Evaluation of the Metabolic Response to Cyclopamine Therapy in Pancreatic Cancer Xenografts Using a Clinical PET-CT System

Hany Kayed*, Patrick Meyer*, Yong He*,†, Bettina Kraenzlin‡, Christian Fink*, Norbert Gretz‡, Stefan O. Schoenberg* and Maliha Sadick*

*Institute of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany; †Department of Nuclear Medicine, Union Hospital, Tongji Medical College, Hua Zhong University of Science and Technology, Wuchang, China; ‡Medical Research Center, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

Abstract

OBJECTIVES: We analyzed the effects of anti-hedgehog signaling on the $^{18}$F-FDG uptake of pancreatic cancer xenografts (PCXs) using a clinically implemented positron emission tomography (PET)–computer tomography (CT) scanner with high-resolution reconstruction.

METHODS: PCXs from two pancreatic cancer cell lines were developed subcutaneously in nude mice and injected intraperitoneally with a low dose of cyclopamine for 1 week. $^{18}$F-FDG PET-CT was performed using a new-generation clinical PET-CT scanner with minor modifications of the scanning protocol to adapt for small-animal imaging. The data set was reconstructed and quantified using a three-dimensional workstation.

RESULTS: MiaPaCa-2 cells, which respond to cyclopamine, showed decreased $^{18}$F-FDG uptake without a change in tumor size. For hip tumors, the maximum standardized uptake value (SUVmax) was reduced by $-24.5 \pm 9.2\%$, the average SUV (SUVavg) by $-33.5 \pm 7.0\%$, and the minimum SUV (SUVmin) by $-54.4 \pm 11.5\%$ ($P < .05$). For shoulder tumors, SUVmax was reduced by $-14.7 \pm 7.5\%$, SUVavg by $-12.6 \pm 6.3\%$, and SUVmin by $-30.3 \pm 16.7\%$ ($P < .05$). Capan-1 cells, which do not respond to cyclopamine, did not show significant SUV changes.

CONCLUSIONS: The new generations of clinically implemented PET-CT scanners with high-resolution reconstruction detect a minimal response of PCX to low-dose short-term cyclopamine therapy without changes in tumor size and offer potential for preclinical translational imaging.

Translational Oncology (2012) 5, 335–343

Introduction

New-generation clinical positron emission tomography (PET)–CT scanners are being used increasingly, particularly at dedicated medical centers. A large field of view (FOV), advanced point-spread function (PSF) reconstruction algorithms, and an advanced high-definition detector technology represent hallmarks of these clinical PET-CT systems. This technology uses millions of accurately measured PSFs for iterative reconstruction of the image to produce high-definition PET images with better full width at half-maximum (FWHM), complete uniformity, high resolution, and superior contrast throughout the entire FOV [1]. In turn, less noise and fewer streak artifacts like those in filtered back-projection reconstructed images are generated [2]. Therefore, a corrected partial volume effect, increased quantification accuracy, and improved sensitivity for detecting small lesions up to 2 mm in size are landmarks of this new technology, rendering PET-CT imaging an optimal diagnostic tool in the diagnosis, therapy planning, and follow-up after treatment of different cancers [3,4].

Small animals such as tumor-bearing mice are usually imaged using a dedicated small-animal (DSA) PET scanner. Similar to the new
generations of the clinical PET-CT scanners, DSA-PET devices use the advanced reconstruction algorithms that model PSF of individual detector elements [1,5–7]. This improves spatial resolution of the small-animal scans. However, DSA-PET scanners are less available, costly, and use complicated operating protocols. These limitations were overcome in successful trials using new generations of PET-CT scanners for imaging of small animals with results comparable to those for DSA-PET scanners [8–10]. Recently, the effects of novel targeted therapies such as the tyrosine kinase inhibitors imatinib and sunitinib in gastrointestinal stromal tumor xenografts in nude mice could be evaluated using a clinical PET-CT scanner [11].

Hedgehog signaling contributes to the pathogenesis and progression of pancreatic cancer. Inhibiting the oncogenic receptor of hedgehog signaling smoothened (Smo) using hedgehog antagonists, such as the steroidal veratrum alkaloid cyclopamine, reduces pancreatic cancer cell growth and metastasis [12–15]. The suppression of hedgehog signaling pathway is associated with decreased accumulation of the hedgehog transcription factor glioma associated antigen (Gli1) in pancreatic cancer cells [12]. Additionally, hedgehog inhibition using cyclopamine prolongs survival in a genetically engineered mouse model of pancreatic cancer [15]. Therefore, clinical PET-CT imaging of pancreatic cancer xenografts (PCXs) after anti-hedgehog therapy might be valuable in simultaneously providing information about the anatomic, functional, and metabolic status of these tumors in preclinical settings. This can be confirmed by analyzing the expression of hexokinase II (HK2), which is a marker for the cellular glucose metabolism [16]. In this study, we quantitatively analyzed the known inhibitory effects of cyclopamine therapy on the 18F-FDG uptake of subcutaneous PCX using a clinically implemented, latest generation PET-CT scanner as an alternative to DSA-PET systems. This might extend the availability and reduce the costs of small-animal imaging and drug testing.

Materials and Methods

Animal Model

MiaPaCa-2 and Capan-1 pancreatic cancer cells, which express high and low Smo mRNA levels, respectively [13], were grown until 50% confluence in RPMI 1640 medium and Dulbecco’s modified Eagle’s medium, respectively (Life Technologies, Karlsruhe, Germany), and supplemented with 10% fetal calf serum (FCS; Sigma-Aldrich, St Louis, MO) and 100 U/ml penicillin/streptomycin (Life Technologies). MiaPaCa-2 and Capan-1 pancreatic cancer cells, which express high and low Smo mRNA levels, respectively [13], were grown until 50% confluence in RPMI 1640 medium and Dulbecco’s modified Eagle’s medium, respectively (Life Technologies, Karlsruhe, Germany), and supplemented with 10% fetal calf serum (FCS; Sigma-Aldrich, St Louis, MO) and 100 U/ml penicillin/streptomycin (Life Technologies). Cells were harvested at a density of 1 × 10^6 cells in 500 μl 1× phosphate-buffered saline (PBS) and then injected subcutaneously into the right shoulder and left hip regions of 8-week-old NMRI-nu (nu/nu) mice (Charles River Laboratories, Maastricht, The Netherlands). Tumor size was determined daily, starting on the fourth day after subcutaneous cell inoculation in all groups, until the tumor reached a diameter of 8 to 9 mm [12]. Cyclopamine (ENZO Life Sciences GmbH, Loerach, Germany) was complexed with 2-hydroxypropyl-β-cyclodextrin (Sigma) and PBS [17]. Then, the cyclopamine suspension or placebo (2-hydroxypropyl-β-cyclodextrin and PBS) was injected intraperitoneally for 7 days at a dose of 0.6 mg per mouse. Treatment with cyclopamine or placebo was initiated simultaneously for all mice. An experienced technician measured tumor size daily under therapy for 7 days. A final total number of 12 mice divided into four groups were used in the study. For each pancreatic cancer cell line, three mice were treated with cyclopamine and three mice were treated with placebo. All animals were maintained in a sterile environment in special cages with filter hoods in a scantainer (Scanbur, Koge, Denmark) on a daily 12-hour light/dark cycle. Cages, bedding, and water were autoclaved and the chow was sterilized by gamma radiation. All manipulations were conducted under aseptic conditions using a laminar flow hood. In accordance with guidelines for animal welfare, all experiments were approved according to federal and local laws (Baden-Wuerttemberg, Karlsruhe, Germany) and complied with Institutional Regulation License No. 35-9185.81/G-178/09.

PET-CT Imaging

At the end of the treatment period, tumor-bearing mice ranging in weight from 34.6 to 39.2 g were fasted for 6 hours before the PET-CT examination. Mice received general anesthesia using a mixture of ketamine (75–100 mg/kg) and xylazine (15–20 mg/kg) [18]. 18F-FDG [ZAG Zyklotron, Karlsruhe, Germany; 7.4 MBq (200 μCi), in 150 μl of 0.9% NaCl solution] was injected intravenously into the tail vein using a G30 needle. Immediately after injecting 18F-FDG, mice were positioned in prone position on a warm polystyrene or plastic pad to maintain their body temperature. Imaging studies were performed with a clinical PET-CT system (Biograph high-definition 40 slice mCT, Siemens Healthcare Sector, Erlangen, Germany). CT scans were acquired using a tube voltage of 80 kV, tube current of 120 mA, slice collimation of 0.6 mm, rotation time of 1 second, and kernel B31f for reconstruction. A high-resolution PET examination was performed five consecutive times using an acquisition time of 10 min/bed position to provide a dynamic curve of FDG uptake over 50 minutes (Table 1). PET images were reconstructed using an iterative mode (attenuation-weighted, three iterations and 24 subsets, matrix size of 512, zoom of 10 isotropic, and CT resolution of 0.24 mm with 2-mm uniform resolution throughout the FOV).

Data Analysis

PET and CT images were loaded to a three-dimensional workstation for data analysis. With the image analysis software, hybrid images acquired over a period of time can be accurately coregistered, compared, and quantified simultaneously on a display. The CT images were coregistered with the PET images to produce fusion images. Data were evaluated using the ellipsoid isocontour icon (volume-of-interest [VOI]), which was determined around PCX where maximum diameter is seen in the fused PET-CT images, to avoid partial volume effects (Figure 1). The uptake index was normalized to the loaded tracer activity, loading time point, and the body weight of the mouse. Tracer uptake by the tumors was quantified as standardized uptake value (SUV) using the following formula: SUV = tissue activity concentration (Bq/ml)/[injected dose (Bq)/body weight (g)]. Consequently, the 18F-FDG uptake was calculated as tracer uptake and quantified as an SUV.

Table 1. Parameters for Image Reconstruction after PET Acquisition.

<table>
<thead>
<tr>
<th>Function</th>
<th>Reconstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition time</td>
<td>10 min/bed</td>
</tr>
<tr>
<td>Output image type</td>
<td>Corrected</td>
</tr>
<tr>
<td>Iteration/subset</td>
<td>3/24</td>
</tr>
<tr>
<td>Size of image</td>
<td>200</td>
</tr>
<tr>
<td>Matrix</td>
<td>512</td>
</tr>
<tr>
<td>Zoom</td>
<td>10</td>
</tr>
<tr>
<td>Gaussian filter</td>
<td>1 mm and 3 mm</td>
</tr>
<tr>
<td>Scatter correction</td>
<td>Active</td>
</tr>
<tr>
<td>Match CT slice location</td>
<td>Active</td>
</tr>
<tr>
<td>Volume scaling</td>
<td>Active</td>
</tr>
</tbody>
</table>
Tissue Sampling and Ex Vivo $^{18}$F-FDG Uptake

Immediately after PET-CT imaging, all mice were sacrificed by cervical dislocation. Major organs such as liver and spleen as well as tumors were excised, and the $^{18}$F-FDG uptake was assessed ex vivo by using an $\alpha$-well gamma counter (PTW Curiementor, Freiburg, Germany). Then, the excised PCX were preserved in 4% paraformaldehyde for 24 hours and transferred into 70% ethanol until tissue processing and embedding in paraffin took place.

Immunohistochemistry

Paraffin-embedded tissue sections of 2- to 3-μm thickness were deparaffinized in xylene and rehydrated in progressively decreasing concentrations of ethanol. Thereafter, the slides were placed in washing buffer (10 mM Tris-HCl, 0.85% NaCl, 0.1% BSA, pH 7.4) and subjected to immunostaining. Antigen retrieval was performed by boiling tissue sections in 10 mM citrate buffer for 10 minutes in a microwave oven. Tissue sections were incubated with the following specific primary antibodies: rabbit anti-human Gli1 (Santa Cruz Biotechnology, Santa Cruz, CA) and rabbit anti-human HK2 (Cell Signaling Technology, Inc, Danvers, MA). The corresponding normal immunoglobulins (IgGs) were used as negative controls. The slides were then rinsed with washing buffer and incubated with the corresponding horseradish peroxidase (HRPO)-conjugated secondary antibodies for 1 hour at room temperature. Tissue sections were then washed in washing buffer, and 3′-3′-diaminobenzidin (DAB) chromogen/substrate mixture (Dako, Hamburg, Germany) was applied to each section. Slides were analyzed by two experienced investigators using a Leica DMRB microscope (Leica, Bensheim, Germany).

Statistical Analysis

For statistical analysis, the two-tailed nonparametric Mann-Whitney test was used to compare SUVs. Significance of results was defined as $P < .05$.

Results

In Vivo Tumorigenicity

On day 7 of treatment before the PET-CT examination, the mean diameter of the hip PCX from the control MiaPaCa-2 cells was 12.3 ± 1.2 mm and from the cyclopamine-treated cells 11.7 ± 0.9 mm. The mean diameter of the shoulder PCX from the control MiaPaCa-2 cells was 10.7 ± 0.5 mm and from the cyclopamine-treated cells 12.0 ± 1.4 mm. In contrast, the mean diameter of the hip PCX from the control Capan-1 cells was 10.6 ± 0.5 mm and from the cyclopamine-treated cells 10.3 ± 0.9 mm. The mean diameter of the shoulder PCX from the control Capan-1 cells was 9.0 ± 0.8 mm and from the cyclopamine-treated cells 8.3 ± 0.9 mm. No significant difference was
observed between the cyclopamine-treated and placebo groups, with $P > .05$ (Figure 2).

**Change in SUV of PCX In Vivo**

The main values obtained were minimum SUV ($SUV_{\text{min}}$), maximum SUV ($SUV_{\text{max}}$), and average SUV ($SUV_{\text{avg}}$) expressed as a percent of control placebos (100%). In MiaPaCa-2 cells, which express Smo receptor and respond to cyclopamine, $^{18}$F-FDG uptake was decreased. Forty-five minutes after injecting $^{18}$F-FDG, $SUV_{\text{max}}$ for the hip tumors had decreased by $-24.5 \pm 9.2\%$, $SUV_{\text{avg}}$ by $-33.5 \pm 7.0\%$, and $SUV_{\text{min}}$ by $-54.4 \pm 11.5\%$ ($P < .05$; Figure 3, A–C). A significant reduction ($P < .05$) in $SUV_{\text{max}}$ by $-14.7 \pm 7.5\%$, $SUV_{\text{avg}}$ by $-12.6 \pm 6.3\%$, and $SUV_{\text{min}}$ by $-30.3 \pm 16.7\%$ could also be observed in the shoulder tumors (Figure 3, D–F). In Capan-1 cells, which do not express the Smo receptor, no significant change in $^{18}$F-FDG uptake could be observed in response to cyclopamine therapy (Figure 3).

**Absolute $SUV_{\text{max}}$ of PCX**

The maximum change in $SUV_{\text{max}}$ of PCX was observed in the PET images obtained after 45 minutes. Therefore, we compared the absolute $SUV_{\text{max}}$ of PCX from the two pancreatic cancer cell lines in vivo and ex vivo. In vivo, the SUV for placebo-treated PCX from MiaPaCa-2 cells in the hip regions ranged from 5.6 to 8.4, but for cyclopamine-treated PCX, SUV ranged from 5.1 to 6.4. Ex vivo, placebo-treated PCX from MiaPaCa-2 cells in the hip regions showed an SUV range of 7.8 to 8.8, but cyclopamine-treated PCX showed an SUV range of 5.6 to 8.2. In vivo, the SUV for placebo-treated PCX from MiaPaCa-2 cells in the shoulder regions ranged from 9.4 to 19.1, but for cyclopamine-treated PCX, SUV ranged from 6.1 to 11.4. Ex vivo, placebo-treated PCX from MiaPaCa-2 cells in the shoulder regions showed an SUV range of 6.6 to 19.8, but cyclopamine-treated PCX showed an SUV range of 2.2 to 18.4. This indicates a decrease in $SUV_{\text{max}}$ of PCX from MiaPaCa-2 cells after cyclopamine therapy both in vivo and, as confirmed by a similar reduction in the calculated SUV of these PCXs, ex vivo (Figure 4A).

In contrast, for placebo-treated PCX from Capan-1 cells in the hip regions, SUV ranged from 7.8 to 12.3, but for cyclopamine-treated PCX, SUV ranged from 9.8 to 12.4 in vivo. Ex vivo, placebo-treated PCX from Capan-1 cells in the hip regions showed an SUV range of 8.6 to 14.8, but cyclopamine-treated PCX showed an SUV range of 10.3 to 12.3. In vivo, for placebo-treated PCX from Capan-1 cells in the shoulder regions, SUV ranged from 6.7 to 21.4, but for cyclopamine-treated PCX, SUV ranged from 16.1 to 18.8. Ex vivo, placebo-treated PCX from Capan-1 cells in the shoulder regions showed an SUV range of 6.7 to 45.6, but cyclopamine-treated PCX showed an SUV range of 12.1 to 60.8. This indicates no change in $SUV_{\text{max}}$ of PCX from Capan-1 cells after cyclopamine therapy either in vivo or, as confirmed by the calculated SUV of these PCXs, ex vivo (Figure 4B).

**PCX/Liver Ratio**

To confirm successful fasting of mice and to validate the data obtained by measuring the absolute $SUV_{\text{max}}$ in PCX, we calculated the $^{18}$F-FDG uptake both in vivo (SUV) and ex vivo (MBq) for both PCX and liver as tumor-to-liver ratio (T/L ratio). In vivo, T/L ratio of placebo-treated PCX from MiaPaCa-2 cells in the hip regions ranged from 0.77 to 0.94, but the ratio of cyclopamine-treated PCX ranged from 0.39 to 0.6 (Figure 5A). Ex vivo, placebo-treated PCX from MiaPaCa-2 cells in the hip regions showed an SUV range of 0.16 to 0.36, but cyclopamine-treated PCX showed an SUV range of 0.03 to 0.19 (Figure 5B). In vivo, T/L ratio of placebo-treated PCX from MiaPaCa-2 cells in the shoulder

![Figure 2](image-url)  
*Figure 2. In vivo tumor growth of PCX during therapy. PCX from MiaPaCa-2 cells (A and B) and Capan-1 cells (C and D) were treated with cyclopamine or placebo. The diameter of the PCX was measured during treatment over 7 days for tumors grown in the hip region (A and C) and shoulder region (B and D) of nude mice as described in Materials and Methods. The data are expressed as the mean diameter ± SEM. $P > .05$.*
regions ranged from 1.4 to 2.5, but the ratio of cyclopamine-treated PCX ranged from 0.9 to 1.6 (Figure 5A).

Ex vivo, placebo-treated PCX from MiaPaCa-2 cells in the shoulder regions showed an SUV range of 0.12 to 0.36, but cyclopamine-treated PCX showed an SUV range of 0.07 to 0.2. This indicates that the range of the T/L ratio in cyclopamine-treated PCX of MiaPaCa-2 cells was lower than in the placebo group.

In vivo, T/L ratio of placebo-treated PCX from Capan-1 cells in the hip regions ranged from 0.9 to 1.4, but the ratio of cyclopamine-treated

Figure 3. $^{18}$F-FDG uptake of PCX with PET-CT. PCX from MiaPaCa-2 cells (continuous line) and Capan-1 cells (interrupted line) were grown in the hip region (A–C) and shoulder region (D–F) of nude mice and treated with cyclopamine and scanned with the clinically implemented PET-CT scanner, and the images were analyzed as described in Materials and Methods. After cyclopamine therapy, SUVs were calculated as a percentage of control placebos (100%). The data are expressed as the mean SUVs in three different experiments ± SEM. $P < .05$.

Figure 4. $^{18}$F-FDG uptake (SUV) in tumors in vivo and ex vivo. SUVs of PCX were measured in vivo in the coregistered PET-CT images. Additionally, the radioactivity uptake of PCX was determined ex vivo and recalculated as SUV as described in Materials and Methods. In vivo and ex vivo results were plotted as individual values for the cyclopamine-treated and its corresponding placebo-treated PCX of both MiaPaCa-2 (A) and Capan-1 (B). The results are plotted as individual values of cyclopamine-treated and corresponding placebo-treated PCX. The experiments were repeated three times.
PCX ranged from 0.9 to 1.2 (Figure 5A). Ex vivo, placebo-treated PCX from Capan-1 cells in the hip regions showed an SUV range of 0.03 to 0.27, but cyclopamine-treated PCX showed an SUV range of 0.23 to 0.27 (Figure 5B). In vivo, T/L ratio of placebo-treated PCX from Capan-1 cells in the shoulder regions ranged from 0.9 to 2.3, but the ratio of cyclopamine-treated PCX ranged from 1.5 to 2.2 (Figure 5A). Ex vivo, placebo-treated PCX from Capan-1 cells in the shoulder regions showed an SUV range of 0.03 to 0.3, but cyclopamine-treated PCX showed an SUV range of 0.18 to 0.5 (Figure 5B). This indicates no change in the range of the T/L ratio in cyclopamine-treated PCX of Capan-1 cells compared to the placebo group.

Figure 5. In vivo and ex vivo measurements of the $^{18}$F-FDG uptake T/L ratio. $^{18}$F-FDG uptake was measured in vivo (A) as SUV and ex vivo (B) as MBq for both PCX and livers of mice. The T/L ratio was calculated and blotted as individual values for the cyclopamine-treated and its corresponding placebo-treated PCX in both MiaPaCa-2 and Capan-1 PCX. The results were plotted as individual values of cyclopamine-treated and corresponding placebo-treated PCX. The experiments were repeated three times.

Figure 6. Immunohistochemistry staining of placebo-treated and cyclopamine-treated PCX from MiaPaCa-2 cells. PCX were treated with placebo (A and C) or cyclopamine (B and D), then excised after PET-CT scanning, and immunohistochemistry was performed using specific antibodies for HK2 (A and B) and Gli1 (C and D) as described in Materials and Methods. Note the moderately increased staining intensity in the placebo-treated PCX compared to cyclopamine-treated PCX. Lower insets are magnified images to demonstrate the cellular localization of both HK2 and Gli1 (arrows), as well as the difference in staining. The specificity of immunostaining was confirmed using a negative control rabbit IgG in a consecutive tissue section (B; upper inset). A magnification scale of 100 μm was used to capture images.
Immunohistochemistry
To confirm the results obtained by PET-CT at the cellular level, we performed immunohistochemical studies for PCX using a marker for glucose metabolism HK2 and Gli1 transcription factor of the hedgehog signaling pathway. The immunostaining for PCX from MiaPaCa-2 cells was qualitatively slightly less intense for both HK2 and Gli1 in response to cyclopamine than in placebo (Figure 6, A–D). In contrast, PCX from Capan-1 cells, with or without cyclopamine therapy, did not show any changes in the immunostaining intensity for either HK2 or Gli1 (Figure 7, A–D). The specificity of immunostaining was confirmed by using the corresponding control IgG (Figure 7, A and inset).

Discussion
DSA-PET is used to study animal models in preclinical research. DSA-PET is a sequential noninvasive imaging technique, providing high-resolution CT images and precise localization of PET findings that can more accurately estimate radioactivity concentration through partial volume corrections. Many recent studies have demonstrated the potential use of clinical PET-CT scanners in small-animal imaging [10,19]. In contrast to DSA-PET imaging systems, less molecular/metabolic information is obtained when imaging small tumors in mice using conventional clinical PET scanners. This is because of the low spatial resolution and low sensitivity of the back-projection techniques during image reconstruction [10]. This problem could be overcome to a large extent by using advanced reconstruction algorithms that modulate the PSF of the PET-CT detectors, increasing the spatial resolution for imaging small lesions in mice [7,8]. Additionally, the new generations of PET-CT imaging systems have been developed to overcome the variability in quantitative results because of software and hardware challenges in conventional scanners. The advanced clinical PET-CT system offers accurate and reproducible quantification in PET-CT imaging by optimizing the volumetric image resolution, daily system calibration, accuracy of attenuation correction using accurate coregistration algorithms, and user-independent reproducible SUV calculation methods. Nowadays, these technical developments are considered to be a cornerstone in radiotherapy planning and follow-up of subtle tumor changes after therapy [20–23].

In the present study, clinical PET-CT served as the basis for translational small-animal imaging, using dedicated clinical imaging protocols established for humans with minor modifications of the scanning protocol. Increasing the zoom to 10 and PET acquisition time to 10 min/bed position has helped greatly to overcome the discrepancy between the body mass of mice and humans, which negatively affects the isotropic and temporal spatial resolution of PET-CT in conventional scanners with a back-projection function [10].

By repeating the 10 min/bed position scan five times instead of the standard 3 min/bed position applied for humans, we could monitor $^{18}$F-FDG uptake dynamically over 50 minutes. We observed a difference in the dynamic uptake between PCX from MiaPaCa-2 cells and Capan-1 cells, which reflects the difference in the metabolic activity in the two cell lines and—in turn—might be attributed to the heterogeneous genetic and epigenetic profile of pancreatic cancer cells and their differing responses to therapy. The investigation of $^{18}$F-FDG uptake in PCX from many different pancreatic cancer cell lines in clinical settings might be investigated in future studies to determine the individual metabolic profile for each cell line in vivo.
To avoid bias in the *in vivo* measurement of the SUVs, we calculated the SUVs of tumors after measuring their radioactivity uptake *ex vivo* using a scintillation counter in both the cyclopamine-treated group and the placebo group in both cell lines. The difference in 18F-FDG uptake of cyclopamine-treated and placebo MiaPaCa-2 PCX *in vivo* matched the calculated SUVs *ex vivo* as well as the radioactivity uptake *ex vivo*. Additionally, to further confirm the reduced 18F-FDG uptake after cyclopamine therapy and to confirm a successful fasting of mice, the T/L ratio was calculated *in vivo* and *ex vivo*. The data obtained from this evaluation corresponded to the absolute SUVs obtained by VOIs determined in the coregistered PET-CT images. Thus, bias of successful fasting was excluded and the effects of cyclopamine therapy were confirmed by using the liver as an internal control *in vivo* and *ex vivo*. Taken together, these data indicate that the clinical PET-CT system is a useful tool for evaluating tumors in small-animal models.

The gold standard for detecting and staging pancreatic cancer is multidetector CT combined with endoscopic ultrasound examination [24]. However, the role of 18F-FDG PET-CT for diagnosis, staging, and follow-up of patients with pancreatic cancer has been thoroughly discussed [25]. Despite these advances, the prognosis for pancreatic cancer has not significantly changed in decades, with a 5-year survival of less than 5% [26]. Therefore, new therapeutic agents that target specific signaling pathways involved in pancreatic carcinogenesis need to be implemented for the treatment of this dismal disease. One of the promising new candidates is antagonizing hedgehog signaling [27]. The inhibition of pancreatic cancer cell growth by antagonizing the hedgehog signaling pathway using cyclopamine has been demonstrated *in vitro* and *in vivo* [12–15]. Most of these studies used high-dose cyclopamine and a long treatment period. The design of the current study aimed at determining the ability of the clinical PET-CT system to detect minimal changes in the metabolic activity of very small PCX treated with cyclopamine at a low dose and over a short period of time. The clinical PET-CT system could detect a subtle decrease in the 18F-FDG uptake of PCX after treatment with cyclopamine. This has been shown at the level of SUVmin, SUVmax, and—in turn—SUVavg of 18F-FDG by PCX, which reflects the potentially high sensitivity of the clinical PET-CT system in detecting the minimal response to anti-hedgehog therapy of PCX, even in the absence of detectable changes in tumor size. Data reporting the high accuracy of the new generations of the clinically implemented PET-CT scanners in monitoring the response of gastrointestinal stromal tumor xenografts after targeted anti-tyrosine kinase therapy support these results [11].

The hedgehog signaling cascade is activated through ligand/receptor binding, causing overexpression and cytoplasmic/nuclear accumulation of Gli1 transcription factor in a feedback mechanism [28,29]. Hedgehog signaling molecules, including Gli1, are overexpressed in pancreatic cancer and could be downregulated after therapy with cyclopamine [12–15]. The expression of hedgehog signaling molecules depends highly on the type of cancer cells. For example, the oncogenic receptor Smo is overexpressed in MiaPaCa-2 pancreatic cancer cells, in contrast to Capan-1 pancreatic cancer cells, which do not express this receptor [13]. As a result, MiaPaCa-2 cells respond to cyclopamine therapy by inhibiting their growth *in vitro*, which is in contrast to Capan-1 cells [13]. Activated hedgehog signaling pathway is associated with the accumulation of Gli1, which could be reversed by cyclopamine in pancreatic cancer cells [12]. We have shown in the current study that both the SUVs and Gli1 accumulation were slightly decreased in MiaPaCa-2 cells of PCX in response to low-dose and short-term therapy with cyclopamine. In contrast, these changes were not seen in Capan-1 cells. Additionally, HK2 is a known marker for glucose metabolism [16]. The slight reduction in metabolic activity and glucose consumption of PCX from MiaPaCa-2 cells was also confirmed by the slight reduction in intracellular accumulation of HK2 after cyclopamine therapy compared to PCX from Capan-1 cells. This again reflects the high sensitivity of the clinically implemented PET-CT system in detecting subtle changes in 18F-FDG uptake by PCX after low-dose and short-term therapy, which is in agreement with the immunohistologic findings. Importantly, these changes occurred in the absence of size changes, which demonstrates the efficacy of the clinical PET-CT in the preclinical assessment of new targeted molecular anticancer drugs.

The main limitation of this study is the lack of baseline clinical PET-CT imaging and repeated PET-CT monitoring of PCX before and during treatment. Regulations for animal housing facilities do not allow mice to be transported for scanning with the clinical PET-CT scanner more than once for hygienic and ethical reasons. Therefore, baseline clinical PET-CT imaging was not possible owing to the different locations of the animal facility and clinical PET-CT system. To overcome this bias, we considered the SUVs of placebo-treated PCX as a 100% uptake of tracer. Then, the SUVs obtained for the cyclopamine-treated PCX were calculated as a percentage reduction of tracer uptake in placebo PCX. Additionally, we compared PCX of the same size from a homogeneous population of the same pancreatic cancer cell line and without any apparent change in the size of PCX owing to a short-term low-dose therapy. Therefore, the metabolic profile is expected to be the same as if baseline imaging had been performed. However, this logistic problem should be resolved in future studies.

In conclusion, we demonstrated that a clinical PET-CT system equipped with PSF could monitor the effects of preclinical therapeutic agents in PCX developed into nude mice. The new generations of the clinically implemented PET-CT scanners with high-resolution reconstruction might be useful tools for translational imaging, especially for evaluating new anti-tumor therapies for pancreatic cancer. However, small-animal PET-CT systems that have a better spatial resolution should be used if available.

**Acknowledgments**

The authors are grateful to the technical support provided by E. Goetz, M. Dinger, S. Losev, B. Kiesslich, V. Skude, E. Wühl, and E. Selinger.

**References**


