

Safety, feasibility and clinical benefit of localized chemotherapy using microencapsulated cells for inoperable pancreatic carcinoma in a phase I/II trial

Research Article

Matthias Löhr^{1,2}, Jens-Christian Kröger⁴, Anne Hoffmeyer^{2,6}, Mathias Freund³, Johannes Hain⁶, Albrecht Holle², Wolfram T. Knöfel⁸, Stefan Liebe², Horst Nizze⁵, Matthias Renner^{6,9}, Robert Saller⁶, Petra Müller^{2,6}, Thomas Wagner¹⁰, Karlheinz Hauenstein⁴, Brian Salmons^{6,9} and Walter H. Günzburg^{7*}

¹Department of Medicine II, Medical Faculty Mannheim, University of Heidelberg, Germany; ²Division of Gastroenterology, ³Division of Hematology & Oncology, Department of Medicine, ⁴Department of Diagnostic and Interventional Radiology and ⁵Department of Pathology, University of Rostock, Rostock, Germany; ⁶Bavarian Nordic GmbH, Martinsried, Germany, ⁷Institute of Virology, University of Veterinary Sciences, Vienna, Austria, ⁸Department of Surgery, University of Hamburg, Hamburg, Germany; ⁹Austrianova, Veterinärplatz 1, Vienna, Austria; ¹⁰Division of Hematology & Oncology, Medical University Lübeck, Lübeck, Germany

***Correspondence:** Prof. Walter H. Günzburg, Institute of Virology, University of Veterinary Medicine, Veterinärplatz 1, A-1210 Vienna, Austria; Tel.: +43-1-25077-2301; Fax: +43-1-25077-2390; e-mail: walter.guenzburg@vu-wien.ac.at

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Summary

Previous preclinical studies suggested that implantation of encapsulated, genetically modified cells converting a chemotherapeutic agent in the vicinity of tumors may represent an effective treatment for pancreatic cancer. A phase I/II clinical trial was performed to determine safety, feasibility and efficacy of such a targeted, low dose, chemotherapy in 14 advanced-stage pancreatic cancer patients. Genetically modified allogeneic cells expressing the enzyme cytochrome P450 2B1 encapsulated in cellulose sulfate polymers were delivered angiographically via catheter into blood vessels leading to the tumor. These cells locally activate systemically administered ifosfamide to its active metabolites, whilst remaining immuno-isolated. Although adverse events were experienced by all patients, none of these were related to the treatment, with the possible exception of increased serum lipase 15 days after CapCell instillation in one patient. According to the NCI tumor response classification, at the final observation within the study, 2 of the 14 patients treated had partial remissions (14.3%), 11 patients had stable disease (78.6%) and one patient died after 8 days. Median survival was doubled compared to a historic control group ($p=0.008$) and 50% more than usually achieved with gemcitabine. One year survival, at 36%, was three fold that of the control group ($p=0.047$) and twice that reported for gemcitabine. Of 13 evaluable patients, 4 patients reported improvements in pain assessment, with 6 remaining unchanged (4 of these experienced no pain) and 3 patients experiencing slightly more pain. Using a worst case scenario, 50% of patients experienced a clinical benefit whereas in a best case scenario benefit was experienced by 71% of patients.

I. Introduction

Pancreatic carcinoma ranks as the eighth most frequent solid cancer in industrialized countries but is the fifth leading cause of cancer-related deaths (Greenlee et al, 2001). Radical surgery can only be applied in about 10% of diagnosed cases (Huguier and Mason 1999; Neoptolemos et al, 2001) and, to date, all efforts to control tumor growth by radiation and/or chemotherapy have not

significantly prolonged survival or reduced tumor load (Heinemann 2002; Rosenberg 2000). Even the newly introduced chemotherapeutic agent gemcitabine only marginally prolongs the survival of patients (Burris et al, 1997). Nevertheless, this agent has rapidly become a standard treatment because of the additional palliative effect and its ability to improve the clinical benefit response and quality of life of patients with pancreatic cancer. Thus, there is a need for new treatment regimes to

treat pancreatic cancer (Hawes et al, 2000). Along with more classical types of treatment, attempts also have been made to employ gene therapy approaches such as using suicide genes encoding enzymes that are able to convert a prodrug to its active, tumor toxic form (Aspinall and Lemoine 1999; Günzburg et al, 2002; Rosenberg 2000).

The chemotherapeutic agent, ifosfamide, has been shown to have potentially therapeutic effects for pancreatic cancer (Loehrer et al, 1985). In a phase II trial in which 1.6g/m²/day ifosfamide was administered for 5 days to 21 evaluable patients, 7 Stable Diseases with mostly none severe, grade 1-2 toxicity was reported (Wils et al, 1993). In another study (Keizer et al, 1995), where up to 1.5g/m²/day ifosfamide was given as a 10 day continuous i.v. infusion to patients with various tumor types, of six patients with pancreatic cancer, one showed a partial response and a second evidenced a tumor reduction of 45%. Major side effects observed were leukopenia with granulocytopenia, whilst subjective side-effects included nausea/vomiting and fatigue (probably related to neurotoxicity). More encouraging clinical effects have been observed in other trials where medium doses of ifosfamide (1-2g/ml) have been investigated, but this is accompanied by medium grade toxicity profiles. In an initial study by Gad-El-Mawla and colleagues, where 2g/m² were given for 5 days, all but two patients developed haemorrhagic cystitis. However there were 6 partial responses in 10 patients (Gad-El-Mawla and Ziegler 1981). A further study revealed that of 25 patients receiving daily doses of 1.8g for five days, 1 patient showed a complete remission and 14 patients showed partial remission (Gad-El-Mawla 1986). However, these patients suffered from grade 3 alopecia (100%), grade 1 anaemia (100%) and leukopenia (30%). Thus, it is to be expected that higher doses (2-3g/ml) of ifosfamide may show even greater efficacy, but that this will be associated with possibly unacceptable levels of toxicity.

Ifosfamide is a prodrug that requires activation by liver specific cytochrome enzymes, such as the 2B1 isoform (CYP2B1) to generate tumor toxic metabolites (Dirven et al, 1996). Unfortunately, the short half-life of these metabolites in plasma (Cerny et al, 1991b; Kurowski and Wagner 1993), coupled with the distance that they have to travel, require high systemic levels of ifosfamide to achieve therapeutic levels in the tumor. Indeed, these levels are so high as to lead to unacceptable side effects (Loehrer et al, 1985). Local activation of ifosfamide at the site of the tumor should, in contrast, result in good local cytotoxic activity, and at the same time low systemic ifosfamide concentrations, thus resulting in only minimal systemic side effects (Chen and Waxman 2002). Local activation may be achieved by introducing encapsulated human 293 cells genetically modified to overexpress CYP2B1 at the site of the tumor. Encapsulation in cellulose sulfate allows allogeneic cells to survive in vivo, by protecting them from host immune attack as well as by physically constraining them to the site where they are required (Dautzenberg et al, 1999). Previous experiments had revealed that injection of such encapsulated CYP2B1 expressing cells into pre-established tumors in a nude mouse model of human pancreatic carcinoma (Löhr et al,

1994), resulted in complete tumor regression in about 20% of mice and a significant anti-tumor effect in the remaining mice (Löhr et al, 1998). Nevertheless, this route of application may not be suitable for patients and so a further study was performed to demonstrate the feasibility of intra-arterial placement of micro-encapsulated cells into blood vessels leading to the pig pancreas (Kröger et al, 1999; Löhr et al, 2003). Based upon these encouraging preclinical data, a phase I/II clinical trial was initiated involving patients with inoperable pancreatic carcinoma to assess the feasibility, safety, and tolerability of this new treatment modality (Löhr et al, 1999). We have recently described the results obtained concerning safety and efficacy in a brief report (Löhr et al, 2001). Here, data is presented concerning the outcome and clinical benefit of this treatment.

II. Patients and methods

A. Patients, trial design and approval

The study was planned as an open, prospective, single-arm, single center phase I/II-study, following the German gene therapy working group (DAG-GT) recommendations. The protocol was approved by the state ethics committee, the gene therapy board of the German Medical Association and published (Löhr et al, 1999), in line with the recommendation of the German working party on gene therapy indicating the approval of all regulatory bodies. The study was opened on the 28th July 1998 and closed on the 20th September 1999. The trial was conducted in full accordance with good clinical practice guidelines (ICH-GCP).

B. Patient enrollment

A total of 17 patients were enrolled in the trial between July 1998 and April 1999 (**Table 1**) from the 51 patients screened during the study period. Reasons for non-enrolment were previous chemotherapy (n = 8), pancreatic surgery (n = 13), poor general condition (n = 18), unwillingness to participate (n = 5), or death (n = 7). Criteria for entering the study included an inoperable pancreatic adenocarcinoma stage III-IV (UICC) (Hermanek et al, 1997), as determined by histology and measured by CAT scan and only patients who had not received prior chemotherapy were enrolled (Löhr et al, 1999). During the preparation period, clinical data were collected and a baseline CAT scan of the abdomen was performed. The patients were scheduled for the initial celiac angiography with capsule placement (day 0). On day 1, the patients were monitored for evidence of any clinically relevant adverse reactions, e.g. allergic, and/or pancreatitis. The levels of serum amylase, lipase, lactate, lactate dehydrogenase, and liver enzymes, as well as complete blood cell count were determined. Systemic chemotherapy commenced on day 2 with 1g/m² body surface of ifosfamide (Holoxan®) in 250 ml 0.9% normal saline being given as a 1-hour intravenous infusion on three consecutive days. This was accompanied by a 60% dose equivalent of the uroprotector MESNA (Uromitexan®) given as three i.v. injections. This regimen was repeated at days 23-25 for all patients except 5 and 17 who only received one round of ifosfamide. Toxicity was measured based on the WHO/NCI guidelines on common toxicity criteria. Control CAT scans were scheduled for weeks 10 and 20, respectively. During the final visit, a control angiography was performed. On the initial CAT scan, the scan demonstrating the largest diameter of the primary tumor was identified and the area measured. Using appropriate

landmarks, an identical scan was used for comparison. CAT scans were evaluated by two unrelated radiologists, one of whom was not involved in the study. Standard NCI criteria for evaluating tumor growth were used to assess stable disease (SD), partial remission (PR), and minor response (MR). After formally finishing the study, patients were followed up on an ambulatory basis with three-monthly visits. Besides measuring tumor size by CAT scan, the need for pain medication and the quality of life was monitored using questionnaires established for pancreatic diseases (Bloechle et al, 1995). A clinical benefit score based upon variables including Karnofsky score, body weight, pain and analgesic consumption was also calculated from this data. Pain intensity was measured on a visual analogue scale ranging from 0 (no pain) to 100 (the most imaginable intensive pain), in steps of 10. Analgesics consumption was assessed using another scale in which 0 indicated no regular administration of analgesic, whereas scores of 25, 50 and 75 indicated administration of non-steroidal anti-inflammatory drugs (NSAID) or opiates several times per year (25), per month (50) or per week (100) (Bloechle et al, 1995).

C. Historical patient collective

Survival data of a retrospective (historic) control group and the treatment group of this study were compared. A historic control group was established from an evaluation of all patients (n=35) with pancreatic carcinoma admitted to the Division of Gastroenterology, Rostock during the years 1996 to 1998 who were not treated by tumor resection. Of these patients, 1 had UICC stage I, 2 stage III, and 33 stage IV pancreatic carcinoma, respectively. Seven underwent palliative surgery, 10 received palliative chemotherapy, 24 needed biliary drainage (ERCP or PTCD), and 19 received best supportive care (in addition to biliary drainage or surgery). One stage IV patient was excluded since no date of death was available for this patient. Though the selection criteria for treated patients could not be applied completely to the historic control group, the historic controls and the treated patients were comparable in clinical diagnoses and initial symptoms of the disease (jaundice, abdominal pain were most frequent) and also with respect to median age (63 years in both cohorts) and gender (male patients: 74.3% in historic controls and 64.3% in treated patients).

D. Production of clinical grade CapCell®

The cytochrome P450 2B1 (CYP2B1) expression construct (Löhr et al, 1998), as well as the good laboratory practice (GLP) production and characterization of the CYP2B1 expressing 293 cell clone (22P1G) (Gunzburg et al, 1999) have been described previously. Cells were amplified under good manufacturing practice (GMP) conditions (Q-One, Glasgow, Scotland, UK) and encapsulated in polymers of cellulose sulphate using an apparatus from Inotech (Dottikon, Switzerland) (Dautzenberg et al, 1999). The encapsulated cells (CapCell®) were washed twice with plain RPMI cell culture medium (Gibco/BRL) and stored at 4°C. Cell viability was determined using the Life&Dead viability kit (MobiTec, Braunschweig, Germany). Necessary quality control tests required for release included sterility and a demonstration that the CapCell® were both mycoplasma and endotoxin free. The mechanical stability of the capsules was determined and the potency of the encapsulated cells was determined in a cell toxicity bioassay (Löhr et al, 2002).

E. Angiography

Visualization of the vasculature leading to the pancreatic tumor was performed by angiography in a standard manner with

the transfemoral approach (Seldinger technique). Digital subtraction angiography of the celiac trunk, superior mesenteric artery, splenic artery, common hepatic artery and, if necessary for identification of tumor leading vessels, of the gastroduodenal artery, was performed with a 4 French introducer system (Terumo), 4 French visceral catheters with a inner diameter of 0,038" (Cordis) and a monomer nonionic contrast medium (Imeron 300, BYK, Gulden). The most appropriate tumor access was determined by relating tumor localization in CAT scans to the vessel anatomy. Supraselective catheterization of an artery leading into the tumor was performed with a coaxial 2.3 French microcatheter system (Cordis) (Kröger et al, 1999; Löhr et al, 2003). The optimal approach to the tumor vasculature was gained through the inferior pancreatoduodenal artery, the dorsal pancreatic artery and/or the superior pancreatic head branches of the gastroduodenal artery. After documentation of the correct microcatheter placement in a non-occluding position, 300 CapCells were instilled slowly one by one with the blood flow in 13 patients. An additional patient received 250 capsules due to limited space in the tumor artery. The patency of the cannulated vessel was controlled periodically by fluoroscopy, followed by a control angiography of the target vessel region. The catheter and introducer systems were then removed, the puncture site compressed for 15 minutes, and a compression tape put in place for 6 hours. Diagnostic angiography visualising the peritumoral vessels was repeated in the same manner during the final visit (week 20).

F. Quality of Life

A quality of life core questionnaire for cancer patients, QLQ-C30, has been validated in several languages (Aaronson et al, 1993; Fayers et al, 1999; Hjermstad et al, 1998; Klee et al, 1997; Sprangers et al, 1993), but the module for pancreatic carcinoma is still under development with respect to reliability, sensibility against changes, and multicultural validation (Fayers et al, 1999). Therefore, in this study an unauthorised version of the core questionnaire and a German quality of life scale for pancreas patients was used which had been published (Bloechle et al, 1995). The quality of life-data were documented independently from the safety and efficacy data by filling-out an independent questionnaire by the patient. Thus, the assessment of the quality of life data did not interfere with the routine documentation of the adverse events that were reported by the patient. The quality of life core questionnaire was analyzed in analogy to the prescriptions of the EORTC (Fayers et al, 1999). Quality of Life data were available from the baseline evaluation for all 14 patients and for analysis of change from 8 patients. The analysis was strictly performed according to the EORTC recommendations (Fayers et al, 1999).

III. Results

Each patient enrolled in the trial received 300 cellulose sulfate capsules (CapCell®) except patient 12 who received 250 CapCell® by angiographic placement (day 0) into a suitable artery feeding a primary, inoperable tumor (stage III-IV). Each capsule had an average diameter of 0.8 mm and contained around 10^4 cells (Löhr et al, 1999). An appropriate artery leading into the tumor could be supraselectively cannulated (**Figure 1**) in 14 of the 17 patients entering the study (**Table 1**). Two patients developed severe infections before the start of the trial and had to be treated by other means, whilst angiography was not successful in one patient.

Table 1: Patients entering the CT-PCA-1 study

Sex	Age	TNM	Stage	1st Symptom	Survival wks	Metastases	Tumor
m	48	T4N1Mx	IV	abd. Pain	102	n	SD
m	76	T4N1Mx	IV	abd. Pain	39	n	PR
m	67	T4NxMx	IV	Jaundice	64	n	MR
m	57	T3NxM1	IV	Diarrhea	29	y	SD
m	74	T3N1M1	IV	abd. Pain	67	y	MR
m	65	T4N1M1	IV	abd. Pain	20	y	SD
f	61	T4N1M0	IV	abd. Pain	65	n	SD
m	65	T4N1M1	IV	incidental1	28	y	PR
f	58	T4N1M0	IV	abd. Pain	-	-	
m	64	T3NxM1	IV	abd. Pain	-	-	
m	53	T3NxMx	IV	Jaundice	44	n	SD
f	57	T3N0M0	III	Jaundice	33	n	SD
f	61	T4N1M0	IV	abd. Pain	112	n	SD
f	68	T4N1M1	IV	abd. Pain	6	y	SD
f	70	T3N0M0	III	abd. Pain	35	y	SD
f	60	T4NxM0	IV	abd. Pain	-	n	
m	52	T4N1Mx	IV	abd. Pain	41	n	SD

¹detected on ultrasound, y = yes, n = no

SD=stable disease, PR=partial response (more than 50% tumor regression), MR=minor response (between 25 and 50% tumor regression). abd.Pain = abdominal pain

Immediately after instillation of the CapCell®, a transient spasm could be observed (**Figure 1D**) but this did not significantly impair blood flow. At the trial endpoint, 20 weeks after CapCell® instillation, angiographic visualisation of the targeted vessels was performed. No or only minor alterations to the tumor vessels, such as reduction of diameter or increased compression as compared to day 0, were observed (data not shown).

Subsequent to CapCell® instillation, each patient received low dose ($1\text{g}/\text{m}^2$ body surface) ifosfamide (Holoxan®) on days 2-4 and 23-25, respectively (Löhr et al, 1999). Although 11 serious adverse events (SAEs) were recorded in 7 patients during the study period, none of these were treatment related (i.e. due to CapCell® instillation or ifosfamide treatment) (Löhr et al, 2001) and were attributed to the underlying disease and/or the effects thereof (**Table 2**). Administration of CapCell® did not result in any obvious allergic or inflammatory response and none of the patients developed pancreatitis at any time during the course of the study. Although elevated amylase levels were detected in some patients, presumably as a result of the tumor infiltration of the pancreas and limited obstructive (chronic) pancreatitis (van Gulik et al, 1997), no further increase was measured after angiography and CapCell® placement (**Figure 2**). Only one AE (increased lipase activity observed on day 15 after instillation) may have been possibly related to CapCell® administration. The concentration of ifosfamide in the patients blood plasma were monitored 30 to 60 minutes after administration and revealed levels of $100\text{--}200\text{ }\mu\text{mol}/\text{L}$ (**Figure 3**).

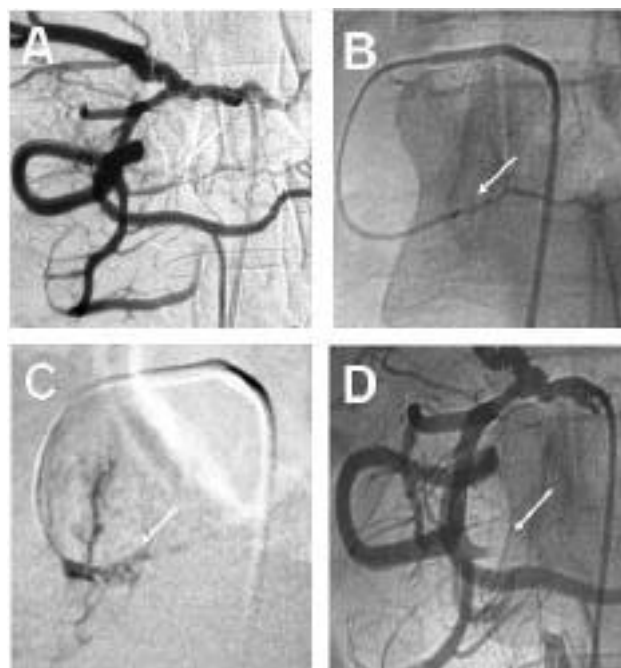


Figure 1. Angiographic placement of microcapsules in patient #2 with pancreatic carcinoma. (A) Digital subtraction angiography of celiac and mesenteric axis (Acunas and Rozanes 1999). (B) Supraselective cannulation of the A. transversalis (indicated by arrow) with the coaxial 2.3 French microcatheter. (C) Injection of the microcapsules. Arrow points to the area of contrast medium exclusion resulting from the capsules. (D) Celiac axis angiography directly after the capsule instillation indicating the spasm in the vessel filled with the capsules (arrow).

Table 2**Percentage of patients experiencing adverse events**

Adverse events	Grade 1*	Grade 2*	Grade 3*	Total
Pain	2 %	12 %	2 %	16 %
Malignant pancreas neoplasm	9 %	5 %	0 %	14 %
Aggravated condition	2 %	7 %	4 %	13 %
Decreased weight	7 %	0 %	0 %	7 %
Cholestatic hepatitis	0 %	2 %	4 %	6 %
Diarrhea	5 %	0 %	0 %	5 %
Vomiting	0 %	5 %	0 %	5 %
Nausea	0 %	4 %	0 %	4 %
Anemia	0 %	4 %	0 %	4 %
Anorexia	0 %	2 %	0 %	2 %
Ascites	2 %	0 %	0 %	2 %
Constipation	2 %	0 %	0 %	2 %
Pulmonary embolism	0 %	0 %	2 %	2 %
Enzyme abnormality	2 %	0 %	0 %	2 %
Gastrointestinal hemorrhage	0 %	0 %	2 %	2 %
Other hemorrhage	0 %	0 %	2 %	2 %
Hypertension	2 %	0 %	0 %	2 %
Subileus	0 %	2 %	0 %	2 %
Fungal infection	0 %	2 %	0 %	2 %
Intestinal obstruction	0 %	0 %	2 %	2 %
Jaundice	0 %	2 %	0 %	2 %
Kidney neoplasm	0 %	2 %	0 %	2 %
Nervousness	2 %	0 %	0 %	2 %
Pleural effusion	0 %	2 %	0 %	2 %
Sepsis	0 %	0 %	2 %	2 %
Vertigo	2 %	0 %	0 %	2 %

* NCI – common toxicity criteria

The chemotherapy regimen was well tolerated with no toxicity beyond grade II being detected in any of the 14 patients treated in this trial (not shown). The data thus strongly suggest that there is no obvious specific treatment-related risk.

In addition to safety and tolerability, the efficacy of the treatment was examined. The size of the primary tumor was measured prior to starting the treatment and at weeks 10 and 20 post treatment (**Figure 4**). The tumor did not grow any further during this observation period in any of the treated patients. In two of the 14 patients, a partial

response (PR), characterised by a more than 50% reduction in tumor volume, was recorded; the remaining 12 patients showed a stable disease (SD) with tumor sizes in the range of 50-125% of initial size (Löhr et al, 2001). Of these 12 patients, 2 demonstrated a minor response (MR), i.e. tumor reduction by 25 to 50%.

Kaplan-Meier analysis of the survival of the patients enrolled in the trial showed that the median survival time from the time of diagnosis is 39 weeks (**Figure 5**). In contrast, data from a historic control group of patients of similar age as well as disease symptoms and stage from the same medical center showed a median survival of 20 weeks (**Figure 5**).

A second survival parameter, the percentage of patients that survived for one year or more was also monitored in long term follow-up of the patients beyond the period defined for the clinical trial. The one year survival for the treatment group was 36 % (i.e. 5 of the 14 patients treated), compared to 11 % (4 out of 35 patients) for the historic control group (Löhr et al, 2001).

Within the 20 week study period, three patients died from disease progression (on days 9, 85 and 132). The patient who died on day 9, from a recurrent pulmonary embolism, underwent a postmortem examination. Gross pathology (**Figure 6A**) revealed tumor necrosis.

This was confirmed by histological examination demonstrating the well differentiated adenocarcinoma and extensive necrotic tissue (**Figure 6B**). The capsules could not be localized in serial sections of the pancreas, spleen and liver. Thus it is not strictly possible to rule out that the effects observed were not due to the placement of the capsules and the local chemotherapeutic conversion of ifosfamide. However, sampling difficulties associated with finding tiny, almost transparent, capsules in such large organs have also experienced in pig preclinical studies (Lohr et al, 2003). In the period beyond the 20 weeks defined as the study period, a further 10 patients died. It should be noted that all three patients who died during the trial as well as three of the patients who died after the 20 week observation already had distant (liver) metastasis (**Table 1**) before beginning the trial.

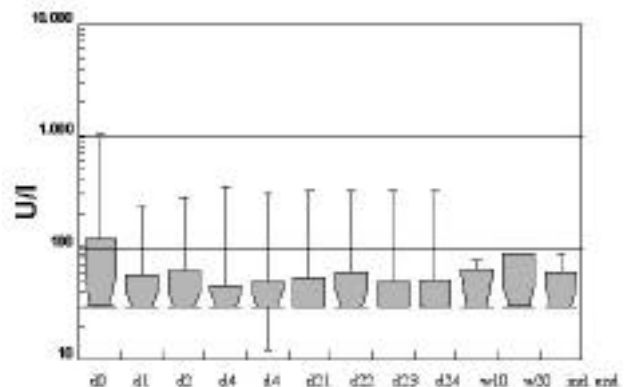


Figure 2 Serum amylase values of the patient cohort. The course of amylase levels in 14 patients before (d0) and after (day 1 to day 24; d1-d24; week 10, 20: w10, w20) instillation of CapCells. The individual endpoints after completion of study are indicated as ind. end. The normal range for amylase is between 20 and 120 U/L.

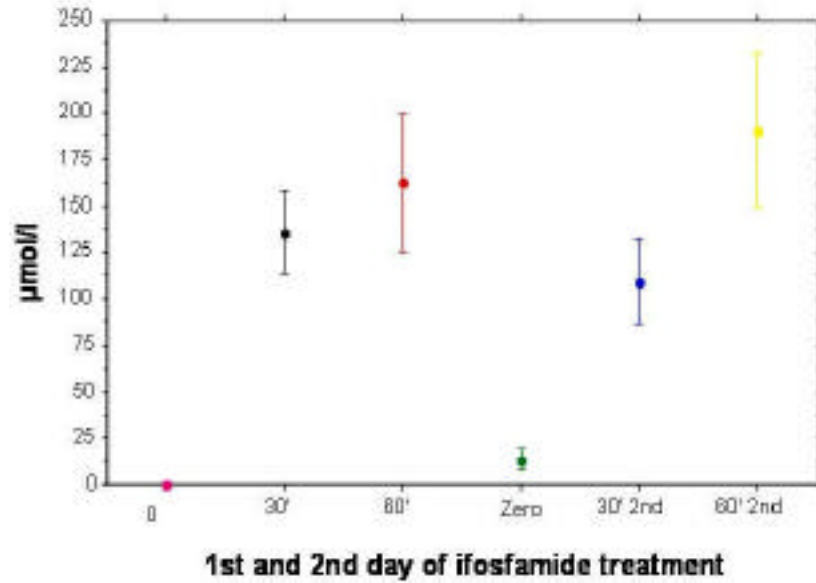


Figure 3. Ifosfamide plasma levels in pancreatic carcinoma patients carrying microencapsulated CYP2B1 producing cells in a tumor vessel after 1 g/m² body surface given IV over 1 hour.



Figure 4. CAT scan of pancreatic tumor (A) before (0), (B) 10 weeks and (C) 20 weeks after instillation of CYP2B1-expressing, microencapsulated cells into the pancreas and low-dose ifosfamide treatment. The area of the tumor is outlined.

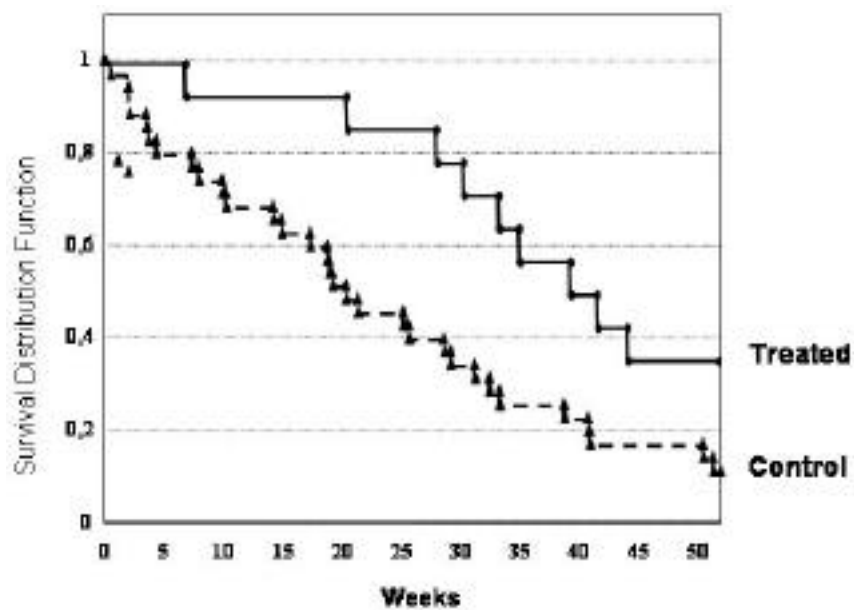


Figure 5. Kaplan-Meier analysis of patients treated with microencapsulated, CYP2B1-expressing cells and low-dose ifosfamide (●; n = 14) vs. a historical control group (▲; n = 33).

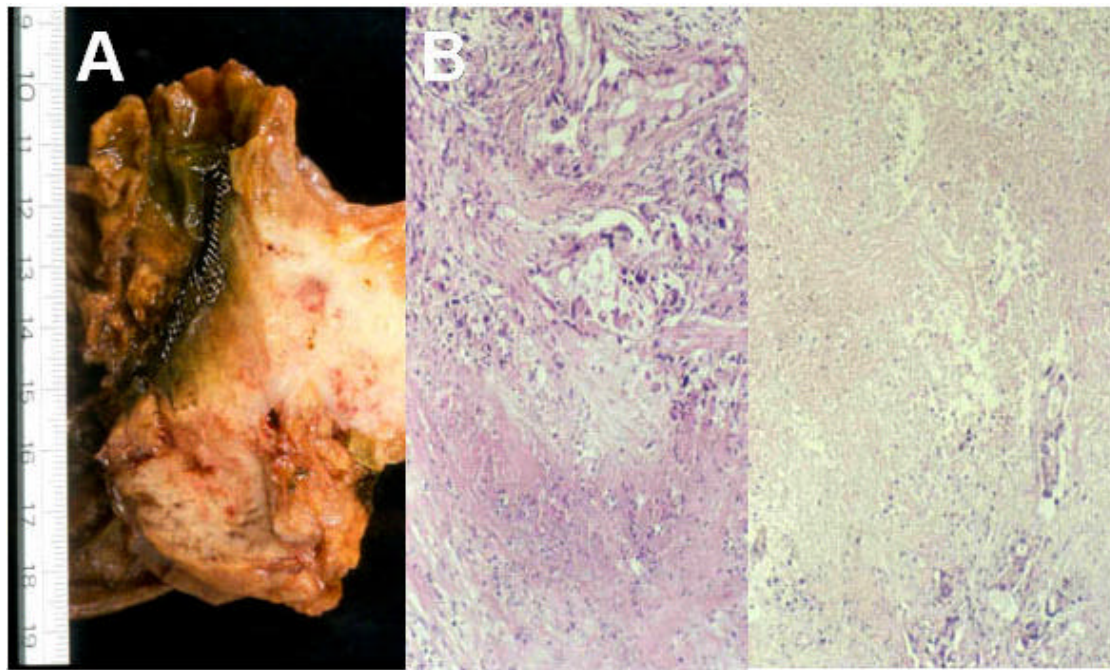


Figure 6. Gross pathology (A) and histology (B) of pancreatic tumor from patient who died on day 9 of treatment from a pulmonary embolism. Gross tumor necrosis (A) was confirmed by histological examination (B) demonstrating the well differentiated adenocarcinoma and extensive necrotic tissue.

A postmortem examination of the patient who died at week 53 (i.e. outside the study period) confirmed a widespread pancreatic tumor.

The information gathered from the patients with respect to clinical benefit is shown in **Table 3**. If a clinical benefit is considered to be either no increase or a decrease in pain intensity then 10 of the 14 patients show a benefit (**Table 3**, Pain Intensity, bold). This could be confirmed for 7 of the patients by analgesic consumption (**Table 3**, Analgesic Consumption, bold).

It should be noted that none of the benefiting patients registered an increase in pain medication both in terms of dosage or WHO level. While none of the patients showed an increased Karnofsky score after treatment, 7 of the 14 patients remained stable at the week 10 assessment and 4 of those were stable even at week 20 (**Table 3**, Karnofsky Score, bold). One patient (patient 1) showed an increase in body weight at week 10 and at week 20 and patient 12 had a weight increase at week 10 (this patient dropped out and so no week 20 weight value could be obtained).

A further two patients (7 and 11) showed stable body weight at week 10 but patient 7 dropped out and patient 11 showed weight loss at week 20 (**Table 3**, Body Weight, bold). Taken together, two of the patients (5 and 11) had stable measurements for all four criteria (italics), with only marginal (4kg) weight loss at week 20.

Two scenarios were made to establish the overall (i.e. integrative) clinical benefit response, where each patient was given a +2 for an improved value, +1 for a stable value and -1 for a worsened value for each of the 4 criteria (pain, analgesic consumption, Karnofsky index and body weight) compared to the relevant week 0 values. The

"worst case scenario" required a pain relief of 20 points or more to be judged as an improvement and a decrease in the Karnofsky index of 10 points or more taken to indicate worsening. In this scenario, 7 patients (50%) experienced a clinical benefit, 3 were neutral (21.4%, benefits were offset by impairments) and 4 patients (including those dying before the average survival time) had no clinical benefit (28.6%). The second, "best case scenario" assumes a pain relief of 10 points or more as an improvement and a decrease in the Karnofsky index of 20 points or more is taken to indicate worsening. Using these criteria, 10 patients (71.4%) had clinical benefit, 2 patients showed no benefit but no deterioration either (14.3%), and 2 patients had definitely no benefit.

IV. Discussion

Chemotherapy has previously only been marginally effective for the treatment of pancreatic carcinoma despite the introduction of new cytotoxic agents such as gemcitabine (Carmichael et al, 1996; Storniolo et al, 1999). Gemcitabine acts by multiple mechanisms, including inhibition of ribonucleoside diphosphate reductase, dCMP deaminase and dCTP incorporation into DNA and RNA thereby disrupting DNA synthesis leading to apoptosis (Rieger et al, 1999). Clinical responses are achieved in 5.4-11% of pancreatic cancer patients, with a median survival time of between 5.6 and 6.3 months (Burris et al, 1997; Carmichael et al, 1996; Casper et al, 1994). However, in the face of a general median survival of patients with pancreatic carcinoma of around 4 months (Carmichael 1997), new treatment modalities for single or combinatorial therapy approaches are desperately needed.

Table 3: Analysis of measures of clinical benefit

Patient		Pain Intensity(0-100)			Analgesics Consumption(0-100)			Karnofsky Score(0-100)			Body weight (Week 0 and Variation (in kg)		
		week 0	week 10	week 20	week 0	week 10	week 20	week 0	week 10	week 20	week 0	week 10	week 20
1	C	40	0	0	100	100	100	100	80	80	63	3	2
2	C	20	40	30	0	100	100	100	100	90	67	-1	-9
3	C	0	0	0	0	0	0	80	80	80	76	-9	-11
4	nC	0	0	nd	0	0	nd	100	90	nd	55	-5	nd
<i>5</i>	<i>C</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>100</i>	<i>100</i>	<i>100</i>	<i>78</i>	<i>-6</i>	<i>-4</i>
6	D	30	30	-	75	75	-	100	70	-	73	-1	nd
7	nC	50	40	40	50	75	100	100	100	90	63	0	0
8	D	0	0	-	0	0	-	100	90	-	73	-1	nd
<i>11</i>	<i>C</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>100</i>	<i>100</i>	<i>100</i>	<i>79</i>	<i>0</i>	<i>-4</i>
12	nC	20	20	nd	50	100	nd	100	90	nd	61	1	nd
13	C	30	20	20	50	75	75	100	100	100	60	-2	-4
14	D	50	-	-	100	-	-	70	-	-	55	nd	nd
15	C	40	50	50	50	100	100	80	80	70	79	-10	-14
17	nC	10	nd	nd	100	nd	nd	80	nd	nd	68	nd	nd

Abbreviations: C: completion of trial; nC: noncompletion of trial; D: discontinued/death; nd: not done

BOLD: fulfils benefit criteria; ***Italic:*** fulfils benefit criteria in all sections with just marginal weight loss (4kg) at week 20

Oxazaphosphorines such as ifosfamide are naturally activated in the liver and also have been used for the chemotherapy of pancreatic cancer (Cerny et al, 1991a). However, the high systemic concentrations required for effective chemotherapy are associated with significant side effects especially in elderly patients (Loehrer et al, 1985). The response rates reported range between 0 and 30% (Cerny et al, 1991a; Loehrer et al, 1985). Nevertheless, preclinical studies on other tumor types have shown the utility of the local expression of cytochrome enzymes such as the isoform 2B1, in combination with oxazaphosphorines, as reviewed by Chen and Waxman (Chen and Waxman 2002). However, in many of the previous studies, the gene encoding cytochrome P450 was introduced directly into the tumor cells prior to establishing tumors in nude mice and subsequent oxazaphosphorine treatment. Thus, although these studies elegantly demonstrate proof of principle, they represent a situation that is clinically not applicable, given that gene transfer efficacies directly to tumors in vivo are relatively poor, regardless of the vector system used.

We have previously demonstrated the efficacy of the intratumoral injection of encapsulated cells expressing cytochrome P450 2B1 into pre-formed pancreatic tumors

in nude mice (Löhr et al, 1998) as well as into mammary tumors in immunocompetent mice (Kammertöns et al, 2000). In these experiments, the activated metabolites that diffused from the encapsulated cells to the surrounding tumor cells were sufficient to result in a clear anti-tumor effect in both instances. Therefore, we reasoned that a similar approach might prove feasible in patients. Local intratumoral activation holds the promise of good efficacy coupled with low systemic side effects due to reduced concentrations of the chemotherapeutic agent. Direct injection of the capsules, however, brings with it the risk of bleeding as well as the danger of metastatic cells seeding along the needle track. The pancreas is located deep in the retroperitoneum, also making it difficult for repeated transcuteaneous injections. The possibility for future repeated intra-arterial instillations of CapCell® was supported by the finding(s) that (i) only 2 patients showed occluded vessels and (ii) only one other patient displayed evidence that the tumor had affected the blood vessels that lead to it. Another potential route of application of CapCell® would be by endoscopic delivery. However, the pancreatic duct is occluded in the majority of tumors, limiting the use of this route of delivery (Schmid et al, 1994).

The relatively non-invasive interventional angiography approach (Dondelinger 1999) was used to deliver 300 CapCell®. Comparable delivery of almost identical size solid particles did not result in substantial occlusion of the blood vessels leading to primary and metastatic hepatic masses (Talamonti et al, 1998; Trinchet 1995). The delivery of CapCell® via the angiographic route was shown to be both feasible and safe. No treatment related Serious Adverse Events (SAEs) were recorded during the study period. The ifosfamide dose was well tolerated, as was the capsule instillation, which could be performed under local anesthesia in 15 minutes on average. Thus the primary objectives of the study, the evaluation of the safety and tolerability of the treatment were met.

The efficacy of the treatment was also examined in this trial. In contrast to the usual progression of the disease, in which the tumor mass continues to grow, all the treated patients showed a stabilization of tumor size and 4/14 (28%) showed a reduction in tumor size of more than 25%, suggesting a clear tumor killing effect. Our cell culture studies suggested that the toxic metabolites of ifosfamide act by inducing cell necrosis, rather than apoptosis (Karle et al, 2001). In this light it is of interest that in our preclinical animal studies extensive necrosis was observed after tumor treatment (Löhr et al, 1998) as well as in the one patient treated in this trial whose tumor could be examined retrospectively at postmortem, although it should also be noted that these tumors have a tendency to be necrotic. Especially for pancreatic cancer patients, a decrease in the primary tumor size is of clinical benefit in terms of increased survival time and decreased pain. This was confirmed in our study in which a comparably large increased median survival time after diagnosis of more than 44 weeks and no impaired or even an increased quality of life including no requirement for increased pain medication was noted. Although this is a phase I/II study with a relatively small patient collective, these results compare very favourably with those obtained with gemcitabine, in which a median survival of around 28 weeks was reported (Burris et al, 1997; Carmichael et al, 1996; Casper et al, 1994). The one-year survival rate was also higher in the treated group (36%) as compared to the historic control group (11%). Although the two cohorts of historic controls and treated patients might differ in potential risk factors for survival (Löhr et al, 1999), the difference in survival rate of 25% cannot be explained only by selection bias, and may indicate a possible superior efficacy of the CapCell® treatment. In comparison, the one year survival rate for gemcitabine in a large compassionate use setting in which a comparable 80% of patients were stage IV (86% of patients in our trial were stage IV) was 15% (Storniolo et al, 1999). A phase III trial of gemcitabine yielded a 1 year survival of 18% (Burris et al, 1997). It is possible that with optimisation and/or additional rounds of treatment, higher survival rates may be obtainable after CapCell® therapy, for example in a phase II or III trial.

In order to uphold the principle laid down in the declaration of Helsinki stating that the interests of the subject must prevail over the interests of science and

society the effects of clinical trials on quality of life should be determined (Hope-Stone et al, 1997). This is particularly applicable to a devastating disease like pancreatic cancer and, in this light, gemcitabine received approval because of its ability to improve the quality of life of patients (Carmichael 1997). Thus clinical benefit responses were also examined in this phase I/II clinical trial. A best case and a worst case scenario were examined. Even in a worst case scenario, some clinical benefit was experienced by 50% of patients and this was extended to 71% in the best case scenario.

It is clear that in its present form, this kind of treatment is directed towards the treatment of the primary tumor rather than metastases and that even if the primary tumor could be effectively treated in those patients without obvious metastases, occult micrometastases may later become a problem. One hypothetical outcome of the treatment that has yet to be analysed is that tumor cell death may lead to better tumor antigen presentation and the induction of anti-tumor and metastases responses. There are a number of ways in which this issue may be dealt with including the use of combination chemotherapies, for example capsules with low dose ifosfamide to deal with nonresectable tumors, followed by gemcitabine to treat potential metastases. Other potential strategies include the use of viral vectors that are ideally targeted to deliver the cytochrome P450 gene to metastatic cells (Chen and Waxman, 2002; Kan et al, 2002) or the implantation of encapsulated retroviral vector producing cells (Saller et al, 2002).

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Walter H. Günzburg

