Combination chemotherapy with gemcitabine with isolated lung perfusion for the treatment of pulmonary metastases

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Objective: Isolated lung perfusion is an experimental technique for the treatment of lung metastases. Single-agent isolated lung perfusion does not result in complete remission. We studied the in vivo and in vitro efficacy of combinations of gemcitabine, cisplatin, and melphalan.

Methods: In vitro, using the sulforhodamine B assay, CC531s cells were incubated with cisplatin, gemcitabine, or melphalan or with a combination of these drugs. One drug was added at concentrations causing 25% growth inhibition, whereas the second drug was added at variable concentrations. In vivo, left pulmonary metastases were induced in Wag/Rij rats by means of intravenous injection of CC531s adenocarcinoma cells. At day 7, rats underwent left isolated lung perfusion with gemcitabine (n = 7), cisplatin (n = 9), melphalan (n = 7), gemcitabine-cisplatin (n = 6), melphalan-gemcitabine (n = 6), and cisplatin-melphalan (n = 7). Death by means of metastatic disease was the end point. Survival and differences in survival were assessed by using Kaplan-Meier and log-rank testing.

Results: In vitro synergistic activity was observed for melphalan-gemcitabine, whereas other combinations showed additive or antagonistic activity. In vivo treated rats lived longer compared with control animals ($P < .0001$). In isolated lung perfusion melphalan resulted in longer survival compared with gemcitabine ($P = .0016$) and cisplatin ($P = .046$). Isolated lung perfusion with melphalan-gemcitabine resulted in 67% survival of the rats after 90 days versus 0% in other groups.

Conclusions: Isolated lung perfusion monotherapy or combination therapy with gemcitabine, cisplatin, or melphalan resulted in significantly longer survival compared with that seen in control animals. Isolated lung perfusion combination therapy with melphalan-gemcitabine resulted in the best survival either in vitro or in vivo.

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Isolated lung perfusion (ILuP) with chemotherapy is an experimental surgical technique for the treatment of pulmonary metastases to improve the current mean 5-year survival of approximately 40% after complete surgical resection of metastases from colorectal carcinoma, renal carcinoma, and sarcoma.¹ On the basis of the finding that cell killing of tumor cells caused by chemotherapeutics is concentration dependent, ILuP aims to achieve higher

TABLE 1. In vivo survival after different combinations

Left ILuP	GCB	Cis	MN	MN + GCB	GCB + Cis	Cis + MN	Control
Days (mean ± SD)	38 ± 1	42 ± 3	44 ± 2	79 ± 7	47 ± 2	56 ± 4	28 ± 1
Survival at day 90 (%)	0	0	0	67	0	0	0
P value vs MN-GCB	.0004	.0015	.0007		.0016	.011	<.00001

ILuP, Isolated lung perfusion; GCB, gemcitabine; Cis, cisplatin; MN, melphalan.

Figure 1. Dose-response curves for the combination of GCB alone or GCB with MN or Cis.

local drug levels without systemic toxicity.² Animal models of ILuP with sarcoma and carcinoma cell lines were set up to test the toxicity, pharmacokinetics, and antitumor activities of several drugs, such as doxorubicin, tumor necrosis factor α (TNF- α), melphalan (MN), cisplatin (Cis), fluorodeoxyuridine, and gemcitabine (GCB).²⁻¹¹ These studies showed significantly longer survivals in rats after ILuP monotherapy with GCB and MN compared with that seen in control rats and intravenously treated rats.^{7,11} Although significantly longer survival was achieved with ILuP monotherapy, only casual complete remissions were seen. MN is an alkylating agent, and it is not cell phase specific, resulting in DNA-interstrand or DNA-protein cross-links and causing inhibition of DNA synthesis. GCB is a deoxycytidine analog with proved clinical activity in ovarian, breast, colorectal, pancreas, bladder, small cell lung, and non-small cell lung cancers.^{12,13} After entering the cell, GCB is phosphorylated and incorporated into the DNA. Subsequently, one nucleotide has to be added until DNA polymerase is not capable of proceeding (masked chain termination). Mainly S-phase cells proved to be sensitive to GCB. In addition to actions on DNA, GCB has some self-potentiating mechanisms within the cell. Cis is a platinum compound and has proved clinical activity against many of the earlier mentioned types of cancer. Cis affects not only the DNA but also the mitochondrial RNA, the phospholipid membranes, and the cytoskeleton.

Because of their different mechanisms of cytotoxicity, we studied the in vitro and in vivo efficacy of combina-

tions of MN, GCB, and Cis for the treatment of pulmonary metastatic colorectal adenocarcinoma in a rat model of ILuP.

Material and Methods

In Vitro

Cell line. In this study the CC531s adenocarcinoma cell line was used to determine sensitivity for the cytotoxic effect of GCB (2',2'-difluorodeoxyuridine, Gemzar), MN (L-PAM, Alkeran), and Cis (cis-diaminedichloroplatinum). CC531s is a weakly immunogenic carcinoma of the colon that is syngeneic for Wag rats. The tumor is a dimethylhydrazine-induced adenocarcinoma.¹⁴

CC531s cells were cultured in RPMI-1640 medium and supplemented with 10% fetal calf serum (Invitrogen, Merelbeke, Belgium). Cultures were maintained in exponential growth in a humidified atmosphere at 37°C under 5% CO₂/95% air.

Chemicals. Cis (Sigma, Bornem, Belgium) solutions were made in 0.9% NaCl and stored at -20°C. Solutions of 2',2'-difluorodeoxycytidine (Eli Lilly, Brussels, Belgium) were made in phosphate-buffered saline (PBS; 2.7 mmol/L KCl, 136.9 mmol/L NaCl, 1.5 mmol/L KH₂PO₄, and 6.5 mmol/L Na₂HPO₄) and stored at -80°C. MN (Wellcome, Waterloo, Belgium) solutions were made in dimethyl sulfoxide/0.9% NaCl (1:1), stored at -80°C, and used for 1 month.

In vitro growth inhibition experiments. Cells were harvested from exponential-phase cultures by means of trypsinization, counted, and plated in 48-well plates. The seeding density was 600 cells per well to ensure exponential growth during the experiments. After plating and a 24-hour recovery period, cells were treated for 30 minutes with Cis alone, with GCB alone, or with MN alone or with a combination of 2 drugs: one drug was added at a concentration causing about 25% growth inhibition (IC25), whereas the other drug was added at variable concentrations. Each concentration was tested 6 times within the same experiment. After 30 minutes of incubation, cells were washed with drug-free medium. Four days after the start of treatment, cell survival was determined by using the sulforhodamine B (SRB) assay.

The SRB assay was performed according to the method of Skehan and colleagues¹⁵ and Papazisis and associates,¹⁶ with minor modifications. Culture medium was aspirated before fixation of the cells by addition of 200 μ L of 10% cold trichloroacetic acid. After 1 hour of incubation at 4°C, cells were washed 5 times with deionized water. Cells were stained with 200 μ L of 0.1% SRB (ICN, Asse, Belgium) dissolved in 1% acetic acid for at least 15 minutes and subsequently washed 4 times with 1% acetic acid to remove unbound stain. The plates were left to dry at room temperature, and bound protein stain was solubilized with 200 μ L of 10 mmol/L unbuffered tris(hydroxymethyl)aminomethane base

TABLE 2. IC50 and IC25 values after in vitro exposure for 30 minutes with different concentrations of MN, GCB, or Cis

	IC50 value (mmol/L)	IC25 value (mmol/L)
MN	8.0 ± 2.2	3.2 ± 0.6
GCB	7.6 ± 1.2	3.5 ± 0.9
Cis	10.3 ± 3.5	4.9 ± 2.0

IC50, Concentration causing 50% growth inhibition; IC25, concentration causing 25% growth inhibition; MN, melphalan; GCB, gemcitabine; Cis, cisplatin.

and transferred to 96-well plates for reading the optical density at 540 nm (550 Microplate Reader; Biorad, Nazareth, Belgium).

Statistical analysis The survival rates were calculated as follows:

$$\frac{\text{Mean optical density of treated cells}}{\text{Mean optical density of treated cells}} \times 100$$

The dose-response curves were fitted to the sigmoid inhibition model as follows:

$$\exp(\text{survival}) = 1 - (C\gamma/C\gamma + IC50\gamma)$$

where C is the concentration of the drug, and γ is a constant, using WinNonlin (Pharsight, Mountain View, Calif) to calculate the concentration of drug causing a 50% growth inhibition value (IC50).

All data are presented as means ± SD. All experiments were performed at least 3 times. A 2-sample *t* test was used to compare IC50 values.

Possible synergism is evaluated with the median drug effect analysis method of Chou and Talalay¹⁷ and processed by the computer program CalcuSyn (Biosoft, Cambridge, United Kingdom). *D_m* values (IC50 values) are calculated by extrapolation. For the separate drugs, the respective growth inhibition parameters, expressed as fraction affected (FA; eg, an FA of 0.25 is a growth inhibition of 25%), were introduced. The combination index (CI) was calculated by using the following formula:

$$CI = [(D)_1/(D_x)_1] + [(D)_2/(D_x)_2] + [a(D)_1(D)_2/(D_x)_1(D_x)_2]$$

where *a* = 1 for mutually nonexclusive drugs, (*D*)₁ and (*D*)₂ are the doses of the separate drugs and their combination, and (*D_x*)₁ and (*D_x*)₂ are the doses resulting in a growth inhibition of *x*%. These doses are calculated by using the following formula:

$$D = D_m[FA/(1 - FA)]^{1/m}$$

where *D_m* is the dose required to produce 50% growth inhibition, FA is the fraction affected, and *m* is the slope of the median plot. Because CIs changed with FA, the average CIs were used. An average CI of less than 1 indicates synergism, an average CI of greater than 1 indicates antagonism, and an average CI of 1 indicates additivity.

In Vivo

Animals. Male inbred Wag-Rij strain rats (weight, approximately 250 g), obtained from Iffa Credo (Charluff River, Belgium), were used for all experiments. Animals were treated in accordance with the Animal Welfare Act and the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health publication No. 86-23, revised 1985). The rats were transported in sterile conditions, housed in suspended mesh wire cages

Figure 2. Dose-response curves for the combination of Cis alone or Cis with GCB or MN.

under standard laboratory conditions, and ad libitum fed a standard pellet diet (standard rat chow; Hope Farms, Woerden, The Netherlands). The experimental protocols were approved by the Ethical Committee of the University of Antwerp.

Left isolated lung perfusion. Left ILuP was performed according to the technique described by Hendriks and coworkers.¹⁸ In brief, rats were anesthetized with isoflurane in a mixture of nitrous oxide (NO₂) and oxygen (O₂). Isoflurane was administered in a concentration of 4%, and the NO₂/O₂ ratio was 3:1. After 5 minutes, rats were intubated with a 16-gauge Insyte-W catheter by means of translaryngeal illumination under direct vision and afterward ventilated with a volume-controlled ventilator (Harvard Rodent Ventilator, South Natick, Mass). Once connected to the ventilator, the NO₂/O₂ ratio was set to 1:1 (0.5 L/min), and the rate of ventilation was 75 strokes/min, with a tidal volume of 10 mL/kg. Subsequently, the left chest was shaved and prepared with a 70% alcohol solution, followed by a left thoracotomy between the fourth and fifth rib. After placing a rib retractor, the left lung was luxated anteriorly, and the hilum was dissected free under microscopic view (magnification 16×; Carl Zeiss, Javalentem, Belgium). The pulmonary artery and vein were clamped with curved microvascular clips (Kleinert-Kurz WK65145). A PE-10 perfusion catheter (Becton Dickinson, Bornem, Belgium) was introduced into the chest through a 16-gauge Angiocath, which was placed through the chest wall. The PE-10 catheter was inserted into the pulmonary artery and fixed with a 4-0 silk tie. Perfusate was delivered through this catheter. The effluent was collected at the venous side by using a catheter in proximity to the venotomy. The left thorax was flushed with 2 mL of saline every 5 minutes.

In all experiments rats were perfused for 25 minutes, followed by a 5-minute washout period with buffered starch and a roller pump at a rate of 0.5 mL/min. At completion of the perfusion, the arteriotomy was repaired with 10-0 nylon sutures (Ethilon; Ethicon, Dilbeek, Belgium). No venotomy repair was performed, but pressure was applied, with gauze placed over the lung to control the bleeding from the venotomy. The thoracotomy incision was closed in layers after introduction of a 16-gauge catheter connected to a 50-mL syringe into the left chest cavity. When animals recovered, the chest tube and endotracheal tubes were removed.

MN, GCB, and Cis solutions were prepared by reconstituting lyophilized powder in the supplied diluent and performing appro-

Figure 3. Dose-response curves for the combination of MN alone or MN with GCB or Cis.

appropriate dilutions with buffered hydroxyethyl starch before the experiments. The optimal dose of MN, GCB, and Cis to be administered by means of left ILuP was determined in previous experiments.^{2,6,10}

Induction of unilateral pulmonary metastases. At day 0, 63 rats were infused with 2.0×10^6 viable CC531s adenocarcinoma cells through the left femoral vein while clamping the right pulmonary artery for 10 minutes for induction of left pulmonary metastases, as described previously.^{4,7} Briefly, anesthesia was induced, as described previously. After a right thoracotomy, the rib retractor was placed in the third intercostal space. After luxating the right upper and middle lobe posteriorly, the right pulmonary artery was dissected free under microscopic view (magnification $16\times$; Carl Zeiss). Subsequently, a longitudinal incision was made along the posterior border of the superior caval vein, and the right main pulmonary artery was identified. Finally, an occluding curved microvascular clamp (Kleinert-Kurz WK65145) was placed over the main pulmonary artery. The rat was repositioned to gain access to the left femoral vein for infusion of the tumor cells. The clamp on the right pulmonary artery was removed after 10 minutes. The groin incision was subsequently closed with a running suture. After full lung re-expansion, the chest was closed in 3 layers with 4-0 Vicryl sutures (Ethicon, Inc, Somerville, NJ), and the animal was allowed to recover from anesthesia.

Experiment: Survival after left ILuP with MN, GCB, and Cis. Data on survival after left ILuP with MN, GCB, and Cis are shown in Table 1. At day 0, 63 rats were infused with 2.0×10^6 viable CC531s adenocarcinoma cells through the left femoral vein while clamping the right pulmonary artery for 10 minutes for induction of left pulmonary metastases, as previously described. At day 7, all rats were randomized into 7 groups. Groups 1 to 3 underwent ILuP with 2 mg/kg MN ($n = 7$), 320 mg/kg GCB ($n = 7$), and 3 mg/kg Cis ($n = 9$), respectively. Groups 4 to 6 had ILuP with 320 mg/kg GCB combined with 3 mg/kg Cis ($n = 6$), 2 mg/kg MN combined with 320 mg/kg GCB ($n = 6$), and 3 mg/kg Cis combined with 2 mg/kg MN ($n = 7$), respectively. Group 7 was the untreated control group ($n = 21$). The end point of the study was death caused by metastatic disease or death at day 90.

Statistical analysis. Survival curves were constructed by using the Kaplan-Meier method, and differences in survival were assessed by using the log-rank test with SPSS version 11.0 for Windows (Chicago, Ill).

TABLE 3. IC50 value after in vitro exposure of CC531s adenocarcinoma cells with different combinations

	IC50	P value
GCB	7.6 ± 1.2	
GCB + IC25 Cis	$9.6 \pm 0.8^*$	.044
GCB + IC25 MN	$4.3 \pm 1.0^*$	.010
Cis	10.3 ± 3.5	
Cis + IC25 GCB	8.4 ± 1.0	.370
Cis + IC25 MN	$17.2 \pm 1.7^\dagger$	.021
MN	8.0 ± 2.2	
MN + IC25 GCB	8.0 ± 2.0	.828
MN + IC25 Cis	9.1 ± 1.0	.626

IC50, Concentration causing 50% growth inhibition; GCB, gemcitabine; IC25, concentration causing 25% growth inhibition; Cis, cisplatin; MN, melphalan. $^*P < .05$ versus 2',2'-difluorodeoxyuridine. $^\dagger P < .05$ vs cis-diaminedichloroplatinum.

Results

In Vitro: Analysis of the Interactions Among MN, GCB, and Cis

The IC50 and IC25 values of MN, GCB, and Cis in CC531s cells are summarized in Table 2. The cells were less sensitive for the cytotoxicity of Cis. However, the values were not significantly different.

On the basis of these sensitivity data, combination experiments were designed in which cells were exposed to the approximate IC25 of one drug and a concentration range of the other drug. Figure 1 shows dose-response curves for the combination of GCB alone or in combination with MN or Cis, Figure 2 shows dose-response curves of Cis alone or in combination with MN or GCB, and Figure 3 shows dose-response curves of MN alone or in combination with GCB or Cis. The IC50 values of the respective combinations are summarized in Table 3.

The combination of GCB with the IC25 of MN resulted in increased growth inhibition. The IC50 value is significantly different from the IC50 value of GCB alone ($P = .010$). Significantly less growth inhibition was observed after combination therapy with Cis and the IC25 of MN ($P = .021$) and after therapy with GCB and the IC25 of Cis ($P = .044$).

Survival remained unchanged after treatment of CC531s cells with the combination of Cis with the IC25 of GCB, the combination of MN with the IC25 of Cis, and the combination of Cis and the IC50 of GCB.

Synergism was analyzed with the median drug effect analysis of Chou and Talalay,¹⁷ and average CIs are shown in Table 4. Only the combination of GCB with the IC25 of MN resulted in synergism. The other combinations resulted in antagonism, and the mean CI was greater than 1.

In Vivo: Survival After Left ILuP With MN, GCB, and Cis

Data on survival after left ILuP with MN, GCB, and Cis are shown in Figure 4. All rats that had left ILuP monotherapy or combination therapy lived significantly longer compared with untreated control animals ($P < .0001$). None of the rats died of contralateral lung metastases but rather because of bulky disease at the perfused lung with direct ingrowth of the mediastinum, thoracic wall, or diaphragm. Rats that underwent ILuP with MN had significantly longer survivals compared with those that underwent ILuP with Cis ($P = .046$) or ILuP with GCB ($P = .0016$). No significant difference in survival was observed between ILuP with GCB or Cis ($P = .096$).

Combination ILuP with MN-Cis resulted in significantly longer survival compared with Cis alone ($P = .029$), whereas no prolongation in survival was observed compared with single-agent MN ($P = .88$). A significantly longer survival was seen after combination ILuP with GCB-Cis compared with GCB ($P = .0004$) and MN-Cis ($P = .028$), whereas no longer survival was observed compared with Cis ($P = .36$). Combination ILuP with MN-GCB resulted in a significant extension of survival (one complete remission) compared with GCB ($P = .0005$), Cis ($P = .0031$), and MN-Cis ($P = .046$), whereas no significant difference was observed compared with MN ($P = .12$). Histologic examination of the lungs of rats that died before 90 days did not show inflammatory or fibrotic changes in the treated left lung compared with the nontreated right lung.

Discussion

ILuP is a surgical experimental technique for the treatment of lung metastases on the basis of the concentration depen-

TABLE 4. Mean combination index value after treatment with different combinations

	Mean CI
GCB + IC25 Cis	1.433
GCB + IC25 MN	0.629
Cis + IC25 GCB	1.800
Cis + IC25 MN	2.124
MN + IC25 GCB	1.274
MN + IC25 Cis	1.226

CI, Combination index; GCB, gemcitabine; IC25, concentration causing 25% growth inhibition; Cis, cisplatin; MN, melphalan.

dency of cytotoxicity of chemotherapeutic drugs.² The technique aims to deliver high local lung levels without systemic toxicity or metabolism by the kidney or liver, resulting in a better tumor-eradicating response. Animal studies investigating the efficacy of left ILuP with several drugs, such as MN, GCB, fluorodeoxyuridine, TNF, doxorubicin, and Cis, were promising but did not result in complete remission, with the exception of a single rat.³⁻¹¹

Because of their different mechanisms of cytotoxicity, synergistic actions were investigated in this study both in vitro and in vivo by using combinations of MN, GCB, and Cis. ILuP with each single drug resulted in significantly longer survival compared with that seen in control animals, whereas survival with MN after ILuP was better compared with that seen with GCB and Cis.

Theoretically, GCB and Cis interact at different levels from cellular uptake until incorporation into the DNA. GCB can interact with the uptake of Cis or with the binding of Cis to DNA. Otherwise, Cis can interact with the cellular uptake, the phosphorylation, or the incorporation of GCB into DNA.¹⁹

Figure 4. Kaplan-Meier survival curves for control animals and animals receiving GCB with MN, GCB with Cis, and Cis with MN.

Van Moorsel and associates²⁰ concluded from in vitro studies that the effect of GCB on the accumulation of Cis or DNA platination by Cis might be important factors in this synergistic action. The best synergistic effect was observed when GCB was administered 4 hours before the addition of Cis. These synergistic effects were confirmed by the in vitro results that showed a significant growth inhibition after exposure of the CC531s cells with GCB and the IC50 of Cis ($P = .044$, CI = 1.433). However, a nonsignificant synergistic tendency was found in vivo when drugs were delivered simultaneously ($P = .0004$ compared with GCB; $P = .36$ compared with Cis). Intravenous pretreatment with GCB before ILuP with Cis might induce synergistic activity¹⁷ but was not tested in these experiments.

The mechanism of cytotoxicity of MN is totally different from that of GCB. MN can be activated to an alkylating agent, resulting in DNA cross-links, whereas GCB is incorporated into the DNA after a phosphorylation step.²⁰ In contrast to these small synergistic actions between GCB and alkylating agents, the present study shows a strong and significant synergistic effect when MN is added to GCB ($P = .0005$ compared with GCB). One rat was in complete histologic remission after death at 90 days. In vitro results confirmed this significant synergistic activity ($P = .01$, CI = 0.629) after treatment with GCB and the IC50 of MN.

Finally, left ILuP with MN and Cis did not result in synergistic action either in vivo ($P = .029$ compared with Cis; $P = .88$ compared with MN) or in vitro.

In conclusion, left ILuP monotherapy with MN, GCB, and Cis enhances the survival of rats with unilateral left lung metastatic disease compared with that seen in control animals, with MN having the best results. A synergistic tendency in prolongation of survival was observed after left ILuP with MN-GCB and GCB-Cis, but the strongest synergistic effects were seen with MN-GCB.

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References

- Pastorino U, Buyse M, Friedel G, et al. Long-term results of lung metastasectomy: prognostic analysis based on 5206 cases. *J Thorac Cardiovasc Surg.* 1997;113:37-49.
- Hendriks JMH, Van Schil PEY, De Boeck G, Van Oosterom A, Van Marck E, Eyskens EJM. Isolated lung perfusion with melphalan and tumour necrosis factor for pulmonary adenocarcinoma. *Ann Thorac Surg.* 1998;66:1719-25.
- Weksler B, Lenert J, Ng B, Burt M. Isolated single lung perfusion with doxorubicin is effective in eradicating soft tissue sarcoma lung metastases in a rat model. *J Thorac Cardiovasc Surg.* 1994;107:50-4.
- Abolhoda A, Brooks A, Nawata S, Kaneda Y, Cheng H, Burt ME. Isolated lung perfusion with doxorubicin prolongs survival in a rodent model of pulmonary metastases. *Ann Thorac Surg.* 1997;64:181-4.
- Weksler B, Blumberg D, Lenert JT, Ng B, Fong Y, Burt ME. Isolated single-lung perfusion with TNF- α in a rat sarcoma lung metastases model. *Ann Thorac Surg.* 1994;58:328-32.
- Van Putte BP, Hendriks JMH, Romijn S, et al. Isolated lung perfusion with gemcitabine in a rat: pharmacokinetics and survival. *J Surg Res.* 2003;109:118-22.
- Hendriks JMH, Van Schil PEY, Van Oosterom AAT, Kuppen PJK, Van Marck E, Eyskens E. Isolated lung perfusion with melphalan prolongs survival in a rat model of metastatic pulmonary adenocarcinoma. *Eur Surg Res.* 1999;31:267-71.
- Nawata S, Abecasis N, Ross HM, et al. Isolated lung perfusion with melphalan for the treatment of metastatic pulmonary sarcoma. *J Thorac Cardiovasc Surg.* 1996;112:1542-8.
- Ng B, Lenert JT, Weksler B, Port JL, Ellis JL, Burt ME. Isolated lung perfusion with FUDR is an effective treatment for colorectal adenocarcinoma lung metastases in rats. *Ann Thorac Surg.* 1995;59:205-8.
- Tanaka T, Kaneda Y, Li T, Matsuoka T, Zempo N, Esato K. Digitonin enhances the antitumour effect of cisplatin during isolated lung perfusion. *Ann Thorac Surg.* 2001;72:1173-8.
- Hendriks JMH, Van Schil PEY, Van Putte B, Vermeulen PB, Van Marck E. Isolated lung perfusion with gemcitabine prolongs survival in a rat model of metastatic pulmonary adenocarcinoma [abstract]. Presented at the 10th Annual Meeting of The European Society of Thoracic Surgeons; 2002; Istanbul, Turkey.
- Storniolo AM, Enas NH, Brown CA, Voi M, Rothenberg ML, Schilsky R. An investigational new drug treatment program for patients with gemcitabine: results for over 3000 patients with pancreatic carcinoma. *Cancer.* 1999;85:1261-8.
- Sandler A, Ettinger DS. Gemcitabine: single-agent and combination therapy in non-small cell lung cancer. *Oncologist.* 1999;4:241-51.
- Marquet RL, Westbroek DL, Jeekel J. Interferon treatment of a transplantable rat colon carcinoma. *Int J Cancer.* 1984;38:689-92.
- Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst.* 1990;82:1107-12.
- Papazisis KT, Geromichalos GD, Dimitriadis KA, Kortsaris AH. Optimization of the sulforhodamine B colorimetric assay. *J Immunol Methods.* 1997;208:151-8.
- Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul.* 1984;22:27-55.
- Hendriks JMH, Van Schil PEY, Eyskens EJM. Modified technique of isolated left lung perfusion in the rat. *Eur Surg Res.* 1999;31:93-6.
- Peters GJ, Bergman AM, Ruiz Van Haperen VWT, et al. Interaction between cisplatin and gemcitabine in vitro and in vivo. *Semin Oncol.* 1995;22(suppl 11):72-9.
- Van Moorsel CJA, Veerman G, Bergman AM, et al. Combination chemotherapy studies with gemcitabine. *Semin Oncol.* 1997;24(suppl):17-23.