

Resident Research Day

June 10, 2021
7:00 - 8:30 am

Location: Virtual Zoom Meeting Room

Moderator: Hien Dang, PhD



Presented by the
Department of Surgery
Division of Surgical Research

Itinerary

- 7:00 – 7:03 Opening Remarks, Charles J. Yeo, MD
Samuel D. Gross Professor and Chair of Surgery
Senior Vice President and Chair, Enterprise Surgery
- 7:03 – 7:10 Primary Non-Function Following Liver Transplant: Optimizing Retransplantation
Strategies through Recipient Characterization by **Peter Altshuler, MD**
- 7:10 – 7:13 Discussion
- 7:13 – 7:20 Determining the Effect of Mesh Width and Fixation Patterns on the Strength of
Prophylactically Reinforced Laparotomy Incisions by **Adrienne Christopher, MD**
- 7:20 – 7:23 Discussion
- 7:23 – 7:30 GUCY2C as a Stem Cell Marker and Target in Gastrointestinal Malignancies by
Madison Crutcher, MD
- 7:30 – 7:33 Discussion
- 7:33 – 7:40 Evaluating the Risk of Peri-Umbilical Hernia after Sutured or Sutureless Gastroschisis
Closure by **James Fraser, MD**
- 7:40 – 7:43 Discussion
- 7:43 – 7:50 SARS-CoV-2 Messenger RNA Vaccine Antibody Response and Reactogenicity in Heart
and Lung Transplant Recipients by **Andrew Hallet, MD**
- 7:50 – 7:53 Discussion
- 7:53 – 8:00 Clinical Significance of a Negative Elongation Factor-E Single Nucleotide
Polymorphism in Hepatocellular Carcinoma by **Ryan Lamm, MD**
- 8:00 – 8:03 Discussion
- 8:03 – 8:10 Above the Cuff Vocalization for Ventilator-Dependent Patients with Tracheostomies by
Lindsay Lynch, MD
- 8:10 – 8:13 Discussion
- 8:13 – 8:20 Employing a CRISPR-CAS9 Screen to Identify Mechanisms of Resistance to Anti-GPC3
CAR T Cell Therapy In HCC by **Keyur Patel, MD**
- 8:20 – 8:23 Discussion

Research Abstracts



Peter Altshuler, MD

Mentors:

Adam Bodzin, MD (Surgery, Thomas Jefferson University),

Pavan Atluri, MD (Surgery, University of Pennsylvania),

Walter Kraft, MD (Pharmacology & Experimental Therapeutics, Thomas Jefferson University)

Awards:

- NIH/NIGMS T32 Clinical Pharmacology Postdoctoral Training Program
- AHA CVSA Vivien Thomas Early Career Investigator Award

Primary Non-Function Following Liver Transplant: Optimizing Retransplantation Strategies through Recipient Characterization

Background: Primary non-function (PNF) occurs in 1 to 8% of deceased donor liver transplants and carries a mortality approaching 50% at 90 days. Retransplantation for PNF improves recipient survival but mortality remains high and additionally risks the potential loss of second allograft. While donor and allograft factors correlated with PNF have been well described, recipient ability to tolerate PNF has not been well defined. Here we uniquely identify pre-transplant recipient characteristics associated with survival following PNF to help guide rescue retransplantation strategies.

Methods: Retrospective review of the United Network for Organ Sharing Organ Procurement and Transplantation Network database from 2002-2020 identified 2,718 adult (≥ 18 year old) liver transplant recipients meeting criteria for PNF who survived beyond post-operative day 0. Patients were stratified by retransplantation (n=936) vs. no retransplantation (n=1,782). Pre-transplant recipient factors predicting mortality were identified in each strata through separate backward stepwise regression models. To better define profiles of recipients with higher likelihood of surviving PNF without retransplantation, a 'PNF Outcome Score' (POS) was derived from the relative sizes of Cox proportional hazard regression coefficients (log-hazard ratios [HRs]) in the group not retransplanted.

Results: One-year survival from index transplant was 37.3% for all patients with post-transplant PNF. Of those retransplanted, 48.9% survived 1 year while 31.9% survived without retransplant (log-rank $p < 0.01$). Donor quality as determined by the Liver Donor Risk Index was similar irrespective of post-transplant survival in both groups. Recipient factors predictive of mortality in the retransplant group included: initial Status 1A listing (HR: 1.65, 95% CI: 1.24-2.20), functional disability requiring significant assistance with activities of daily living (HR: 1.42, 95% CI: 1.07-1.89), ICU disposition at time of transplant (HR: 1.45, 95% CI: 1.05-1.99), pre-transplant dialysis (HR: 1.33, 95% CI: 1.05-1.68), pre-transplant portal vein thrombosis (HR: 1.48, 95% CI: 1.11-1.96) and re-transplantation beyond 10 days from index transplant (HR: 1.32, 95% CI: 1.01-1.74). The POS, derived from recipient factors predictive of mortality in the non-retransplant group, demonstrated an AUC of 0.72. Points were assigned for the following recipient characteristics: Age (55-65: 2.8 points / 65+: 4.8), sex (male: 1.0), diagnosis (NASH: 3.5 / acute hepatic necrosis: 1.4), MELD (40+: 1.5), Status 1A (3.9), functional disability (2.4), ICU (3.1), portal vein thrombosis (3.5) and multiorgan transplant (2.7). Patient survival was incrementally and significantly decreased in each successive POS quartile in the non-retransplant group.

Conclusions: While donor factors contribute significantly to PNF, ability to rescue remains largely dependent on recipient profiles. Early retransplantation provides the greatest probability of survival, however organ shortages remain. Allograft optimization is critical, and the POS may be used to help guide pursuing rescue retransplantation.



Adrienne Christopher, MD

Mentor:

John P Fischer, MD (Plastic Surgery, University of Pennsylvania)

Awards:

- Center for Human Appearance Grant Diastasis-Q

Determining the Effect of Mesh Width and Fixation Patterns on the Strength of Prophylactically Reinforced Laparotomy Incisions

Background: The rate of incisional hernia (IH) continues to affect as many as 1 in 5 patients after midline laparotomy. Recent literature has supported that prophylactic mesh placement (PMP) during an index abdominal procedure may reduce the development of postoperative IH by shielding the fascia from excessive tension. In this study, we compare the failure loads and biomechanical stiffness of 35 porcine abdominal wall laparotomy incisions reinforced with suture and/or meshes of various widths and fixation distances. Specimens were tested with a ball-burst apparatus, the Instron Electropulse e3000, in order to simulate postoperative multi-axial abdominal wall forces.

Methods: Porcine abdominal wall specimens were dissected free of skin and excess adipose tissue, leaving only the rectus muscle and rectus sheaths. Ten-centimeter (cm) incisions were made and subsequently closed in a standard fashion using continuous 1-0 Maxon suture. Specimens were randomized to tack separation distance (1.5cm or 2cm apart), and mesh width (none, 2.5cm, 3cm, 4cm, 6cm, or 8cm). Bard Phasix mesh was secured to the specimens in an onlay fashion using the Bard Optifix tacker. Baseline failure load (410N) was established using two suture-only specimens, and utilized to calculate the oscillating cyclic forces (15N-140N) to apply to the remaining specimens for 500 cycles at a rate of 1 Hertz. After cyclic testing to stress the incision, the mesh was removed and the specimens loaded to failure. The peak of the load vs force curve was used to determine failure load (N, Newtons), and the slope of the load versus force curve used to determine the biomechanical stiffness (N/mm). Failure load and stiffness were comparatively analyzed between specimens based on mesh width and tacker distance.

Results: Each specimen failed via suture pull-through during failure load testing. The average failure load was lowest in specimens with no mesh reinforcement ($421.43 \text{ N} \pm 117.83 \text{ N}$). In both 7-tack and 9-tack fixated mesh groups, the failure load was highest for specimens reinforced with a 4cm wide mesh ($557.03 \text{ N} \pm 156.54 \text{ N}$ and $577.98 \pm 86.40 \text{ N}$, respectively). Failure loads of specimens re-enforced with 4cm mesh were significantly higher than no mesh ($p=0.04$), 2.5cm mesh ($p=0.04$), and 3.0cm mesh ($p=0.04$). Failure loads in specimens with 9 tacks per side were consistently higher than those specimens with only 7 tacks per side, although these did not reach statistical significance. Biomechanical stiffness was highest in specimens re-enforced with 4cm meshes (21.85 N/mm), and significantly higher than specimens with no mesh ($p=0.003$), 2.5cm mesh ($p=0.01$), and 3.0cm mesh ($p=0.02$).

Conclusions: Mesh re-enforcement is superior to suture only closure at preserving strength in a laparotomy closure in the early stages of healing. Meshes 4cm in size are superior to smaller mesh widths in terms of withstanding cyclic stress to the abdominal wall, but larger meshes (6cm and 8cm) do not provide additional benefit. Meshes with more fixation points may provide further benefit, but additional data is needed to make definitive conclusions.



Madison Crutcher, MD

Mentor:

Scott Waldman, MD/PhD (Biochemistry and Molecular Pharmacology, Thomas Jefferson University)

Awards:

- NIH/NIGMS T32 Clinical Pharmacology Postdoctoral Training Program

GUCY2C as a Stem Cell Marker and Target in Gastrointestinal Malignancies

Background: Gastrointestinal malignancies account for a quarter of global cancer incidence and 35% of all cancer-related deaths. While treatments for localized cancer are well established with generally favorable 5 year relative survival rates, overall survival for all combined SEER stages are decreased due to poor control in malignancies with regional spread and distant disease. In that context, cancer stem cells directly contribute to tumor metastasis and treatment resistance, eluding conventional therapies. Guanyl cyclase C (GUCY2C), a transmembrane protein selectively expressed by normal intestinal epithelial cells, has emerged as a tumor suppressor in colorectal cancer. Compartmentalization of GUCY2C expression in normal intestine, confined to the lumen, but its expression by metastatic tumors emerging from the colorectum, stomach, esophagus and pancreas, offers a unique target for directed therapies that could eliminate tumor stem cells, providing durable cures for disseminated disease.

Methods: Gastric cancer specimens from the National Cancer Institute were obtained and propagated in immunodeficient mice. Tumors were harvested from these mice and used to produce stem cell-containing spheroids on low attachment plates. Spheroids were harvested and stem cell markers were quantified using RT-PCR. A sample of the same tumor also was dissociated into single cells and sorted by flow cytometry into stem, and non-stem, cell populations, based on aldehyde dehydrogenase (ALDH) expression. These cells were injected into mice at low concentrations (50, 500, 1000, 3000 cells) and monitored for tumor growth.

Results: Established stem cell markers (ALDH, cyclin D, sox2) were upregulated in stem cells arising from spheroids. GUCY2C also was upregulated in spheroids, compared to whole tumor. Moreover, none of the mice injected with ALDH⁻ (non-stem) cells grew tumors. However, the mouse injected with 3000 ALDH⁺ stem cells formed a tumor, confirming ALDH as a marker of tumorigenicity and stemness.

Conclusions: GUCY2C is widely expressed by GI malignancies. Cancer stem cells drive tumor treatment resistance, recurrence and metastasis. GUCY2C appears to be upregulated in spheroids, structures comprised by cancer stem cells. We have previously established the efficacy of our GUCY2C-targeted chimeric antigen receptor-expressing (CAR) T cells to kill intraperitoneal colorectal cancer, as well as subcutaneous gastric and esophageal cancers, in mice. Moving forward, we will explore the efficacy of our GUCY2C-directed CAR T cells to eliminate orthotopic models (pancreas, colon) of human GI cancer stem cells to establish the proof of principle that this adaptive cell therapy approach can eradicate treatment-resistant tumor-initiating cells to ultimately create durable responses in patients.



James Fraser, MD

Mentor:

Shawn St. Peter, MD (Pediatric Surgery, Children's Mercy Hospital)

Awards:

- Avery's Angels Gastroschisis Research Fellowship

Evaluating the Risk of Peri-Umbilical Hernia after Sutured or Sutureless Gastroschisis Closure

Introduction: Gastroschisis management protocols have undergone considerable study in the past decade, with increasing emphasis on the benefits of a sutureless approach to closure, including fewer general anesthetics, less ventilator use and time, a shorter time from birth to final closure, and fewer surgical site/deep space infections than those who undergo a sutured closure. The selection of closure method typically incorporates consideration of defect size, viscerico-abdominal disproportion, and associated anomalies to dictate primary or silo assisted closure. Umbilical hernia is cited as a complication of gastroschisis, with significantly higher rates of peri-umbilical hernia in those who undergo sutureless closure, thought to be secondary to a lack of formal fascial closure. Post-closure peri-umbilical hernias are approached as complications that require intervention without clear indications for repair. The purpose of the present study is to examine and compare the rate and timing of spontaneous closure and repair of peri-umbilical hernias after varying closure methods in infants with uncomplicated and complex gastroschisis across a large regional cohort in the United States.

Methods: A retrospective follow-up study of neonates with gastroschisis who underwent closure at 11 children's hospitals from 2013 to 2016 was performed. All patient encounters were reviewed through 2019 to identify the presence of peri-umbilical hernia, time to spontaneous closure or repair, and associated complications.

Results: 375 patients met inclusion criteria. Sutured closure was performed in 305 (81%). 310 (83%) infants had uncomplicated gastroschisis. Median follow-up was 2.5 years [IQR 1.3, 3.9]. Peri-umbilical hernia incidence after gastroschisis closure was 23%, significantly higher in the sutureless vs sutured cohort (50% v 16%, $p < 0.01$) and higher in those with uncomplicated gastroschisis who underwent primary vs. silo assisted closure (53% v 17%, $p < 0.01$). Spontaneous closure was observed in 39% of patients within a median of 17 months [9, 26], and most frequently observed in those who underwent a sutureless primary closure (52%). 27 patients (32%) underwent operative repair within a median of 13 months [7, 23.5]. Rate and interval of spontaneous closure or repair were similar between the sutured and sutureless closure groups, with no difference between those who underwent primary vs. silo assisted closure. Indication for repair was most commonly an unrelated procedure, including inguinal hernia repair and stricturoplasty.

Conclusion: Peri-umbilical hernias after gastroschisis closure may be safely observed, as spontaneous closure is common, with minimal complications. The age at repair is lower than the recommended age for umbilical hernia repair in the normal population and frequently less than the interval to spontaneous closure. Although the high rate of umbilical hernia is often described as a complication and potentially adverse effect of the sutureless technique, our data suggest that this concern should not influence the closure approach, as the rate of spontaneous closure is higher than conventional sutured techniques. Given the high rate of spontaneous closure observed and low complication rate, early elective repair of gastroschisis peri-umbilical hernias may not be required, and these data suggest that repair to prevent complications is not an appropriate indication for surgery. Our data support that in those who have no additional indication for operative intervention, repair may be reasonably be delayed and observed as umbilical hernias in the general pediatric population. Appropriate anticipatory guidance for families and reassurance to providers is needed to emphasize the safety of observation for peri-umbilical hernias after gastroschisis closure.



Andrew Hallett, MD

Mentor:

Dorry Segev, MD (Transplant Surgery, Johns Hopkins University Hospital)

Awards:

- NIH/NIDDK T32

SARS-CoV-2 Messenger RNA Vaccine Antibody Response and Reactogenicity in Heart and Lung Transplant Recipients

Background: Early studies have observed that solid organ transplant recipients (SOTRs) experience diminished antibody responses to SARS-CoV-2 mRNA vaccines. However, these studies have been dominated by abdominal transplant recipients with different immunomodulation and perhaps different safety profiles than heart and lung transplant recipients.

Methods: US adult heart and lung transplant recipients completed their vaccine series between 1/7/2021-3/26/2021. Reactogenicity and SARS-CoV-2 anti-spike antibody were assessed after a priming dose (D1) and booster dose (D2). Modified Poisson regression with robust variance estimator was used to evaluate associations between participant characteristics and antibody development.

Results: Of 90 heart recipients, there were 44% non-responders (D1-/D2-), 47% booster responders (D1-/D2+), and 9% priming dose responders (D1+/D2+). Of 62 lung recipients, 58% were non-responders, 32% were booster responders, and 10% were priming dose responders. Priming dose antibody response was associated with transplant-to-vaccination time ≥ 6 years ($p=0.047$), lack of anti-metabolite maintenance immunosuppression ($p<0.01$), and receipt of the mRNA-1273 vaccine ($p<0.01$). Median semiquantitative D2 immunoassay titers for non-responders, booster responders, and priming dose responders were 0 U/mL (IQR, 0-0 U/mL), 21.3 U/mL (IQR 3.1-238.4 U/mL), and 250 U/mL (IQR 250-250 U/mL) (Roche Elecsys). No serious adverse events were reported.

Conclusions: While safe, these vaccines may be less effective at preventing COVID-19 in heart and lung transplant recipients. Lung recipients may exhibit a more impaired humoral response than heart recipients. While current recommendations are to vaccinate eligible candidates and recipients, further studies characterizing the cell-mediated immune response and clinical efficacy of these vaccines in this population are needed.



Ryan Lamm, MD

Mentors:

Hien Dang, PhD (Surgical Research, Thomas Jefferson University)

Awards:

- Saligman Family Surgery Pilot Grant
- 2021 Abdomina Core Health Quality Collaborative Resident Research Grant

Clinical Significance of a Negative Elongation Factor-E Single Nucleotide Polymorphism in Hepatocellular Carcinoma

Background: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer deaths in the US and worldwide. Multiple etiologies, including hepatitis B and C, alcoholic cirrhosis, and non-alcoholic steatohepatitis (NASH) lead to progressive inflammation, fibrosis, cirrhosis and are associated with HCC. Due to these diverse etiologies and their heterogeneous nature, current therapies provide only modest benefits. Therefore, it is important to distinguish biological differences at the molecular and genetic level to pursue more accuracy in diagnosis and targeted therapy. Previous work has identified that negative elongation factor E (NELFE) promotes tumor progression in more than 38% of all HCC tumors and patients with high NELFE levels have significantly lower overall survival. NELFE is part of the NELF complex which regulates transcription. Further studies identified a germline single nucleotide polymorphism (SNP) of NELF-E, rs79208225 (G>A), at a rate of 1% in the general population, with a 9% prevalence in HCC patients. We found that the NELFE SNP affects splicing to decrease its mRNA expression. Thus, our hypothesis is HCC patients with the NELFE SNP have better overall outcomes. Our current work sought to identify the clinical significance of rs79208225 in HCC.

Methods: To determine the clinical significance of the SNP, genomic DNA from a diverse patient population was analyzed between 2016 and 2021 in 78 HCC patients from China as well as 95 fresh frozen paraffin embedded (FFPE) and 35 patient leukocyte samples from Thomas Jefferson University Hospital (TJUH). Patients from TJUH were predominantly Caucasian and Korean-American. Samples were Sanger sequenced to detect presence of the SNP. T-tests, Kaplan-Meier analyses, and Cox regression analyses were performed on patients with clinical data to determine whether the predictive value of the SNP is independent of other clinical variables. All statistics were performed on STATA 17.

Results: The rs79208225 SNP was found in 3.8% of patients in our population. When broken down by race 8.5% of Chinese patients had the SNP and were 3.38 times more likely to have the SNP compared to other races (OR 3.38, 95% CI: 1.002-11.398, p=0.05). 7.1% of patients whose HCC etiology was HBV had the SNP compared to no patients from all other etiologies. No correlation was found between age and sex. Patients with SNP survive significantly longer (52.75 months v 30.52 months, p=0.0168). Patients with SNP have significantly longer time to recurrence (52.75 v 25.04 months, p=0.0012).

Conclusions: There is a correlative relationship between race, etiology, and improved outcomes in HCC patients with the SNP. Specifically, patients with the SNP survived on average 22.23 months longer and disease-free survival was 27.71 months longer. Further studies will work towards investigating the SNP's function role in cells, which is hypothesized to involve exon skipping. Antisense oligonucleotide (ASO) technology will be developed to mimic the effects of the NELFE SNP, which would provide the protective effects as seen in patients who carry the mutation.



Lindsay Lynch, MD

Mentor:

John Mah, MD (Surgical Critical Care, Hartford Hospital)

Above the Cuff Vocalization for Ventilator-Dependent Patients with Tracheostomies

Background: As many as 10-15% of patients requiring ICU admission require a temporary tracheostomy. These airways are required for prolonged mechanical ventilation and use an inflated cuff to allow for positive pressure ventilation as well as protect the airway from aspiration of gastric and oral secretions. As a result, the inflated cuff allows material to accumulate above the cuff prompting creation of subglottic suction devices. A downside to the inflated cuff is that there is no air flow through the upper airway, which renders patients unable to phonate. The impact on patients is significant because their ability communicate with providers and family members is compromised. This has been shown to directly relate to how patients perceive their quality of life.

Using tracheostomy tubes which include in-line subglottic suctioning, has generated significant interest in using this port to instill air into the upper airways, allowing for phonation. In particular, the Smith-Medical (Ashford, Kent, UK) Blue Line Ultra Suctionaid tracheostomy tube includes a subglottic suction channel. This tracheostomy is marketed as having the suction port to allow for above the cuff vocalization, but little data exists supporting its use. We will evaluate the safety and efficacy of above the cuff vocalization by assessing for successful phonation after capping of the subglottic port with instillation of air, if necessary, to aid in vocalization.

Methods: A retrospective review of quality improvement data collected from October 2019 to October 2020, with prospective data collected from November 2020 onward. Eligible patients included all genders, aged 18 years and older admitted to one of the intensive care units at Hartford Hospital. Phonation was measured on a 5-point National Outcomes Measurement System for communication and speech pathology (NOMS scale). Attention was also given to potential complications such as discomfort, excessive oral secretions, stomal air leak, gagging, and development of subcutaneous emphysema or patient request for removal.

Results: A total of 23 patients were collected retrospectively and of those collected, several had multiple attempts at vocalization. In those cases, we took the patient's highest score on the NOMs scale. We did exclude a significant number of patients if they had tracheostomy placed for head and neck cancer, ventilator settings > 60% FiO₂ or 12 PEEP, unresponsive patient, tolerated speaking valve, upper airway obstruction, copious secretions, and tracheostomy for < 48 hours. All individuals included in the study were able to achieve some degree of phonation. A total of 5 out of 23 patients achieved level 4, 3 at level 3, 12 at level 2 and 3 at level 1. Only 4 patient experienced complications, the most common being excessive oral secretions which was managed with suctioning.

Conclusions: Above the cuff vocalization provides a safe and effective strategy to allow patients with a tracheostomy and ventilator dependence to phonate. All of our patients were able to achieve some degree of vocalization. In many cases, the trials were timed during a family visit or meeting to allow patients the opportunity to participate in decision making. This has the potential to dramatically improve patient's perceived quality of life. With continued data collection, we hope to expand our sample size to demonstrate statistically significant improvements in vocalization among our patients.



Keyur Patel, MD

Mentors:

Ashesh Shah, MD (Transplant Surgery, Thomas Jefferson University)

Awards:

- Saligman Family Surgery Pilot Grant

Employing a CRISPR-CAS9 Screen to Identify Mechanisms of Resistant to Anti-GPC3 CAR T Cell Therapy

Background: Hepatocellular carcinoma (HCC) is a leading cause of morbidity and mortality with a projected increase in both categories. HCC is often diagnosed at advanced stages with limited treatment options available in these advanced cases. Chimeric Antigen Receptor (CAR) T cells have shown clear promise in the treatment of hematologic malignancies. They provide a patient-derived specific treatment for cancer with fewer side effects than traditional chemotherapy. While CAR T cells are being used in liquid cancers, there have been additional hurdles barring their quick adaptation in the treatment of solid cancers. Besides the physical barriers, solid malignancies have multiple mechanisms of resistance to immune detection. Of the tumor associated antigens in HCC, glypican 3 (GPC3) has shown to be widely expressed in HCC, specific to HCC and involved in its tumorigenesis, making it an ideal candidate as a target for CAR T cells. Anti-GPC3 CAR T cells have been generated to target HCC cells, but current data do not show the same level of efficacy as CAR T cells have in the treatment of other malignancies. Our goal is to identify mechanisms of resistance of HCC cells to anti-GPC3 CAR T cells using a genome wide CRISPR-CAS9 screen.

Methods: HCC cell lines (Hep3B, Huh-1 and HLE) were transduced with lentivirus carrying a plasmid for the doxycycline-inducible CAS9 protein. Single clones of these cells were transduced with lentivirus carrying a luciferase enzyme plasmid with GFP reporter and were subsequently single cell cloned via sorting. Validation studies for CAS9 expression and GPC3 expression were done in replicate. Cells were grown in preparation for the genome wide CRISPR-CAS9 screen. Preparation of the lentivirus carrying the genome wide guide library was done as previously described. Once the anti-GPC3 CAR T cells are generated, they will be co-cultured with the HCC cell line for the screen to determine the appropriate effector-target ratio prior to conducting the screen.

Results: Expression of GPC3 in our HCC cell lines was first determined using Western Blot. Surface expression of GPC3 was then confirmed by flow cytometry using the YP7 antibody generated by the Mitchell Ho Lab. CAS9 expression was confirmed *in vitro* via Western Blot over multiple time points after doxycycline supplementation in cell culture media. Luciferase activity was determined by the Luciferase Assay Kit and GloMax machine.

Conclusions: Using these modified HCC cell lines with anti-GPC3 CAR T cells, we will perform a genome wide CRISPR-CAS9 screen to identify potential mechanisms of resistance to anti-GPC3 CAR T cell treatment. These cell lines will also be used in *in vitro* validation studies as well as the basis for our future *in vivo* screen.