Interception of pancreatic cancer metastasis by targeting tissue wound repair

In order to maintain a safe work environment during the COVID-19 pandemic, the Salk Institute has continued to work at partial capacity since March 2020. While this delayed the initiation of these studies due to restricted mouse ordering, we were able to acquire mice starting in August and have the first round of chemotherapies underway. Below is a summary of progress in the last 5 months.

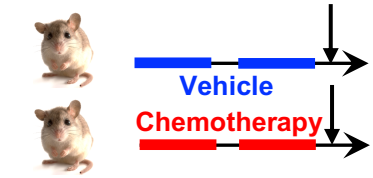

Figure 1. Experimental Schematic. Non-tumor bearing C57Bl/6J adult mice are treated with vehicle (blue) or chemotherapy (red) i.p. 3x weekly. Treatment is given for 4 weeks, stopped for 2 weeks, and given for 4 more weeks. 5 days after the final dose, mice are either euthanized for tissue collection or transplanted with oxaliplatin-treated PDAC organoids via portal vein injection.

Aim 1: Determine to what extent chronic chemotherapy impacts pancreatic cancer metastasis to the liver. Nearly all pancreatic cancer patients receive chemotherapies such as Gemcitabine, Fluorouracil, Irinotecan, and Oxaliplatin. Some of these agents can cause liver damage referred to as CASH (chemotherapy-associated steatohepatitis). It is unknown how CASH impacts metastatic progression in the liver. In this aim, I will determine which chemotherapies can increase risk of liver metastasis by transplanting chemotherapy-treated organoids by portal vein injection into recipient mice that have been chronically treated with either chemotherapy or vehicle (**Fig 1**). The only variable that could alter metastatic progression is the chemotherapy being tested.

Table 1 summarizes the progress made thus far for each chemotherapy. For these studies, I utilize an organoid line established from an orthotopic KPC pancreas tumor. At passage 8, I determined the IC-50 of each agent by Cell Titer-Glo 3D assay (72-hour treatment, n=2 biological replicates, performed in triplicate). Prior to transplantation, organoids were treated with the IC-50 dose for each respective agent for 72 hours (**Table 1**). Organoids were then harvested and partially dissociated into cell clusters that closely resemble circulating cell clusters observed in patients.

Table 1	**In vitro**	**In vivo**	**# mice transplanted**	
Chemotherapy	**IC50**	**Dose**	**Vehicle-treated**	**Chemo-treated**
Oxaliplatin	78.9 uM	3 mg/kg	8	5
5-FU	4.94 uM	20 mg/kg	10	12
Gemcitabine	56.5 nM	120 mg/kg	9	9
Irinotecan	12.1 nM	30 mg/kg	10	11

In vivo chemotherapy doses were selected based on publications in pancreatic cancer mouse models as well as studies looking at maximum tolerated doses. However, long-term chronic dosing is rarely studied, so we were unable to anticipate tolerability over a 10-week period (**Fig 1**). We tracked body weight weekly and performed complete blood counts 1 week prior to transplantation (**Fig 2**). Mice were excluded if they lost >20% of their body weight or displayed overt signs of illness. While 5-FU and Irinotecan were well tolerated, 7 Oxaliplatin-treated and 4 Gemcitabine-treated mice died or had to be excluded. Those mice that did survive treatment gained significantly less weight and had ~75% reduction in circulating white blood cells as compared to vehicle-treated control mice (**Fig 2**). <u>For these reasons, I am repeating the Oxaliplatin and Gemcitabine studies at 50% dose.</u> Of note, oxaliplatin-treated mice had a significantly greater loss in circulating lymphocytes while gemcitabine-treated mice had a proportionate loss in neutrophils, lymphocytes, monocytes, eosinophils, and basophils (data not shown). Similarly, the smaller loss in white blood cells observed in 5-FU and Irinotecan-treated mice are also due to proportionate loss of all cell types (data not shown).

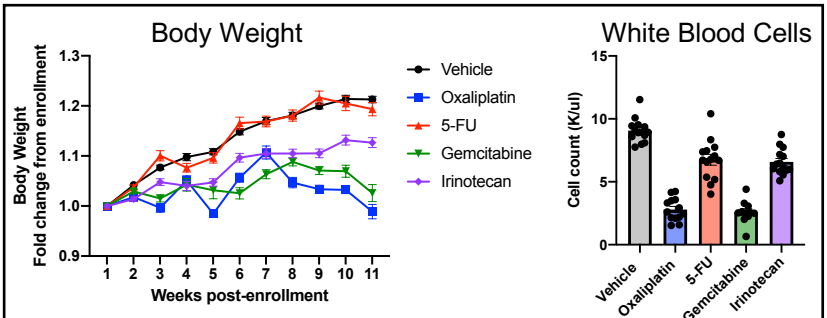

Figure 2. Impact of chronic chemotherapy treatment on body weight (left) and circulating white blood cell count (right) prior to organoid transplantation. Regimen and doses are described in Figure 1 and Table 1.

Study Continuation: I expect the survival data from the first round of transplanted mice to be completed by February. The second round of oxaliplatin and gemcitabine mice (with reduced dose) will be transplanted in early February. I will then assess level of liver damage for each treatment and analyze changes in secreted liver proteins, with focus on treatments that have the greatest impact on metastasis/survival. Due to the immunosuppression observed in all 4 agents, I have also started a small cohort of mice that will be analyzed for liver-resident immune populations as a collaboration with Dr. Susan Kaech's lab at Salk Institute.