Selective pulmonary artery perfusion with melphalan is equal to isolated lung perfusion but superior to intravenous melphalan for the treatment of sarcoma lung metastases in a rodent model

Willem A. Den Hengsta, Jeroen M.H. Hendriksa, Tom Van Hoofa, Karel Heytensb, Gunther Guetensb, Gert de Boeckb, Filip Lardona and Paul E.Y. Van Schila,*

a Department of Thoracic and Vascular Surgery, Antwerp University Hospital, Edegem, Belgium
b Department of Oncology, University of Antwerp, Wilrijk, Belgium
* Corresponding author. Department of Thoracic and Vascular Surgery, Antwerp University Hospital, Wilrijkstraat 10, B-2650 Edegem, Antwerp, Belgium.
Tel: +32-3-8214360; fax: +32-3-8214396; e-mail: paul.van.schil@uza.be (P.E.Y. Van Schil).

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Abstract

OBJECTIVES: Isolated lung perfusion (ILuP) and selective pulmonary artery perfusion (SPAP) are experimental surgical techniques to deliver high-dose chemotherapy selectively to the lung for the treatment of lung metastases. ILuP with melphalan (MN) has shown to be feasible in clinical studies but can only be used once because it is invasive. SPAP as an endovascular technique can be repeated several times, but no results have been reported so far. Pharmacokinetics and efficacy of SPAP with MN were studied in a rodent lung metastasis model and compared it with ILuP and intravenous (IV) therapy.

METHODS: Pharmacokinetics: forty-five Wag-Rij rats were randomized into three groups: IV 0.5 mg MN, ILuP 0.5 mg MN and SPAP 0.5 mg MN. Every treatment group was again randomized in three groups: 15 min treatment, 30 min treatment and 30 min treatment with 30 min reperfusion. Blood and tissue samples were taken for MN concentrations. Efficacy: twenty-five Wag-Rij rats were randomized into five groups: control, sham thoracotomy, IV 0.5 mg MN, ILuP 0.5 mg MN and SPAP 0.5 mg MN. At day 0, bilateral lung metastases were induced, and treatment followed at day 7. At day 28, rats were sacrificed and pulmonary metastases counted. Survival: thirty Wag-Rij rats were randomized into five groups: control, sham ILuP, IV 0.5 mg MN, ILuP 0.5 mg MN, SPAP 0.5 mg MN. At day 0, left-sided lung metastases were induced with treatment at day 7. Endpoints were death due to disease or survival up to 90 days.

RESULTS: Pharmacokinetics: SPAP and ILuP resulted in significantly higher left lung MN concentrations compared with IV (P = 0.05). Efficacy: SPAP (30 ± 22 nodules) and ILuP (20 ± 9 nodules) resulted in significantly less nodules compared with IV (113 ± 17 nodules; P < 0.01). Survival: median survival of SPAP (74 ± 8 days) was equal to ILuP MN (71 ± 10 days) but significantly longer compared with IV (54 ± 7 days; P < 0.01 both).

CONCLUSIONS: SPAP with MN for the treatment of sarcoma lung metastases in rats is equally effective to ILuP but resulted in a significantly better survival compared with IV MN. As SPAP can be applied as a minimally invasive endovascular procedure, continued research with this technique is warranted.

Keywords: Isolated lung perfusion • Melphalan • Pulmonary metastases • Selective pulmonary artery perfusion • Rhabdomyosarcoma

INTRODUCTION

Despite the advances in diagnostic techniques, in knowledge about tumour growth and behavior, and better chemotherapeutic regimens, the prognosis for pulmonary metastases from certain solid tumours remains poor, even after complete surgical resection. For these patients, the 5-year overall survival rate remains between 18 and 50% during the past 20 years [1–5]. This low overall survival rate is probably due to micrometastatic disease already present during the initial procedure and resistance to current chemotherapy [1]. In order to prevent local recurrence in the treated organ, techniques that selectively deliver chemotherapy in a high dose to the organ can be applied. Examples in case of the lung are isolated lung perfusion (ILuP) and selective pulmonary artery perfusion (SPAP) with blood flow occlusion (BFO). ILuP is an invasive surgical procedure where the lung is isolated from the systemic circulation by cannulating both pulmonary artery and veins [6, 7]. This allows the delivery of a high dose of chemotherapy to the lungs with minimal to no
systemic exposure [6, 7]. However, because this technique is invasive, it can only be applied during the thoracotomy done for metastasectomy [6, 7]. SPAP combined with BFO is an endovascular technique by which a balloon catheter is introduced through the femoral vein into the pulmonary artery. When the balloon is insufflated (which is BFO), chemotherapy can be given through the central lumen of the balloon catheter into the selected pulmonary artery at a determined rate and volume, allowing the chemotherapy to diffuse slowly into the selected right or left lung. In animal models, SPAP has shown to be a feasible technique [8–10]. As already mentioned, the SPAP technique has the advantage that it can be repeated several times before or after a pulmonary metastasectomy allowing to destroy metastatic disease in different cycles of their growth [8–10]. For MN, ILuP has proven to be superior to intravenous (IV) therapy in rodent models of lung metastases of a colorectal adenocarcinoma and of sarcoma [11–13]. The aim of the present study is to investigate the uptake of MN into the lung and its efficacy in a rodent model with SPAP, and compare it with ILuP and IV injection.

**MATERIALS AND METHODS**

**Animals**

Male inbred Wag-Rij strain rats, weighing between 200 and 250 g obtained from Charles River (Elsené, Belgium), were used for all experiments. The animals were treated in accordance with the Animal Welfare Act and the ‘Guide for the Care and Use of Laboratory Animals’ (NIH Publication 86-23, revised 1985). The experimental protocols were approved by the Ethical Committee of the University of Antwerp.

**Cell line and cell preparation**

The cell line used in this study was kindly provided by Dr A. Raabe (Laboratory for Radiobiology and Experimental Radiation Oncology, Department of Radiotherapy and Radiation Oncology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany). The R1H cell line is a rhabdomyosarcoma cell line of the rat. R1H cells were cultured in Dulbecco’s Modified Eagle’s Medium supplemented with 10% foetal calf serum, 2 mM l-glutamine and 1% penicillin/streptomycin (Life Technologies, Invitrogen, Merelbeke, Belgium). Cultures were maintained in exponential growth in a humidified atmosphere at 37°C under 5% CO2/95% air.

For subsequent experiments, cells were harvested by trypsinization. The cell suspension was filtered using a cell strainer (Becton Dickinson, Erembodegem, Belgium), counted and twice washed with 0.9% sterile NaCl. A solution of 2.5 × 10⁶ cells/ml NaCl or 1.0 × 10⁶ cells/ml NaCl was prepared for injection, depending on the experiment. This cell suspension was injected in the rat within 30 min after preparation.

**Chemicals**

Melphalan solution (Alkeran®, Aspen medical Europe, Redditch, Worcestershire, UK, 50 mg/vial) was diluted at a concentration of 0.5 mg/ml adding saline 0.9% NaCl and stored within 10 min after preparation at −20°C and used for 2 weeks.

**Analysis of melphalan in plasma and tissue homogenates by high-performance liquid chromatography**

A validated high-performance liquid chromatography assay with ultra violet detection was used for the quantification of melphalan (L-PAM) in plasma, perfusion and tissue samples.

**Sampling**

Perfusion and tissue samples were immediately frozen in liquid nitrogen for later measurement of L-PAM and stored at −70°C. Blood samples were first centrifuged to separate plasma from the other blood compartment. The plasma was also stored at −70°C.

**Plasma and perfusion samples**

Plasma and perfusion samples were stored at −70°C until analysis. L-PAM and the internal standard, L-dansyl-arginine, were isolated from the samples by solid phase extraction over trifunction C18 cartridges. Samples were clarified by centrifugation before the supernatant was brought on the cartridges. After the samples were completely absorbed by the column, the columns were washed and dried. Finally, the compounds of interest were eluted and the extract was evaporated to a volume of approximately 200 µl, filtered and ready for injection. Solid phase extraction efficiencies exceeded 85% in all specimen types (tissue and plasma). Separation was performed isocratic on a Waters Symmetry C18 column (5 µm, 4.6 × 250 mm) using an aqueous NH₄-acetate buffer (pH 3)/methanol (55/45) mobile phase containing SDS (400 mg/l). The analytes were detected by fluorescence detection at an excitation wavelength of 265 nm and an emission wavelength of 360 and 575 nm for L-PAM and L-dansyl-arginine, respectively.

**Tissue samples**

Tissue samples were stored at −70°C until analysis. After addition of internal standard (L-dansyl-arginine), the samples were homogenized mechanically. The emulsions of cellular debris were clarified by centrifugation and the supernatant was extracted as described above.

**Induction of lung metastases**

**Efficacy study.** To determine the appropriate time of treatment in relation to the amount of injected cells, 18 rats were injected with 1 ml of 1.0 × 10⁶ R1H cells/ml or 2.5 × 10⁶ R1H cells/ml in the femoral vein and sacrificed at day 14, 21 or 28 after injection.

**Survival study.** R1H cells were prepared for injection at a concentration of 1.0 × 10⁶ cells/ml NaCl. Induction of lung metastases was performed by injecting 1 ml of cell suspension in the femoral vein right after performing a right-sided thoracotomy with clamping of the right pulmonary artery with a
curved micro-vascular clamp. This resulted in left-sided pulmonary metastases only [12, 14].

**Isolated lung perfusion**

ILuP was performed according to the technique as described by Hendriks et al. [15]. In short, anaesthesia was induced by a mixture of isoflurane (4%) with nitrous oxide and oxygen. Intubation was performed by transaryngeal illumination with a 16-gauge Insyte-W catheter after which the rat was connected to a volume-controlled ventilator. Immediately after induction, the rat was placed on a heating pad and body temperature was kept between 34 and 37°C. A left thoracotomy was performed and after clamping the pulmonary artery and vein with curved micro-vascular clamps, a PE-10 perfusion catheter was introduced into the pulmonary artery. The perfusion fluid consisted of hydroxyethyl starch (Voluven®, Fresenius Medical Care Belgium, Wilrijk, Belgium) with or without 0.5 mg MN at a total volume of 12.5 ml. If MN was administered, it was at a concentration of 0.04 mg/ml. Perfusion was performed using a perfusion pump at a rate of 0.5 ml/min. A pulmonary venotomy was performed to remove the effluent by suction. During the perfusion, the lung was ventilated and kept moist with normal saline. Perfusion was performed till all the 12.5 ml of the hydroxyethyl starch with or without 0.5 mg melphalan was administered, about 25 min, followed with a 5 min washout with hydroxyethyl starch alone. The perfusate was kept on 37°C throughout the duration of the perfusion.

**Selective artery perfusion**

This procedure follows the same steps as the ILuP procedure; however, no clamp is placed on the pulmonary vein. Total hydroxyethyl starch perfusion volume is 6 ml with 0.5 mg of melphalan at a rate of 0.2 ml/min. Concentration of MN is 0.083 mg/ml. Total perfusion time was 30 min and no washout was performed.

**Experiment 1: Pharmacokinetics**

Forty-five male Wag-Rij rats (200–250 g) were randomized into three groups of 15 rats each: SPAP with 0.5 mg MN, ILuP with 0.5 mg MN and IV injection of 0.5 mg MN. Every treatment group (N = 15) was again randomized in three groups of five rats each: 15 min of treatment with immediate sacrifice, 30 min of treatment with immediate sacrifice and 30 min of treatment with 30 min of reperfusion. For the latter, in the IV treatment group, a total of 65 min after IV injection was taken as the time point of sacrifice (similar to 30 min of treatment + 5 min of closure of the PA + 30 min of reperfusion). Femoral vein puncture was performed before the start of the procedure to measure the hematocrit (Hct). Immediately after the start of the experiment, the remaining melphalan was snap frozen to determine the exact starting dose with an acceptable error of 0.125 mg/ml. A cardiac puncture was performed at the end of the experiment to measure systemic MN concentration. The left perfused lung was divided in an upper, middle and lower part for MN concentration in the tissue. In addition, the MN concentration was also measured in the right lung. These biopsies were immediately snap frozen in liquid nitrogen. Remaining blood was stored for measurement of the Hct after treatment. To correct for possible errors by dilution between the different treatment groups in the systemic circulation, correction was made using the pre- and postoperative Hct in the following equation: Corrected $MN_{concentration} = measured \ MN_{concentration} \times \frac{(Hct_{pre}}{Hct_{post})}$.

**Experiment 2: Efficacy study**

Twenty-five Wag-Rij rats were randomized into five groups of five rats each; control rats for cell growth only, sham thoracotomy, IV 0.5 mg MN, ILuP 0.5 mg MN and SPAP 0.5 mg MN. At day 0, bilateral lung metastases were induced by IV injection of $2.5 \times 10^6$ viable R1H rhabdomyosarcoma cells into the femoral vein. Treatment was performed at day 7. At day 28, the rats were sacrificed. Left and right lungs were weighted. The number of pulmonary metastases was visualized by the method of Wexler, using a Fekete’s solution, resulting in a black-stained lung with white pulmonary metastases [16]. Both left and right sides were counted; the number of left-sided metastases was corrected for the number of right-sided metastases for precise statistical evaluation.

**Experiment 3: Survival study**

Thirty Wag-Rij rats were randomized into five groups of six rats each; control rats for cell growth only, IV 0.5 mg melphalan, sham ILuP (perfusion with hydroxyethyl starch alone), ILuP 0.5 mg melphalan and SPAP 0.5 mg melphalan. At day 0, left-sided pulmonary metastases were induced with IV injection of $1 \times 10^6$ viable R1H rhabdomyosarcoma cells into the femoral vein after clamping of the right pulmonary artery. Treatment was performed at day 7. The weight of the rat was assessed two times a week. Endpoints of the study were:

1. **Weight loss of more than 10%**. The rats needed to be killed.
2. **Death due to metastatic disease**.
3. **Sacrifice at day 90**.

**Statistical analysis**

All data are presented as mean ± standard deviation besides the pharmacokinetic data which are presented as median ± standard deviation. The pharmacokinetic data are analysed by using the Mann–Whitney U-test for comparison between groups. Efficacy data are evaluated by using the Mann–Whitney U-test for comparison between left and right lungs between groups. Survival plots are generated by the Kaplan–Meier method and statistical comparison between groups is made using the log-rank test. Significance is defined as a $P \leq 0.05$.

**RESULTS**

**Pharmacokinetics**

A total of eight rats were excluded from the analysis because of a starting MN concentration outside the 0.5 ± 0.125 mg error...
range: three rats from the BFO group, three from the ILuP group and two from the IV group. The MN concentration in the systemic circulation of the different treatment groups is shown in Table 1. This concentration was significantly lower for the ILuP group when compared with IV injection or SPAP. No significant difference was found between the maximum concentration of MN in the systemic circulation for the IV-treated rats or SPAP. The tissue concentrations of MN in the left lung are depicted in Table 2 and for the right non-treated lung in Table 3. The maximum concentration for the three different treatment groups in both left lung tissue (the target organ) and systemic circulation are illustrated in Fig. 1. At 15 min, the concentration of MN in the left lung is significantly higher for the ILuP group when compared with IV injection or SPAP. No significant difference was found between IV and SPAP. At 30 min of treatment, SPAP resulted in a significantly higher MN concentration in the left lung compared with IV injection and ILuP, and ILuP had a significantly higher concentration compared with IV injection. No significant difference was found between the peak concentration of melphalan for ILuP at 15 min and SPAP at 30 min. At 30 min of reperfusion, no significance was found between the treatment groups.

**Efficacy**

A preliminary study showed that the appropriate time without treatment to wait until sacrifice was 28 days after injection and that an injection of 2.5 × 10^6 R1H cells was needed for sufficient growth (data not shown). Two rats, one from the sham thoracotomy and one from the control group died between injection of the tumour cells and treatment, and these rats were excluded from the analysis. The data from the efficacy study are shown in Table 4. Both SPAP and ILuP resulted in a significant reduction in the number of left-sided pulmonary metastases compared with IV injection (P < 0.01), sham thoracotomy and control rats (P = 0.01). No significant reduction between the animals was found in the right non-treated lung. IV administration of 0.5 mg MN also resulted in a significant reduction in left-sided pulmonary metastases compared with control (P = 0.05) and sham thoracotomy (P = 0.01); however, no significant reduction was found on the right side.

**Survival**

The survival graph is displayed in Fig. 2. No rats died during the procedure. The mean survival of SPAP with MN (74 ± 8 days) was almost equal to ILuP with MN (71 ± 10 days) but significantly longer compared with IV MN (54 ± 7 days; P < 0.01 both), sham ILuP (45 ± 6 days; P < 0.01 both) and control rats (47 ± 6 days; P < 0.01 both). IV MN prolonged survival in comparison to sham ILuP (P = 0.03). No difference in survival was found between control and sham ILuP group (P = 0.53).

**Table 1:** Results pharmacokinetic study; blood concentration of melphalan (L-PAM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 min treatment</th>
<th>30 min treatment (end perfusion)</th>
<th>30 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>N = 4</td>
<td>L-PAM (µg/ml)</td>
<td>N = 4</td>
</tr>
<tr>
<td></td>
<td>14.05 ± 2.83</td>
<td>P = 0.02</td>
<td>9.85 ± 1.14</td>
</tr>
<tr>
<td>SPAP</td>
<td>10.55 ± 1.59</td>
<td>P = 0.38</td>
<td>15.00 ± 6.53</td>
</tr>
<tr>
<td>ILuP</td>
<td>0.00 ± 1.50</td>
<td>P = 0.00d</td>
<td>0.00 ± 2.25</td>
</tr>
</tbody>
</table>

IV: intravenous injection; SPAP: selective pulmonary artery perfusion; ILuP: isolated lung perfusion; N: number of subjects; Sig: significance.


dILuP in comparison to IV injection in the same timeframe.

cILuP in comparison to SPAP in the same timeframe.

Table 2: Results pharmacokinetic study; left-lung tissue concentration of melphalan (L-PAM)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 min treatment</th>
<th>30 min treatment (end perfusion)</th>
<th>30 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L-PAM (µg/g)</td>
<td>Sig</td>
<td>L-PAM (µg/g)</td>
</tr>
<tr>
<td>IV</td>
<td>11.02 ± 4.13</td>
<td>P = 0.16</td>
<td>4.30 ± 4.2</td>
</tr>
<tr>
<td>SPAP</td>
<td>24.63 ± 9.11</td>
<td>P = 0.08</td>
<td>67.03 ± 12.36</td>
</tr>
<tr>
<td>ILuP</td>
<td>57.9 ± 8.16</td>
<td>P = 0.03c,d, P = 0.86</td>
<td>34.54 ± 10.21</td>
</tr>
</tbody>
</table>

IV: intravenous injection; SPAP: selective pulmonary artery perfusion; ILuP: isolated lung perfusion; Sig: significance; /: not significant with any group in the timeframe.

aIV injection in comparison to SPAP in the same timeframe.
bSPAP at 15 min in comparison to ILuP at 30 min.
cILuP in comparison to IV injection in the same timeframe.
dILuP in comparison to SPAP in the same timeframe.


DISCUSSION

Since the large retrospective database of surgically treated lung metastases in 1997 by Pastorino et al. [1]. The 5-year overall survival rate has not increased and remains low between 20–50% [1–5]. One of the reasons is poor local control with a high, mainly intrathoracic, recurrence rate for colorectal and sarcoma pulmonary metastases: 44% for adenocarcinoma and 66% for sarcoma metastases. It is explained by micrometastases that are already present during the initial procedure, and insufficient adjuvant systemic chemotherapy [1]. ILuP and SPAP with BFO are two experimental surgical techniques with the intention to deliver a high dose of chemotherapy directly into the pulmonary circulation with or without control of the venous effluent at the time or around the time of surgical resection. During the ILuP procedure, the lung is isolated from the systemic circulation by cannulating the pulmonary artery and veins, resulting in a closed circuit in which a high dose of chemotherapy can be perfused without systemic exposure [17]. ILuP has shown to be feasible in both animal and human clinical studies [17–20]. A prolonged survival could be demonstrated for rodents with pulmonary metastases from colorectal carcinoma when ILuP was performed with melphalan, gemcitabine or the combination of these two drugs [12, 21]. We recently reported the long-term follow-up results of our clinical phase I study of ILuP with melphalan for resectable lung metastases, with no long-term toxicity and an encouraging 5-year overall survival in comparison to recent literature [22]. However, ILuP requires a thoracotomy and repeated application is not possible. SPAP combined with BFO could be a possible solution for this problem as it is an endovascular technique. With SPAP, a modified balloon catheter is introduced into the femoral vein and moved up in position in the selected left or right pulmonary artery. This allows injection of the chemotherapy through the balloon catheter directly into the lung resulting in an increased uptake of chemotherapy in the lung tissue, which is further enhanced by insufflating the balloon (BFO) immediately after injection [8–10]. Especially, drugs with an initially high uptake by the lung parenchyma should be used but so far only gemcitabine has been tested. Both ILuP and SPAP with gemcitabine give rise to an increased lung tissue concentration compared with IV administration [8, 23]. Until now, no pharmacokinetic data have been published on SPAP with MN, as well as survival or efficacy data of SPAP with MN for rodents with sarcoma lung metastases. Nawata et al. [13] reported in 1996 efficacy data of ILuP with MN in a rodent model with sarcoma lung metastases, with significantly better results compared with IV MN; however, no survival data have been reported.

### Table 3: Results pharmacokinetic study; right-lung tissue concentration of melphalan (L-PAM)

<table>
<thead>
<tr>
<th></th>
<th>15 min treatment</th>
<th>30 min treatment (end perfusion)</th>
<th>30 min reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-PAM (µg/g)</td>
<td>Sig</td>
<td>L-PAM (µg/g)</td>
<td>Sig</td>
</tr>
<tr>
<td>IV</td>
<td>10.20 ± 5.17</td>
<td>P = 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.20 ± 3.27</td>
</tr>
<tr>
<td>SPAP</td>
<td>3.20 ± 3.40</td>
<td>P = 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.00 ± 13.45</td>
</tr>
<tr>
<td>ILuP</td>
<td>0.00 ± 0.00</td>
<td>3.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

IV: intravenous injection; SPAP: selective pulmonary artery perfusion; ILuP: isolated lung perfusion; Sig: significance; /: not significant with any group in the timeframe.

<sup>a</sup>ILuP in comparison to IV injection in the same timeframe.

<sup>b</sup>ILuP in comparison to SPAP in the same timeframe.

### Figures

**Figure 1:** Maximum concentration of the three different treatment groups in the left lung (µg/g) and the systemic circulation (µg/ml). ILuP: isolated lung perfusion; IV: intravenous injection; SPAP: selective pulmonary artery perfusion; Llung: left lung; syst.: systemic circulation.

**Figure 2:** The Kaplan–Meier curve of the overall survival rate for the different treatment groups. MN: melphalan; ILuP: isolated lung perfusion; IV: intravenous injection; SPAP: selective pulmonary artery perfusion.
In this study, pharmacokinetics (uptake in the lung tissue and systemic exposure), efficacy and survival were analysed for SPAP and compared with ILuP and IV injection of MN in a rodent model with rhabdomyosarcoma lung metastases.

The study shows that SPAP and ILuP with MN are well tolerated with no operative deaths. Both techniques result in an increased survival and both are equally efficient by reducing the number of lung metastases compared with IV MN, demonstrating an improved local control. As expected, ILuP showed a good separation of systemic and perfusion circuit. Also, the highest MN concentration in the systemic circulation is found for IV injection at 15 min and for SPAP at 30 min. Despite this higher systemic exposure (similar as for IV) compared with ILuP, the SPAP technique was able to induce tissue concentrations in the target organ that were significantly higher compared with IV injection (around six times higher), while the peak concentration was even similar to ILuP (five times higher than IV injection), although at different time points and unexplained. The higher peak concentration of MN for the SPAP group showed its effect both in the efficacy study and in the survival study where a significantly better result was seen for the SPAP group when compared with IV injection.

In conclusion, this study showed that both SPAP and ILuP with MN results in 5–6 times higher tissue concentrations in the target organ without increased systemic exposure, and a prolonged survival when compared with IV MN, suggesting better local control.

Because of its less invasive character, SPAP is a promising experimental surgical technique and should be compared to ILuP as an alternative, less invasive technique.

**ACKNOWLEDGEMENTS**

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**Conflict of interest:** none declared.
APPENDIX. CONFERENCE DISCUSSION

Dr T. Krueger (Lausanne, Switzerland): Once it is established, selective pulmonary artery perfusion for high-dose chemotherapy would indeed facilitate its clinical application and widespread use. As you know, Dr Furrer from our group had compared ILuP, SPAP, in 1998, and I think your pharmacokinetic study confirms his results very nicely in a different animal model. This brings me to my first question. Your pharmacokinetic study assesses tissue levels in normal tissue but not in the target, which is the tumour. Could you speculate about drug levels in tumour, and do you think that the perfusion mode could influence the ratio of tumour and normal tissue uptake? The second issue of interest is toxicity. A potential advantage of SPAP would be repeated application of SPAP in patients. In your preclinical study in the rat you used the SPAP only once. Do you have any idea about what happens to normal lung tissue if you apply high-dose chemotherapy repeatedly?

Dr Den Hengst: To answer the second question first, it’s very difficult to do SPAP multiple times in rats because you always have to do a thoracotomy, and it’s very hard to do several treatments in one rat to evaluate the damage done over multiple treatments. This should be done in a bigger animal model, and we don’t have the facilities at our university for that. If you use what is used for systemic chemotherapy, if you use that concentration for SPAP, I don’t think you will have any more toxicity than you will find in the systemic chemotherapy.

Dr Krueger: You may perform contralateral pneumonectomy, then you can easily assess the toxicity of the treatment you’ve done in the other lung.

Dr Den Hengst: One of my co-workers before me had already done a toxicity study for isolated lung perfusion itself with the same concentration of melphalan used here and also with a pneumonectomy on one side, and there was no decrease in survival for those rats compared to control groups. There was no toxicity found when comparing the dose of isolated lung perfusion I used in this study, when comparing to both SPAP and isolated lung perfusion. So I don’t think that that will diminish the survival in rats. There is no more toxicity for SPAP compared to isolated lung perfusion. Sorry, your first question?

Dr Krueger: The first question was about drug levels in tumour. You have assessed the drug level in normal tissue and not in the tumour. What do you know about tumour drug uptake?

Dr Den Hengst: The rat lung itself is very small and it’s not possible to excise the tumour itself from the lung tissue of the rat, so we have to do it in a larger model or in humans. Just now, in our phase 2 trial, we are looking at how much the concentration is in the normal lung tissue and in the tumour tissue. So at the present time, it’s not possible to give any thoughts about it. At the moment, we think it’s lower in the tumour tissue than in the lung tissue.

Dr P. Sardari Nia (Nieuwegein, Netherlands): Could you explain why you did not have any concentration difference of melphalan between the left and right lung?

Dr Den Hengst: For the right side, the concentration of melphalan in the tissue, in the lung tissue, you can clearly see that the isolated lung perfusion had no concentration measurable. However, there was such a wide range in the measurements of melphalan for both SPAP and i.v. injection in the right lung tissue that it gave a wide range of the mean concentration, resulting in a tendency towards significance but not a clear significant difference in the concentration, although the tissue concentrations would suggest it, because I think you can clearly see that when you look at the right lung tissue concentration with i.v. injection on the left side, there’s no difference with the right side. So the measurements are the same because there is more of a wide variance in the measurements. There was no significance.

Dr Sardari Nia: So you think that this is dependent on the sample size?

Dr Den Hengst: Yes. Probably with an increased sample size, we would have met significance, yes.

Dr M. Loubani (Hull, UK): You demonstrated a 4-day improvement in survival. Do you see this being applied in humans, and how could that translate into improved survival in humans?

Dr Den Hengst: We already did a phase 1 clinical trial where isolated lung perfusion was used in patients with resectable lung metastasis from colorectal carcinoma, sarcoma, and renal cell carcinoma, and we saw that we had a 5-year overall survival rate of 54.8%. Of course, it’s a phase 1 clinical trial, so there are different doses used with lung perfusion, but this result suggests at least a clinical benefit for long-term survival for patients when isolated lung perfusion is used for resectable lung metastasis.