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Imaging Tumor Variation in Response to Photodynamic Therapy in Pancreas Cancer Xenograft Models

Kimberley S. Samkoe, PhD^{1,*}, Alina Chen¹, Imran Rizvi, MS^{1,2}, Julia A. O'Hara, PhD¹, P. Jack Hoopes, DVM^{1,3}, Stephen P. Pereira, MD⁴, Tayyaba Hasan, PhD², and Brian W. Pogue, PhD^{1,2,3}

¹Thayer School of Engineering, Dartmouth College, Hanover NH 03755

²Wellman Center for Photomedicine, Massachusetts General Hospital, Boston MA 02114

³Department of Surgery, Dartmouth Medical School, Hanover NH 03755

⁴The Institute of Hepatology, University College London Medical School, London WC1E 6HX

Abstract

Purpose—A treatment monitoring study investigated the differential effects of orthotopic pancreatic cancer models in response to interstitial photodynamic therapy (PDT), and the validity of using magnetic resonance (MR) imaging as a surrogate measure of response was assessed.

Methods and Materials—Different orthotopic pancreas cancer xenograft models (AsPC-1 and Panc-1) were used to represent the range of pathophysiology observed in humans. Identical dose escalation studies (10, 20 and 40 J/cm) using interstitial verteporfin PDT were performed and MR imaging with T2-weighted and T1-weighted contrast were used to monitor the total tumor volume and the vascular perfusion volume, respectively.

Results—There was a significant amount of necrosis in the slower growing Panc-1 tumor using high light dose, although complete necrosis was not observed. Lower doses were required for the same level of tumor kill in the faster growing AsPC-1 cell line.

Conclusions—The tumor growth rate and vascular pattern of the tumor affects the optimal PDT treatment regime, with faster growing tumors being relatively easier to treat. This highlights the fact that therapy in humans will show a heterogeneous range of outcomes, and suggests a need for careful individualized treatment outcomes assessment in clinical work.

Keywords

photodynamic therapy; pancreas cancer; magnetic resonance imaging; tumor aggressiveness; orthotopic tumor

*Correspondence to: Thayer School of Engineering; Dartmouth College; 8000 Cummings Hall; Hanover, NH; 03766 USA; phone - 1-603-646-9602; fax - 1-603-646-3856; samkoe@dartmouth.edu or pogue@dartmouth.edu.

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Introduction

Survival rates in pancreatic cancer (PCa) have remained low, with curative treatment only possible with complete surgical removal of the tumor and/or pancreas; even with complete resection only 15-20% of patients survive to 5 years.¹ The current standard-of-care for patients presenting with locally advanced or metastatic disease is gemcitabine, which has a higher one-year survival rate (18%).² Integration of photodynamic therapy (PDT) with gemcitabine or antibody therapy has been examined with potential value in certain populations.³⁻⁵ This paper examines the range of outcomes from PDT in PCas, testing response in a dose escalation trial, and utilizing optimized magnetic resonance imaging (MRI) as a surrogate marker.

PDT is a treatment that induces localized cellular death through combination of photosensitizing drug, oxygen and light;⁶ with interest in the use of PDT for localized treatment in PCas.⁷⁻⁹ Bown *et al*¹⁰ (2002) undertook a phase I clinical trial treating PCa with mTHPC. Although successful within acceptable morbidity rates, there were many drawbacks to mTHPC-PDT. The drug-light-interval was long (3 days), patients remained sensitive to direct sunlight for 1-2 months after treatment and regrowth occurred at the edges of the treated area.

The use of verteporfin for the treatment of PCa is appealing due to its very short metabolic half-life, near-infrared absorption and it is clinically approved for PDT of age-related macular degeneration and some cancers.¹¹ Only two verteporfin PDT studies for PCa have been approached: Yusuf *et al.* (2008)¹² with endoscopic ultrasound-guided PDT of normal swine pancreas and Ayaru *et al.* (2007)¹³ in normal pancreas of the Syrian golden hamster. Minimal normal tissue damage occurred in both studies. Verteporfin PDT is now entering a Phase I/II clinical trial at University College London Hospital, UK.¹³ Standard imaging techniques for diagnosis and treatment monitoring of PCa include computed tomography, endoscopic ultrasound, MRI,^{14,15} and contrast-based imaging; the latter two have potential to track response to PDT.

The first goal of this study was to explore the feasibility of verteporfin-PDT in a dose escalation trial using interstitial-PDT in two orthotopic xenograft PCa models with fast and slow growth rates to track the universality of the response. The second goal was to determine whether standard imaging techniques such as contrast-enhanced and T2-weighted MR could be used to gauge effects of PDT within 48hrs of treatment.

Materials and Methods

Cell Culture

AsPC-1¹⁶ and Panc-1 tumor cells^{17,18} were suspended in culture medium at 4×10^7 cells/mL and mixed 1:1 with Matrigel® (*BD Biosciences*, San Jose, CA) for orthotopic implantation. Sterile insulin syringes (½cc U-100 Lo-Dose Insulin Syringe 28G½, Becton Dickinson & Co., Franklin Lakes, NJ) were loaded with the mixture and kept on ice until implantation.

Orthotopic Pancreas Model

Animal procedures were approved in a protocol by the local Institutional Animal Care and Use Committee (IACUC). Fur was removed from the left side of male SCID mice, ~6 weeks in age, and the surgical site was sterilized with iodine. A small (<1cm) incision was made through which the spleen was pulled out, allowing access to the pancreas. Fifty micro-liters of the cell-Matrigel® solution was implanted into the pancreas tail. Once Matrigel® solidified (~10sec) the needle was removed and incision site closed with 3-4 sutures (Ethilon 5-0 PS-3, *Ethicon*, Piscataway, NJ). There was 100% success rate of tumor growth.

Magnetic Resonance Imaging

MRI was used to monitor both tumor development and therapeutic response to PDT. At 24-48hrs pre-PDT and 48hrs post-PDT, each mouse underwent MRI to determine pre-treatment total tumor and tumor vascular perfusion volumes. Grimm *et al* (2003) reported that motion artifacts for mouse abdomen imaging were greatly reduced by placing the mice supine (minimizing breathing motions), and fasting the mice for 6-12 hours (reducing peristaltic motions).¹⁹ A tail vein catheter (MTV-01, Braintree Scientific Inc, Braintree, MA) filled with gadolinium (Gd)-DTPA (*Magnevist*TM) was placed in the intraperitoneal cavity of the mouse. The mice were anesthetized with 1-1.5% isoflurane during imaging with an oxygen flow rate of 1L/min.

MRI was performed in a *Phillips Achieva* 3.0T X-series MRI with a modified rodent coil (*Philips Research Europe*, Hamburg, Germany) as previously described.²⁰ The MR sequence protocols are described in Table 1. After a preliminary survey, a native T1-weighted turbo-spin echo (T1W) was performed, Gd-DTPA (0.03mg/kg) was administered and allowed to localize during the T2-weighted turbo spin echo (T2W) image acquisition. A post-contrast T1W image sequence was collected 10min after gadolinium injection. Post-imaging tumor volume analysis was performed using *Mimics Software* (Materialise, Version 11.11).

PDT

PDT was performed when the tumors were $\sim 60(\pm 15)\text{mm}^3$, as determined by T2W MR image analysis (Figure 1); this corresponded to two-weeks growth for the AsPC-1 and five-weeks for Panc-1. The following total doses are described as energy (J) per length of diffusing fiber (cm). For both AsPC-1 and Panc-1 tumors there were four treatment groups: low-dose (10J/cm with verteporfin), medium-dose (20J/cm with verteporfin), high-dose (40J/cm with verteporfin) and control (40J/cm without verteporfin). Three mice were in each group.

Prior to PDT, fur that had re-grown was removed. Verteporfin for injection was administered intravenously via the tail vein (75 μL total volume, 1mg/kg) one hour prior to light administration. Before PDT was initiated, an incision was made in the left side of the mouse to expose the pancreas and tumor. A diffusing fiber (320 μm diameter, 1cm length) was inserted into the center of the longest axis of the tumor using a 20-gauge needle. Interstitial-PDT was performed with a 690nm wavelength laser (Applied Optronics, South Plainfield NJ) at a linear irradiance of 74mW/cm. The treatment time was varied (2.25, 4.5, and 9min) to achieve total energy of 10, 20, and 40J/cm. After the light treatment, the fiber was removed from the tumor and the surgical incision was closed with 4-5 sutures. The mice were administered an analgesic i.p. (0.1mg/kg, Buprenorphine hydrochloride, *Hospira Inc.*) immediately after surgery and every 12hrs thereafter if physical signs of pain persisted. After the 48hr post-PDT MR imaging session, the mice were anesthetized with 100:10mg/kg ketamine:xylazine (i.p.) and then injected i.v. with 1.0mg/kg 3,3'-diheptyloxacarbocyanine iodide (DiOC₇(3) (*Molecular Probes*, Eugene, OR) in a 4:1 mixture of dimethyl sulfoxide:water). After 1min, the animal was sacrificed by cervical dislocation. The tumor and surrounding tissues were removed for *ex vivo* fluorescence and histological analysis. A time line describing the full experimental procedure is presented in Figure 1B for easy reference.

Ex Vivo Tissue Analysis

The tissues immediately surrounding the AsPC-1 or Panc-1 tumor (pancreas, liver, spleen, kidney, stomach and intestine) were removed and fixed in a 10% buffered formalin solution. Collateral damage to the tissues caused by PDT was assessed on H&E stained sections with the aid of a research pathologist (author PJH). Images were acquired with an Olympus-BX50 microscope and a SPOT Insight (Diagnostic Instruments Inc.) mounted digital camera at varying magnifications (1, 2, 10 and 20 $\times$).

The orthotopic tumors were removed, placed in Tissue Tek® optimum cutting temperature compound and flash frozen in a mixture of methylbutane and dry ice. The frozen samples were stored short term at -20°C or long term at -80°C. Frozen specimens were cryotome-sectioned perpendicular to the treatment fiber axis and two consecutive 10µm sections were taken every 500µm throughout the entire tumor. One slice was used for DiOC₇(3) fluorescence blood vessel analysis and the other was used for H&E staining.

Functional blood vessel status in treated tumors was analyzed using DiOC₇(3) fluorescence (excitation:480/20nm; emission:540/40nm). Each frozen slice had six fluorescent images (682×512 pixels) recorded in different microscopic fields using a Nikon Diaphot-TMD fluorescence microscope equipped with a Qimaging Micropublisher Imaging System (Burnaby, BC, Canada). Of the six images, one was from the center of the tumor, one contained the edge of the tumor and four were random images from the outer segments. Image processing was performed with *NIH ImageJ*, and blood vessel parameters were determined with thresholded image data.

Results

Orthotopic Tumor Lines

The two tumor lines displayed very different growth rates (Figure 1): AsPC-1 tumors grew to 60mm³ by 14 days, while the Panc-1 line required 35 days to reach the same volume. The difference between the tumors was evident in the vascular structure of the tumors (observed by DiOC₇(3) localization, data not shown), which may be related to the difference in growth rates. The AsPC-1 tumors had large, chaotic, and highly perfusing blood vessels indicative of a rapidly growing tumor, whereas the blood vessels in the Panc-1 tumors were smaller, organized and demonstrated less significant amounts of perfusion.

PDT Outcomes

The AsPC-1 and Panc-1 PCa had different response to PDT. A summary of the PDT treatment parameters is shown in Table 2. The control and 10J/cm treated groups appeared to be affected very slightly. There was one unexplained fatality in the Panc-1 10J/cm by 36hrs, likely due to surgical complications. In both AsPC-1 and Panc-1 implanted mice, there was a strong physical reaction to PDT in the 20J/cm and 40J/cm; however, the response was most pronounced in the 40J/cm group. One mouse in the Panc-1 40J/cm group died within the first 36hrs after treatment and all three mice in the AsPC-1 40J/cm group died within 48hrs post-treatment. All of these deaths were thought likely to be PDT related. Extreme blood loss during treatment (several hundred microliters) was observed in two of the AsPC-1 mice: one mouse from the 40J/cm and one from the 20J/cm treatment groups. In both cases it was not believed that any large vessels were punctured during fiber implantation as the bleeding did not occur until several minutes into treatment (5-10min after fiber implantation). In these two cases, the mouse receiving the 20J/cm dose survived until the experimental endpoint of the 48hr post-PDT observation period, while the mouse that received the 40J/cm dose died within 36hrs.

MRI Determined Tumor Volumes

Post-MR image analysis was performed to determine the total tumor and vascular perfusing volumes of the orthotopic tumors. Figures 2 and 3 illustrate representative pre- and post-PDT coronal images for Panc-1 and AsPC-1 orthotopic tumors, respectively. The total tumor volume was determined from T2W images (Figures 2A and 3A) while the vascular perfusing volumes were determined from the gadolinium enhanced T1W contrast difference (T1WCD) images (Figures 2B and 3B). In addition, 3D representations (Figures 2C and 3C) of the tumor's total and perfusion volumes were displayed for direct comparison.

The tumor volume results are summarized in Figure 4. Figures 4A and 4C summarize the total tumor volumes and the vascular perfusion volumes taken 24-48hrs pre-PDT and 48hrs post-PDT in box-and-whisker plots for AsPC-1 and Panc-1, respectively. In both tumor lines, there is an increasing trend in the total tumor and the vascular perfusion volumes as the light dose was increased, while the control groups were similar to the pre-PDT volumes. This overall volume increase is most likely due to an acute inflammatory response of the tumor. The one deviation was the 40J/cm AsPC-1 group; both the total tumor and the perfusion volumes were less than that of the 20J/cm AsPC-1 group. It is important to note however, that the volume of the AsPC-1 tumors increased more rapidly with dose as compared to the Panc-1 tumors; the maximum tumor volume for AsPC-1 was achieved from the 20J/cm dose while approximately the same volume was reached in the 40J/cm dose in the Panc-1 tumors. Figures 4B and 4D show that the non-perfusing region of the tumor (i.e. [T2W volume]-[T1WCD volume]) follow the same trend. The size of the necrotic core in the AsPC-1 tumors grew more rapidly with light dose as compared to Panc-1 tumors, indicating that the two tumor lines responded differently to the same treatments.

Tumor Blood Vessel Analysis

DiOC₇(3) fluorescence data was used to sample the number of blood vessels within each tumor. The results for both AsPC-1 and Panc-1 tumors are summarized in Figure 5. As the number zero could not be displayed in a log graph, the lowest values of the log graph were replaced with zero for representation purposes only; this neither affected values reported nor the t-test values calculated.

In both the Panc-1 and the AsPC-1 PDT trials (Figures 5A and 5B), the effect of the 10J/cm dose was not statistically different from that of the control. However, both the 20J/cm and the 40J/cm dose groups had significantly fewer blood vessels than the control (Panc-1 20J/cm – $p < 0.03$; AsPC-1 20J/cm, 40J/cm and Panc-1 40J/cm – $p < 0.00001$). The mean of each group was inversely proportional to the light dose and the decrease in the average number of blood vessels corresponded to an increase in the volume of non-perfusing tumor observed in Figures 4B and 4D.

Generally, the number of blood vessels (Figure 5A and 5B) in the control group, as well as the low and medium dose treatment groups was essentially the same for both the AsPC-1 and Panc-1 tumor lines. This was not the case in the 40J/cm group, where there was no detectable fluorescence in the tumor samples of the AsPC-1 tumor lines, while in the Panc-1 group some functioning vasculature persisted.

Histological Analysis

The 10 μ m frozen AsPC-1 and Panc-1 tumor tissue slices were stained for histological analysis. A representative image for each treatment group is displayed in Figure 6; the necrotic region increased in size with increasing light dose. It was progressively more difficult to cryo-section the tissues that received 20J/cm and 40J/cm doses due to the increasing inflammation and water content in the tissues – an effect often observed for several days following PDT. This was shown by the small tears that were visible in the 20J/cm and 40J/cm treated tumors, while this effect was minimal in the 40J/cm and control tumors. The necrotic region in the AsPC-1 and Panc-1 control tumors had a much smaller apparent necrotic core than the 10J/cm treatment group. However, the necrotic region of the Panc-1 10J/cm treated tumor had intermittent patches of tissue that maintain cellular integrity and were stained dark purple indicating that some cellular viability remains. In all but the 40J/cm group, the normal pancreas surrounding the AsPC-1 and Panc-1 tumors appeared to be relatively unaffected by PDT. The normal pancreas retained the native cellular composition but some minor inflammation was observed; the one exception to this was in the AsPC-1 40J/cm group where complete necrosis of the entire

tumor and surrounding normal pancreas could be observed. This indicates that the 40J/cm dose was too strong for the more aggressive AsPC-1 tumor, but not for the Panc-1 tumor.

The tissues immediately surrounding the pancreas were analyzed for PDT damage. In all treatment groups there was no damage observed to the stomach and duodenum, while the normal pancreas, kidney, liver and spleen had varying levels of damage. The control groups only had minor inflammation to the pancreas, most likely caused by mechanical damage during fiber placement. In the Panc-1 mice there were focal zones of necrosis in one or all of the spleen, kidney and liver with inflammation in the normal pancreas. This trend was observed in the low dose AsPC-1 mice. However, in the 20J/cm and 40J/cm groups there was diffuse damage in one or more organ. This was especially noteworthy in the 40J/cm group where all organs were observed to be entirely necrotic, most likely due to post-treatment shock symptoms.

The histological analysis suggests that the medium light dose group was the most promising treatment group for the aggressive AsPC-1 tumor line, while the 40J/cm group was optimal for the less aggressive Panc-1 tumor line. The tumors responded well to therapy, displaying a large necrotic core surrounding the diffusing fiber axis and some surrounding tissues 48hrs post-PDT; however, most damage was minimal and was unlikely to affect the long term survival of the animals.

Discussion

The AsPC-1 and Panc-1 PCa cell lines were chosen for this study because of differences in both growth rates and vasculature structure, indicating different levels of aggression. In addition to observable variations in the physical composition of the tumors, AsPC-1 cells have significantly higher levels of vascular endothelial growth factor²¹ and epidermal growth factor²² than Panc-1 cells. It was expected that the AsPC-1 line, being the more aggressive tumor line with ill formed blood vessels, would respond more favorably than the Panc-1 line to PDT. Both *in vivo* and *ex vivo* data analysis reveal that both tumor lines responded to PDT, however it is apparent that different doses are required to achieve maximal effect.

The use of MR imaging as a measure of surrogate response was successful in determining PDT effect 48hrs after treatment. The same trends in MR images of mouse orthotopic pancreas tumor enhancement seen by Grimm *et al.* are also observed here.¹⁹ The T2W images indicate heterogeneous enhancing areas of the tumor, correlating well with amount of fibrosis and edema found in the central region of tumor (Figures 2 and 3, A1 and B1). T1W images (Figures 2 and 3, A2 and B2) without gadolinium contrast were non-enhancing while the outer regions enhanced significantly with contrast; additionally, T1WCD images provided well defined areas vascular perfusion. If the vascular perfusion regions are subtracted from the total tumor volume the resultant volume would be the non-perfusing region corresponding to therapeutic response areas. There are two prior examples of using the non-enhancing region in the T1W contrast MRI sequence to track necrosis.²³

T2W and T1WCD volumes (Figure 4A and C) showed therapeutic response resulting in increased volume and increased non-perfusing tumor core. Although the tumors do contain small, non-perfusing central cores of necrosis prior to PDT, the large change in the non-perfusion volume with increased light dose (Figure 4B and D) suggests that vessel occlusion or necrosis in the area surrounding the interstitial fiber occurred. The overall increase in total tumor volume was likely due to an acute edematous immune response typical of PDT induced necrosis in tumors²⁴ and has been observed in other studies monitored by MR imaging.²⁵ Acute inflammation occurs as a response to cytosolic content in the extracellular space due to cellular necrosis and membrane rupture from PDT.²⁴ It was probable that inflammation within

the treated tissue was responsible for the observed increase in perfusion volume, thus the non-perfusing necrotic core was a more valuable measure of PDT success than the T1WCD volume. The largest response in the Panc-1 tumor line was observed at the 40J/cm dose, and in the AsPC-1 tumor at 20J/cm light dose. The 40J/cm light dose in AsPC-1 had a less significant increase, as a result of massive tissue necrosis from both the tumor and surrounding tissues. Many tumor adjacent tissues (i.e. kidney, liver, normal pancreas, spleen) accumulate more verteporfin; however, collateral damage was likely due to the size restriction of the mouse abdomen relative to the interstitial fiber as these effects have not been seen in previous studies.^{12,13} The difference between the PDT-induced tumor volumes in the AsPC-1 and Panc-1 tumor lines signifies that response to therapy may depend on tumor aggressiveness and therefore inherent biophysical properties.

Ex vivo analysis techniques confirmed that seen with *in vivo* MR imaging study. DiOC₇(3), a fluorescent vascular perfusion marker, was used to analyze the functional blood vessels remaining after PDT.²⁶ Vascular occlusion occurs either within the time frame of verteporfin PDT treatment, or for several hours afterwards.²⁷ Therefore, blood vessel occlusion can mark areas of successful treatment. In both tumor lines there was a dose response in decreasing number of blood vessels with light. At the highest light dose there were no blood vessels remaining in AsPC-1 tumors. This was not the case for the Panc-1 tumors, suggesting complete vascular occlusion occurred in the more aggressive AsPC-1. It is important to note here that the decrease in the DiOC₇(3) fluorescence corresponds with the increase observed in non-perfusing volume, as the increase in the T1WCD volume is most likely due to acute inflammatory response of the tissue.

Analysis of the H&E of the frozen tumor sections indicates a dose response of the necrotic core with light dose. This correlates well with the non-perfusing tumor volume (Figure 4B and D) determined from the T2W and the T1WCD images. In the case of the 40J/cm dose, the AsPC-1 tumors and the normal pancreas were completely necrotic, suggesting that the treatment dose exceeded the maximum therapeutic threshold. This result agrees with the MR tumor volume data with a sudden decrease in the tumor volume and a high rate of mortality between the 20J/cm and 40J/cm doses. This was not true for the Panc-1 tumors, where normal surrounding pancreas was unaffected by PDT at all doses. All tissues displayed signs of inflammation including the presence of leukocytes, high water content in the frozen samples and increased extracellular spaces as compared to normal or control tissue, further supporting our hypothesis that the overall tumor volume increase is due to acute inflammation.

Conclusions

This study indicates that PDT can treat PCa albeit with variation in the outcome, based upon the tumor pathophysiology. The two orthotopic pancreas tumors tested had different growth rates and vascularity and it was shown that the more aggressive AsPC-1 tumor line responded well at lower light doses than the less aggressive Panc-1 tumor line.

Non-perfusing tumor volumes determined by MR imaging were successful as a measure of surrogate response; however, the total tumor and perfusion volume information gained would be better interpreted if a molecular marker could be implemented to delineate between the PDT-treated tumor and the surrounding inflamed tissues. *Ex vivo* fluorescence and H&E were also used to measure therapeutic response and confirmed the results observed with *in vivo* MR imaging. PDT of PCa will most likely be successful as an adjuvant therapy considering that complete necrosis was only observed in the highest light dose and was associated with a high mortality rate.

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Figure 1.

Growth curves for the AsPC-1 and Panc-1 tumors, based on the total tumor volume determined by MR image analysis (A). An experimental time-line is presented in B.

Figure 2.

Representative AsPC-1 MR images for tumor volume determination: pre-PDT MR images (A) and post-PDT MR images (B) are of the same mouse. Total tumor volume was segmented in yellow, determined from the T2W image sequence (A1 and B1), while the vascular perfusion volume was segmented in blue and is determined from the T1WCD images (A2 and B2). A3 and B3 - 3D representations of the volumes are displayed on the same scale, demonstrating inflammation of the tumors from pre- to post-PDT.

Figure 3.

Representative Panc-1 MR images for tumor volume determination: pre-PDT MR images (A), and the post-PDT MR (B). Segmentation of the T2W (A1 and B1, yellow) give the total tumor volume, while segmentation of the T1WCD (A2 and B2, cyan) give the vascular perfusion volume. The 3D representations of the tumor volumes are shown in A3 and B3 in corresponding colors.

Figure 4.

The effect of PDT light dose on the MRI determined volumes for AsPC-1 (A and B) and Panc-1 (C and D) tumor lines. A and C - for each PDT treatment parameter the total (T2W) tumor volume is shown at left (black) and vascular perfusion (T1WCD) volume is shown at right (gray). There is an overall increase in tumor volume proportional to the increase in light dose for both tumor lines. The one exception was the 40J/cm AsPC-1 treatment group. B and D illustrate a proportional change in the non-perfusing (necrotic/edemic) region of the tumor with an increase in light dose. The AsPC-1 control was the same as the pre-PDT, while the control was slightly increased in the Panc-1 tumors.

Figure 5.

Sampling the number of blood vessels per tumor from *ex vivo* analysis shows a decreasing trend with increased light dose (AsPC-1-A and Panc-1-B). The 10J/cm groups were not statistically different from the negative control; however, both the 20J/cm and 40J/cm groups were significantly different from the control (* $p < 0.00001$, # $p = 0.025$)

Figure 6.

Representative samples of H&E stained tumor slices display increasing necrotic/edemic core with increasing light dose. Black arrows frame the necrotic/edemic regions; note that almost the entire sample in the AsPC-1 40J/cm group is necrotic. Small tears appear in the treated tumors due to large areas of necrosis and fluid.

Table 1

Summary of MR pulse sequences used for pancreatic carcinoma imaging

Sequence Parameter	MR Image Sequence Protocol	
	T1W-TSE	T2W-TSE
TE (ms)	20.0	80.0
TR (ms)	521.2	4704.1
Flip angle	90°	90°
Number of averages	2	3
FOV (mm)	90	90
Echo train length (ms)	3	15
Matrix size	256 × 256	256 × 256
Percent sampling (%)	100	100
Slice thickness (mm)	0.75	0.75
Number of slices	24	24
Acquisition time (min:sec)	2:24	4:52

Abbreviations: TE – echo time, TR – repetition time, FOV – field of view.

Table 2

Summary of each trial group, number of mice, treatments, and surviving mice.

Treatment Group	Verteporfin (mg/kg)	Total Energy Delivered (J/cm)	Number Surviving
AsPC-1(10)	1.0	10	3
AsPC-1(20)	1.0	20	2
AsPC-1(40)	1.0	40	0
AsPC-1(Control)	0	40	3
Panc-1(10)	1.0	10	2
Panc-1(20)	1.0	20	3
Panc-1(40)	1.0	40	2
Panc-1(Control)	0	40	3