ASRA Carl Koller Memorial Research Award titled: **"Lidocaine Infusions in Pancreatic Cancer: Translational Studies in a Preclinical Model and Human Subjects"**

Major Activities and Specific Objectives:

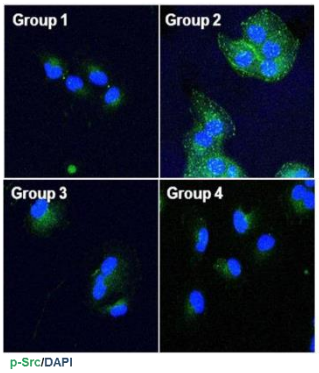
Pancreatic cancer (PDAC) is the fourth leading cause of cancer death and expected to be second most lethal cancer by 2030. The 5-year survival rate of less than 9% and a median survival of less than 6 months after diagnosis, places PDAC in one of the most fatal disease. In 2010, there were an estimated 43,140 new cases, of which 36,800 mortality attributed to the disease, over the same period in USA. Despite the progress made in understanding pancreatic tumor biology, considerable challenges still remain for PDAC treatment and novel therapeutic interventions are urgently needed for this clinically difficult to manage disease with very high mortality. The standard treatment for localized PDAC is pancreatectomy, however majority of the patients present metastatic disease and surgery is not an option for these patients. Therefore, there is a need to identify alternative means to treat PDAC patients, specially presenting a metastatic disease. The circulating tumor cells (CTC) are reported to be in high numbers in circulation of PDAC patients and therefore there is a valid concern that surgery alone might enrich the micro metastatic process in the immediate post-operative period.

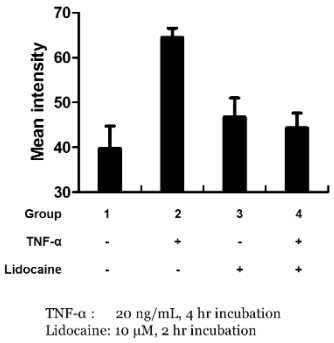
The overall objective of our funded ASRA Carl Koller Memorial Research proposal is to elucidate the action of lidocaine in regulating Src activity and associated pathways in CTCs. We expect that by downregulating the pathways by lidocaine might control CTCs numbers and would benefit the patients with PDAC, either localized or metastatic disease. To address our hypothesis, we proposed three aims:

Specific aim 1. To elucidate lidocaine infusion efficacy for suppression of Src in preclinical pancreatic cancer model with and without survival surgery.

Specific aim 2. To determine the effect of IV lidocaine infusion on Src tyrosine kinase activity in isolated CTCs, as well as the number of CTCs during the perioperative period in patients undergoing pancreatectomy for pancreatic cancer.

Specific aim 3. To determine the global gene expression in human CTCs from pancreatic cancer patients with and without lidocaine infusion.





In the past one year, we made major accomplishments to address aim1 and aim2. However, our main focus was to establish the preclinical and clinical role of lidocaine on Src activity in CTCs. The outcomes of pre-clinical mouse model of pancreatic

Fig. 1: In vitro effect of lidocaine in Panc-1 cells.

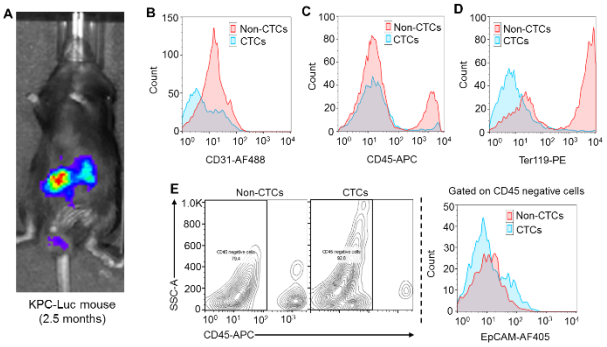


Fig. 2: Characterization of CTCs isolated from KPC mice.

ductal adenocarcinoma (PDAC) and clinical study have been very promising. Below we present only key findings in brief:

Specific aim 1. To elucidate lidocaine infusion efficacy for suppression of Src in preclinical pancreatic cancer model with and without survival surgery. The overall goal of this aim is to delineate the action of lidocaine in pancreatic cancer cell lines and in vivo model using genetically engineered KPC mice model of PDA. As a proof of concept we have examined the effect of lidocaine on phospho-Src activity *in vitro* using Panc-1 cells. Our data shows that lidocaine at the clinically relevant concentration of 10 μM inhibited TNF-α-induced Src activation, at a single cell level **(Fig. 1).** After in vitro study, we standardized protocol for the isolation of CTCs from mouse model of PDAC (i.e. KPC) The CTCs were isolated from a 3.5 month old KPC mouse using tumor cell isolation kit and LS column by QuadroMACS system (all products from Miltenyi Biotec). The purified CTCs were stained with CD31, CD45, Ter119 and EpCAM and analyzed by flow cytometry. Results suggest that CTCs cells were low in CD31, CD45 and Ter119, while expression of

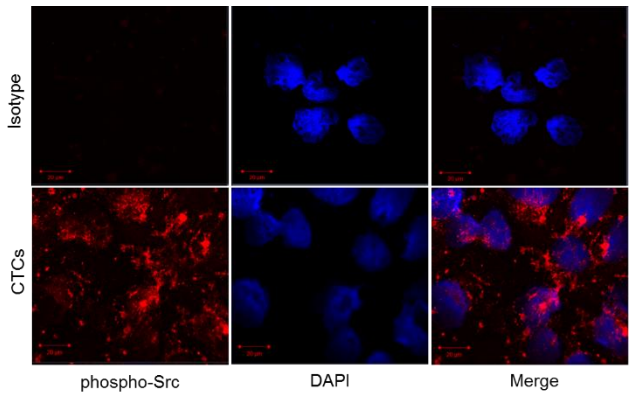


Fig. 3: Src activity in CTCs isolated from KPC mice.

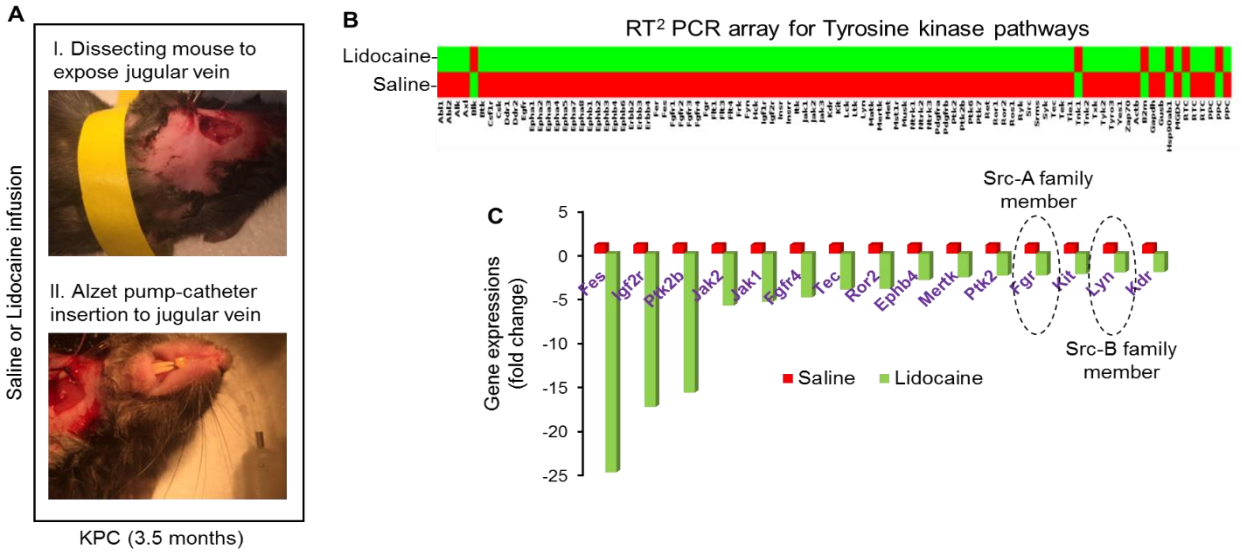


Fig. 4: (A) Procedure of saline or lidocaine infusion in KPC mice. (B) RT2 PCR array for Tyrosine kinase gene expressions in CTCs.

EpCAM were high **(Fig. 2).** We also determined expression of phospho-Src in isolated CTCs and noticed that CTCs were loaded with active Src **(Fig. 3).** Further, we established a method of lidocaine or saline infusion in jugular vein of KPC mice using Alzet pump and catheter **(Fig. 4A)**. Following 24 h infusion of saline or lidocaine, KPC mice were sacrificed and CTCs were isolated and used to determine impact of lidocaine on gene expressions, associated with Tyrosine kinases including Src A and Src B pathways by RT^2 PCR array. Result showed down-regulation of Src family members and interestingly, lidocaine infusion also significantly down regulated other tyrosine kinase members: Fes, Igf2r, Ptk2b and Jak2 **(Fig. 4B)**. We are in the process to repeat these experiments in various conditions in coming days.

Specific aim 2. To determine the effect of IV lidocaine infusion on Src tyrosine kinase activity in isolated CTCs, as well as the number of CTCs during the perioperative period in patients undergoing robotic pancreatectomy for pancreatic cancer. Original IRB approval was obtained on 06/10/18 for 46 subjects (23 in each group) to be recruited in this double blinded case control study. The authorization to use hospital and/or clinic services was obtained on 08/30/18. Starting in November of 2018, five subjects, who met inclusion criteria were successfully recruited. (See demographics table). We were able to conclude the study of one subject. For the other 4 subjects the studies were aborted, since upon the initial surgical exploration it was discovered that 3 of 5 subjects had metastatic disease and for 1 of 5 subjects the robotic procedure was converted to open. These studies were aborted since the subjects no longer met inclusion criteria. During the progression of the study, the protocol was modified in order to expand the inclusion criteria to successfully complete the study. An IRB amendment was submitted (approved 1/6/19) to include short form Spanish consent to recruit Spanish speaking subjects. Another IRB amendment was submitted (approved 3/20/19) in order to expand the inclusion criteria and include cases that were found to be metastatic upon initiation of the surgical procedure and cases that were converted from robotic to open surgeries.

Table 1: Demographics:

Number	Status	Age	Gender	Race	Ethnicity	Note
1	Aborted	56	M	White/Caucasian	Non-Hispanic/ Latino	Metastasis
2	Aborted	58	M	White/Caucasian	Non-Hispanic/ Latino	Metastasis
3	Aborted	62	M	White/Caucasian	Non-Hispanic/ Latino	Metastasis
4	Completed	75	M	White/Other	Hispanic/ Latino	
5	Aborted	61	M	Black/African American	Non-Hispanic/ Latino	Open surgery

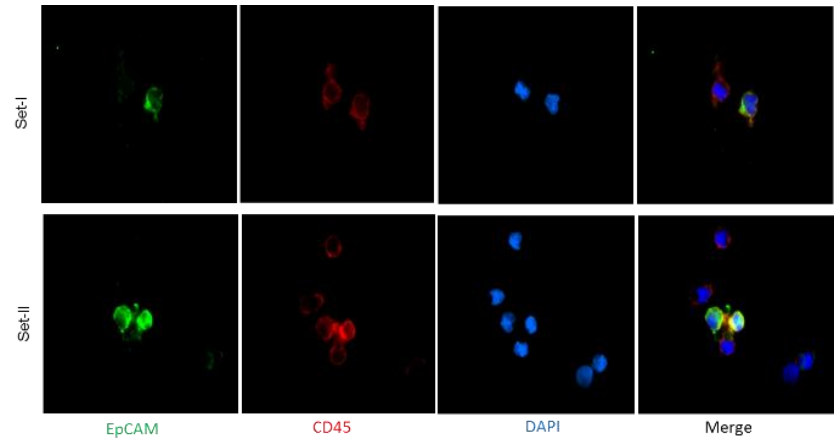


Fig. 5: Characterization of CTCs isolated from patient blood.

We standardized protocol for the enrichment of CTCs from human patients. The CTCs were characterized by using CD45 – ve and EpCAM +ve staining **(Fig. 5)**. The effect of lidocaine treatment on Src activity of human CTCs was determined by treating isolated CTCs with either PBS (pH 7.4) or lidocaine for 30 min. Immunofluorescence staining results showed a robust decrease in Src activity of CTCs **(Fig. 6).** From double blind human study we isolated CTCs during perioperative procedure and studied Src activity. Result showed a decrease in Src activity upon infusion (Placebo??? vs. Lidocaine????) **(Fig. 7).** By broadening our inclusion criteria and include patients with metastatic disease/PDAC, Endocrine/PDAC we expect to recruit the number of patients required to demonstrate the effects of lidocaine infusion on the Src activity of the CTCs.

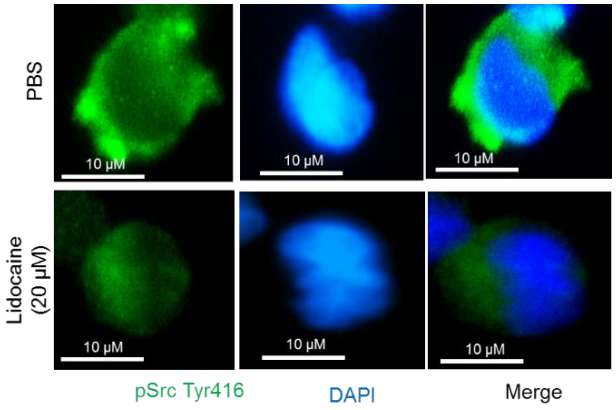


Fig. 6: Src activity in CTCs isolated from patients and treated with lidocaine or PBS. (ex-vivo)

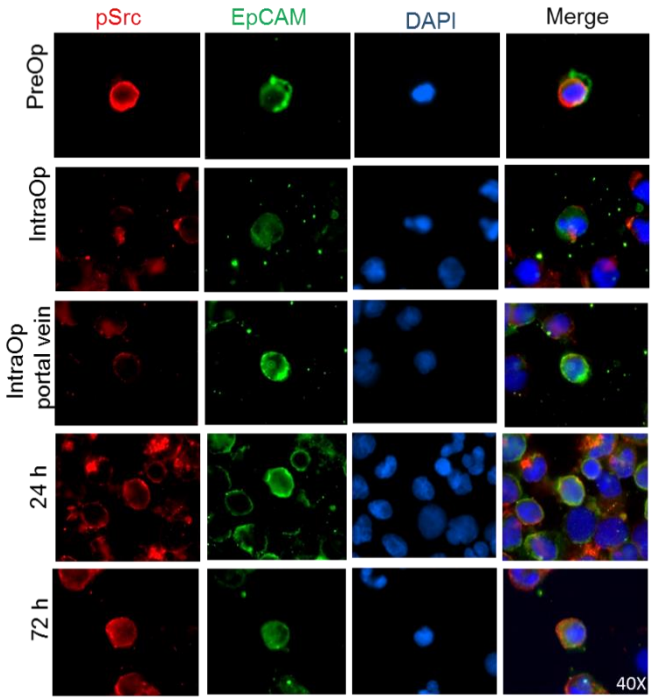


Fig. 7: Src activity in CTCs isolated from patients during perioperative procedure.

Budget summary

Please see the attached file showing the ASRA grant year 1 budget (column B, with column A representing each category of budget/expense) and year 1 expenses (column C). The remaining balance for year 1 (column D) is the result of subtracting the year 1 expenses from the year 1 budget with a remaining balance in year 1 of $68,068.27. In column E you can find the year 2

budget and column F represents the overall remaining balance of this grant for each category with an overall balance $167,761.27 In addition, $100,000 for this project was received from a private donor over the 3-year period. $20,000 was already received for our ongoing project expenses.