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Therapeutic responses in a novel patient-derived xenograft mouse model for rare pancreatic acinar cell carcinoma

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

[1]Department of Cancer Biology, [3]Department of Laboratory Medicine and Pathology, [5]Division of Hematology/Oncology; Mayo Clinic, 4500 San Pablo Rd S. Jacksonville, FL 32224; and [2]Charles River Discovery Services, 3300 Gateway Centre Blvd. Morrisville, NC 27560; [4]The 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[5].

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

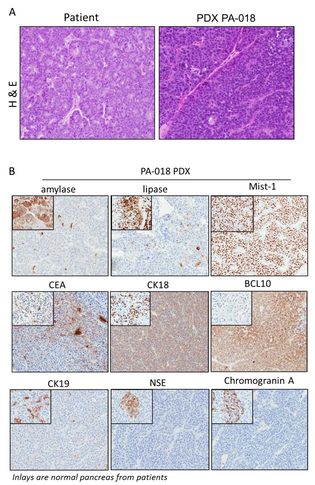

Figure 1. Characterization of the PACC PDX mouse model.
A. Hemotoxylin and eosin (H&E) staining of paraffin embedded patient and PDX ACC tumor tissues (magnification 20X) **B.** Immunohistochemistry (IHC) for acinar cell markers [amylase, lipase, Mist-1], acinar cell carcinoma markers [carcinoembryonic antigen (CEA), cytokeratin 18 (CK18), BCL10], ducts [cytokeratin 19 (CK19)] and neuroendocrine markers [neuron specific enolase (NSE), chromogranin A] at 20X magnification.

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

		Treatment Regimen				Median		Statistical Significance			Mean BW	Deaths	
Group	n	Agent	mg/kg	Route	Schedule	TTE	T-C	chi-square	p value	summary	Nadir	TR	NTR
1	10	placebo	-	-	-	32.7	---	---	---	---	-0.1% Day 14	0	0
2	10	5-FU	100	ip	qwk x 3	37.3	4.6	0.3966	0.5288	ns	-5.9% Day 21	1	0
3	10	irinotecan	100	ip	qwk x 3	40.8	8.1	0.5419	0.4616	ns	-7.9% Day 21	0	0
4	10	oxaliplatin	10	ip	qwk x 3	**74***	**41.3***	14.82	**0.0001**	***	-9.8% Day 21	0	0
5	10	gemcitabine	120	ip	q3d x 4	37.8	5.1	1.265	0.2607	ns	-5.2% Day 39	1	0
6	9	bevacizumab	5	ip	biwk x 5	**68.8**	**36.1**	4.165	**0.0413**	*	-4.3% Day 21	0	1
7	9	erlotinib	80	po	qd x 15	54.4	**21.7**	1.277	0.2585	ns	-15.3% **Day 14**	2	1
8	10	doxorubicin	3	iv	qwk x 3	65.8	**33.1**	2.525	0.112	ns	-5.1% Day 42	0	0
9	9	imatinib	100	po	qd x 28	33.3	0.6	0.09389	0.7593	ns	-0.8% Day 21	0	1

n = number of animals in a group not dead from accidental or unknown causes, or euthanized for sampling
TTE = time to endpoint
T-C = difference between median TTE (days) of treated group versus control group
Statistical Significance (Logrank test, df=1): ne = not evaluable, ns = not significant, * = P < 0.05, ** = P < 0.01, *** = P < 0.001, compared to Group 1
Mean BW Nadir = lowest group mean body weight, as % change from Day 1
TR = treatment-related death; NTR = non-treatment-related death
* = Time of sacrifice (74 days) was artificially used at TTE for oxaliplatin group

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

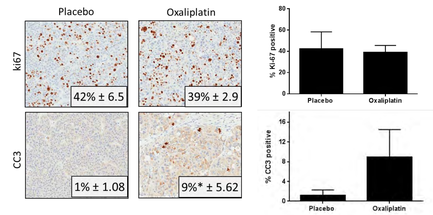

Figure 4. Oxaliplatin's effects on proliferation and apoptosis in PACC.
Immunohistochemistry (IHC) was examined for ki67 for proliferation index and cleaved caspase-3 (CC3) for apoptotic index. Ki67 was scored by positive counts per core section and plotted as mean percent ± standard deviation. CC3 was scored by positive pixel count over area and plotted as mean percent ± standard deviation**.** * indicates p< 0.05 for treatment group as compared to placebo, n = 5.

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

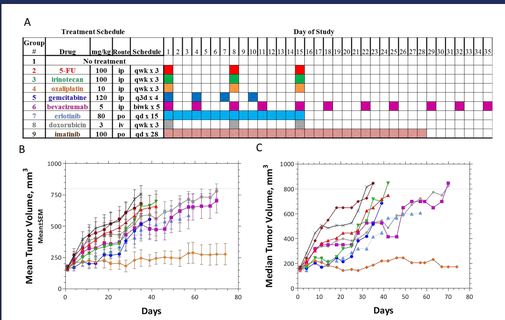

Figure 2. Therapeutic response in PA-018 PACC PDX mouse model.
A. Reference table for chemotherapy doses used along with route and treatment schedule. **B.** Group mean volumes ± standard error (SEM) were plotted as a function of time. **C**. Group median tumor volumes were plotted as a function of time. 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.

Figure 3. Evaluation of expression of pancreatic digestive enzymes

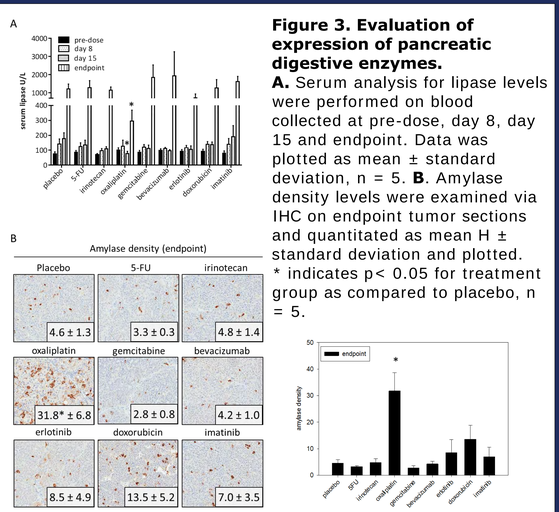

Figure 3. Evaluation of expression of pancreatic digestive enzymes.
A. Serum analysis for lipase levels were performed on blood collected at pre-dose, day 8, day 15 and endpoint. Data was plotted as mean ± standard deviation, n = 5. **B**. Amylase density levels were examined via IHC on endpoint tumor sections and quantitated as mean H ± standard deviation and plotted. * 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[5], we conclude that treatment with oxaliplatin may be a viable option for patients.

References

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