Establishment of a Metastatic Orthotopic Model of Pancreatic Ductal Adenocarcinoma (PDAC) for Drug Development

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) has the worst survival prognosis (<5%) of all common gastrointestinal malignancies. It is typically diagnosed at a very late stage when the tumor has already metastasized to other organs, at which point the treatment can no longer prolong the survival of the patients. To date, surgical resection is the only curative approach present, provided that the cancer is detected at a very early stage. Nonetheless, less than 20% of diagnosed patients qualify for the surgery and majority of these patients will eventually develop recurrence. Despite our advancing knowledge of the tumor biology of PDAC as well as recent improvements in diagnosis, the prognosis remains strikingly poor. The median survival observed after surgery followed by chemotherapy is about 20 months.

The aim of this study was to establish an orthotopic model of PDAC that could be used to study efficacy of new potential treatments.

Materials and Methods

Female (Hsd: Athymic Nude-Foxn1nu, Envigo) mice were used in this study. MiaPaCa-2-Luc human PDAC cells were injected to surgically exposed caudal part of the pancreas. At the time of inoculation, the animals were 4-5 weeks of age. To validate the model, the current standard-of-care (SOC) treatment, combination of Abraxane and Gemcitabine, was used (Nab-paclitaxel, Abraxane, 10 mg/kg, i.p. and Gemcitabine, 60 mg/kg, i.p.). The SOC treatment was initiated two weeks post inoculation and administered twice a week over a period of 4-5 weeks. During the study, tumor burden was quantified by imaging the bioluminescence signal emitted by the MiaPaCa-2-luc cells using IVIS Lumina II imaging system (PerkinElmer). The mice were stratified to treatment groups based on similar intensity of the bioluminescent readout. Imaging was performed every second week after inoculation of the cells. After 30 days in study, tumor growth in surgical area was observed. At sacrifice, orthotopic tumors and metastatic tissues were dissected and processed for histology and immunohistochemical (IHC) stainings (Vimentin, Clone SP20, Abcam). Stained sections were scanned with digital slide scanner (3DHISTECH).

Study design

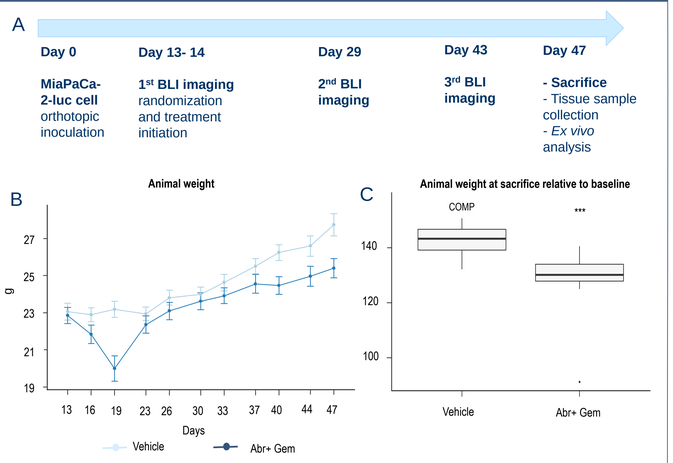


FIGURE 1. Schematic layout of the orthotopic PDAC model. (A) Cancer cells were inoculated orthotopically into intact mice at study day 0. Tumor growth rate was assesed with BLI during the study. (B) Mice were weighted twice a week following tumor cell injection (day 0). The data are presented as mean ± SEM (bars). (C) Body weight at sacrifice relative to baseline (%); COMP = Study group used for statistical comparison, *** p < 0.001.

Ex Vivo tumor weight and volume

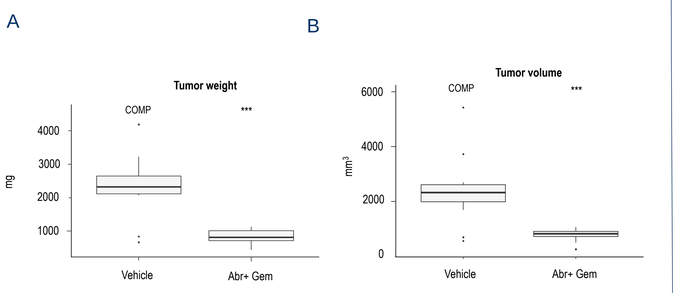


FIGURE 3. *Ex Vivo* tumor weight and volume. (A) Tumor weight (mg, median ± 25% IQR ± min/max) at sacrifice. COMP = Comparison group. (B) Orthotopic tumor volume (L*W*H)*π/6, mm3, median ± 25% IQR ± min/max) at sacrifice. COMP = Study group used for statistical comparison, *** p < 0.001.

Tumor burden measurements

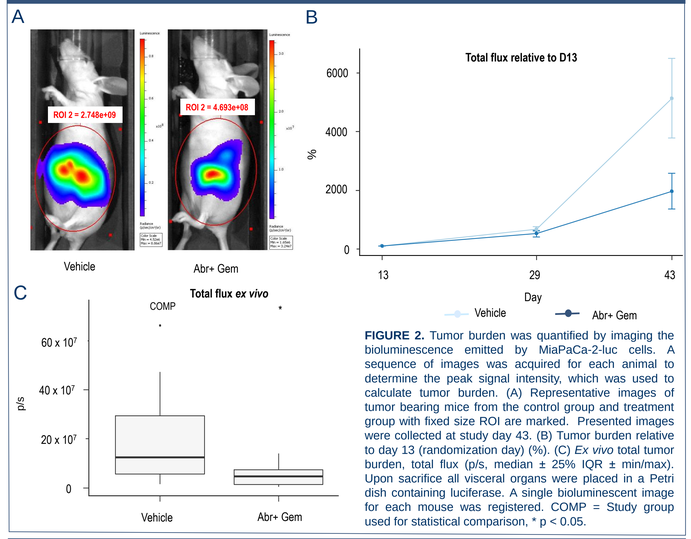
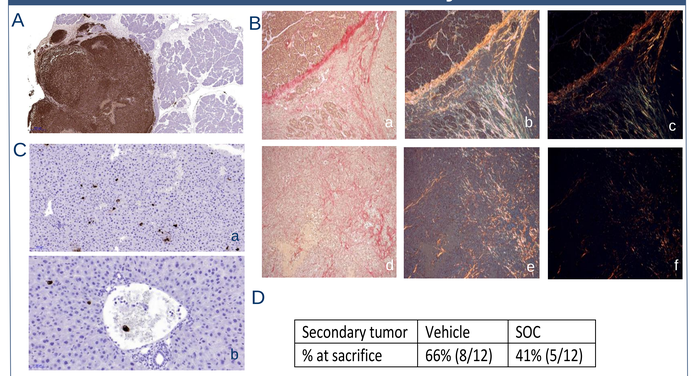


FIGURE 2. Tumor burden was quantified by imaging the bioluminescence emitted by MiaPaCa-2-luc cells. A sequence of images was acquired for each animal to determine the peak signal intensity, which was used to calculate tumor burden. (A) Representative images of tumor bearing mice from the control group and treatment group with fixed size ROI are marked. Presented images were collected at study day 43. (B) Tumor burden relative to day 13 (randomization day) (%). (C) *Ex vivo* total tumor burden, total flux (p/s, median ± 25% IQR ± min/max). Upon sacrifice all visceral organs were placed in a Petri dish containing luciferase. A single bioluminescent image for each mouse was registered. COMP = Study group used for statistical comparison, * p < 0.05.

IHC of micrometastasis formed by MiaPaCa-2-luc



Secondary tumor	Vehicle	SOC
% at sacrifice	66% (8/12)	41% (5/12)

FIGURE 4. Immunohistochemical and histological analysis of tumor samples and metastatic tissue. (A) Primary tumor stained with Vimentin. The staining is specific for human pancreatic carcinoma cells and shows no cross reactivity to mouse vimentin. (B) Weigert Picro-Sirius red stained primary tumor, (a-c) peritumoral area, (d-f) intratumoral area. Images a and d were collected using bright field imaging, and polarization imaging was used for images c and f. Images b and e were taken with the blend of the polarization and bright field. (C) Vimentin staining of the liver. All liver samples contained circulating tumor cells. Circulating tumor cells in sinusoids (a) and in the portal vein (b) are shown. (D) After 30 days in study, tumor growth in surgical area was observed. Corresponding % at sacrifice is presented.

Summary

- The observed tumor take rate in the presented model was 100%
- Despite no differences in body weights, mice receiving the SOC treatment gained less weight when comparing the body weights obtained at endpoint relative to baseline
- *Ex vivo* tumor burden showed decrease in BLI intensity in the group receiving the SOC treatment as compared to the vehicle group
- At sacrifice, tumor weight and volume were lower in the group receiving the SOC treatment as compared to the vehicle group
- MiaPaCa-2-luc cells induced micrometastasis to other visceral organs including liver
- At the end of the study 66% of mice in the vehicle group versus 41% in the SOC group had palpable tumor in the surgical area.

Conclusions

A metastatic orthotopic PDAC model was established successfully and validated with the SOC treatment that slowed down disease progression. This orthotopic model provides a promising tool for testing new treatments against PDAC *in vivo.*

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