

¹Jason C. Hall, ¹Laura A. Marlow, ²Adam C. Mathias, ²Louis K. Dawson, ²William F. Durham, ²Kenneth A. Meshaw, ²Robert J. Mullin, ²Aidan J. Synnott, ²Daniel L. Small, ³Murli Krishna, ⁴Daniel von Hoff, ⁵Gerardo Colon-Otero and ¹John A. Copland.

¹Department of Cancer Biology, ³Department of Laboratory Medicine and Pathology, ⁵Division of Hematology/Oncology; Mayo Clinic, 4500 San Pablo Rd S. Jacksonville, FL 32224; and ²Charles River Discovery Services, 3300 Gateway Centre Blvd. Morrisville, NC 27560; ⁴The Translational Genomics Research Institute (TGen), 445 N 5th St. Phoenix, AZ.

Abstract

Acinar cell carcinoma of the pancreas (PACC) is an uncommon malignancy, accounting for less than 1% of all pancreatic neoplasms. Because of its rarity, only a few retrospective studies are available to help guide management. We previously reported the case of a patient with metastatic PACC who achieved prolonged survival with doxorubicin as a result of personalized treatment designed in part on the basis of molecular and in-vitro data collected on analysis of the tumor and primary cells in culture developed from the liver metastasis. We now report the characterization of a patient derived xenograft (PDX) mouse model originating from this patient's PACC liver metastasis tissue. Antitumor activity of multiple drugs (5-FU, irinotecan, oxaliplatin, gemcitabine, bevacizumab, erlotinib, doxorubicin and imatinib) used as a single agent therapy is demonstrated. Of the targeted and cytotoxic therapies used, oxaliplatin produced a dramatic and prolonged response to therapy even after withdrawal of treatment. The effective therapy induced tumor cell death, decreased serum lipase levels and the pancreatic tissue began expressing cytoplasmic amylase, an indication of healthy acinar cells. Thus, we have developed and characterized a PACC PDX model that may be used in drug discovery for the treatment of this rare cancer for which no standard-of-care exists.

Background

Pancreatic acinar cell carcinoma; (PACC) is a unique disease that is not well understood. The disease accounts for ~1% of the pancreatic cancer cases diagnosed every year⁽¹⁾. Nevertheless PACC is a lethal disease that is likely to become metastatic to the liver or lung⁽²⁾. The molecular signals that drive PACC are still a mystery, and investigators have been trying to test existing cancer treatments to find the most effective therapy. Since little is known about the disease, an understanding of the molecular components is imperative to finding a targeted therapy. Past studies have found that genes that regulate DNA repair may be mutated⁽³⁻⁵⁾. Genomic profiling on 44 PACC patients uncovered DNA repair mutations in 45% of the patients, BRCA2 being the most common gene, followed by BRCA1 and ATM⁽⁶⁾.

Our group was presented with a highly motivated patient who enrolled in a personally designed treatment based on the genetic and molecular profile of his tumor, which was cultured into primary cells and treated with a group of chemotherapeutics⁽⁶⁾. Our lab also established a PACC pancreas derived xenograft (PDX) mouse model from his tumor biopsy and we have proceeded in treating mice with therapies that target specific molecules and DNA replication, in order to find effective targets. We hypothesized that this rationally designed treatment will yield effective therapies.

Figure 1: Characterization of PACC PDX mouse model

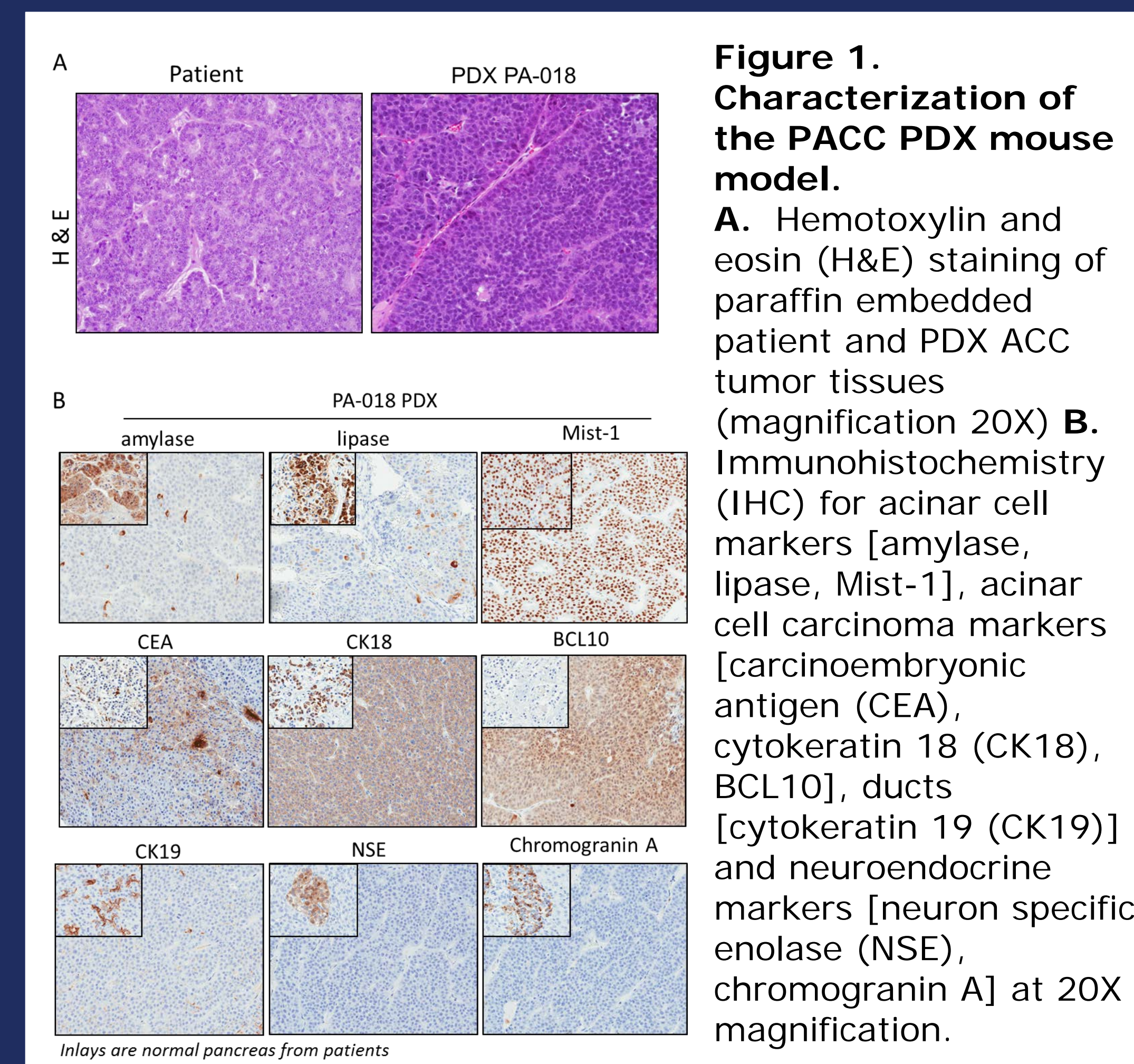


Figure 2. Therapeutic response in PA-018 PDX PACC mouse model

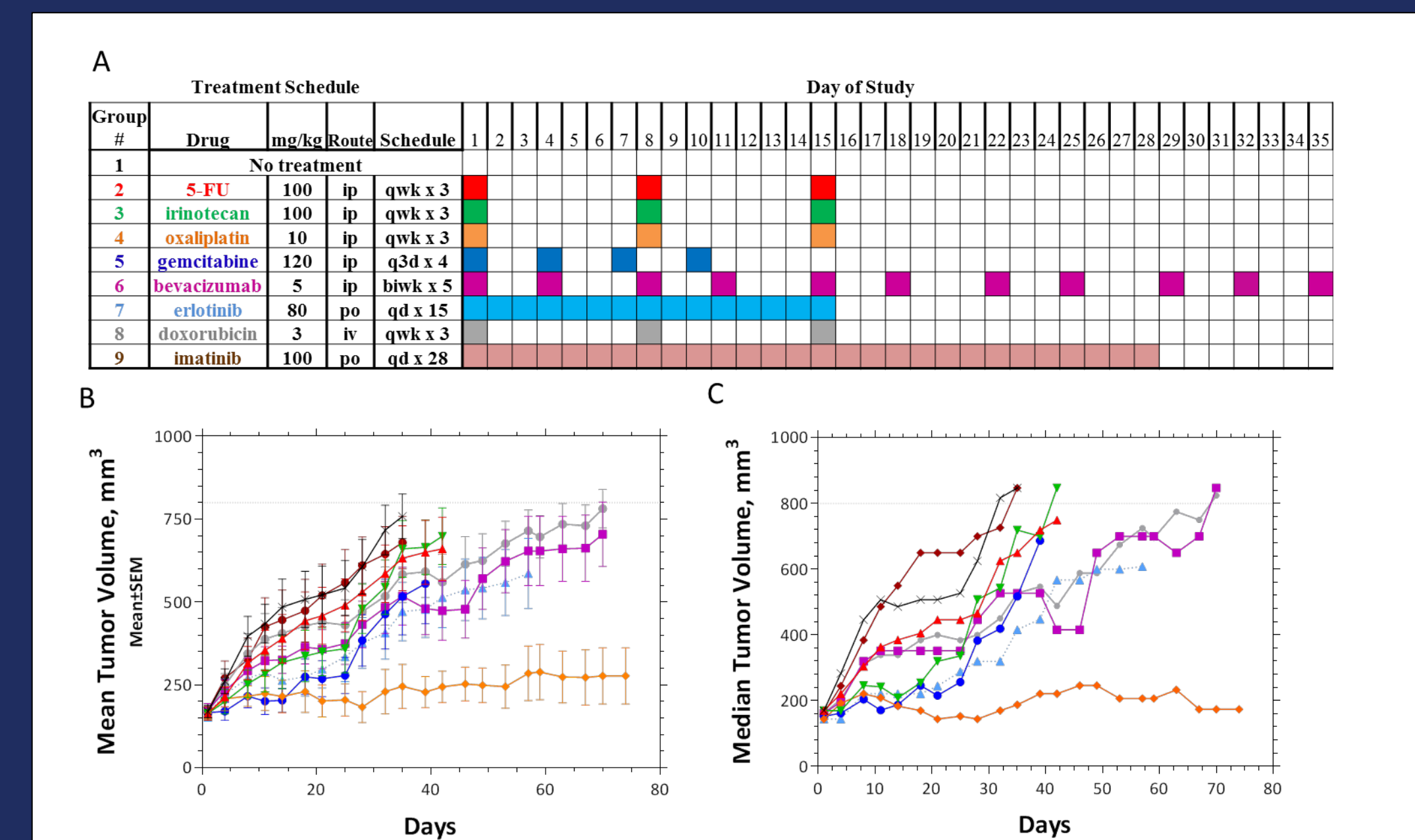


Figure 2. Therapeutic response in PA-018 PDX PACC mouse model.
A. Reference table for chemotherapy doses used along with route and treatment schedule. **B.** Group mean volumes \pm standard error (SEM) were plotted as a function of time. **C.** Group median tumor volumes were plotted as a function of time. When an animal exited the study due to tumor size, the final tumor volume recorded for the animal was included with the data to calculate volume at subsequent time points as indicated by dashed lines.

Table 1. Result summary of PA-018 therapeutic responses and toxicities

Group	n	Treatment Regimen	Median TTE	T-C	chi-square	p value	summary	Mean BW Nadir	TR	NTR
1	10	placebo	32.7	---	---	---	---	0	0	0
2	10	5-FU	37.3	4.6	0.3966	0.5288	ns	-0.1% Day 14	1	0
3	10	irinotecan	40.8	8.1	0.5419	0.4616	ns	-5.3% Day 21	0	0
4	10	oxaliplatin	74*	41.3*	14.82	0.0001	***	-9.8% Day 21	0	0
5	10	gemcitabine	37.8	5.1	1.265	0.2607	ns	-5.2% Day 39	1	0
6	9	bevacizumab	68.8	36.1	4.165	0.0413	*	-4.2% Day 21	0	1
7	9	erlotinib	54.4	21.7	1.277	0.2585	ns	-15.3% Day 14	2	1
8	10	doxorubicin	65.8	33.1	2.525	0.112	ns	-5.1% Day 42	0	0
9	9	imatinib	33.3	0.6	0.09389	0.7593	ns	-0.8% Day 21	0	1

Table 1. The therapies used included DNA synthesis inhibitors (5-FU, gemcitabine), a DNA alkylating agent (oxaliplatin), a DNA intercalating agent (doxorubicin), a topoisomerase inhibitor (irinotecan), an EGFR inhibitor (erlotinib), a c-kit inhibitor (imatinib) and an angiogenesis inhibitor (bevacizumab). Tumor growth was continually observed after treatment regimen ceased in order to determine time-to-endpoint (TTE) and difference between TTE medians (T-C). Body weight (BW) nadir was shown as percent change and deaths were divided into treatment-related deaths (TR) and non-treatment related deaths (NTR).

Figure 3. Evaluation of expression of pancreatic digestive enzymes

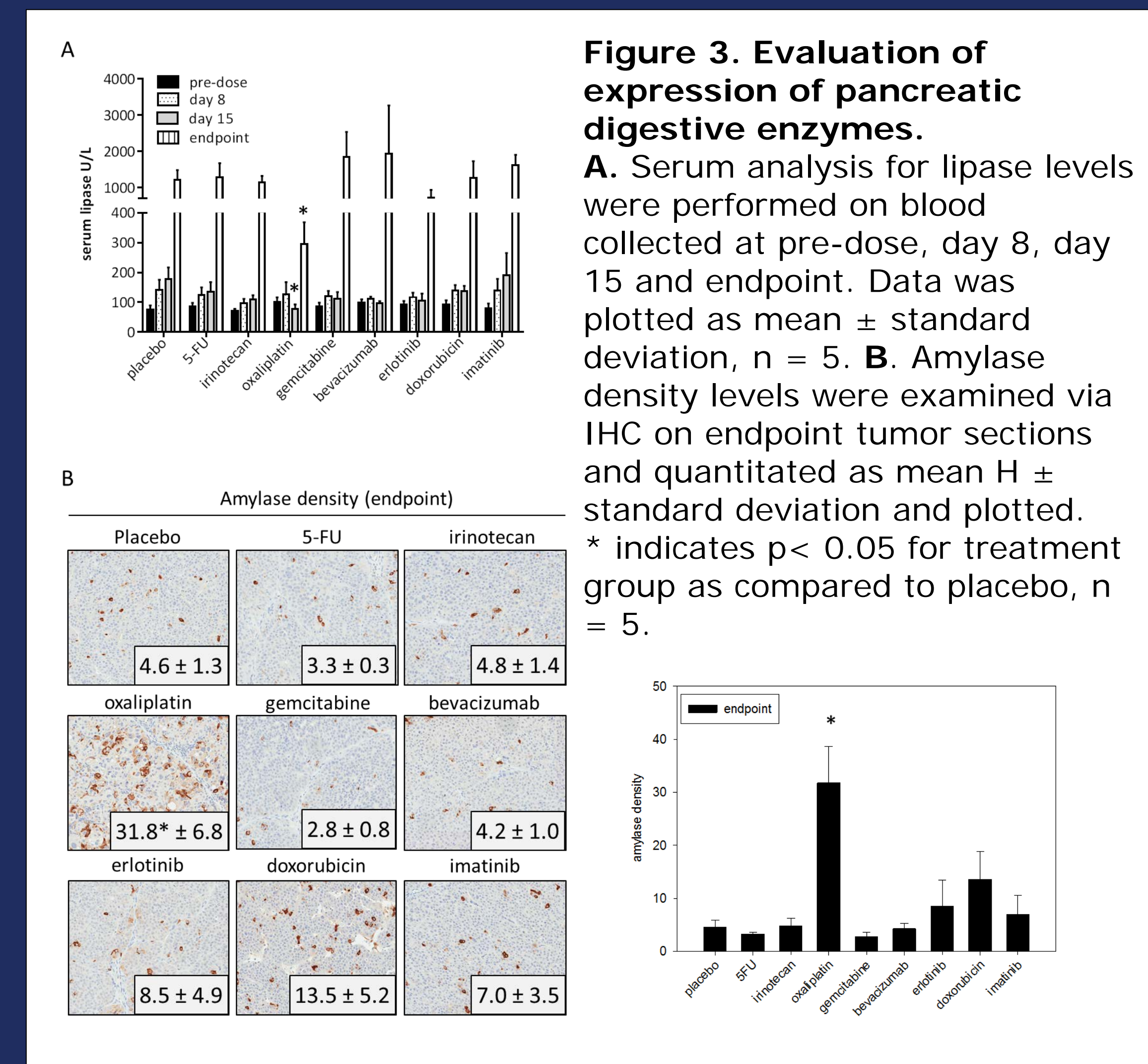


Figure 4. Oxaliplatin's effects on proliferation and apoptosis in PACC

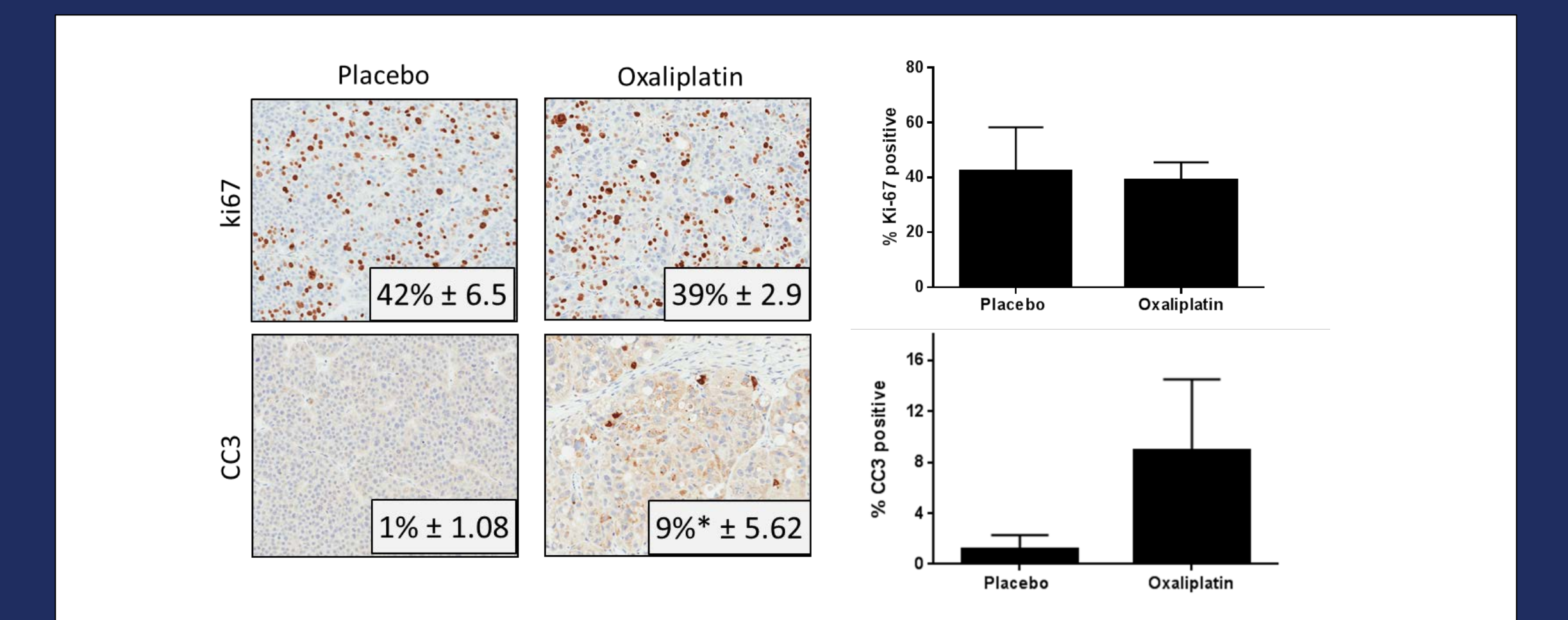


Figure 4. Oxaliplatin's effects on proliferation and apoptosis in PACC.
 Immunohistochemistry (IHC) was examined for Ki67 for proliferation index and cleaved caspase-3 (CC3) for apoptotic index. Ki67 was scored by positive counts per core section and plotted as mean percent \pm standard deviation. CC3 was scored by positive pixel count over area and plotted as mean percent \pm standard deviation. * indicates p < 0.05 for treatment group as compared to placebo, n = 5.

Conclusions

Our studies show that we have established a reliable PDX model for PACC. This model is homogenous, as it is negative for markers of ductal carcinoma and neuroendocrine tumors. We tested nine separate cancer therapeutics to evaluate the benefits induced by each treatment. Overall, oxaliplatin yielded the best anti-tumor effect, without a toxic response. The PACC tumors halted serum lipase secretion and began to histologically appear like normal acinar cells. The therapy induced cell death, but did not stop proliferation. Since oxaliplatin induces DNA damage it may be worth understanding which DNA repair genes could be mutated in this model of PACC. Since previous research shows that DNA repair is an important component of PACC⁽⁶⁾, we conclude that treatment with oxaliplatin may be a viable option for patients.

References

- Holen et al. *J Clin Oncol*. 2002; 20(24):4673-4678
- Wisnoski NC, et al. *Surgery* 2008 ; 144 : 141 – 8.
- Lowery MA, et al. *Oncologist* 2011 ; 16(12):1714-1720.
- Furukawa T, et al. *Scientific Reports* 2015
- Chmielecki J, et al. *Cancer Discovery* 2014 (12): 1398-405.
- Armstrong, MD et al. *Journal of Cancer* 2011; 2:142-152