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TITLE: Optimizing a Novel Intraductal Delivery of Calcineurin Inhibitors as a Radiocontrast Infusion Formulation to Prevent Post-ERCP Pancreatitis

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14. ABSTRACT

An endoscopic retrograde cholangiopancreatography (ERCP) procedure is a common and life-saving gastrointestinal procedure that is performed in about half a million American each year, among which about 3-15% of patients were found to develop post-ERCP pancreatitis (PEP), the most common adverse effect of ERCP without effective preventative modalities.

The present project funded by Award W81XWH-19-1-0683 was proposed based on our recent discovery that calcineurin (Cn) signaling pathway contributes to the development of PEP. It aims to optimize the delivery of calcineurin inhibitors to prevent PEP. In the first project year, we conducted mouse experiments designed for Aim 1, including pancreatic and systemic safety testing of intraductal infusion of Cn inhibitors Tacrolimus (Tac) and cyclosporine A (CsA) within the radiocontrast.

Our proposed project has been progressing smoothly. Due to the COVID-19 pandemic and related social distancing policy, we postponed rabbit safety testing (which usually requires two or more researchers working together) and efficacy studies to third project years. This allowed us to perform mouse studies, which were originally proposed for the third project year in the grant proposal.In this reporting period (the second project year), we have successfully developed rectal suppository delivery of Cn inhibitor (Tac) alone or in combination with non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and diclofenac. We also have defined the pharmacokinetic features of rectal Tac suppositories. In efficacy testing, our data demonstrate that Tac suppositories significantly decrease pancreatic damage and systemic inflammation in PEP models elicited in mice. It was further found that rectal suppository delivery of Tac incombination with NSAID diclofenac achieved synergistic interaction in the prevention of pancreatic damage in a severe pancreatits models elicited in mice. Taken together, these data suggest that rectal suppository delivery of Cn inhibitor/NSAID formulations is a promising prevention against PEP.

In this project year, we held a project retreat recaping the project background and goals, reviewing data for Aims 1 and 2 as well as newer data, and discussing timeline and next steps.

In the next project year, we plan to perform the rest of safety testing and efficacy studies as proposed in the “Summary and timeline for the proposed work”. We believe that our preclinical IND-enabling studies will suggest the effective formulations and optimal delivery of Cn inhibitor in the prevention of post-ERCP pancreatitis and in the treatment of pancreatic disorders.

15. SUBJECT TERMS

Calcineurin inhibitor, post-ERCP pancreatitis, intrapancreatic duct delivery

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1. INTRODUCTION:

Post-ERCP pancreatitis (PEP) remains a significant iatrogenic challenge. Employing mouse models, we found that the development of PEP depends upon the activation of calcineurin (Cn) signaling, which could be prevented by intraductal pharmacological administration of the Cn inhibitor (CnI) tacrolimus or cyclosporin A, along with ERCP radiocontrast (RC) dye. Given the debate on the efficacy of current preventative modalities against PEP, our research aims to develop and optimize the delivery of novel formulations of CnI to prevent PEP. Specifically, we will (1) evaluate the safety profile and efficacy of the CnI-RC formulations delivered via an intraductal route and (2) further explore the optimal delivery (e.g. involving rectal suppositories) and formulations of CnI (involving non-steroidal anti-inflammatory drugs [NSAIDs]). Our proposed preclinical IND-enabling studies will suggest the effective formulations and optimal delivery of Cn inhibitor in the prevention of post-ERCP pancreatitis.

2. KEYWORDS:

Calcineurin, calcineurin inhibitor, ERCP, pancreatitis, mouse models, post-ERCP pancreaitits, Tacrolimus, islet toxicity, systemic toxicity, intraductal infusion, radiocontrast, rectal suppository

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Due to covid-19, we postponed rabbit safety and efficacy testing to the third project year. Instead, we have conducted mouse studies, which were originally proposed for the second and third project years in the grant proposal. Therefore, mouse studies we have performed during this reporting period (the second project year) incule:

Aim 1: We have performed systemic safety testing of intraductal administration of the Cn inhibitor-RC formulations in mice, and found that neither Tacrolimus (Tac)-RC nor Cyclosporin A (CsA)-RC formulation caused systemic toxicity. (Aim 1, 75% copleted during project years 1 and 2)

Aim 2: We have conducted experiments to optimize Cn inhibitor dosing and sterile preparation, assess the pharmacokinetic parameters, and we also have performed efficacy testing (Aim 2, 65% completed during project years 1 and 2).

In addition, we have developed rectal suppository delivery of Cn inhibitor formulations with non-steroidal anti-inflammatory drugs for prevention of post-ERCP pancreaitits.

Regulatory work:

Pre-IND meeting preparation. (60% finished)

What was accomplished under these goals?

Please see the appendix A.

What opportunities for training and professional development has the project provided?

Nothing to report.
This project was not intended to provide training or professional development opportunities.

How were the results disseminated to communities of interest?

The results were disseminated to communities of interest through presenations at conferences and talks at department and division levels. We are alo working on a manuscript that will describe in details the scope and the impact of our research findings. For example, we presented our findings in 2020 Annual Conference of American Pancreatic Association as a oral poster presentation with the title “Preclinical toxicology evaluation of a novel calcineurin inhibitor formulation to prevent post-ERCP pancreatitis demonstrates endocrine safety”.

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

We plan to accomplish the following goals for the next reporting period:

- Complete the rest experiments of safety and efficacy testing of the intraductal administration of Cn inhibitor formulation in support of IND filing.
- Further optimize the dosing and delivery (intrapancreatic ductal, oral, and ductal suppository) of Cn inhibitor formulations involving non-steroidal anti-inflammatory drugs and related PK/PD assessment.
- Validate findings in mouse studies using ERCP model elicited in rabbits.

4. **IMPACT:**

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

We established that intraductal administration of calcineurin inhibitor and radiocontrast dye formulations do not cause any systemic or pancreatic toxicities. We also discovered the advantages of rectal suppository delivery of calcineurin inhibitors, especially in comnination with non-steroidal anti-inflammatory drugs, in the prevention of post-ERCP pancreatitis. Such findings significantly support our next step IND filing and the ensuing clinicl trials. Products that may result from this research could provide an efficacious and safe preventative for post-ERCP pancreatitis.

What was the impact on other disciplines?

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Nothing to Report

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:

Nothing to Report

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

- The relocation of our lab University of Pittsburgh, Pittsburgh, PA to Stanford University, Palo Alo CA in 2019 significantly delayed the initiation of the project as it took about a year to get all compliance protocols set up and to recruit lab members to work on the project.
- COVID-19 pandemic-related California shelter-in-place policy and safety measures during this project year, including social distancing with in the lab, animal facilities, and campus core facilities significantly slowed down our research activities.
- We now are working on the project at max speed and employing CROs when possible to make up for the lost time.

Changes that had a significant impact on expenditures

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

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.

We presented our research findings in form of poster or oral poster presentation at 2020 Annual Conference of Americn Pancreatic Associaton (APA) and North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) comferences in November 2020.

- **Website(s) or other Internet site(s)**

www.husainlab.org: The Husain Lab website gives an overview of our research work and our accomplishments. The home page has a video for a poster presentation that dissemninates the some of the work we have accomplished on this project We presented this video at 2020 Americn Pancreatic Associaton (APA) annual conference and North American Society of Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) comference. The direct links for the videos relating to the abstracts for the current work at Link1 (https://stanford.zoom.us/rec/share/oD5GtWg1sJ0FInXnpJ9fnLmWewA_swYNEGctylE1YJtEc0hfrddwVZQgJ2oLJZTw.pH_oCziHRYcldKtT) and Link2 (https://office365stanford-my.sharepoint.com/:v:/g/personal/szh_stanford_edu/EVP4gykUoTlIsGPumn944IwBoL1LrUi8pQJ7WsCZjaJOYg?e=i1rLtl)

- **Technologies or techniques**

Nothing to Report

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

We filed the following utility patent:
For: Compositions and Methods for Preventing Post-ERCP Pancreatitis
Incentor: Sohail Z. Husain, Monique T. Barakat
U.S. PCT Patent Application No. PCT/US2021/023144
Date Filed: March 19, 2021
Stanford Ref.: S20-149
KTS Ref.: 079445-1234339-005810PC
Declaration with the World Intellectual Property Organization: July 16, 2021

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

1. Sohail Husain, MD
Project Role: Principal Investigator
Nearest person month worked: 12
Contribution to Project: Dr. Husain oversees the overall scientific and administrative leadership of the project. He oversaw the experimental plans and reviewed each of the data outcomes. He presides over the main meetings of the projects and directly interfaces with the CROs and other collaborators.

2. Mang Yu, MD, PhD
Project Role: Senior Research Scientist
Nearest person month worked: 12
Contribution to Project: Dr. Yu assisted Dr. Husain in overseeing and implementing the project. He also conducted mouse studies proposed in the proposal.

3. Jianbo Ni, MD/PhD
Project Role: Visiting Scholar
Nearest person month worked: 12
Contribution to Project: Dr. Ni performed surgical procedures to generate post-ERCP pancreatitis models.

4. Asna Khalid, B.Sc
Project Role: Life Science Research Professional
Nearest person month worked: 12
Contribution to Project: Ms. Khalid managed IACUC, ACURO, and IRB of this project, and performed safety testing.

5. Yu-Chu Daisy Lin, M.S.
Project Role: Life Science Research Professional
Nearest person month worked: 12
Contribution to Project: Ms. Lin performed safety/efficacy testing, as well as surgical procedure to generate post-ERCP pancreatitis.

6. Jing Wang, MD
Project Role: Senior Research Scientist
Nearest person month worked: 1
Contribution to Project: Dr. Wang provided trainings for islet isolation and related experiments.

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

Nothing to Report

In this project year, we identified a regulatory CRO company RPI, which offers strategic and tactical regulatory expertise in all phases of drug development. We would take advantages of their comprehensive experience to strategically guide us to achieve our drug development goals.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: .

Appendix A

1) Major activities:
(1) Systemic safety profile of intrapancreatic duct infusion of the calcineurin (Cn) inhibitor (CnI)-radiocontrast (RC) formulations in mice.
(2) Pharmacokinetic analysis of rectal suppository administration of CnI.
(3) Efficacy assessment of rectal suppository administration of CnI alone or CnI-NSAID (non-steroidal anti-inflammatory drug) formulations in mouse models fo pancreatitis
Regulatory work:
Pre-IND meeting preparation.

2) Specific objectives:
(1) Performing chemistry analysis of serum markers of nephrotoxicity and hepatotoxicity to define the systemic toxicity of intrapancreatic duct administration with RC-CnI (Tacrolimus [Tac] or cyclosporine A [CsA]) formulations;
(2) Analysing the pharmacokinetic features and rectal suppository administration of Tac in mice and evaluating the efficacy of rectal Tac suppository in the prevention of post-ERCP pancreatitis in mice;
(3) Assessing the efficacy of rectal suppository delivery of Tac or Tac+Diclofenac in the prevention of pancreatitis in mouse models.

Regulatory work:
Seeking for strategic and tactical assistance throughout regulatory process, from pre-IND (investigational new drug) meeting preparation to NDA (new drug application) approval proceedings.

3) Significant results or key outcomes:
(1) Influence of intrapancreatic duct infusion of Tac-RC or CsA-RC formulation on serum markers of nephrotoxicity (**Figure 1**).

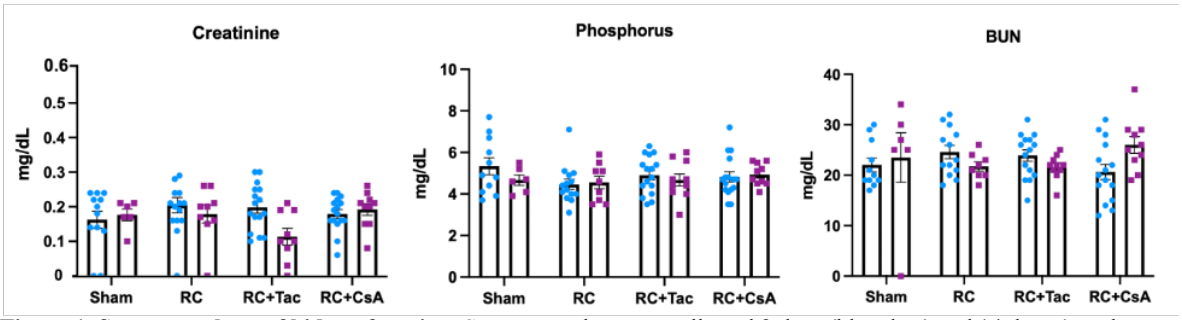

Figure 1. Serum markers of kidney function. Serum samples were collected 2 days (blue dots) and 14 days (purple dots) after the ERCP procedure. N = 6 to 15 mice per group. **Sham**: mice underwent ERCP procedure without intrapancreatic duct infusion; **RC**: mice underwent ERCP procedure with intrapancreatic duct infusion with radiocontrast alone; **RC+Tac**: mice underwent ERCP procedure with intrapancreatic duct infusion with formulation of tacrolimus and radiocontrast; **RC+CsA**: mice underwent ERCP procedure with intrapancreatic duct infusion of formulation of cyclosporin A.

(2) Influence of intrapancreatic duct infusion of Tac-RC or CsA-RC formulation on serum markers of hepatotoxicity (**Figure 2**).

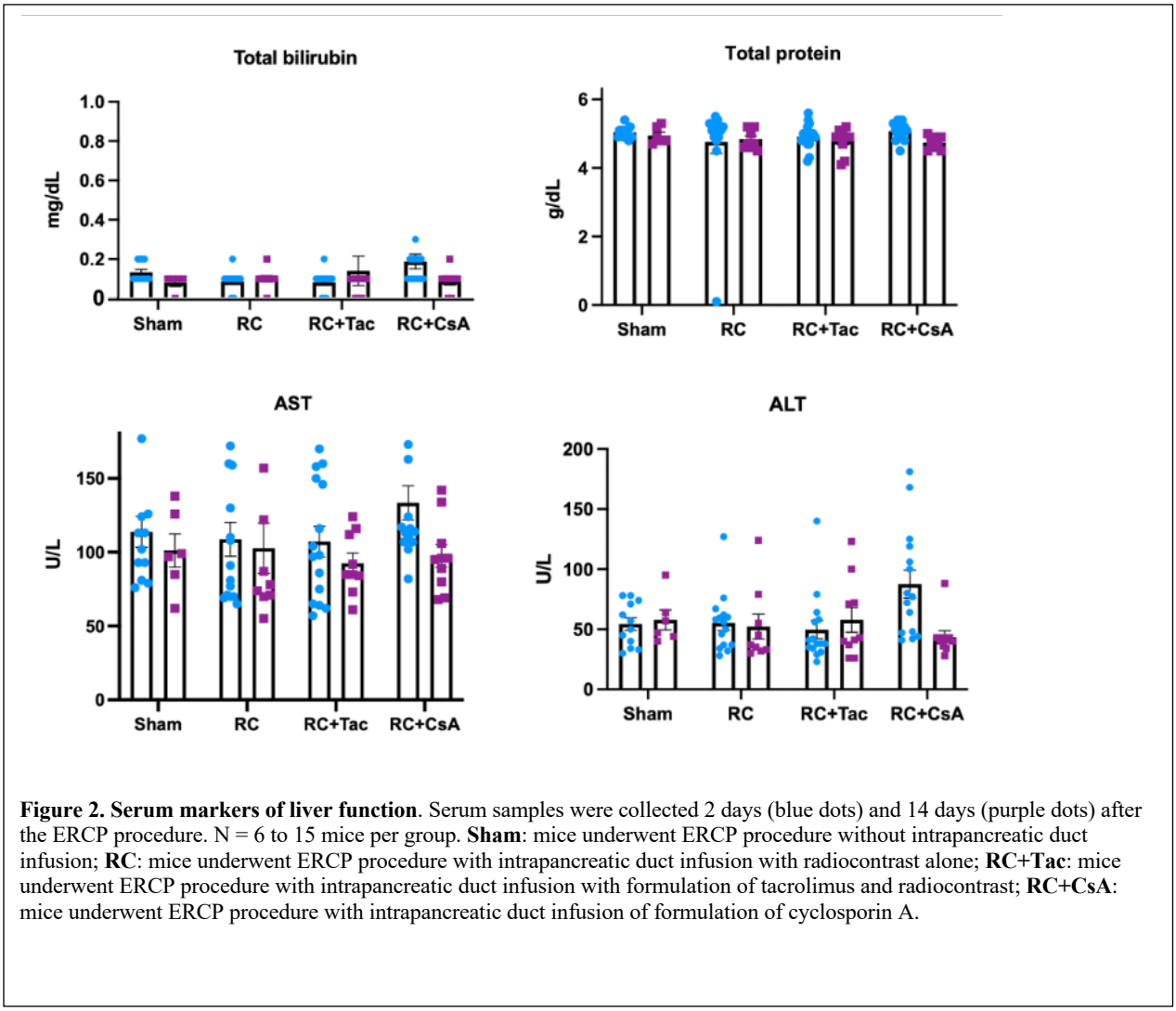

Figure 2. Serum markers of liver function. Serum samples were collected 2 days (blue dots) and 14 days (purple dots) after the ERCP procedure. N = 6 to 15 mice per group. **Sham**: mice underwent ERCP procedure without intrapancreatic duct infusion; **RC**: mice underwent ERCP procedure with intrapancreatic duct infusion with radiocontrast alone; **RC+Tac**: mice underwent ERCP procedure with intrapancreatic duct infusion with formulation of tacrolimus and radiocontrast; **RC+CsA**: mice underwent ERCP procedure with intrapancreatic duct infusion of formulation of cyclosporin A.

(3) Pharmacokinetic features of rectal Tac suppositories and the preventive effect of rectal suppository administration of Tac against psot-ERCP pancreatitis (**Figure 3**).

(4) Prophylactic effect of rectal suppository formulation of Tac and NSAID diclofenac on cerulein-elicited acute pancreatitis in mice (**Figure 4**)

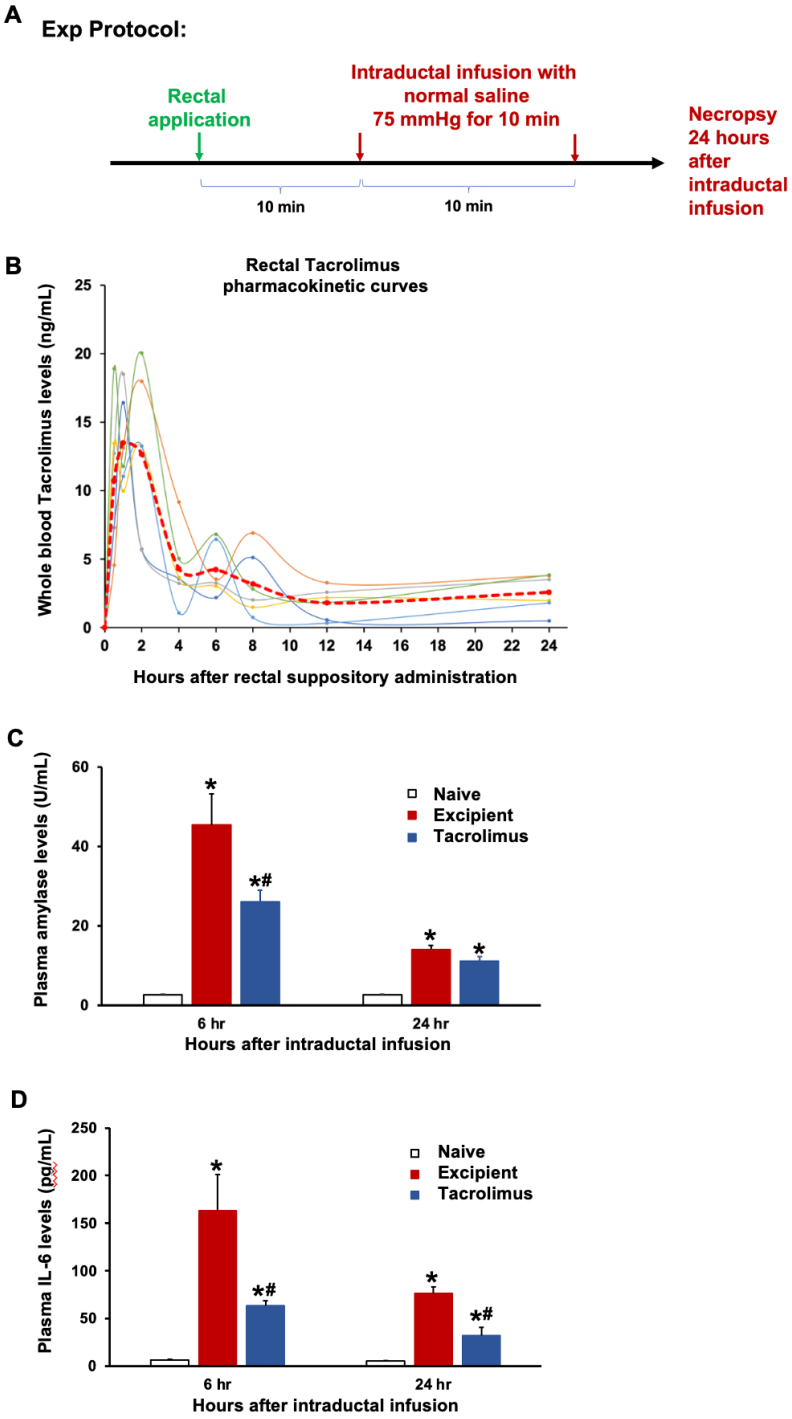

Figure 3. Pharmacokinetic features and prophylactic effect of rectal Tacrolimus suppositories on post-ERCP pancreatitis. (**A**) Experimental protocol of rectal suppository administration and elicitation of post-ERCP pancreatitis models in C57BL/6J mice. (**B**) Pharmacokinetic curves of rectal Tacrolimus suppositories. Solid thin curves represent changes of Tacrolimus levels in whole blood sampled at 0.5, 1, 2, 4, 6, 12, and 24 hours after the time of rectal administration, and the dotted thick curve represents the average values of whole blood Tacrolimus levels at each time points. (**C**-**D**) Plasma levels of amylase or IL-6 measured in samples collected 6 and 24 hours after intrapancreatic duct infusion. N = 3 to 5 mice per group. * $P < 0.05$, compared with naïve mice; # $P < 0.05$, compared with mice that underwent intrapancreatic duct infusion and rectal administration of suppository excipient.

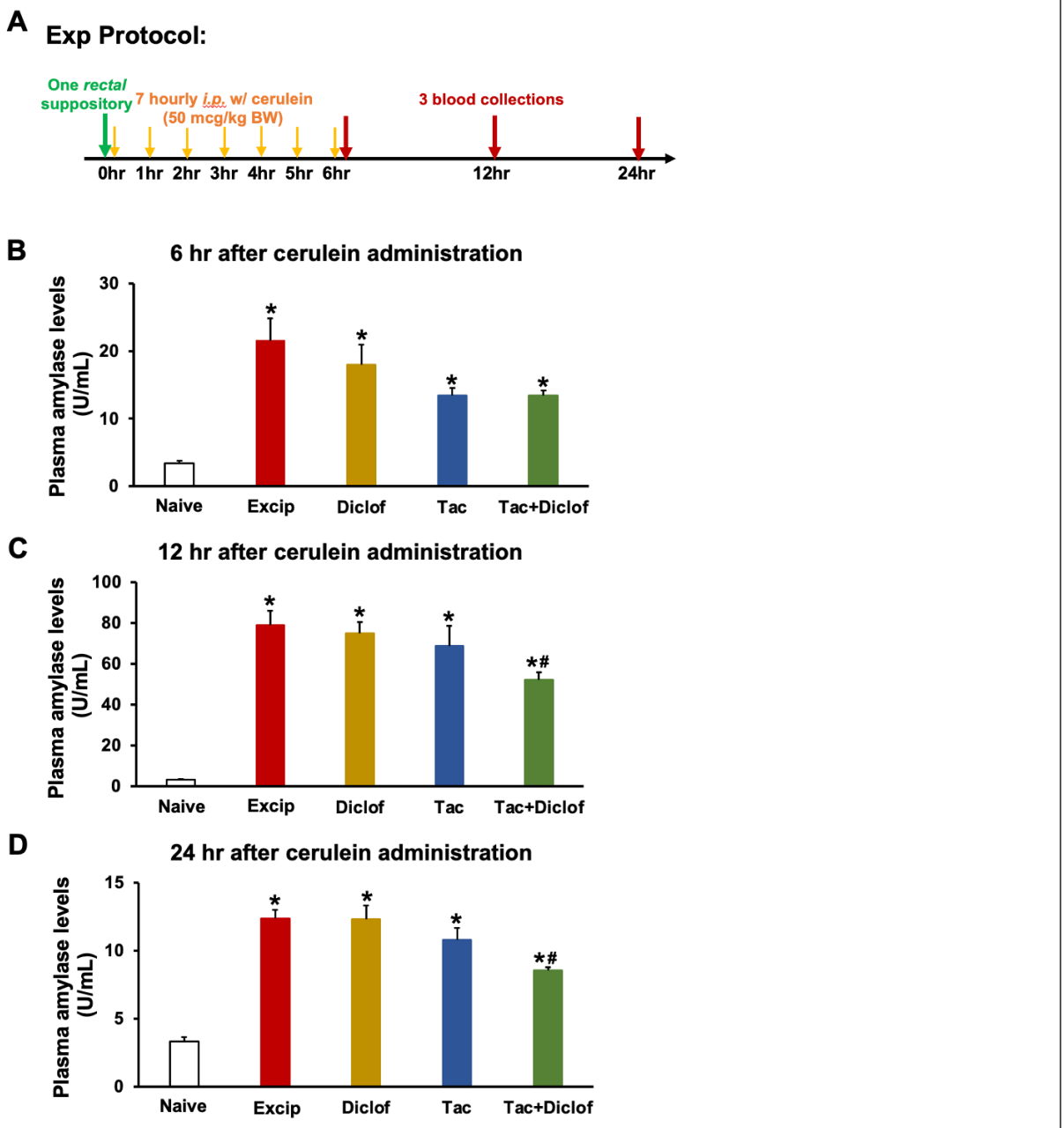

Figure 4. Prophylactic effect of rectal suppository formulation of Tacrolimus and diclofenac on cerulein-elicited pancreatitis in mice. (**A**) Experimental protocol of prophylactic rectal administration in cerulein-elicited models of acute pancreatitis in FVB/N mice. (**B-D**) Plasma levels of amylase measured in samples collected 6, 12, and 24 hours after the time of intraperitoneal cerulein adminisintration. N = 3 to 10 mice per group. * $P < 0.05$, compared with **Naive** mice; # $P < 0.05$, compared with **Excip** mice. **Naive**: mice underwent no treatments; **Excip**: mice underwent cerulein administration and rectal delivery of suppository base; **Diclof**: mice underwent cerulein administration and rectal delivery of diclofenac suppositories; **Tac**: mice underwent cerulein administration and Tacrolimus suppositories; **Tac+Diclof**: mice underwent cerulein administration and rectal delivery of Tacrolimus+diclofenac suppositories.

Conclusions:

(1) Serum markers of kidney and liver function tests were not elevated acutely or subacutely by high doses of intraductal tacrolimus (Tac) or cyclosporin A (CsA), suggesting that our intrapancreatic duct formulus of calcineurin inhibitors are safe in mice.

(2) Prophylactic rectal administration of Tac suppository alone can reduce the levels of key biomarkers of post-ERCP pancreatitis in mouse models

(3) A rectal Tac+diclofenac suppository combination can synergistically reduce the levels of plasma amylase (a key biomarker of acute pancreatitis) in cerulein-induced pancreatitis models in mice.

Regulatory work:

Pre-IND meeting preparation: We identified a regulatory contract research organization (CRO) RPI, a division of Premier Research, which offers strategic and tactical regulatory expertise in all phases of drug development. We would take advantages of their comprehensive experience in regulatory process to guide us to achieve our drug development goals.

4) Other achievements:

Executive Summary of Grant Meeting

Theme: DOD Post-ERCP Pancreatitis Grant Meeting

Date/time: September 29, 2021, 2:00PM - 3:30 PM PST

Attendees: Dr. Sohail Husain - PI, Stanford
Dr. Monique Barakat - Clinical Investigator Lead, Stanford
Dr. Mang Yu - Senior Scientist, Stanford
Dr. Judy-April Murayi - Military Consultant, Fort Drum
Dr. Ying Ding - Biostatistician, University of Pittsburgh
Dr. Thottola Jayaraman - Consultant, University of Pittsburgh
Dr. Georgios Papachristou - Consultant, The Ohio State University
Dr. Ronald Poropatich - Military Consultant, University of Pittsburgh

Broad Goals: (1) Recap the project background, goals and evolution of the plan
(2) Review data for Aim 1 and newer data
(3) Discuss timeline and next steps

Key Points: For the first half of the meeting, we reviewed the data from pre-clinical pancreatitis models. Initial safety testing of intraductal administration of the common radiocontrast iohexol + tacrolimus or cyclosporine A demonstrated safety of intraductal

calcineurin inhibitor administration in mice even at high concentrations of the calcineurin inhibitors. Ongoing work in the Husain lab has, however, revealed that local/intraductal administration of a calcineurin inhibitor may deprive the subject/patient of systemic benefits of the calcineurin inhibition for prevention of post-ERCP pancreatitis and sequelae. Additionally interventional endoscopy approaches to cannulation during ERCP have evolved to favor guidewire-based exploration rather than injection of contrast to confirm intraductal location, making pancreatic ductal contrast injection far less likely during a typical ERCP.

The typical endoscopy unit workflow involves rectal indomethacin administration for many patients undergoing ERCP, and we have pivoted our calcineurin inhibitor formulation and modality of delivery to a rectal route. We have established a patent for a combined rectal NSAID/calcineurin inhibitor rectal formulation. Based on recent clinical data evaluating patients who were serendipitously taking calcineurin inhibitors and did or did not receive indomethacin at the time of ERCP, we hypothesize that rectal tacrolimus + rectal indomethacin will be more efficacious at preventing post-ERCP pancreatitis than rectal indomethacin alone. Note: Diclofenac would be used in mice models because indomethacin can be inflammatory in mice.

Feedback: A synthesis of the feedback was that the transition to a rectal formulation was more clinically feasible for several reasons including: (1) ERCP moved to a guidewire cannulation approach rather than injection and (2) always a concern that the volume that can be injected into the pancreatic duct is relatively limited. We discussed that this novel rectal formulation could be easily integrated into the endoscopy unit workflow, since it would replace the current standard of care which is also a rectal drug. For the clinical trial, the clinical arms should compare rectal indomethacin with rectal indomethacin + tacrolimus in order to investigate the additive benefit. Future studies should also stratify groups by etiology for ERCP. Post-ERCP is still prevalent, so this work is clinically important.

Finally, we discussed combining the work with the ongoing NIH-funded clinical trial called the SVI (Stent vs. Indomethacin) tiral. We could use the indomethacin patients as historic controls. This would then require smaller numbers for clinical safety testing and be more cost-effective.

Next steps

- Circulate the draft of the manuscript for the Intraductal Tacrolimus Manuscript and submit for publication (Jan 2022)
- Circulate the draft of the manuscript for the Rectal Tacrolimus Review and submit for publication (Dec 2021)
- Draft the Rectal Tacrolimus Pre-clinical Studies Manuscript (Feb 2022)
- Drs. Barakat and Papachristou will discuss SVI trial collaboration/clinical parameters (by Dec 2021)

- Formulation work sub-contracted to Tergus pharmaceuticals and ongoing (contract successfully negotiated between Stanford and Tergus, in Oct 2021)
- Move toward pre-IND Meeting with FDA (filing by Sept 2022)
- Next meeting in 6 months (March 2022)