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***In Vitro* and *In Vivo* Comparison of Gemcitabine and the Gemcitabine Analog 1-(2'-deoxy-2'-fluoroarabinofuranosyl) Cytosine (FAC) in Human Orthotopic and Genetically Modified Mouse Pancreatic Cancer Models**

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Abstract

Purpose—Although gemcitabine is a mainstay of pancreatic cancer therapy, it is only moderately effective, and it would be desirable to measure drug uptake in patients. 1-(2'-deoxy-2'-fluoroarabinofuranosyl) cytosine (FAC), is an analog of gemcitabine, and when labeled with F-18, it may be a potential surrogate PET tracer for the drug.

Procedures—[¹⁸F]FAC was synthesized to a radiochemical purity of >96 %. The human tumor lines AsPC1, BxPC3, Capan-1, Panc1, and MiaPaca2 were grown orthotopically in nude mice. KPC mice that conditionally express oncogenic K-ras and p53 mutations in pancreatic tissue were also used. The intra-tumoral distributions of [¹⁴C]gemcitabine and [¹⁸F]FAC were mapped with autoradiography. The inter-tumor correlation between [¹⁴C]gemcitabine and [¹⁸F]FAC was established in the orthotopic tumors. Expression of the equilibrative and concentrative nucleoside transporters (ENT, CNT) *in vitro* was detected by western blotting. Drug uptake was characterized *in vitro* using [³H]gemcitabine and the effect of transporter inhibition on gemcitabine and FAC uptake was investigated. The relative affinity of cells for gemcitabine and FAC was tested in competition assays.

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The cell lines differed in sensitivity to transport inhibitors and in competition studies. There was a good *in vivo* correlation between the total uptake of [^{18}F]FAC and [^{14}C]gemcitabine, measured across all orthotopic tumors. Using the KPC and BxPC3 models, we found that [^{14}C]gemcitabine and [^{18}F]FAC were largely co-localized.

Conclusions—In the lines examined here, [^{18}F]FAC uptake correlates well with gemcitabine *in vivo*, supporting the notion that [^{18}F]FAC can serve as a PET radiotracer surrogate to determine the uptake and distribution of gemcitabine within pancreatic tumors.

Keywords

FAC; Gemcitabine; Pancreatic cancer

Introduction

Gemcitabine (2'-deoxy-2',2'-difluorocytidine) is a fluorinated cytosine analog prodrug used in the treatment of various malignancies, particularly pancreatic disease. It is transported into cells by equilibrative and concentrative nucleoside transporters, (ENT, CNT) [1] where it is either phosphorylated by deoxycytidine kinase (dCK) [2] leading to activation or deaminated by cytidine deaminase (CDA) leading to elimination. The deaminated form is less or non-toxic [3], and is exported from the cell by members of the ABC transporter family [4]. The monophosphate of gemcitabine undergoes further phosphorylations creating di- and tri-phosphate derivatives that are trapped in the cell. The diphosphate inhibits dCTP synthesis and the tri-phosphate is incorporated into DNA. Gemcitabine toxicity is thus dependent on drug delivery to the tumor site, activation by dCK to gemcitabine-monophosphate and evading deactivation by CDA.

In pancreatic cancer, drug access to tumor is a major issue, and clinical trials have been held with anti-stromal agents to enhance tumor drug uptake (reviewed in [5]). The ability to image gemcitabine could improve clinical practice, but the radiochemistry of substituting F-18 onto gemcitabine has proven to be too difficult. Fluorothymidine (FLT) has been proposed as a surrogate for gemcitabine, as they share a common uptake mechanism, and a recent study found a good correlation between the *in vitro* uptake of [^3H]FLT at tracer level with gemcitabine response in a panel of six pancreatic tumor lines [6]. Like gemcitabine, FLT is a substrate for ENT1, and in meta-analysis, ENT1 levels are generally found to be the major predictor of gemcitabine resistance in pancreatic cancer [7].

However, a potentially valuable imaging agent may be 1-(2'-deoxy-2'-fluoroarabinofuranosyl) cytosine (FAC) [8–11]. FAC is the mono-fluorinated analog of gemcitabine (difluorodeoxycytidine). Like gemcitabine, it is a substrate for CDA and dCK, and therefore is able to qualitatively identify gemcitabine-sensitive tumors (CDA negative, dCK positive) in mice. A related probe, (1-(2'-deoxy-2'-fluoroarabinofuranosyl) 5-methyl cytosine, FMAC), is phosphorylated by dCK but not deaminated by CDA and therefore can act as a surrogate for purine nucleoside drugs such as clofarabine that are not substrates for CDA [9].

In this paper, we sought to address a different question: whether [^{18}F]FAC could function as a tracer to predict tumor gemcitabine levels. A first requirement for this is that the relationship between [^{18}F] FAC and gemcitabine should be consistent across different tumor models. To test for this, we used five different human tumor lines grown orthotopically in the mouse pancreas, to establish if there was a correlation between tracer and drug across distinct genetic backgrounds. We also explored the spatial correlation between gemcitabine and [^{18}F]FAC, and how the distribution of drug and tracer were affected by the presence of tumor stroma.

Materials and Methods

Radiochemistry

The synthesis of [^{18}F]FAC was performed manually using the method developed by Radu et al. and Wu et al. [12, 13]. Briefly, F-18 in the form of [^{18}F]HF, trapped on an anion exchange resin was eluted with a 90 % solution of acetonitrile in water containing 1 mg of K_2CO_3 and 10 mg of kryptofix into a 10 ml reactivial. The water acetonitrile azeotrope was dried by heating the react to 110 °C under reduced pressure, while maintaining a slow stream of argon gas. One milliliter of anhydrous acetonitrile was added and dried at 110 °C under argon stream. The process was repeated twice again. The activity was dissolved in 0.5 ml of anhydrous acetonitrile and added to a completely sealed vial containing 15 mg of 2-O-(trifluoromethylsulfonyl)-1,3,5-tri-O-benzoyl- α -D-arabinofuranose and the reaction mixture was heated at 160 °C for 30 min. The reaction mixture was cooled and passed through silica Sep-Pak® plus column (pre-conditioned with 5 ml of hexane) under argon atmosphere and eluted with ethyl acetate (2 $\times$ 1.5 ml). The ethyl acetate solution was dried at 90° C under a slow stream of argon. 1,2-dichloroethane (0.4 ml) and HBr (30 % in acetic acid, 0.1 ml) were added to the residue and heated for 10 min at 80 °C. Excess reagents and solvent were removed by azeotropic distillation with 1 ml toluene, after which 20 mg of silyl ether in 0.7 ml of 1,2-dichloroethane was added under argon. The reaction mixture was heated in a microwave at 160 °C for 30 min.

The reaction mixture was cooled and passed through a silica Sep-Pak® plus column (pre-conditioned with 5 ml of hexane) under argon and eluted with 10 % methanol in CH_2Cl_2 (2 $\times$ 1.5 ml). The solvent was removed as described earlier, 2 ml of 0.5 M sodium methoxide in methanol was added and heated at 110 °C for 10 min. The reaction mixture was neutralized with 60 μl of glacial acetic acid and the solvent was removed under reduced pressure. The product was purified using reverse phase HPLC using a C-18 column (Waters Atlantic C-18, 5 μ , 100 A, 250 $\times$ 10 mm) with 3 % acetonitrile in water containing 0.1 % TFA. Under the conditions above the required β -anomeric product elutes as a broad peak with retention time of about 14 min. The radiochemical purity of the product was >96 % with decay corrected yields averaging 5.2 % and ranging from 1.3 to 9.6 %, over eight runs. The specific activity was about 35 GBq/ μmol .

Cell Culture

Five human pancreatic cell lines were used in this study, cultured as follows: AsPC1, RPMI +10 % fetal bovine serum (FBS) +1 mM sodium pyruvate; BxPC3, RPMI +10 % FBS;

Capan-1, Iscove's medium +20 % FBS; MiaPaca2 and Panc1, DMEM +10 % FBS. Cells were maintained in a humidified incubator at 37 °C, 5 % CO₂, and split according to ATCC recommendations. All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Media and sodium pyruvate were obtained from Cellgro (Manassas, VA, USA). Fetal bovine serum (FBS) was purchased from Gemini Bioproducts (West Sacramento, CA, USA).

Gemcitabine uptake studies were conducted in 24-well plates with cells grown to confluence. Cells were exposed to tracer in serum-free medium for 1 h, at activity concentrations of 7.4 kBq/ml ([³H]gemcitabine) equivalent to a concentration of 167 pmol/ml. Wells were rinsed with phosphate-buffered saline (PBS) and lysed with 1 % sodium dodecyl sulfate, 0.3 % sodium hydroxide, and counted on a Tricarb 2910 TR liquid scintillation counter (Perkin Elmer, Waltham, MA, USA) using Scintisafe cocktail (Fisher Scientific, Fair Lawn, NJ, USA). The transport inhibitors 6-[(4-nitrobenzyl) thio]-9-β-D-ribofuranosylpurine, (NBMPR) and phloridzin (Sigma-Aldrich, St Louis, MO, USA) were used at 100 nM and 100 μM, respectively.

Animal Models

Female athymic mice (6–8 weeks) were purchased from Harlan Laboratories (Indianapolis, IN, USA). Animal husbandry conformed to the Guide for the Care and Use of Laboratory Animals, in an AAALAC accredited institute. All the work described here was approved by the institutional IACUC. Mice were acclimated for a minimum of 3 days prior to surgery. Anesthesia was with isoflurane. Carprofen (5 mg/kg, s.c.) was given pre-emptively for analgesia. An incision was made in the left flank, allowing the spleen to be retracted, revealing the pancreas. Tumor cells were injected (10⁶ in 30 μl, 50 % Matrigel, BD Biosciences, Franklin Lakes, NJ, USA). The wound was closed with absorbable suture in the muscle. Wound clips were applied for 7 days to allow skin healing.

The *Pdx1-Cre* [14], *LSL-Kras^{G12D}* [15], and *LSL-p53^{R172H}* [16] mouse strains have been previously described: Male *LSL-Kras^{G12D/+}* mice were bred with female *Pdx1-Cre^{+/+}*, *LSL-p53^{R172H/R172H}* mice to generate KPC mice. Strains were maintained on mixed background. Mice were genotyped by polymerase chain reaction analysis as described previously [17]. Animals were kept in conventional animal facilities and monitored daily. All animal experiments were performed in accordance with protocols approved by the Memorial Sloan Kettering Institutional Animal Care and Use Committee.

Western Blotting

Total protein was extracted from cells using RIPA buffer (Boston-Bio-Product, Ashland, MA, USA) in the presence of protease inhibitor cocktail (Roche, Nutley, NJ, USA), and protein concentration measured (BCA, Thermo Scientific, Waltham, MA, USA). Sample was prepared in Laemmli Sample Buffer (Bio-Rad, Hercules, CA, USA) and 2-mercaptoethanol (Sigma), resolved by electrophoresis in a Mini-Protein TGX 4–15 % gels (Bio-Rad) and transferred onto nitro-cellulose transfer membrane (Bio-Rad). The membranes were blocked with 5 % non-fat milk in Trisbuffered saline and then incubated with primary antibodies. Antibodies against ENT1, CNT1, and CNT3 were purchased from

Novus Biologicals (NBP1-50508, Littleton, CO, USA) and Santa Cruz (sc48460 and sc135429, Santa Cruz, CA, USA), respectively. Anti-actin was obtained from Sigma (A5316). After three washes with Trisbuffered saline-Tween followed by appropriate horseradish peroxidase-conjugated secondary antibodies (1/1000, Santa Cruz Biotechnology), signal was detected using ECL-Chemiluminescence (Thermo Scientific).

Ex Vivo Determination of Tumor Uptake

When orthotopic tumors had reached approximately 8–10 mm in diameter, (determined by ultrasound imaging using the VeVo 2100 (VisualSonics, Toronto, ON, Canada)), they were co-injected with 74 kBq [^{14}C]gemcitabine, specific activity 2.2 GBq/mmol, (Moravek Biochemicals), and [^{18}F]FAC. While FAC activities were variable depending on the synthesis reaction, the median injected activity was 850 kBq, equivalent to 6 ng of tracer. The mass amount of [^{14}C]gemcitabine injected was approximately 9 μg , or approximately 0.45 mg/kg, assuming a 20 g mouse. This is substantially less than an experimental therapy dose. Injected activity was quantified by counting F-18 in the filled syringe before and after injection; the fraction of F-18 injected was also taken as the fraction of C-14 injected. Injections were administered intraperitoneally. One hour later, animals were sacrificed and the tumors removed. Three samples were cut from each tumor, weighed and F-18 activity was counted; subsequently, they were digested in Scintigest (Fisher Scientific, 500 μl ; tissue samples were approximately 50 mg) for 2 h at 55 $^{\circ}\text{C}$, followed by addition of 200 μl H_2O_2 and a further incubation for 2 h. With vortexing, the tissue was completely solubilized. Further H_2O_2 was added to remove color, the sample was allowed to cool, and counted by liquid scintillation counting. F-18 and C-14 counts were converted to activity by established calibration factors; F-18 activity was corrected for decay, based on its half-life of 110 min. Activity in the tumor samples was related to the injected activity, and expressed as percent injected dose/gram (%ID/g).

Immunohistochemistry and Autoradiography

The [^{18}F]FAC and [^{14}C]gemcitabine distribution were compared using digital autoradiography in two orthotopic BxPC3 tumors and tumors arising in two KPC mice. Animals received pimonidazole (Hypoxypore, Burlington, MA, USA, 100 mg/kg i.p.) 20 h prior to radiochemical injection. Animals were co-injected with 185 kBq [^{14}C]gemcitabine and 5 MBq [^{18}F]FAC. One hour later, animals received Hoechst 33342 (Sigma-Aldrich, St Louis MO, USA, 50 mg/kg, i.v.), and 1 min later were euthanized by CO_2 inhalation. Tumors were excised, frozen, embedded in OCT, and immediately sectioned. Ten-micrometer sections were exposed on phosphor screens for 16 h to collect F-18 images; repeat exposures made 24 h later (after complete decay of F-18) confirmed that the contribution of C-14 to the images was negligible. A third set of exposures was performed of much longer duration (120 h) to collect sufficient signal from the [^{14}C]gemcitabine decays. Prior to the first exposure, markers for spatial alignment were placed on each slide. For these, 1 μCi of C-14 was mixed in 1 ml of nail varnish, along with crystal violet. Three spots of this mixture were placed on each slide, so that the autoradiograph and microscope images could be overlaid. Autoradiographic images were collected on the Typhoon FLA 7000 laser scanner (GE Healthcare Life Sciences, Pittsburgh, PA, USA). Microscopic images were obtained using an Olympus BX60 microscope and Microsuite Biological Suite imaging

software (Olympus America, Center Valley PA, USA). A brightfield microscope image of the slide was collected at low power, showing the relation of the tissue to the marker spots. Pimonidazole, Hoechst, and hematoxylin and eosin (H&E) images were registered to the brightfield image using the tumor outline as a reference. Pimonidazole was imaged by fixing in cold methanol for 30 min. Blocking was for 1 h in 10 % bovine serum albumin, followed by 1 h exposure to FITC conjugated anti-pimonidazole (Hypoxyprobe) diluted 1:20 in blocking solution.

Statistics

Two-tailed *t* tests and correlation coefficients were calculated in Excel. A web calculator from GraphPad [[http:// www.graphpad.com/quickcalcs/](http://www.graphpad.com/quickcalcs/)] (La Jolla CA, USA) was used to determine the 95 % confidence interval on a ratio, (Fieller's theorem) and to calculate *p* from *r*. For competitor binding studies, dose-response curves were generated in Sigmaplot (Systat Software, San Jose CA, USA) along with the concentration required for 50 % inhibition (IC₅₀).

Results

In Vitro Uptake of Gemcitabine

A main objective of this study was to determine the correlation between gemcitabine and [¹⁸F]FAC in a panel of human pancreatic tumors. By employing several cell lines, we hoped to capture some of the inter-tumor variability that is representative of patient tumor diversity. We therefore sought to establish *in vitro* how much the lines differed in terms of mechanisms of gemcitabine uptake. Gemcitabine uptake in pancreatic cancer is primarily through the equilibrative nucleoside transporter 1 (ENT1), though it is also a substrate for concentrative nucleoside transporters 1 and 3 [18]. Western blotting (Fig. 1a), revealed that all lines expressed the ENT1 and CNT3 transporters. CNT 1 could only be detected in Panc1 and AsPC1. When we measured gemcitabine uptake in the presence of NBMPR and phloridzin, inhibitors of ENT1 and concentrative nucleoside transporters respectively [19, 20] we discovered differences between the lines, though not well correlated with the western data. Capan-1 cells were inhibited by phloridzin (Fig. 1b). Panc1 cells were almost completely inhibited by NBMPR, while AsPC1 and BxPC3 cells were partially inhibited by NBMPR (Fig. 1c). This suggests that gemcitabine uptake in Panc1 cells is driven largely by ENT1, and in Capan-1 by one of the concentrative nucleoside receptors. The incomplete inhibition of uptake in AsPC1 and BxPC3 is surprising. There may be a contribution from ENT2, since it is known that ENT2 (which is insensitive to NBMPR inhibition) also transports gemcitabine, though with much lower affinity than ENT1 [21]. However, we were unable to detect ENT2 with western blotting. ENT1 is known to have multiple phosphorylation sites, suggesting the possibility for post-translational modification [22, 23], and it may be that this accounts for the discrepancy between the inhibitor studies and protein expression. RNA interference could very usefully discriminate between receptors.

Differences in transporter activity were also apparent in competition studies (Table 1, Supplementary Fig. 1). Uptake of [³H]gemcitabine was measured in the presence of excess cold gemcitabine or FAC. For Capan-1 cells gemcitabine and FAC were equally effective as

competitors, whereas the other lines all preferred gemcitabine. Relative to the other three lines, uptake in AsPC1 cells was inhibited at relatively high concentrations of gemcitabine. Thus, when judged in terms of sensitivity to NBMPR/phloridzin and competitive inhibition, none of the cell lines were identical in drug uptake mechanism.

In Vivo Uptake of FAC and Gemcitabine

We then explored whether FAC uptake would correlate with gemcitabine on an inter-tumor basis. Figure 2a shows a plot of the relative uptake (%ID/g) of [^{14}C]gemcitabine *versus* [^{18}F]FAC in five human orthotopic pancreatic tumor models, co-injected with each tracer. If both radiotracers were taken up equally by the tumor models, then all of the data points would fall upon the line of identity illustrated by the dashed line. However, the [^{18}F]FAC uptake is found to be lower than [^{14}C]gemcitabine by a factor of approximately 4. Normal tissue (liver and muscle) also exhibited a 4 to 5 enhanced uptake of [^{14}C]gemcitabine relative to [^{18}F]FAC (Fig. 2b). This suggests that FAC is cleared more quickly from the mouse. Importantly however, across all the tumor samples there was a high correlation (solid line) between [^{14}C]gemcitabine and [^{18}F]FAC, $R^2 = 0.718$, $p < 0.0001$.

Results were obtained 1 h after tracer injection, but no special value attaches to the 1 h time point. The object of the experiment was to determine whether all tumor models behaved similarly in terms of the ratio of tracer to drug, as the degree of inter-tumor consistency at one time point would surely be repeated at all other points.

Tumor Distribution of FAC and Gemcitabine

Our next experiment was to determine whether FAC and gemcitabine would co-localize in pancreatic tumors at the histologic level. We examined mice bearing BxPC3 tumors and spontaneous tumors arising in KPC mice. Clinically, pancreatic tumors are typically infiltrated with very high levels of desmoplastic stroma, which interferes with drug delivery. KPC tumors model this histology much more successfully than most orthotopic human xenografts [17]. However, BxPC3 tumors also display significant levels of stroma, and perfused vessels are confined to the stromal regions, which surround islands of tumor cells (Supplementary Fig. 2). Animals were co-injected with [^{18}F]FAC and [^{14}C]gemcitabine. In order to identify hypoxic and perfused regions in the tumor, animals had received pimonidazole 20 h previously, and were given Hoechst 1 min before sacrifice. Animals were sacrificed 1 h after FAC/gemcitabine administration. Figure 3 shows co-registered images from a BxPC3 orthotopic tumor as follows: the autoradiographs of (a) [^{14}C]gemcitabine and (b) [^{18}F]FAC, (c) a combined Hoechst-33342/pimonidazole image (d) H&E staining. There is good spatial agreement across the entire tumor tissue section between the two radioactive markers providing further support for the equivalence of [^{18}F]FAC to [^{14}C]gemcitabine. The C-14 image exhibits a higher resolution compared to the F-18, an expected consequence of the shorter path length of C-14 beta particles. The distribution of gemcitabine and its surrogate marker are heavily localized in the well-perfused areas on the normal pancreas and tumor margins (arrow X) with little penetration into the dense tumor areas (arrow Y) and hypoxic zones.

Images from the KPC tumors are shown in Fig. 4. Neither of the KPC tumors showed pimonidazole uptake suggesting no or minimal hypoxia. The KPC tumors were considerably smaller than the BxPC3s, and were surrounded by normal tissue (outlined in yellow). H&E staining revealed concentrations of tumor cells (outlined in blue) as well as regions of stroma that were minimally cellular. The location of the [^{14}C]gemcitabine and [^{18}F]FAC in the small emerging tumor (Fig.4), shows an almost uniform radiotracer uptake, but with a more selective focal uptake within the proliferating tumor cells.

Discussion

The object of this investigation was to determine whether [^{18}F]FAC has the potential to be an imaging agent capable of predicting gemcitabine uptake in pancreatic tumors. We sought to investigate the inter-tumor correlation between the drug and the tracer, an important factor if [^{18}F]FAC is to serve as an accurate surrogate of gemcitabine drug uptake in pancreatic tumor cells. Our data revealed that pancreatic tumors preferred gemcitabine over FAC. However, FAC and gemcitabine uptake was correlated across five different pancreatic tumor models (Fig. 2). This correlation was established despite 4 out of the 5 cell lines having different gemcitabine uptake mechanisms, as judged by inhibitor studies (Fig. 1 and Table 1).

As shown in Fig. 2, the amount of FAC taken up was low relative to gemcitabine: when the injected radiotracer amounts were normalized to the injected dose, [^{14}C]gemcitabine exceeded [^{18}F]FAC by a factor of four. This ratio was found both in tumor and in normal tissue. Low levels of [^{18}F]FAC tissue incorporation may be due to the very rapid deamination of [^{18}F]FAC by cytidine deaminase [11]. We attempted PET imaging of the tumors, but the results were unsatisfactory, since high uptake in the intestines, as reported by Radu et al. [12] made it difficult to identify the tumor signal. Even with Capan-1 tumors, which had the highest FAC uptake, the tumors could not be distinguished from the surrounding intestines. Whereas this result demonstrates that [^{18}F]FAC is unsuitable as a diagnostic PET agent to identify disease, this is not a requirement of [^{18}F]FAC PET serving its purpose as a gemcitabine surrogate. With the emergence of PET/MR scanners, the MRI scan can be used to outline a volume of interest (VOI) of the tumor and the corresponding PET VOI used to make a quantitative assessment of the gemcitabine tumor uptake.

Regarding the spatial distribution [^{14}C]gemcitabine showed poor penetration into BxPC3 xenografts (Fig. 3), with much of the activity being associated with the stroma rich periphery of the tumor. There was little activity found in the bulk tumor regions or hypoxic zones. This is in contrast to previous results with AsPC1 tumors [24] where we found gemcitabine to be more evenly distributed throughout the tumor with significant accumulation in hypoxic tissue. However, AsPC1 tumors grow with very little stroma and this may lead to increased drug accessibility. The limited penetration of [^{14}C]gemcitabine into the BxPC3 tumors was mirrored by [^{18}F]FAC, with the distribution of the two radiolabels being similar in both the human xenografts and the murine KPC model (Fig. 4).

Conclusions

[¹⁸F]FAC correlates well with gemcitabine uptake *in vivo*, at both the macroscopic (whole specimen) and microscopic (autoradiographic) levels and therefore may serve as a quantitative surrogate of gemcitabine uptake and distribution in pancreatic tumors. However, neither drug nor surrogate tracer may reflect tumor cell uptake alone but consist of a complex mixed signal comprising of tumor and stromal tissue entrapment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Nucleoside transporter expression and inhibition. **a** expression of transporter protein levels in the same lines. **b** the effects of 100 μ M phloridzin, a CNT inhibitor on [^{3} H]gemcitabine uptake in four pancreatic cell lines. Capan-1 is significantly more inhibited than the other lines (all p values <0.003, two-tailed t test). **c** effect of 100 nM NBMPR, an ENT1 inhibitor on [^{3} H]gemcitabine uptake in four pancreatic cell lines. Panc1 is significantly more inhibited than the other lines (all p values <0.02). Additionally, Capan1 is significantly less inhibited than AsPC1 ($p = 0.05$). Error bars represent 1 standard deviation.

Fig. 2.

Uptake of [^{14}C]gemcitabine and [^{18}F]FAC *in vivo*. **a** drug and tracer were co-administered to tumor bearing mice i.p. 1 h before sacrifice. Each point represents an individual tumor, sampled at three different locations. The *dashed line* has slope 1, and corresponds to the line of identity i.e., FAC = gemcitabine uptake. The *solid line* represents the regression slope. **b** tracer uptake in Panc1 tumors and normal tissues derived from the same mice, $n = 4$. Data is the ratio of uptake, calculated as percent injected dose/gram. *Error bars* represent 95 % confidence intervals.

Fig. 3. Distribution of [^{14}C]gemcitabine and [^{18}F]FAC in a BxPC3 orthotopic tumor. **a** [^{14}C]gemcitabine autoradiograph; **b** [^{18}F]FAC autoradiograph; **c**. Hoechst 33342 (*perfused vessels, blue*) and pimonidazole (*hypoxic tissue, green*), are superimposed on each other. **d**. H&E staining. The autoradiographic signal of both radiotracers corresponds more closely with the pancreatic tissue stroma and the peripheral growing tumor edge (*arrow X*). There is little radiotracer uptake in the bulk tumor (*arrow Y*), hypoxic or necrotic areas. *Scale bar*=1 mm.

Fig. 4. Distribution of $[^{14}\text{C}]$ gemcitabine and $[^{18}\text{F}]$ FAC in a small KPC tumor. **a** $[^{14}\text{C}]$ gemcitabine autoradiograph; **b** $[^{18}\text{F}]$ FAC autoradiograph; **c** Hoechst 33342 (*perfused vessels, blue*), **d** H&E. Both drug and surrogate radiotracer co-localize with tumor cell clusters outlined in orange; normal tissue is outlined in green. *Scale bar* = 1 mm.

Table 1
Competitive inhibition of gemcitabine uptake by either gemcitabine or FAC

IC ₅₀ (μM) when [³ H]GEM uptake is inhibited by excess			
	GEM (SD)	FAC (SD)	<i>p</i> (<i>t</i> test)
AsPC1	7.3 (0.7)	25.4 (4.7)	0.003
BxPC3	2.1 (0.2)	4.9 (1.0)	0.025
Capan-1	2.7 (1.9)	1.4 (0.5)	0.303
Panc1	2.3 (0.4)	3.1 (0.2)	0.035