Review

Use of cell therapy as a means of targeting chemotherapy to inoperable pancreatic cancer[*]

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Although approved for the treatment of pancreatic cancer, the chemotherapeutic agent ifosfamide is not an effective therapy for this type of tumour. Ifosfamide must be activated by cytochrome P450 (P450) enzymes in the liver, initially to a short lived intermediate and then to toxic metabolites that are subsequently distributed by the circulatory system. Particularly for pancreatic cancer, this liver-mediated conversion results in relatively high systemic toxicities and poor therapeutic concentrations at the liver-distant site of the tumour. Activation of ifosfamide at the site of the tumour may allow lower doses to be used, while increasing the therapeutic index due to the resultant active concentrations generated locally. A cell-based therapy has been conceived where encapsulated, 293-derived cells genetically modified to overexpress a cytochrome P450 enzyme, are implanted near solid tumours. The cells are encapsulated in polymers of cellulose sulphate in order to provide a means of immunoprotection *in vivo* as well as to physically constrain them to the vicinity of the tumour. A major advantage of this strategy is that it allows one standard cell line to be applied to all patients and this approach can be extended to the treatment of other tumour types. After proof of principle studies in animal models, a phase I/II clinical trial was initiated in patients with stage III/IV nonresectable pancreatic cancer. Encapsulated cells were angiographically placed into the tumour vasculature of 14 patients and followed by systemic low dose ifosfamide treatment. Angiographic delivery of encapsulated cells proved feasible in all but one patient, and was well tolerated with no capsule or ifosfamide treatment-related adverse events. Four of the treated patients showed tumour regressions after capsule delivery and ifosfamide treatment in computer-tomography scans. The other 10 patients showed no further tumour growth (i.e. stable disease) during 20 weeks observation period. The median life expectancy of the patient collective was extended two fold as compared to age and status matched historical controls, with a 3-fold improvement in one year survival being attained. Evidence for a clinical benefit of the treatment was also obtained on the basis of standard parameters for quality of life. This approach has been evaluated by the European Medicines Evaluation Agency (EMEA) and orphan drug status has been granted. A pivotal clinical trial is now being planned with the help of the EMEA. Taken together, the data from this clinical trial suggest that encapsulated cytochrome P450-expressing cells combined with chemotherapy may be useful for the local treatment of a number of solid tumours and support the performance of further clinical studies of this new treatment.

Keywords: cell therapy, encapsulated cells, pancreatic cancer, cytochrome P450, genetically modified cells, chemotherapy

Since its market debut at the end of the 1990's, gemcitabine has rapidly become the treatment of choice for inoperable pancreatic carcinoma (Günzburg *et al.*, 2002; Diaz-Rubio, 2004). Gemcitabine was approved primarily because patients showed an improved quality of life, rather than because of classical parameters such as reduced tumour burden and prolonged survival time. Indeed, recent studies have shown that gemcitabine has only extended the lifespan of patients by a few weeks (Burris *et al.*, 1997). Thus, there is still a need for more efficacious treatments for this devastating

form of cancer. This is reflected in the increasing number of novel approaches over the last few years for pancreatic carcinoma both in the preclinical area as well as in clinical trials (Günzburg *et al.*, 2002; Diaz-Rubio, 2004). Strategies have included, but are not limited to, novel chemotherapeutic agents, either alone or in combination chemotherapies (often with gemcitabine), topoisomerase inhibitors that interfere with DNA synthesis, immune response stimulators such as virulizin, granulocyte-macrophage colony-stimulating factor, monoclonal antibodies as well as antisense, gene and cell therapy approaches (Günzburg *et al.*, 2002; Diaz-Rubio, 2004).

A common gene therapy approach for the treatment of cancer involves the use of genes encoding enzymes that activate nontoxic drugs, or prodrugs to toxic anti-tumour forms (Table 1). These genes have been named suicide genes, and the combination of gene and prodrug is often referred to as GDEPT (gene-directed enzyme prodrug therapy) (for a review see McKeown *et al.*, 2004). The first and most popular system has been the thymidine kinase gene from herpes simplex virus (Block *et al.*, 1997; Carrio *et al.*, 1999; Howard *et al.*, 2000; Makinen *et al.*, 2000) and, more recently, cytosine deaminase (Shiau *et al.*, 1998; Haack *et al.*, 2000; Kanyama *et al.*, 2001). The gene encoding deoxyribonucleoside kinase has also been shown to function as a suicide gene for pancreatic tumour therapy, at least in cell culture (Zheng *et al.*, 2000). Although impressive, the results with these suicide gene/prodrug combinations were obtained *in vitro* or in immunocompromised mouse models, whereas in immunocompetent animals cells expressing these nonmammalian enzymes will eventually be eliminated by the host immune system because they are not of mammalian origin.

RESULTS

We have been developing a cell-based therapy system that utilises a mammalian cytochrome P450 enzyme as a suicide gene in combination with a prodrug that is a commonly used chemotherapeutic agent. The prodrug that we have chosen is ifosfamide, a drug that has proved valuable in the clinic for the treatment of a number of different types of tumours and that initially showed some promise for the treatment of pancreatic carcinoma. In standard
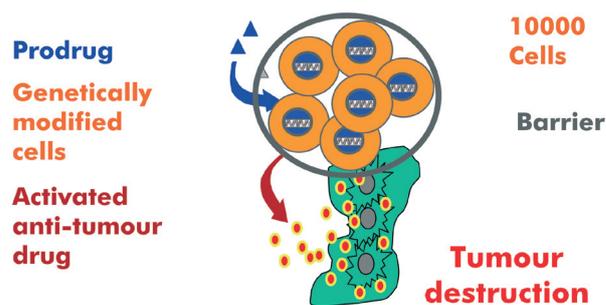

Figure 1. Principle of tumour proximal activation of ifosfamide.

chemotherapy, ifosfamide is first activated in the liver by various cytochrome P450 enzymes, after which the activated transport form, 4-hydroxy-ifosfamide phosphoramide mustard, is distributed by the circulatory system. This short-lived intermediate then decays to the active anti-tumour compounds, phosphoramide mustard and acrolein. Because the half-life of the transport form in plasma is of the order of minutes (Kurowski & Wagner, 1993), effective therapeutic concentrations cannot be reached without life-threatening side effects (Cerny *et al.*, 1991; Keizer *et al.*, 1995). Thus, despite a documented anti-tumour effect (Table 2), ifosfamide has not been evaluated further.

One way to circumvent this problem would be to locally activate ifosfamide at the site of the tumour, allowing lower doses of ifosfamide to be applied, thereby resulting in good local cytotoxic activity and minimal side effects. Local activation could be achieved by placing encapsulated human 293 cells stably transfected with a cytochrome P-450 isoform 2B1 (CYP2B1) expression construct at the site of the tumour (Fig. 1). Encapsulation in cellulose sulphate allows allogeneic cells to survive *in vivo*, because they are physically confined and protected from the host immune system (Löhr *et al.*, 2002; Hauser *et al.*, 2004). Previous experiments had revealed that injection of such encapsulated CYP2B1-expressing cells into pre-established human pancreatic tumours in a nude mouse model resulted in complete tumour regression in about 20% of mice and a significant anti-tumour effect in the remaining mice (Löhr *et al.*, 1998). These encouraging results suggested that a similar strategy may have a therapeutic effect in patients. A phase I/II clinical trial was designed involv-

Table 1. Suicide gene/prodrug combinations already in use in clinical trials for cancer gene therapy

Gene	Source	Prodrug
Thymidine kinase	Herpes simplex virus	ganciclovir
Cytosine deaminase	*E. coli*	5-fluorocytosine
Nitroreductase	*E. coli*	5-(aziridin-1-yl)-2,4-dinitrobenzamide
Cytochrome P450 2B1/3A4	Human, rodent	ifosfamide, cyclophosphamide

For further details see http://www.wiley.co.uk/genmed/clinical/

Table 2. Clinical experience with ifosfamide in pancreatic cancer

	Low dose[1]	Medium dose[2]	Usual dose[3]
	Dose (g/m^2)		
Daily	1.6	1.8–2.0	2.0–3.0
Cycle	< 16 (10 d)	9–10 (5 d)	nt
Total	< 32	< 90	nt
Response			
Stable disease	0	20%	nt
Partial remission	0–33%	56%	nt
Complete	0	4%	nt
Toxicities			
Alopecia	50%	100%	unacceptable
Haematotoxicity	50%	100%	unacceptable
Haemorrhagic cystitis	0%	4%	> 4%
Fatigue	50%	not known	> 50%
Nausea/vomiting	75%	100%	unacceptable

[1](Wils *et al.*, 1993; Keizer *et al.*, 1995); [2](Gad-el-Mawla & Ziegler, 1981; Gad-el-Mawla, 1986); [3]Concentration used for other tumour types. This concentration has not been tested for pancreatic cancer; nt, indicates not tested

ing patients with inoperable pancreatic carcinoma to assess the feasibility, safety, and tolerability of this new treatment modality (Löhr *et al.*, 1999). During the planning of the clinical trial it was decided not to directly inject capsules into the tumour to avoid the possibility of generating needle tract metastases. Another possible route of delivery of the capsules would be *via* the blood supply. Intra-arterial angiographic placement of microencapsulated cells into pig pancreas was shown to be feasible and well tolerated (Kröger *et al.*, 1999; Löhr *et al.*, 2003) and thus was chosen as the route of delivery. Each patient enrolled in the trial received 300 cellulose sulphate capsules with an average diameter of 0.8 mm, each containing 10^4 cells, by angiographic placement (day 0) into a suitable artery feeding a primary, inoperable tumour (stage III/IV). Subsequently, each patient received low-dose (1 g/m^2 body surface) ifosfamide (Holoxan) on days 2–4 and 23–25 after capsule implantation (Löhr *et al.*, 1999).

A total of 17 patients were enrolled in the study and 14 of the 17 patients received encapsulated cells delivered by a supraselective catheter that was inserted into an appropriate artery leading into the tumour. Two of the 3 patients who were not treated had to be excluded from the study because they developed severe infections before the trial was initiated. The third patient did not receive encapsulated cells because an unusual blood vessel architecture did not allow successful angiographic placement. Typically, a transient spasm could be observed immediately after instillation of the encapsulated cells but the vessel quickly returned to normal and patency was observed. Moreover, follow-up angiography of the targeted vessels 20 weeks after capsule instillation revealed no or only minor changes to the vessels, suggesting the possibility of administration of a further dose of encapsulated cells.

Although 11 serious adverse events (SAEs) were recorded in 7 of the patients, none of these SAEs were due to treatment with encapsulated cells or ifosfamide and were clearly attributable to progression of the underlying disease. Moreover, there was no evidence of pancreatitis or allergic responses at any time during the study period. Documented adverse events (AEs) were most often due to pain, underlying disease, or an aggravated health condition, with the possible exception of increased lipase activity which was observed in 1 patient on day 15 after instillation.

The chemotherapy regimen was also well tolerated, with litle toxicity beyond grade II being detected. Plasma levels of ifosfamide were measured and 30–60 min after administration shown to be in the range of 100–200 µmol/l (Lohr *et al.*, 2003). Thus, in summary, the phase I related goals of the study were achieved in that no specific treatment-related risk could be demonstrated in the 14 patients treated.

As well as safety and tolerability, the efficacy of the treatment was also monitored. The size of the primary tumour was measured before starting the treatment and set at 100%. The tumour size was then measured by computer tomography (CT) scans at weeks 10 and 20 post-treatment and the size related to the pre-treatment value. Interestingly, none of the tumours showed any significant growth during the whole observation period in any of the treated patients.

Indeed, in 2 of the 14 patients a reduction of 50% or more (i.e. a partial response) was documented (Table 2). A further 2 patients showed a minor response, i.e. a tumour reduction of 25–50%, with the other 8 patients showing stable disease. Two of the patients were not available for the CT analysis at week 10.

Table 3. Tumour response of patients (n = 14) receiving encapsulated cells and low dose ifosfamide

	n	Percentage
Partial response (PR)	2	14.3%
Minimal response (MR)	2	14.3%
Total responders (PR + MR)	4	28.6%
Stable disease	8	71.4%
Progressive disease	0	0%
No follow-up	2	14.3%

The median survival time of the patients treated in this study was 40 weeks from the time of diagnosis. This compares favourably both with respect to the 20 weeks survival achieved by an age- and status-matched control group consisting of 35 stage III–IV patients from the same study centre as well as with published data on patient survival after gemcitabine treatment (Burris *et al.*, 1997). The average survival time of the treatment group was more than 45 weeks compared to 30 weeks for the control group. The percentage of patients surviving for 1 year or more was 35.7%, compared to 11.1% for the control group. One of the patients from the treated group survived almost two years. Within the 20 week study period, 3 patients died of disease progression (on days 9, 85, and 132). The patient who died on day 9, as a result of recurrent pulmonary embolism, underwent post-mortem examination. This patient had a history of pulmonary embolism, even before commencing the trial and no capsules could be found in the lesions. Macroscopic examination of the tumour in this patient revealed remarkable tumour necrosis and this was confirmed by histological examination. Necrosis associated with the encapsulated cell/ifosfamide therapy has also been observed in the mouse model system (Löhr *et al.*, 1998) and, at least for some cell types in cell culture, seems to be the mechanism of cell killing (Karle *et al.*, 2001). Beyond the 20-week study period, a further 10 patients died. The 3 patients who died during the trial and 3 of the patients who died after the 20-week observation had metastases in the liver before commencement of the trial, which is know to be associated with a poorer prognosis. One patient who died at week 53 (i.e., outside the study period) was examined by post-mortem, and a wide-spread pancreatic tumour was confirmed.

An integrative score of clinical benefit was calculated in which a stable condition was deemed a clinical benefit. In a worst case scenario (any worsening means no benefit), seven patients (50%) experienced a clinical benefit, three were neutral (21.4%; benefits were offset by impairments), and four patients (including those dying before the average survival time) had no clinical benefit (28.6%). In a best case scenario (clinically relevant worsening required), ten patients (71.4%) had clinical benefit, two patients showed no benefit but no deterioration (14.3%), and two patients had definitely no benefit. None of the patients registered an increase in pain medication in terms of either analgesic dosage or WHO scale.

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 and causing apoptosis (Rieger *et al.*, 1999). Clinical responses after treatment with gemcitabine are achieved in 5.4–11% of pancreatic cancer patients, with a median survival time of between 5.6 and 6.3 months (Casper *et al.*, 1994; Carmichael *et al.*, 1996; Burris *et al.*, 1997). The general median survival of patients with pancreatic carcinoma is only around 4 months so the improvement afforded by gemcitabine treatment is at best modest. New treatment modalities for pancreatic cancer are thus desperately needed. The chemotherapeutic agent ifosfamide requires activation in the liver. In the late 1970's and early 1980's, ifosfamide was used for the chemotherapy of pancreatic cancer and gave response rates of up to 30% (Loehrer *et al.*, 1985; Ajani *et al.*, 1988; Cerny *et al.*, 1991; Keizer *et al.*, 1995). However, relatively high systemic concentrations were required and this was associated with significant side effects, particularly in elderly patients (Loehrer *et al.*, 1985).

A number of preclinical studies, using tumour cells of nonpancreatic and pancreatic cancer origin, have shown the utility of the local expression of cytochrome P450 enzymes, in combination with oxazaphosphorines like ifosfamide or cyclophosphamide (Wei *et al.*, 1994; Chen & Waxman, 1995).

Table 4. Survival data

	n	Mean survival	Median survival	1 year survival (%)
Control	36	> 30 weeks	20 weeks	11%
Encapsulated cells + ifosfamide	14	> 45.4 weeks	40 weeks	36%
Gemzar[1]	63		28 weeks	18%

[1]Data from pivotal phase III trial (Burris *et al.*, 1997)

Most previous studies, however, report that the cytochrome P450 gene was introduced directly into the tumour cells before the establishment of tumours in nude or SCID mice, followed by oxazaphosphorine treatment. Thus, although these studies convincingly demonstrate proof of principle, they represent an artificial system that is not clinically applicable.

Encapsulation of genetically modified cells is a powerful technology that allows cells to be safely implanted. Encapsulation of cells in biocompatible polymers protects them from the host immune system and localises them to the site where their action is required (Hauser *et al.*, 2004) (Fig. 1). We have previously reported that the encapsulation of cells genetically modified to overexpress cytochrome P450 and the intratumoral injection of these encapsulated into preformed human pancreatic tumours in nude mice, followed by ifosfamide treatment, results in tumour reductions (Löhr *et al.*, 1998). Similar results were obtained when these encapsulated cells were injected into mammary tumours in two immuno-competent strains of mice (Kammertöns *et al.*, 2000; Uckert *et al.*, 2003). Based upon all of this data, we reasoned that a similar approach might prove feasible in humans. Local intra-tumoral activation holds the promise of good efficacy coupled with low systemic side effects due to reduced concentration of the chemotherapeutic agent. In contrast to mouse model studies, it was planned to deliver the encapsulated cells *via* blood vessels leading to the tumour since direct intra-tumoral injection may cause the formation of needle track metastases as well as causing unnecessary bleeding, both of which can potentially seed metastatic cells. The pancreas is located deep in the retroperitoneum, making repeated transcutaneous injections difficult. Delivery *via* the pancreatic duct is also limited in the majority of tumours due to occlusion. In contrast, interventional angiography is a relatively noninvasive approach that is becoming popular for the treatment of non-resectable tumours (Dondelinger, 1999). Thousands of smaller solid microspheres delivered by angiography have been used to deliberately occlude blood vessels leading to the primary and metastatic hepatic masses in previous studies (Trinchet, 1995; Talamonti *et al.*, 1998). This contrasts with our study in which only 300 cell-filled capsules were delivered, without causing blood vessel occlusion (although a momentary spasm lasting a few minutes was observed) (Löhr *et al.,* 2001; 2003). This was confirmed in experiments where blood flow was measured in isolated perfused porcine pancreas that had received encapsulated cells (Löhr *et al.*, 2003). That the mode of action is not simply blood vessel occlusion is also supported by the finding that a therapeutic effect was obtained after direct injection of encapsulated cells into tumours followed by ifosfamide administration in two different tumour model systems (Karle *et al.*, 1998; Löhr *et al.*,

1998; Kammertöns *et al.*, 2000). It seems likely that arterial blood flow is an important requirement in our study both to ensure adequate survival of the encapsulated CYP2B1 cells as well as to allow good intra-tumoral diffusion of the active metabolites.

The encapsulation material consists of inert cellulose polymers (Dautzenberg *et al.*, 1999; Löhr *et al.*, 2002), and there was no evidence for an allergic response or inflammatory reaction either to the material or to the genetically modified cells. Treatment-related serious adverse events were not observed during the study period. The ifosfamide dose was well tolerated, as was the capsule instillation, which could be performed under local anaesthesia on average over a 15-min period. Thus the primary objectives of the study, namely the evaluation of the safety and tolerability of the treatment, have been met.

In addition, all the treated patients showed a stabilization of tumour size and 4/14 (28%) showed a reduction in tumour size of more than 25%, in contrast to the normal progression of the disease in which the tumour mass continues to grow. The ability to effect a reduction in tumour size may allow originally non-resectable tumours to become eligible for surgical resection. Even if subsequent surgery is still not feasible, a decrease in the primary tumour size is of clinical benefit in terms of increased survival time and clinical response data such as decreased pain. This benefit was confirmed in our study in which an increased median survival time after diagnosis of more than 40 weeks and 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 favourably with those obtained with gemcitabine, in which a median survival of about 25 weeks was reported (Burris *et al.*, 1997; Storniolo *et al.*, 1999). Even more impressive was the increase in the 1-year survival rate from 11.1% in the control group to 35.7% in the treated group. The 1-year survival rate for gemcitabine in a pivotal phase III trial was 18% (Burris *et al.*, 1997).

New treatment strategies for pancreatic cancer may qualify for orphan drug status since the incidence is relatively low. The use of encapsulated cells overexpressing cytochrome P450 has been designated an orphan medicinal product by the European Medicines Evaluation Agency and a pivotal phase III clinical trial is now planned for 2006 with a view to market approval, should the data warrant it.

Even if encapsulated cells plus ifosfamide is efficacious enough in a phase III trial to be approved as a marketable product for primary pancreatic cancer, it seems unlikely that the treatment described here will affect metastases. The use of encapsulated cells producing retroviral vectors that can seek out and deliver cytochrome P450 to metastatic cells may

represent a viable strategy for this purpose. One prerequisite for this approach has been met in that encapsulated retroviral vector producing cells have been reported to be able to infect surrounding cells after transplantation (Saller *et al.*, 2002). The use of infection and/or expression targeted retroviral vectors (Salmons & Gunzburg, 1993; Hlavaty *et al.*, 2004a; 2004b) may contribute further to the success of such an approach.

In summary, we report the feasibility, tolerability, and safety of intra-tumour placement of CYP2B1-expressing encapsulated cells combined with low doses of systemic ifosfamide as a means to treat local, solid tumours such as pancreatic cancer without major side effects. More importantly, considering the circumstances of a phase I/II study, a substantial anti-tumour effect on the primary pancreatic tumour was observed. Clearly, however, there is room for optimization of the treatment, for example, by multiple administrations of ifosfamide and, later, possibly capsules.

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