

Video Article

# Bioluminescent Orthotopic Model of Pancreatic Cancer Progression

Ming G. Chai<sup>1</sup>, Corina Kim-Fuchs<sup>1,2</sup>, Eliane Angst<sup>2</sup>, Erica K. Sloan<sup>1,3</sup>

<sup>1</sup>Monash Institute of Pharmaceutical Sciences, Monash University

<sup>2</sup>Department of Visceral Surgery and Medicine, University of Bern

<sup>3</sup>Cousins Center for Neuroimmunology, University of California Los Angeles

Correspondence to: Erica K. Sloan at [Erica.Sloan@monash.edu](mailto:Erica.Sloan@monash.edu)

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## Abstract

Pancreatic cancer has an extremely poor five-year survival rate of 4-6%. New therapeutic options are critically needed and depend on improved understanding of pancreatic cancer biology. To better understand the interaction of cancer cells with the pancreatic microenvironment, we demonstrate an orthotopic model of pancreatic cancer that permits non-invasive monitoring of cancer progression. Luciferase-tagged pancreatic cancer cells are resuspended in Matrigel and delivered into the pancreatic tail during laparotomy. Matrigel solidifies at body temperature to prevent leakage of cancer cells during injection. Primary tumor growth and metastasis to distant organs are monitored following injection of the luciferase substrate luciferin, using *in vivo* imaging of bioluminescence emission from the cancer cells. *In vivo* imaging also may be used to track primary tumor recurrence after resection. This orthotopic model is suited to both syngeneic and xenograft models and may be used in pre-clinical trials to investigate the impact of novel anti-cancer therapeutics on the growth of the primary pancreatic tumor and metastasis.

## Video Link

The video component of this article can be found at <https://www.jove.com/video/50395/>

## Introduction

Pancreatic cancer is the fourth leading cause of cancer-related death, with a 5-year survival rate of 4-6%.<sup>1,2</sup> Only 15% of patients are diagnosed early enough in the disease to be eligible for surgery, and tumors recur in >80% of those patients.<sup>3,4</sup> Gemcitabine is used for treatment of pancreatic adenocarcinomas, however, chemoresistance is common and often the drug has little impact on overall survival.<sup>5</sup> New pharmacological strategies to treat pancreatic cancer are critically needed. Their development depends on significantly improved understanding of the key steps of disease progression that may be sensitive to therapeutic intervention.

Orthotopic models of pancreatic cancer emulate key aspects of the human disease, making them ideal tools for studying the biology of pancreatic cancer.<sup>6-9</sup> In contrast to *in vitro* cell-based assays of pancreatic cancer cell behavior and subcutaneous *in vivo* models of pancreatic cancer, orthotopic models allow investigation of tumor cell interactions with the pancreatic microenvironment. The kinetics of disease progression are highly reproducible in orthotopic models and occur over a short time frame (weeks), which makes them well suited to pre-clinical testing of novel therapeutics. This is in contrast to transgenic models where disease onset occurs over a longer and more variable time frame (months to 1 year).<sup>10</sup> When used with more aggressive cell lines, orthotopic models of pancreatic cancer have patterns of spontaneous metastasis similar to those seen in patients.<sup>8</sup> Expression of bioluminescent reporter genes such as firefly luciferase facilitates longitudinal monitoring of tumor growth, metastatic dissemination, recurrence and response to therapeutics.<sup>6,11</sup>

Here we describe an orthotopic model of pancreatic cancer that utilizes Matrigel for localized cell delivery and *in vivo* bioluminescence imaging for non-invasive monitoring of tumor progression. This orthotopic model of pancreatic cancer allows non-invasive analyses of disease progression and response to therapeutic interventions in syngenic or xenograft models.

## Protocol

The protocol being demonstrated is performed under the guidance and approval of the author's institution's animal care and use committee. All experiments are executed in compliance with all relevant guidelines, regulations and regulatory agencies.

## 1. Transducing Pancreatic Cancer Cell Lines

1. Transduce pancreatic cancer cells to express luciferase as previously described.<sup>12,13</sup> Panc-1 and Capan-1 pancreatic cancer cell lines transduced with firefly luciferase are used here.

**Note:** Renilla luciferase or bacterial luciferase may also be used.

## 2. Pancreatic Cancer Cell Preparation

1. Culture transduced pancreatic cancer cells until 70% confluent.
2. Lift the pancreatic cells and ensure viability is greater than 90%.
3. Resuspend at  $2 \times 10^7$  cells/ml in a 3:2 mixture of chilled Matrigel:Phosphate buffered saline (PBS).
4. Keep the Matrigel-cell suspension on ice prior to injection into the pancreas.

**Notes:** To ensure rapid solidification of Matrigel, reduce the PBS volume to account for the volume of the cell pellet. Handle Matrigel using ice-cold instruments and syringes at all times to prevent solidification prior to injection. The suggested cell number is a guide and should be determined empirically for each cell line.

## 3. Mouse Preparation

1. Anesthetize the mouse using inhaled 2-3% isoflurane. Determine depth of anesthesia by lack of pedal reflex to a gentle toe pinch.
2. Apply lubricant to the eyes to prevent desiccation.
3. Position the mouse on its back on a 37 °C heating pad and gently turn the mouse to raise the left side of the abdomen.
4. Prepare the abdomen with a 10% povidone iodine solution.

**Notes:** Injectable anesthesia may be used instead of inhaled anesthesia. Pre-operative fasting is not necessary.

## 4. Laparotomy

1. Using sterile surgical instruments make a 1.5 cm incision in the skin approximately 1 cm left lateral from the midline.
2. Make a 1.5 cm incision in the underlying abdominal muscle.
3. Locate the spleen using the forceps and gently remove the spleen from the abdominal cavity. Secure the spleen along a sterile cotton bud to expose the underlying pancreas.
4. Locate the tail of the pancreas adjacent to the spleen.
5. Using a 29 G 0.3 ml insulin syringe, inject 20  $\mu$ l of the Matrigel-cell suspension into the pancreas.
6. Following injection, hold the syringe in the pancreas for 30-60 sec until the Matrigel has solidified. This important step minimizes cell leakage.
7. Inspect the site of injection to ensure no leakage occurred.
8. Return the spleen and pancreas to the abdominal cavity.

**Note:** Take care to avoid puncturing the dorsal side of the pancreas which may be thin.

## 5. Abdominal Wall Closure

1. Close the abdominal musculature of the mouse with an absorbable braided 4-0 suture with a round needle using a continuous stitch.
2. Close the external skin with a non-absorbable monofilament 6-0 suture with a cutting needle using a continuous stitch.
3. Remove the mouse from the inhaled anesthetic and injected 0.05-0.1 mg/kg buprenorphine subcutaneously.
4. Allow the mouse to recover in its cage placed on a 37 °C heating pad with free access to food and water. If mice demonstrate signs of pain such as hunching or reduced mobility, buprenorphine may be given every 12 hr over a 36 hr period.
5. After wound healing (7-10 days), anesthetize the mouse and remove the external sutures.

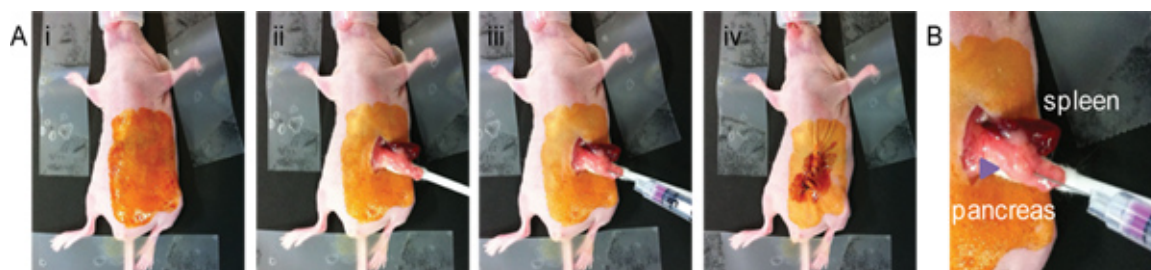
## 6. Bioluminescent Tracking of Pancreatic Cancer Progression

1. Anesthetize the mouse using inhaled isoflurane.
2. Inject 150 mg/kg D-luciferin via tail vein.
3. Place the mouse in the bioluminescent imaging system and capture white-light and bioluminescence images as previously described.<sup>14,15</sup>
4. Remove the mouse from the inhaled anesthetic and allow it to recover in its home cage.

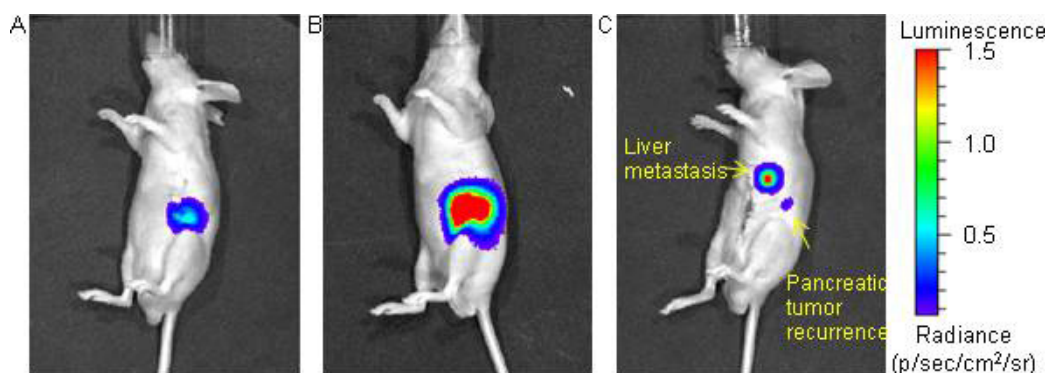
**Note:** Bioluminescent imaging is non-invasive and can be conducted periodically to investigate tumor growth kinetics. To image tumor in the pancreatic tail it is important to put the mouse on its left side, so the tumor points towards the camera. We imaged once per week with the frequency increased up to three times per week prior to the experimental endpoint using a Lumina II imaging system (Perkin-Elmer, formerly Caliper Life Sciences) running Living Imaging 4.3.1 software with binning 4, FOV 12.5, F-stop 1, exposure 1 - 60 sec (determined by the highest exposure without pixel saturation).

## Representative Results

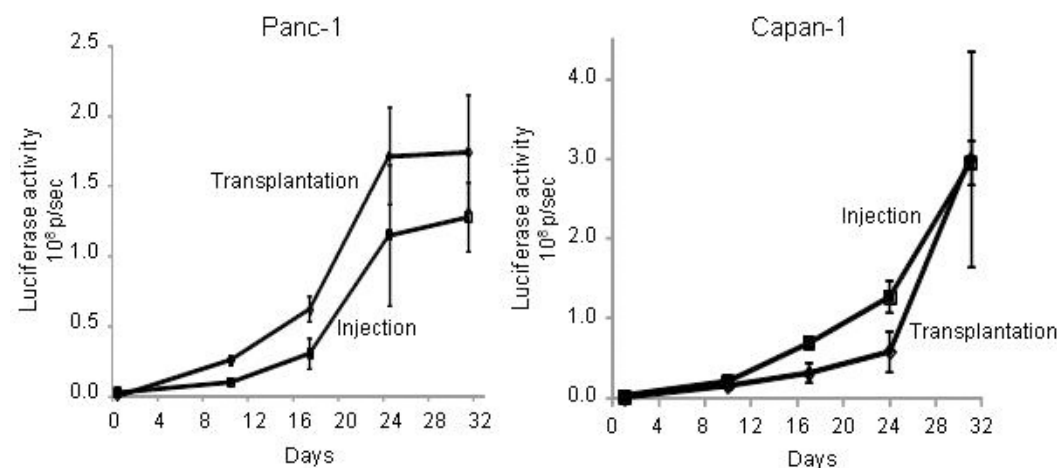
This method describes an orthotopic model of pancreatic cancer using surgical procedures, including induction of anesthesia, laparotomy, injection of cancer cells in Matrigel and abdominal closure (**Figure 1A**). The injected cells form a bubble in the surface of the pancreas (**Figure 1B**). Pancreatic cancer progression may be non-invasively monitored using *in vivo* bioluminescence imaging to track cancer cell proliferation and dissemination (**Figure 2**). Liver metastasis was indicated by observation of bioluminescence in the liver during surgical resection (**Figure 2**) and confirmed *ex vivo* by histology (**Figure 4B**). Primary tumors were resected with the pancreatic tail and spleen at 6 weeks after implantation. Tumor growth dynamics in this orthotopic injection model are reproducible and closely resemble the kinetics of orthotopic transplantation models of pancreatic cancer (**Figure 3**). Tumor growth in the pancreas is locally invasive and metastasizes to organs including liver (**Figure 4**).



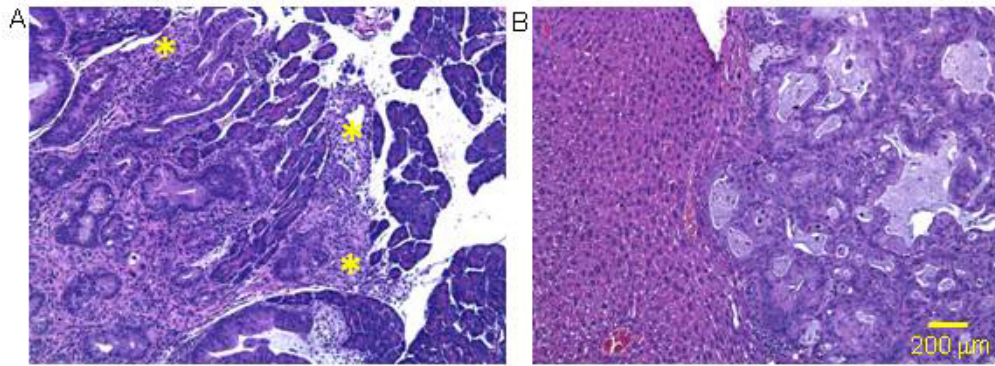
**Figure 1. An orthotopic mouse model of pancreatic cancer progression.** **A)** i. The anesthetized mouse is fixed in place using tape and the abdomen is disinfected. ii. After longitudinal laparotomy the spleen and pancreatic tail are gently exteriorized and held in place on a sterile cotton bud. iii. Matrigel-embedded pancreatic tumor cells are injected into the pancreas tail. iv. The abdomen is closed in two layers. **B)** Magnified image showing exteriorized spleen and pancreas. The injected cells form a bubble (arrowhead). [Click here to view larger figure.](#)



**Figure 2. Bioluminescent imaging of orthotopic pancreatic tumor (A) ten days after injection, (B) 31 days after injection, and (C) five days following tumor resection.**



**Figure 3. *In vivo* primary tumor growth of Panc-1 and Capan-1 cell lines was monitored over time (days after injection) by bioluminescence imaging (n = 4).** The kinetics of tumor growth after injection of Matrigel-embedded pancreatic tumor cells are similar to orthotopic models using transplantation of pancreatic tumor pieces.



**Figure 4. Hematoxylin and eosin-stained sections of (A) Panc-1 primary pancreatic tumor, and (B) liver metastasis from Panc-1 primary pancreatic tumor. \* Local invasion of pancreatic cancer cells into the surrounding organ.**

## Discussion

Here we describe an orthotopic model for longitudinal assessment of pancreatic tumor development and progression. Primary tumor growth kinetics are reproducible (**Figure 3**) and may be non-invasively monitored using bioluminescence imaging of luciferase-tagged cells, *e.g.* for analyses of tumor response to novel anti-pancreatic cancer therapeutics. Consistent with the human disease, the model shows local pancreatic invasion (**Figure 4A**) which allows investigation of tumor cell interactions with the pancreatic microenvironment. The use of luciferase-tagged cell lines allows analyses of frequency, location and kinetics of metastatic dissemination. Use of luciferase-tagged cell lines facilitates detection of metastases before they become visually apparent and tissue localization of metastases may be confirmed by *ex vivo* imaging and histology. As in the clinical situation, the model shows metastasis to organs including mesenteric lymph nodes, liver, gastrointestinal tract and peritoneal cavity (**Figure 4**).<sup>8</sup> Bioluminescence imaging may also be used to determine successful resection, and incidence and kinetics of primary tumor recurrence (**Figure 2C**).<sup>16</sup>

Careful preparation and handling of pancreatic cancer cells is essential for reproducibility of the model. The ratio of Matrigel to PBS has been optimized for rapid solidification of the cell pellet after injection. The volume of pelleted cells (approximately 35 µl for  $10^7$  tumor cells) should be subtracted from the volume of PBS for a final 3:2 ratio of Matrigel:cells+PBS. During injection, cell leakage is prevented by maintaining the needle *in situ* for 30-60 sec until the Matrigel has solidified (**Figure 1**). If leakage does occur it is easily detected as Matrigel is colored and those mice may be excluded from further analyses. Preventing tumor cell leakage ensures that metastasis occurs from tumor cell dissemination rather than as an artifact of the injection technique.

The kinetics of tumor development will be influenced by the cell number injected and should be determined empirically for each cell line. We found reproducible tumor growth patterns after injection of  $4 \times 10^5$  Panc-1 or Capan-1 cells (**Figure 3**). The threshold for bioluminescent detection of metastases will be influenced by characteristics of the expression construct used including the promoter strength and the use of codon-optimized luciferase. In addition to the xenograft models described here, the techniques may also be used with syngenic pancreatic cell lines to examine pancreatic progression in an immunocompetent setting. Similar tumor dynamics were observed in male or female mice (data not shown).

The method described here has been modified for an orthotopic transplantation model of pancreatic cancer. In that model, tumors are grown subcutaneously in donor mice, then a 1 mm<sup>3</sup> piece is transplanted into a small pocket in the pancreas tail of a recipient mouse.<sup>6-8,17</sup> Primary tumor growth may be monitored using bioluminescence imaging using donor tumors derived from luciferase-tagged tumor cells. In contrast to the orthotopic model described here, transplantation models require additional time (up to 1 month) and additional mice for generation of donor tumors. Results using transplantation models may be influenced by heterogeneous composition of transplanted tumor pieces. We showed here that the kinetics after injection of Matrigel-embedded cells are similar to the transplanted model. Transplantation models have been used to study and expand clinical patient samples.<sup>18-21</sup>

The orthotopic models described here complement transgenic models of pre-invasive and invasive pancreatic cancer.<sup>10,22,23</sup> Transgenic models recapitulate key aspects of human pancreatic cancer including oncogene mutations but show significant variability in disease onset (7 weeks to > 1 yr).<sup>10,23</sup> Most transgenic models do not express luciferase and so are not suitable for *in vivo* bioluminescence imaging of disease progression. In contrast, orthotopic models of pancreatic cancer show reproducible kinetics of tumor progression and allow non-invasive monitoring, making them well suited to pre-clinical therapeutic studies.

New therapeutic approaches are critically needed to combat the exceptionally low survival rates for pancreatic cancer. By permitting visualization of metastatic and recurrent disease, the orthotopic models of pancreatic cancer described here are relevant to the clinical setting, where most current treatments are palliative. In addition, orthotopic models are highly valuable for investigating pancreatic tumor biology and evaluating novel therapeutic strategies in the *in vivo* setting.

## Disclosures

The authors declare that they have no competing financial interests.



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