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Sensitization of therapeutic-resistant pancreatic cancer by cancer cell-specific drug delivery

**PRINCIPAL INVESTIGATOR:** Stephen J Pandol, MD

**CONTRACTING ORGANIZATION:** Cedars-Sinai Medical Center, Los Angeles, CA

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**PREPARED FOR:** U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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**14. ABSTRACT**
In the third year of this project, we have completed most of the tasks following the timeline of our Statement of Work, although the COVID-19 pandemic severely affected progress in the second year of the project by interrupting the proposed animal studies. We have partially characterized the two new pancreatic cancer cell lines by verifying their tumorigenicity in athymic mice, and confirmed dose-dependent killing of all the six pancreatic cancer cell lines by HMCD-SIM, all in doses below 12.5 μM. In the first test, HMCD-SIM inhibited UN-KPC-960 intrasplenic tumor growth and prolonged 129 mice host survival. Modified experimental protocol will be used in repeated studies to consolidate effect of the conjugate in immune intact mice. In GASP-1 ELISA assays with a wide spectrum of patient samples, it is revealed that substantial amount of GASP-1 was detected in pancreatitis, indicating that the GASP-1 biomarker lacks tumor cell-specificity. We have now confirmed that HMCD-SIM could indeed kill pancreatic cancer cells in vitro and inhibit pancreatic tumor growth in mouse, and completed a manuscript to report this finding. Additionally, with the support of the ongoing DoD PRCRP TTSA Award, we have identified a new entity from pancreatic cancer patient blood samples.

**15. SUBJECT TERMS**- Pancreatic ductal adenocarcinoma, heptamethine carbocyanine, simvastatin, conjugate, anti-tumor therapy, chemotherapeutic sensitization

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## 1. INTRODUCTION:

This project was based on our previous finding that a specific group of heptamethine carbocyanine dyes (HMCD) has tumor cell-specificity. When it was synthesized as chemical conjugate with simvastatin, the HMCD-SIM became a highly tumor-specific cytocidal agent. In the proposed project, HMCD-SIM will be used as an anti-tumor agent and a sensitizer in the treatment of pancreatic cancers. We hypothesized that the tumor-specific HMCD-SIM targets pancreatic cancer cells through abnormally expressed OATP channel proteins on cancer cell surface. Inside cancer cells, HMCD-SIM is localized in subcellular organelles including mitochondria, where HMCD-SIM impairs mitochondrial integrity to cause organelle leakage, and apoptotic cell death. We proposed to validate HMCD-SIM as a promising new drug for pancreatic ductal adenocarcinoma (PDAC) targeting and therapeutic sensitization; and to determine the mechanisms of HMCD-SIM-mediated cancer cell killing and therapeutic sensitization. Pancreatic cancer cells will be subjected to HMCD-SIM treatment in the presence or absence of other conventional chemotherapeutic agents to evaluate therapeutic efficacy. A series of molecular and cellular studies will be used to elucidate the mechanism of HMCD-SIM action. Xenograft tumor formation, patient derived xenograft tumor formation, and KPC transgenic pancreatic cancer models will be used to validate therapeutic efficacy of the HMCD-SIM.

## 2. KEYWORDS:

Pancreatic ductal adenocarcinoma, heptamethine carbocyanine, simvastatin, conjugate, anti-tumor therapy, chemotherapeutic sensitization

## 3. ACCOMPLISHMENTS:

i. With the support of the ongoing DoD PRCRP TTSA Award, we have now confirmed that HMCD-SIM could indeed kill pancreatic cancer cells in vitro and inhibit pancreatic tumor growth in mouse. These experimental data support our previous hypothesis that HMCD-SIM is a potent new drug for pancreatic cancer treatment. We have now completed a manuscript to report this finding.

ii. With the support of the ongoing DoD PRCRP TTSA Award, we have now identified a new entity from pancreatic cancer patient blood samples. Presence of this aberrant object could be an alternative cause of cancer-associated thrombosis, a therapeutic target to improve cancer patients from thrombosis- induced death. We have now completed a manuscript to report this finding.

### What were the major goals of the project?

Major goals of the project are: 1) To validate HMCD-SIM as a new drug for PDAC targeting and therapeutic sensitization; and 2) To determine the mechanisms of HMCD-SIM for cancer cell kill and sensitization.

**Aim 1:** To validate HMCD-SIM as a promising new drug for PDAC targeting and therapeutic sensitization.

**Major task 1: Determining the therapeutic efficacy of HMCD-SIM.** We have synthesized and purified enough amount of HMCD-SIM conjugate for the entire study in this proposal (months 1 – 2), and we have determined efficacy of the HMCD-SIM on BXPC-3, MIA PaCa-2, UN-KPC-960 and UN-KPC-961

cell lines (months 3 – 6). We now established two new human pancreatic cancer cell lines: CS-P-2 and CS-P-124. All the 6 cell lines have been tested for HMCD-SIM treatment (Figure 1). The results were in good agreement with our previous finding. HMCD-SIM effectively killed these pancreatic cancer cells. IC50 values were around 5 μM after 24 hours of treatment. Mice bearing BXPC-3 and MIA PaCa-2 xenograft tumors have been tested for efficacy on inhibiting tumor formation by HMCD-SIM alone. Preliminary analysis indicated significant inhibition of tumor growth.

We have recently published a paper showing a study using the same KPC pancreatic cancer mouse model we plan to use in our project. The measurements performed in this study include fibrosis, inflammation, epithelial to mesenchymal transition and metastasis, cancer stemness and drug resistance, glucose metabolism and cytokine secretions (Edderkaoui M, et al., Gastroenterology; 2018 Dec;155(6):1985-1998). All these measurements represent key mediators of tumor growth and promotion and we plan to perform most of them in the present study. We have generated enough KPC mice to treat them with HMCD-SIM. KPC mice are now ongoing the treatment for a survival study.

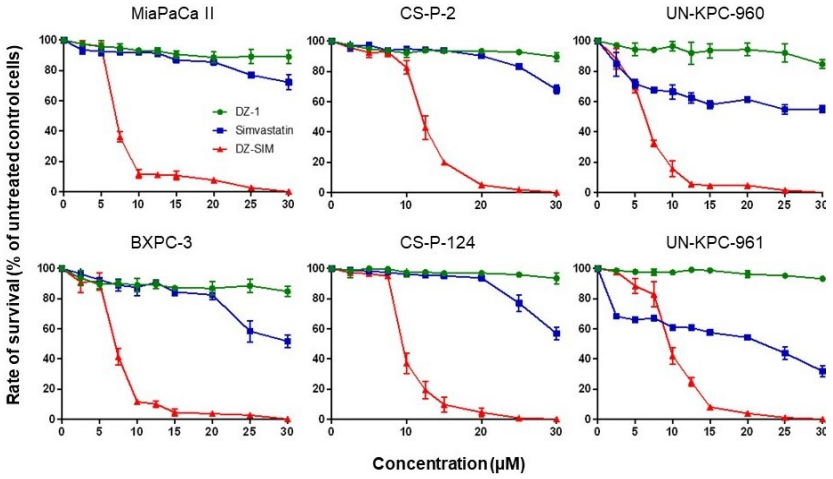


**Figure 1.** Dose-response curves as determined with MTT assay at 24 hours after treatment.

We have now successfully established new pancreatic cancer cell lines from surgical tumor specimens of two patients (months 7 – 18). Eight cell lines (clones CS-P-2-1 to CS-P-2-8) have been established from a single tumor of the first patient. Though these cell lines are mutually divergent in terms of their growth pattern and cellular morphology, all have been found to have tumorigenicity as assayed with xenograft tumor formation. A single cell line from the second patient (CS-P-124) has now been cultured continuously to passage 60. Immortality of the cell line is thus demonstrated. We have determined that like the CS-P-2 cell line, CS-P-124 can also form xenograft tumors. These cell lines have now been tested for short tandem repeat (STR) analysis, which revealed unique DNA fingerprint, conforming that these cells are indeed newly established cell lines of human origin. These two new cell lines have now been tested with HMCD-SIM treatment and therapeutic efficacy assessment has been completed. We are using commercial contract service now for karyotyping analysis to detect aneuploidy.

We have obtained HRPO approval for acquiring fresh patient surgical specimens for ex vivo culture to establish and characterize two new human PDAC cell lines. Surgical tumor specimens will be acquired for tumor tissue culture starting next month (assuming a rate of 1 cell line successfully established from every 6 specimens) (months 7 – 18).

We have obtained ACURO approval for determining anti-tumor efficacy of HMCD-SIM by treating human BXPC- 3, MIA PaCa-2, CS-P-2, CS-P-26 and mouse UN-KPC-960 and UN-KPC-961 xenograft tumors, and spontaneous PDAC tumors in KPC mice (months 7 – 18). We are now testing whether the CS-P-26 cell line is tumorigenic and metastatic with xenograft tumor formation in athymic nude mice.
To establish UN-KPC-960 and UN-KPC-961 tumor models, we found that these cells could not form tumors in C57BL/6 mice after subcutaneous inoculation. We are now doing two experiments: 1) inoculating to nude mice to make sure that these cell lines can form tumors at all after subcutaneous inoculation or pancreatic orthotopic inoculation; and 2) inoculating to 129 mice to make sure that these cell lines can form tumors at all after subcutaneous inoculation or pancreatic orthotopic inoculation. Due to COVID-19 pandemic, we were not able to purchase nude mice for about 2.5 months. We only recently obtained permission from Vivarium for starting to purchase mice for this study.

We have established image-guided approaches to evaluate the cancer specificity of HMCD-SIM and to monitor therapeutic efficacy with near infrared fluorescence (NIRF) and bioluminescence (BL) imaging. We are developing a LC-MS/MS protocol for quantitative analysis of HMCD-SIM for blood clearance and biodistribution studies.

For performing lymphocyte depletion in tumor-bearing mice to investigate the role of host immunity in HMCD- SIM-induced inhibition (months 19 - 24), the proposed study has not been started due to COVID-19 pandemic. KPC mice are being bred for this study.

We now conducted an alternative study, in which syngeneic 129 mice were used as host for intra-splenic inoculation of UN-KPC-960 mouse pancreatic cancer cells ($5\times10^5$ cells/injection). In this study, tumor-bearing mice treated with HMCD-SIM (n = 6) were found with prolonged survival.

In the current reporting quarter, we are performing the third study. Each B6 129 momice are inoculated intrasplenically with $5\times10^5$ cells (n=6). Mice are being treated with HMCD-SIM (10 mg/kg, intraperitoneally, twice a week). After 2 months, all 6 mice in the control group have died of cancer metastasis, whereas, all 6 mice treated with HCMD-SIM were still alive (Fig. 2). These results strongly indicate effectiveness of the HMCD- SIM in inhibiting pancreatic tumor growth and metastasis. This study is now completed. Histopathologic staining is being carried out to confirm tumor metastasis in various mouse host organs.

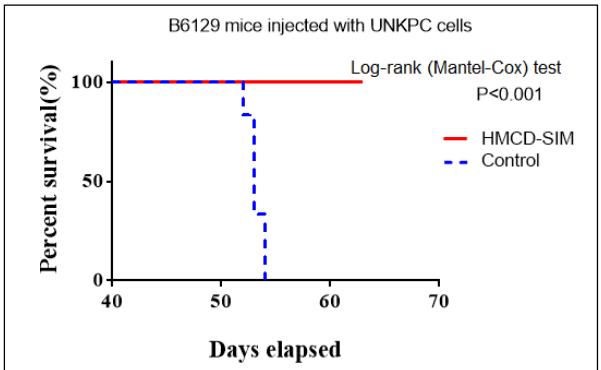

**Figure 2.** HMCD-SIM significantly improve survival in syngeneic mice with pancreatic cancer cells.

To further determine anti-tumor efficacy of HMCD-SIM (months 7 – 18), KPC mice were bred. The first study using low dose of HMCD-Sim (5mg/Kg) did not show any improvement in KPC mice survival. We then increased the dose to 25mg/kg and we have now a significant increase in KPC mice survival in the HMCD-SIM treatment group. The median survival time increased significantly (*p=0.03*) from 115 days in control group to 145 days in DZ-SIM group (Fig. 3).

**Major task 2: Confirming the ability of HMCD-SIM to sensitize chemotherapies.** We have determined IC50 of gemcitabine (GEM), cisplatin (CDDP) and paclitaxel (PTX) on BXPC-3 and MIA PaCa-2 cells, individually and in combination with HMCD-SIM (months 7 – 18). Experiments are currently underway for reduced IC50 of GEM, PTX, CDDP, and mTOR inhibitors in combinatory treatment with HMCD-SIM (months 7 – 18). Control xenograft tumor formation with CS- P-2 cell lines has been completed. Control xenograft tumor formation with CS-P-26 cells will be started in the next month, followed by treating mice bearing BXPC-3, MIA PaCa-2, CS-P-2 and CS-P-26 tumors with GEM in combination with a low dose HMCD-SIM to improve animal survival (months 13 – 24). The study of treating GEM-resistant human MIA PaCa-2 xenograft tumors with HMCD-SIM to exhibit xenograft tumor growth and metastasis to improve animal survival (months 19 – 30) has just been started. Histopathologic analysis and GASP-1 detection on HMCD-SIM-treated xenograft tumors (months 13 – 36) is halted at the moment till the cross-reactivity issue of GASP-1 ELISA in pancreatitis specimens is resolved.

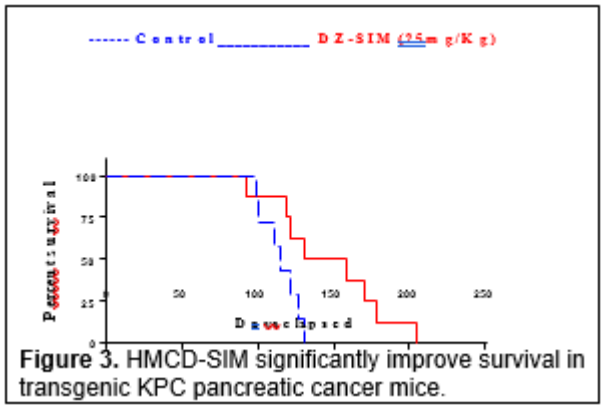

**Figure 3.** HMCD-SIM significantly improve survival in transgenic KPC pancreatic cancer mice.

We conducted a series of analyses to assess whether a low dose HMCD-SIM treatment could make pancreatic cancer cells become sensitive to conventional chemotherapeutic drugs (months 13 – 24). HMCD-SIM at 1 μM, 3 μM and 5 μM did not significantly make pancreatic cancer cells more sensitive to gemcitabine treatment. This study will be repeated to confirm that HMCD-SIM does not sensitize pancreatic cancer cells to other anti-tumor treatments. Through repeated experiments, we noticed a conspicuous issue on time difference between HMCD- SIM-induced cancer death and the death by other anti-tumor treatments. While HMCD-SIM kills cancer cells within 24 hours, the effect of other treatments was much slower. We will repeat the combinatory treatment in the next with extended time (96 hours) and to assess the effect of sensitization.

It turned out difficult to study HMCD-SIM sensitization with cultured cancer cells. The main hurdle is probably the difference in the time of action between this conjugate and chemotherapeutics. Time of action by HMCD- SIM is within 24 hours, while chemotherapeutics such as GEM, CDDP and PTX inhibits cancer cell growth in a much slower pace, beyond 48 hours. We decided to use xenograft tumor models to assess the effect of HMCD- SIM sensitization.

To treat GEM-resistant human MiaPaCa II xenograft tumors with HMCD-SIM to inhibit xenograft tumor growth and metastasis to improve animal survival (months 19 – 30), we conducted two preliminary and pioneer animal studies during the last reporting quarter to assess *in vivo* experimental conditions. In the first study, nude mice (n=3) were inoculated with human pancreatic cancer MiaPaCa II cells ($2\times10^6$/site, subcutaneously) and treated with HMCD-SIM (5 mg/kg, intraperitoneally and twice a week) for 4 weeks. Compared to the controls, tumor size in the treatment group was reduced, although the reduction did not reach statistical significance, most likely due to the small group size. Through this study, we determined that a 10 mg/kg dose was to be used to a group of 10 mice (n=10) in the next study in order to reach statistically significant difference between control and treatment groups.

**Major task 3: Examining serum GASP-1 peptide levels as a PDAC companion biomarker.** In preparation for the current study, we have used legacy samples to optimize an ELISA method for measuring GASP-1 peptide in patient serum samples (months 1 – 12). We have recently tested the hypothesis that GASP-1, a G protein coupled receptors (GPCRs)-associated sorting protein-1, a 156 kDa cytosolic protein, as a serum marker for human bladder cancer. This approach allows us to standardize our technology and protocol. When overexpressed in cancer cells, DASP-1 directs ligand-bound GPCRs to plasma membrane as a signal enhancer promoting cell proliferation. We collected serum samples from 13 healthy donors as control and 30 bladder cancer patients with IRB approval. Serum GASP-1 was assessed by ELISA (Proplex Technologies, Dresher, PA). Data were analyzed with Graph-pad prism 6.0 for statistical clinical correlation. GASP-1 protein was also measured in 8 cultured bladder cancer cell lines with or without Gemcitabine treatment by western blot and 12 archived cancerous and 5 normal bladder tissues by immunohistochemistry. Our results reveal that higher serum GASP-1 expression was found in bladder cancer patients than controls ($p<0.001$). The area under the ROC curve (AUC) for GASP-1 to discriminate bladder cancer from normal was 0.8096 (95% confidence interval [CI], 0.7202 to 0.899; $P<0.0001$). GASP1 expression in stage- Ta, -T1 and -TIS and PUNLMP, low- and high-grade were all higher than controls statistically (all

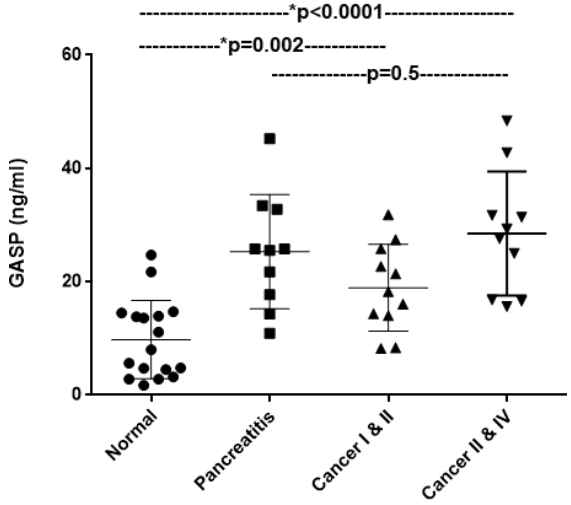


**Figure 2.** The level of GASP-1 in the serum of healthy donor, pancreatitis patients, and patients with pancreatic cancer (stage I and II) and (stage III and IV).

p<0.05). GASP-1 was detected in clinical bladder cancer tissue specimens and cultured bladder cancer cell lines. At tissue level, semi- quantitative IHC expression of cytoplasmic GASP-1 was comparable in normal and cancerous bladder epithelial cells but nuclear membrane GASP-1 expression in the cancer group was significantly higher than control (p=0.0036) also higher in metastatic and Gemcitabine-treated bladder cancer cells. This method will be used for this DoD study using serum samples collected from pancreatic cancer patients, in comparison to specimens collected from normal controls and also from patients with confirmed pancreatitis.

We have now obtained the first 40 plasma samples of pancreatic cancer patients. GASP-1 ELISA of these samples will be performed in the coming month. We are also collecting more patient plasma from clinical pancreatic cancer patients. After testing the plasma samples, however, it was found that only serum samples were suitable for the ELISA platform. Additional patient serum samples have now to be prepared. Alternatively, we are testing whether commercial GASP-1 ELISA kit could be used to detect plasma samples. We have now obtained the first 40 serum samples of pancreatic cancer patients. GASP-1 ELISA of these samples has been completed and the data indicate a significant two-fold increase in the GASP-1 level in the serum of patients with pancreatic cancer (stages I and II) and a 3-fold increase in patients with stages II and IV pancreatic cancer (Figure 2). However, there was no significant difference between pancreatitis and cancer patients. This lack of specificity may prevent development for clinical application.

We have obtained HRPO approval for conducting GASP-1 IHC on 28 tumor specimens and statistical correlation with PDAC progression and metastasis (months 7 – 33). In primary tests, tumor sections from 4 PDAC cases were tested for GASP-1 level, indicating that the detection protocol was appropriate. Interpretation of the results is halted now until the cross-reactivity issue of GASP-1 ELISA in pancreatitis specimens is resolved.

**Aim 2:** To determine the mechanisms of HMCD-SIM for cancer cell kill and sensitization.

**Major task 4: Examining the toxic effect of HMCD-SIM conjugate.** We have documented impaired mitochondrial function using 3 methods: 1) immunohistochemical staining for cytochrome C release from mitochondria to cytosol following HMCD-SIM treatment; 2) Quantitative flow cytometry for reduced mitochondrial membrane potential with HMCD-SIM-treated cancer cells whose mitochondrial were pre-loaded with either rhodamine 123 of JC1 (months 7 – 18). The same methods will be used to examine toxic effect on UN-KPC- 960, UN-KPC-961 and the two newly established PDAC cell lines, which will be analyzed in the next month (months 7 – 18).

We recently determined that the cancer cell death caused by HMCD-SIM could be alleviated by Mdivi-1, an inhibitor for mitochondrial division (mitochondrial fission). In the presence of Mdivi-1, MiaPaCa II and BXPC-3 cells became resistant to HMCD-SIM-induced death. We will generalize this finding with CS-P-2, CS-P-124, UN- KPC-960 and UN-KPC-961 cells to affirm that mitochondrial dynamics of fusion and fission play a critical role in cancer cell survival.

**Major task 5: Assessing the mechanism of HMCD-SIM-mediated sensitization.** We are currently locating an isolated cell culture incubator for culture cells with radioactive gemcitabine-$^{13}C$-$^{15}N2$ (months 7 – 18). For mutagenesis studies, we determined to obtain a human full length OATP1B3 cDNA clone from commercial sources. The mutagenesis work will be started as soon as COVID-19 pandemic recedes (months 13 – 18). The study of the mechanism of HMCD-SIM for promoting cancer cell entry of the conventional chemotherapeutic drugs, using the 6 HMCD-SIM treated human PDAC cell lines (19 – 24) is under way.

We noticed in the literature reporting that HMCD forms adduct with albumin (Osama *et al*., 2018, 2020), suggesting that OATP may not be the only thoroughfare for tumor cell uptake. We initiated a project assessing the role of albumin in HMCD-SIM uptake, by comparing adduct protein formation between HMCD and HMCD-SIM groups. Our results thus far suggest that HMCD binds with certain proteins in cell culture (Figure 3). When whole cell lysates were analyzed on SDS-PAGE, a protein adduct could be seen with a molecular weight around 60 kD. Interestingly, this analysis revealed that HMCD-SIM may affect additional proteins (Figure 3), as several additional bands became visible. These results suggest that, different from its precursor HMCD, the conjugate of HMCD-SIM may form adducts with other cellular proteins to compromise their biological function. Protein adducts formation may be an explanation for cytotoxicity of the HMCD-SIM. Identification of these proteins may elucidate the mechanism of HMCD-SIM-induced cancer cell death.

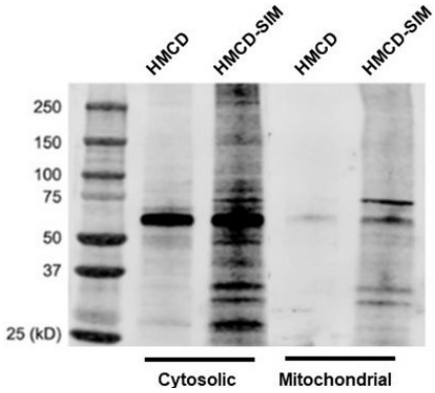


**Figure 3.** Differential protein adduct formation between HMCD and HMCD-SIM. In this study, cancer cells were treated with HMCD or HMCD-SIM. Whole cell lysates and mitochondrial lysates were fractionated on SDS-PAGE and subjected to near infrared detection of adduct proteins.

In this reporting quarter, we started to isolate the bands revealed in the SDS-PAGE by cutting out the gel pieces for proteomic identification of the adduct protein by mass spectroscopy.

**Major task 6: Investigating HMCD-SIM-induced cholesterol loss and shh signaling inhibition.** We have confirmed that HMCD-SIM inhibits cholesterol level significantly in treated cancer cells, with more prominent effect on cholesterol level of the mitochondria (months 7 – 12). A luc reporter driven by a Smo promoter will be transfected to BXPC-3 and MiaPaC2 cells starting in the next month. This work is currently under way (months 13 – 18). The study for altered cancer-stromal interaction in HMCD-SIM-treated human MIA PaCa-2 and BxPC- 3 PDAC cells under 2-D and 3-D con-culture conditions is under way (19 – 24).

We have obtained CS-P-2 xenograft tumor specimens by inoculating the cells to SCID mice for 2 months. Following inoculation with our protocol, CS-P-2 cells yielded 100% tumor formation. These tumors are fixed in formalin. Part of the tumor tissue was embedded and sectioned for histopathology assessment.

## Human Use Regulatory Protocols

**TOTAL PROTOCOLS:** 2

**SUBMITTED TO AND APPROVED BY:**

- Cedars-Sinai Medical Center IRB

**PROTOCOL (1 of 2):**

Protocol [HRPO Assigned Number]: E00502.1a

Title: Sensitization of therapeutic resistant pancreatic cancer by cancer cell-specific drug delivery- biomarkers.
Local Approval: 23-JAN-2019
Continuing Review: Pending

**PROTOCOL (2 of 2):**

Protocol [HRPO Assigned Number]: E00502.2a

Title: Sensitization of therapeutic resistant pancreatic cancer by cancer cell-specific drug delivery- tumor tissue.

Local Approval: 18-JAN-2019

Continuing Review: Pending

## Animal Use Regulatory Protocols TOTAL PROTOCOL(S):

**TOTAL PROTOCOL(S):** 2

**SUBMITTED TO AND APPROVED BY:**
Cedars-Sinai Medical Center IACUC.

**PROTOCOL 1 of 1):**

Protocol [ACURO Assigned Number]: CA170974.e001

Title: Sensitization of therapeutic-resistant pancreatic cancer by cancer cell-specific drug delivery.

Local Approval: 01-MAY-2018
De Novo Renewal: 01-MAY-2021

**What do you plan to do during the next reporting period to accomplish the goals and objectives?**

1. To continue to finish subtasks in chronological order as planned in SOW.

2. To get our ACURO approval for animal studies.

3. To get our HRPO approval for using clinical specimens in the proposed study.

4. We will invite Dr. Haiyen E. Zhau as a co-Investigator (10% effort) starting April 1, 2019. Her expertise in biomarker validation is sought for critically validating specific proteolytic peptide of GASP-1 in patient blood as an early sign of PDAC development, progression and metastasis.

## 4. IMPACT

**What was the impact on the development of the principal discipline(s) of the project?**

In the first year of this three-year project, we have completed tasks following the timeline of our Statement of Work. These works laid a solid foundation for second year mechanistic investigation of the molecular mechanism of HMCD-SIM mediated pancreatic cancer cell killing.

In the second year of this project, we have validated the efficacy of HMCD-SIM on all the six representative pancreatic cancer cell lines. Studies on the role of HMCD-SIM in sensitizing cancer cells to conventional therapy are under way both *in vitro* with cancer cell lines and *in vivo* with xenograft tumors and transgenic mouse spontaneous pancreatic cancer models.

In the third year of this three-year project, we have completed most of the tasks following the timeline of our Statement of Work. Although the COVID-19 pandemic has severely affected progress of the project during this year by interrupting some of the proposed studies, we are excited to report our studies performed confirms that HMCD-SIM demonstrates cytotoxic activity in pancreatic cancer cells in vitro and furthermore demonstrates tumor growth inhibition in pancreatic cancer xenografts. These experimental data obtained strongly support our main hypothesis that HMCD-SIM is a potential potent new anti-tumor agent drug for pancreatic cancer. This was our main goal of our third year of experimentation.

**What was the impact on other disciplines?**

Through our studies, HMCD-SIM is determined to have a cytotoxic effect on pancreatic cancer cells. These results support broader hypothesis that HMCD-SIM may be cytotoxic to other human cancer cells. At the same time when this project is going on, our colleagues have tested HMCD-SIM on prostate and lung cancer cell lines. As anticipated, HMCD-SIM was found to induce rapid death of these cancer cells.

**What was the impact on technology transfer?**

Nothing to report.

**What was the impact on society beyond science and technology?**

Nothing to report.

## 5. CHANGES/PROBLEMS

### Changes in approach and reasons for change

** The pandemic severely impacted our ability to carry on animal studies as well as other studies secondary to university/lab closure/work from home policies that were implemented throughout various times during the year in the name of public health & safety. Additionally, the illness and passing of the pioneering PI, Dr. Chung, this past year also caused a delay as would be expected.

As a result, a No Cost Extension (NCE) was applied for and granted.

### Actual or anticipated problems or delays and actions or plans to resolve them

During the high COVID-19 pandemic season, vivarium in our institution was shut down for almost three months. Laboratory research of the project was also delayed because of the pandemic. A one-year NCE for the project was applied for and granted.

### Changes that had a significant impact on expenditures

Nothing to report.

### Significant changes in use or care of vertebrate animals

Nothing to report.

### Significant changes in use of biohazards and/or select agents

Nothing to report.

## 6. PRODUCTS

### Publications, conference papers, and presentations
### Journal publications

Nothing to report

### Books or other non-periodical, one-time publications

Nothing to report

### Other publications, conference papers and presentations

Development of agents targeting membrane cholesterol and mitochondria to accelerate pancreatic cancer cell kill. Accelerating the pipeline for improving pancreatic cancer, Translational Symposium, Digestive Disease Week (DDW) 2019, San Diego Convention Center, 33ABC, May 18. 2019

### Website(s) or other Internet site(s)

Nothing to report.

### Technologies or techniques

Nothing to report.

**Inventions, patent applications, and /or licenses**

Nothing to report.

**Other products**

Nothing to report.

## 7. Participants & Other Collaborating Organizations

| | | |
|---|---|---|
| Leland W. K. Chung (Initiating PI) | Experimental design | 6 months |
| Yi Zhang | HMCD-SIM synthesis and purification | 2 months |
| Chia-Yi Chu | Cell culture and cytotoxicity testing | 2 month |
| Ruoxiang Wang | Culture of patient samples | 4 months |
| Haiyen E. Zhau | GASP-1 ELISA development and evaluation | 2 months |
| Stephen Pandol (Partnering PI) | Patient recruitment and protocol preparation | 1 month |
| Edderkaoui Mouad | Animal breeding | 1 month |
| Nicholas Nissen | Patient recruitment | 6 months |
| Andrew Hendifar | Patient recruitment | 2 month |
| James Tomlinson | Patient recruitment and protocol preparation | 2 month |
| Michael Lewis | GASP-1 biomarker evaluation | 2 month |

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Gina Chia-Yi Chu has left for another job opportunity.

**What other organizations were involved as partners?**

Nothing to report

## 8. Special Reporting Requirements:

Nothing to report.