Only one patient (4 mg) needed circulatory support with dopamine infusion because of a 'sepsis like' syndrome. Five patients (2, 3 or 4 mg) developed low systolic blood pressure requiring fluid management. No haematological, hepatic, pulmonary toxicity or allergic reaction over grade I was observed. No compartment syndrome was observed. Local toxicity did not differ according to treatment arm (Table 2), gender (42% for women versus 37% for men), pre-irradiation (37% for pre-irradiated patients versus 40%) or tumour location (34% upper limb versus 43% lower limb).

Surgical morbidity after ILP consisted of two arterial thromboses, one treated surgically and another percutaneously. All patients were discharged from hospital, able to walk, after a median stay of 7 days after ILP (range 4–15).

Tumour response

All but two patients were evaluated for response (one patient did not have ILP, another was amputated outside the referring centre before evaluation). MRI and histological responses observed in the four groups are shown in Tables 3 and 4. The percentages of OR are 68%, 56%, 72% and 64% in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively ($P=0.93$). OR did not differ significantly according to tumour grade (53%, 56% and 77% in grades I, II and III), pre-irradiation (52% for pre-irradiated patients versus 70%) or

Table 3. MRI response

Group	0.5 mg ($n=25$)	1 mg ($n=25$)	2 mg ($n=25$)	3 mg/4 mg ($n=25$)	Total ($n=100$)
CR, *n*	8	10	8	10	36
%	32%	40%	32%	40%	36%
(IC 95%)	(15%–54%)	(21%–61%)	(15%–54%)	(21%–61%)	$P=0.71$
PR, *n*	9	4	10	6	29
Stable, *n*	5	8	2	7	22
PD, *n*	3	2	5	1	11
Not available, *n*	0	1	0	1	2
OR (CR + PR), *n*	17	14	18	16	65
%	68%	56%	72%	64%	65%
(IC 95%)	(46%–85%)	(35%–76%)	(51%–88%)	(43%–82%)	$P=0.93$

Table 4. Pathological response and quality of surgery (R)

Group	0.5 mg	1 mg	2 mg	3 mg/4 mg	Total
No. operated patients[a]	($n=23$[b])	($n=21$[b])	($n=21$)	($n=17$)	($n=82$)
Percentage of viable cells					
0	4	3	1	5	13 (16%)
1–10	1	3	7	3	14 (17%)
11–50	5	7	6	3	21 (26%)
>50	13	8	7	6	34 (41%)
R (UICC) (conservative surgery)	($n=21$)	($n=17$)	($n=18$)	($n=15$)	($n=71$)
R0	8	9	10	8	35 (49%)
R1	12	8	6	6	32 (45%)
R2	1	0	2	1	4 (6%)

[a]Operated patients: amputations + conservative surgery.
[b]Two pathological responses missing.

topography (61% upper limb versus 68% lower limb). Correlation between radiological (CR or not on MRI) and pathological responses (less than 11 viable cells or not) was good (82%) (Table 5).

Post ILP surgery

Sixteen patients were not operated (nine metastasis progression, four patient refusals, one second cancer, two other reasons), 71 underwent conservative surgery and 13 were amputated. The percentages of conservative surgery (Table 6) are 84%, 68%, 72% and 60% in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively ($P=0.10$). Conservative surgery required a myocutaneous flap in 24 patients (two MacGregors, two pedicled latissimus dorsi, 20 latissimus dorsi free flaps) and four vascular grafts. Quality of surgery in the 71 patients with conservative surgery was: R0 in 35 patients, R1 in 32 patients and R2 in four patients. Two patients were amputated after vascular complications (one occlusion of a vascular graft associated with a muscular free flap 1 month after surgery, and one vascular rupture). Rates of limb salvage were 88%, 80%, 88%, 92% in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively (NS).

Further treatments

Thirty-seven patients received post-operative radiotherapy at a median dose of 50 Gy (range 15–65) concerning 10, 10, nine and eight patients in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively. Adjuvant chemotherapy was delivered in 18 patients: three, six, three and six patients in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively.

Late toxicity

Late toxicity consisted of a muscular atrophy (5%), oedema (11%), grade 1 sensitive disorders (6%), grade 3 nerve palsy (3%) and stiffness (16%). Late toxicity was not significantly different whether a patient received post-ILP radiotherapy (21%) or not (33%). However, three post-ILP irradiated patients experienced a spontaneous fracture between 6 and 12 months after the end of treatment.

Table 5. Correlation between MRI and pathological responses

	MRI complete responses		
% viable cells	Yes	No	Total
0–10	21 (26%)	6	27
>11	9	46 (56%)	55
Total	30	52	82%

Table 6. Post-ILP surgery

Group	0.5 mg ($n=25$)	1 mg ($n=25$)	2 mg ($n=25$)	3 mg/4 mg ($n=25$)
No surgery	1	3	4	8
Amputation	3	5	3	2
Conservative surgery	21 (84%)	17 (68%)	18 (72%)	15 (60%)
Limb salvage[a]	22 (88%)	20 (80%)	22 (88%)	23 (92%)

[a]Limb salvage: no surgery + conservative surgery.

Survival and local recurrence

No patient was lost to follow-up. With a median follow-up of 24 months, the 2-year overall and disease-free survival rates (95% CI) were 82% (73%–89%) and 49% (39%–59%), respectively (Figure 1), with no significant difference between the four groups. Median disease-free survival was 24 months. Twenty-four local recurrences were recorded with a median time to progression of 13 months: 6, 5, 8 and 5 months in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively, with no statistical difference between the four groups (Figure 2). The 2-year local recurrence rate (95% CI) was 27% (18%–38%; Figure 3). Recurrences were correlated with quality (R) of post-ILP surgical resection (log rank $P<0.00\,001$).

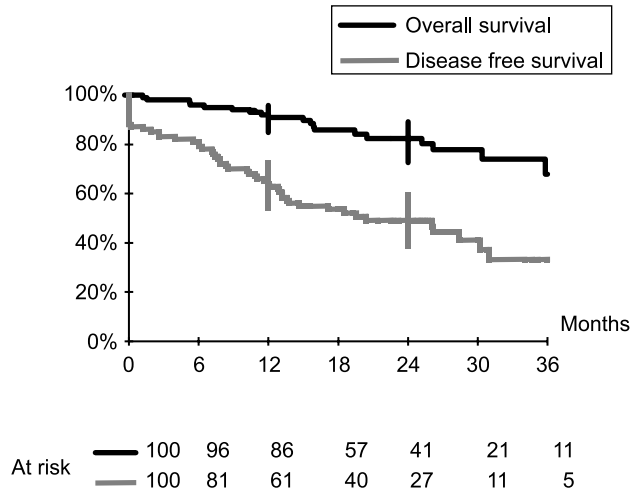

Figure 1. Overall and disease-free survival.

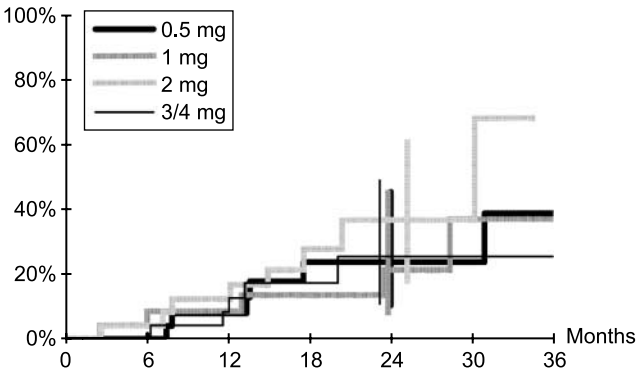

Figure 2. Local recurrences by group.

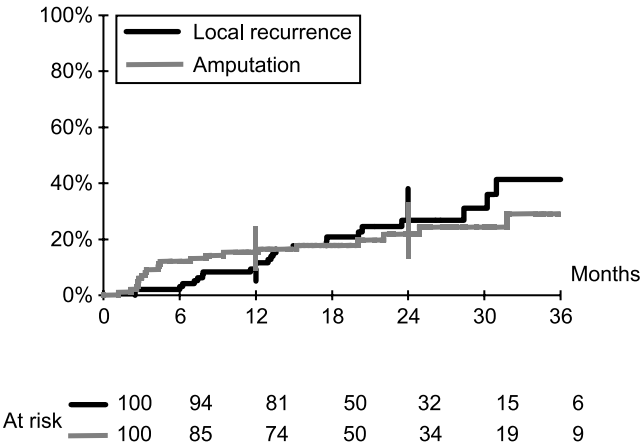

Figure 3. Local recurrences and amputations.

A total of 13 amputations were initially performed (Table 6) and eight additional amputations were performed during the follow-up due to recurrence (six patients) or complication of post-ILP surgery (two patients). The amputation rate at 2 years (95% CI) was 22% (14%–32%; Figure 3). A total of 21 amputations were thus performed: five, five, six and five amputations in the 0.5 mg, 1 mg, 2 mg and 3 mg/4 mg groups, respectively.

Discussion

This trial was conducted over a short period of time (3 years inclusion), with a high homogeneity of the ILP procedures, as well as in the indications and evaluation of results. In Gustave Roussy Institute, the 83 patients included represented 12% of the operated sarcomas by the same surgeon during this period, reflecting the strict criteria of inclusion.

The present study is the first to randomise different doses of TNF-α with the registered dose of melphalan in sarcomas. Animal models and kinetic findings suggested that very high doses of TNF-α used traditionally in the clinical setting may well be reduced. In a pilot clinical study [32], nine sarcoma patients experienced a CR with a reduced dose of TNF-α (0.5–0.925 mg) albeit with significant local toxicity. In 20 patients with melanoma [33], low-dose TNF-α ILP achieved tumour responses comparable with those historically reported with a higher dose.

Our primary end point was radiological response, which is the main criterion to make the correct decision about limb salvage or amputation. Our study failed to show a 10% difference between the different dose groups, which was the statistical prerequisite to find a TNF-α dose effect (see Patients and methods) at least at this range of TNF-α doses. The rates of conservative surgery, which is the final issue, were equivalent in the four groups. These results suggest that synergism between low-dose TNF-α and melphalan was conserved. Moreover, overall response rates and limb salvage rates are similar to those of previous studies with high doses of TNF-α [10, 34, 35].

There was no significant difference in terms of CR according to tumour grade, although there was a trend for better responses in patients with high-grade sarcoma. This is supported by the observation of better clinical responses correlated with high mitotic activity and a low amount of apoptosis in tumour samples taken prior to ILP with TNF-α and melphalan [36]. It should be emphasised that the rate of CR in low-grade sarcomas is far higher than that obtained with systemic chemotherapy.

Correlation between radiological and pathological responses was good (82%). Nevertheless, assessment of the percentage of viable tumour cells is difficult because its evaluation in soft tissue sarcoma after pre-operative treatment is based on the residual volume of the tumour. If the tumour shrinks but still contains viable tumour cells, the percentage of residual tumour is often overestimated. This is why our main objective was to compare the clinical OR of each therapeutic arm on MRI, which is more reliable for deciding further surgery.

Systemic toxicity, mostly cardiovascular, is significantly correlated with the administrated dose of TNF-α. Systemic toxicity with melphalan is rarely severe, with nausea and vomiting being the most frequently encountered side-effects

[37]. It is remarkable that no patient with low-dose TNF-α had systemic toxicity. This is in contrast with previous studies with high-dose TNF-α [10, 34] where all patients developed a hyperdynamic state and went through a phase of lowered blood pressure that may require fluid loading and vasopressor drugs [38]. These cardiovascular side-effects [39] increase with the exposure of the patient to systemic circulation of TNF-α [21]. In a review of studies using TNF-α [40], 4.2% of patients had a shock, 2.7% acute pulmonary oedema and 1.7% anuria. The possible occurrence of such serious adverse events until now has demanded the use of isotopic leakage monitoring in order that corrective measures can be taken if a systemic leak should occur. Insertion of a Swan Ganz catheter was recommended with 24 h observation in an intensive care unit [38, 41]. With the current use of 1 mg TNF-α, patients are now directly admitted to the surgery ward in two of the centres.

The increase of the TNF-α dose does not add any regional toxicity. The local toxicity was low compared with other series; probably because mild hyperthermia (38–40°C) was used [16]. In our study, grade 4 toxicity was only observed in one of 99 patients compared with 15/186 grade 4 and 5 in the multicentric European study [10]. This toxicity was not different for males and females and was not increased when ILP was performed in a pre-irradiated field. The main disturbing local toxicity was neurological (paraesthesia and nerve palsy) which has been reported in a wide range of perfused patients [42]. Nerve palsy was observed mainly in the upper limb at the beginning of this study. These neurological side-effects are observed with melphalan alone; TNF-α clearly does not add any regional toxicity. Therefore, after inclusion of the first five upper limb sarcomas, we decided to modify the protocol and use the same concentration of melphalan (10 mg/l) for upper and lower limbs. From that moment on, no more paralysis was observed and there was no statistical difference in terms of local toxicity or response rates between upper and lower limbs in the whole study.

Patients who received post-ILP radiotherapy did not experience more significant morbidity. Olieman [43] demonstrated that adjuvant radiotherapy after ILP with TNF-α and melphalan and delayed tumour resection was feasible and could increase local tumour control without increasing treatment morbidity.

The complete surgery rate with free margins is high regarding criteria of inclusion, but many of them (24/71) required reconstructive surgery to achieve these results. The rate of local recurrences is obviously higher than those observed in primary non-selected patients [44, 45], but all the patients of our study had recurrences or locally advanced high-grade primary. Median disease-free survival (24 months) was similar to that reported in the Stojadinovic study evaluating the outcome of recurrent soft tissue sarcoma of the extremity where no significant difference in OS was found between patients undergoing amputation and limb sparing surgery [46]. In our study, all local failures or progressions after ILP occurred after incomplete surgery or no surgery at all. This underlines the aim that post-ILP surgery should be a wide resection whenever possible.

Conclusion

ILP with TNF-α and melphalan is an effective neo-adjuvant treatment with high response rates that can achieve limb salvage for most patients with locally advanced soft tissue sarcoma. At the range of TNF-α doses tested, there was no dose effect detected for OR and the rate of conservative surgery was similar, but systemic toxicity was significantly related to high doses of TNF-α. Thus, 1 mg TNF-α might be an effective dose in ILP for advanced soft tissue sarcoma. Such a decrease of the recommended dose of TNF-α might greatly facilitate ILP procedures in the near future.

Acknowledgements

The authors thank Dr Arriagada for reviewing the script and Mrs M. Luboinski and Mrs G. Goma for their help with data management.

References

1. Creech O, Krementz ET, Ryan RF, Winblad JN. Chemotherapy of cancer: regional perfusion utilizing an extracorporeal circuit. Ann Surg 1958; 148: 616–632.
2. Lienard D, Eggermont AM, Kroon BB et al. Isolated limb perfusion in primary and recurrent melanoma: indications and results. Semin Surg Oncol 1998; 14: 202–209.
3. Krementz ET, Carter RD, Sutherlan CM, Hutton I. Chemotherapy of sarcomas of the limbs by regional perfusion. Ann Surg 1977; 185: 555–564.
4. Abdel-Wahab OI, Grubbs E, Viglianti BL et al. The role of hyperthermia in regional alkylating agent chemotherapy. Clin Cancer Res 2004; 10: 5919–5929.
5. Carswell EA, Old LJ, Kassel RL et al. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 1975; 72: 3666–3670.
6. Hersh EM, Metch BS, Muggia FM et al. Phase II studies of recombinant human tumor necrosis factor alpha in patients with malignant disease: a summary of the Southwest oncology Group experience. J Immunother 1991; 10: 426–431.
7. Lienard D, Ewalenko P, Delmotte JJ, Renard N, Lejeune FJ. High-dose recombinant tumor necrosis factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. J Clin Oncol 1992; 10: 52–60.
8. Creagan ET, Kovach JS, Moertel CG et al. A phase I clinical trial of recombinant human tumor necrosis factor. Cancer 1988; 62: 2467–2471.
9. Old LJ. Tumor necrosis factor. Science 1985; 230: 630–632.
10. Eggermont AM, Schraffordt Koops H et al. Klausner JM. Isolated limb perfusion with tumor necrosis factor and melphalan for limb salvage in 186 patients with locally advanced soft tissue extremity sarcomas. The cumulative multicenter European experience. Ann Surg 1996; 224: 756–764.
11. de Wilt JH, ten Hagen TL, de Boeck G et al. Tumour necrosis factor alpha increases melphalan concentration in tumour tissue after isolated limb perfusion. Br J Cancer 2000; 82: 1000–1003.

12. Renard N, Nooijen PT, Schalkwijk L et al. Early endothelium activation and polymorphonuclear cell invasion precede specific necrosis of human melanoma and sarcoma treated by intravascular high-dose tumour necrosis factor alpha (rTNF alpha). Int J Cancer 1994; 57: 656–663.
13. Eggermont A, Schraffordt Koops, Liénard J, Ouderk M. Destruction of tumor associated vessels by isolated limb perfusion with TNF: angiographic observations in sarcoma patients. Eur J Surg Oncol 1994; 20: 403–404.
14. Fraker DL, Alexander HR, Andrich M, Rosenberg SA. Treatment of patients with melanoma of the extremity using hyperthermic isolated limb perfusion with melphalan, tumor necrosis factor, and interferon gamma: results of a tumor necrosis factor dose-escalation study. J Clin Oncol 1996; 14: 479–489.
15. Lienard D, Lejeune FJ, Ewalenko P. In transit metastases of malignant melanoma treated by high dose rTNF alpha in combination with interferon-gamma and melphalan in isolation perfusion. World J Surg 1992; 16: 234–240.
16. De Wilt JH, Manusama ER, van Tiel ST et al. Prerequisites for effective isolated limb perfusion using tumour necrosis factor alpha and melphalan in rats. Br J Cancer 1999; 80: 161–166.
17. Broukaert P, Libert C, Everaerdt B, Fiers W. Selective species specificity of tumor necrosis factor for toxicity in the mouse. Lymphokine Cytokine Res 1992; 11: 193–196.
18. Curnis F, Sacchi A, Corti A. Improving chemotherapeutic drug penetration in tumors by vascular targeting and barrier alteration. J Clin Invest 2002; 110: 475–482.
19. Ten Hagen TL, Van Der Veen AH, Nooijen PT et al. Low-dose tumor necrosis factor-alpha augments antitumor activity of stealth liposomal doxorubicin (DOXIL) in soft tissue sarcoma-bearing rats. Int J Cancer 2000; 87: 829–837.
20. Vrouenraets BC, Kroon BB, Ogilvie AC et al. Absence of severe systemic toxicity after leakage-controlled isolated limb perfusion with tumor necrosis factor-alpha and melphalan. Ann Surg Oncol 1999; 6: 405–412.
21. van Ginkel RJ, Limburg PC, Piers DA et al. Value of continuous leakage monitoring with radioactive iodine-131-labeled human serum albumin during hyperthermic isolated limb perfusion with tumor necrosis factor-alpha and melphalan. Ann Surg Oncol 2002; 9: 355–363.
22. Trojani M, Contesso G, Coindre JM et al. Soft tissue sarcoma of adults, study of pathological variables and definition of histopathological grading system. Int J Cancer 1984; 33: 37–42.
23. Thompson JF, Lai DT, Ingvar C, Kam PC. Maximizing efficacy and minimizing toxicity in isolated limb perfusion for melanoma. Melanoma Res 1994; 4 (Suppl 1) 45–50.
24. Vanel D, Bonvalot S, Guinebretiere JM et al. MR imaging in the evaluation of isolated limb perfusion: a prospective study of 18 cases. Skeletal Radiol 2004; 33: 150–156.
25. Tumor of bone and soft tissues. R classification. TNM Classification of Malignant Tumours UICC. In Sobin LH, Wittekind Ch (eds): 6th edition. New York: Wiley Liss 2002; 110.
26. World Health Organisation (WHO). WHO handbook for reporting results of cancer treatment. Geneva: WHO offset publication No. 48, 1979.
27. 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: 905–910.
28. Nam J. A simple approximation for calculating sample sizes for detecting linear trend in proportions. Biometrics 1987; 43: 701–705.
29. Kaplan EL, Meier P. Non parametric estimation from incomplete observations. J Am Statist Assoc 1958; 53: 457–481.
30. Rothman KJ. Estimation of confidence limits for the cumulative probability of survival in life table analysis. J Chron Dis 1978; 31: 557–560.
31. Schemper M, Smith T. A note on quantifying follow-up in studies of failure time. Controlled clinical trials 1996; 17: 343–346.
32. Hill S, Thomas JM. Low-dose tumour necrosis factor-alpha (TNF-alpha) and melphalan in hyperthermic isolated limb perfusion. Results from a pilot study performed in the United Kingdom. Melanoma Res 1994; 4 (Suppl 1) 31–34.
33. Rossi CR, Foletto M, Mocellin S, Pilati P, Lise M. Hyperthermic isolated limb perfusion with low-dose tumor necrosis factor-alpha and melphalan for bulky in-transit melanoma metastases. Ann Surg Oncol 2004; 11: 173–177.
34. Lejeune FJ, Pujol N, Lienard D et al. Limb salvage by neoadjuvant isolated perfusion with TNFalpha and melphalan for non-resectable soft tissue sarcoma of the extremities. Eur J Surg Oncol 2000; 26: 669–678.
35. Gutman M, Inbar M, Lev-Shlush D et al. High dose tumor necrosis factor-alpha and melphalan administred via isolated limb perfusion for advanced limb soft tissue sarcoma results in a >90% response rate and limb preservation. Cancer 1997; 79: 1129–1137.
36. Plaat BE, Molenaar WM, Mastik MF et al. Hyperthermic isolated limb perfusion with tumor necrosis factor-alpha and melphalan in patients with locally advanced soft tissue sarcomas: treatment response and clinical outcome related to changes in proliferation and apoptosis. Clin Cancer Res 1999; 5: 1650–1657.
37. Sonneveld EJ, Vrouenraets BC, van Geel BN et al. Systemic toxicity after isolated limb perfusion with melphalan for melanoma. Eur J Surg Oncol 1996; 22: 521–527.
38. Sigurdsson GH, Nachbur B, Lejeune F. Anesthesiologist's management of isolated limb perfusion with 'high' doses TNFα. Anesthesiology 1993; 79: 1433–1437.
39. Zwaveling JH, Maring JK, Clarke FL et al. High plasma TNF concentrations and a sepsis like syndrome in patients undergoing hyperthermic isolated limb perfusion with recombinant TNF, interferon-gamma and melphalan. Crit Care Med 1996; 24: 765–770.
40. Eggermont A, Lejeune F, Mann B. Isolated limb perfusion with Beromun: a neoadjuvant induction bio-therapy for the treatment of irresectable soft tissue sarcoma of the extremities. Boehringer Ingelheim Pharma GmbH 2003.
41. Christoforidis D, Chassot P-G, Mosimann F et al. Isolated limb perfusion: distinct tourniquet and tumor necrosis factor effects on the early hemodynamic response. Arch Surg 2003; 138: 17–25.
42. Vrouenraets BC, Eggermont AL, Klaase JM. Long term neuropathy after regional isolated perfusion with melphalan for melanoma of limbs. Eur J Surg Oncol 1994; 20: 681–685.
43. Olieman AF, Pras E, van Ginkel RJ et al. Feasibility and efficacy of external beam radiotherapy after hyperthermic isolated limb perfusion with TNF-alpha and melphalan for limb-saving treatment in locally advanced extremity soft-tissue sarcoma. Int J Radiat Oncol Biol Phys 1998; 40: 807–814.
44. Pitcher ME, Ramanathan RC, Fish S, A'Hern R, Thomas JM. Outcome of treatment for limb and limb girdle sarcomas at the Royal Marsden Hospital. Eur J Surg Oncol 2000; 26: 548–551.
45. Pisters PW, Leung DH, Woodruff J, Shi W, Brennan MF. Analysis of prognostic factors in 1,041 patients with localized soft tissue sarcomas of the extremities. J Clin Oncol 1996; 14: 1679–1689.
46. Stojadinovic A, Jaques DP, Leung DH, Healey JH, Brennan MF. Amputation for recurrent soft tissue sarcoma of the extremity: indications and outcome. Ann Surg Oncol 2001; 8: 509–518.