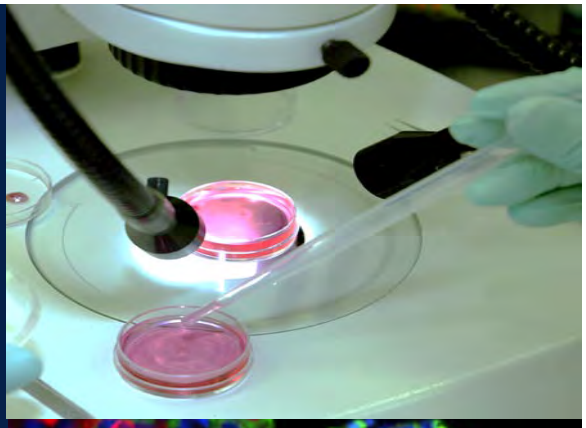
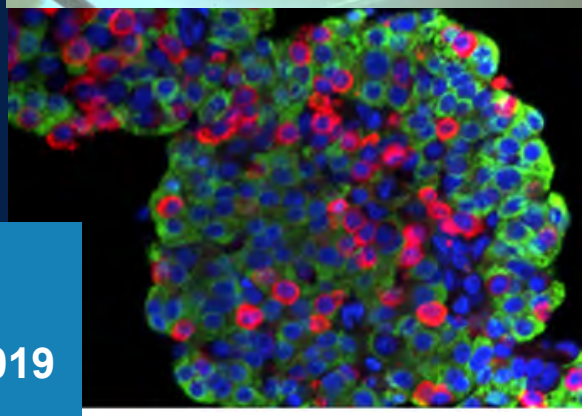
UCSF Department of Surgery

32nd Annual

Resident Research Symposium





April 17, 2019

In Memoriam

Thomas K Hunt, MD (1930-2019)

This year's symposium is dedicated to the memory of Thomas K. Hunt, MD

Lillian S. Kao, MD, MS, FACS
Dunphy Visiting Professor

UCSF Mission Bay
William & Susan Oberndorf Auditorium
1855 4th Street, Room A1602B
San Francisco, California

UCSF Department of Surgery Education Office
Telephone: (415) 476-1239 **Email:** EducationOffice@ucsf.edu **Website**: www.surgery.ucsf.edu
For more information about this event, visit: http://tiny.ucsf.edu/RRD or scan QR code on this page.

In Memoriam

This year's Research Symposium is dedicated to the memory of Thomas K. Hunt, MD, who passed away in February 2019 at the age of 88.

Dr. Hunt was an internationally known and respected surgeon, professor and researcher at UCSF from 1964 until 2003. He is best known for helping to develop the Trauma Unit at San Francisco General Hospital and for his research on the cellular biology of wound healing. His easy-to-implement ideas shaped the standard of care for the prevention of infections after surgery.

Dr. Hunt's ideas were notable for their simplicity and practicality: he proved that simply applying oxygen, warmth, fluids, vasodilation and pain relief could substantially reduce infections and improve healing. These inexpensive and easy-to-implement ideas eventually became fundamental to post-surgical infection prevention programs.

Research was one of Dr. Hunt's passions and he loved teaching residents, fellows and medical students. Dr. Hunt remained an active member of the UCSF Department of Surgery Research Committee, where he reviewed abstracts for the annual J. Englebert Dunphy, MD Resident Research Symposium, which he attended every year until 2018.

Patients knew Dr. Hunt as a compassionate and understanding physician with a calm and caring bedside manner. Colleagues often described him as brilliant yet modest; kind, funny and straightforward. "Tom Hunt was a giant in the world of wound healing," said Annette Wysocki, past President of the Wound Healing Foundation. "Equally as important as the research and teaching that Tom did, was the way he provided mentorship and inclusivity to the next generation of junior postdocs, faculty and scholars."

Born in Evanston, Ill., Dr. Hunt grew up in Depression-era poverty with his beloved sister Eleanore. His mother, a Swedish émigrée, was a schoolteacher and his father was a US Coast Guard Lieutenant and builder of church organs. During those years, he decided, while operating on his teddy bear, that he would grow up to be a surgeon.

Dr. Hunt was such a gifted student that in his senior year of high school the principal called him in to announce that he should go to Harvard. When Dr. Hunt protested they couldn't possibly afford a private college, the principal merely replied, "Son, have you heard of scholarships? You apply and I'll take care of the rest."

That same year Dr. Hunt joined his father, by then a Master Mariner, as First Mate aboard a US Navy destroyer escort, which had been refashioned into a luxury yacht. The job was to bring the ship from Lake Huron down the Mississippi River to New Orleans, out the Gulf Coast, then around South America to Rio de Janeiro for the new owner, a wealthy Brazilian. It was a wild adventure that included stormy seas off the coast of Cuba, which nearly swept him overboard, a knife fight on shore, a visit to post-revolutionary Haiti and finally, to Carnaval in Rio.

At the end of the journey, he flew back to Chicago aboard a prop plane to find a letter inviting him to interview for Harvard the following morning, with a dozen of the city's biggest names. Without a proper suit, he showed up in khakis and told the tales of where he had just been. "I apologized for my clothing and told the story. I saw smiles on their faces and after half an hour, I came out and said to myself, 'If I didn't know better, I'm going to be at Harvard next year'", he later recalled.

Dr. Hunt earned his undergraduate degree at Harvard in 1952, where he remained for medical school, earning his medical degree in 1956. During medical school, he met Evelyn Maria Schnabel on a blind date. Two years later, under a full moon, he proposed marriage. They were married at St. James Church in New York City in June and remained together for 62 years, raising three children and a succession of golden retrievers.

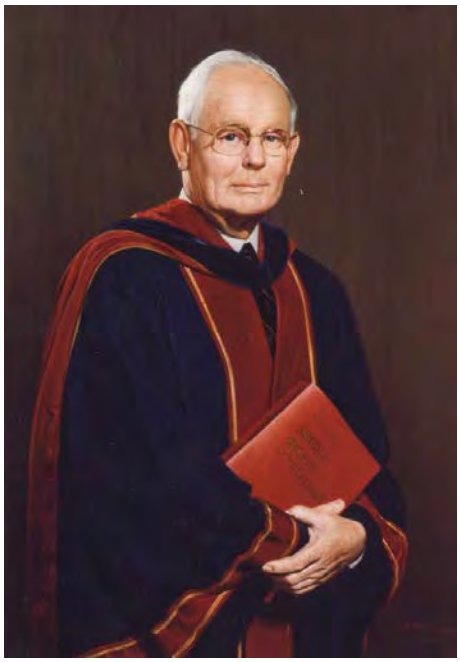
Dr. Hunt did his internship at Boston City Hospital under famed surgeon J. Englebert Dunphy, MD. He followed Dr. Dunphy to the University of Oregon and completed his surgical residency there in 1964, and then a year-long research fellowship in Glasgow, Scotland, where he worked on methods to infuse hyperbaric oxygen into tissue to aid the healing of surgical wounds.

Dr. Hunt followed Dr. Dunphy once again, and joined the UCSF Department of Surgery as a faculty member in 1965. He became Director of the Wound Healing Laboratory and Vice-Chairman for Research. In addition, he was an Adjunct Professor of Surgery at Ohio State University and a Consulting Surgeon at the University of Tübingen in Germany. He was the founding President of the Wound Healing Society, served on the Board of Directors of the Wound Healing Foundation, Undersea and Hyperbaric Medical Society, and as President of the American Trauma Society, in addition to countless honors from universities and organizations across the globe.

Despite his demanding professional workload, Dr. Hunt made time for family backpacking trips in the summer and ski weekends in the winter. Until back pain prevented it, he was usually up for a game of tennis with the kids; and some of his proudest moments were when each of his children beat him in swimming races across the pool or performed in their school band concerts.

He was a warm and loving father who taught by example that hard work is its own reward, and that honesty and integrity are the cornerstones of self-respect. At times, the Hunt household was like an international hotel for Postdoctoral Fellows, bringing the entire family an awareness of other worlds and cultures that his children honor to this day.

Dr. Hunt served on the Department of Surgery Resident Research Committee since its inception (at which time he was Committee Chair), and was an active participant in our department's annual J. Englebert Dunphy, MD Resident Research Symposium – including last year. At the end of that symposium, he commented that he was amazed and impressed at the quality of presentations, which was such a meaningful compliment coming from the leader in perpetuity for this committee. This year marks the first that his name will be missing from the Research Committee roster, and he will be sorely missed. To some he was a mentor, to others an esteemed colleague, and to most (if not all), a role model, a true gentleman in every sense of the word. His incredible legacy will continue to escalate the research achievements of all members of our surgery team at UCSF to new heights. It is an honor and a privilege to dedicate this year's symposium to the memory and legacy of Dr. Thomas K. Hunt.



J. Englebert Dunphy, MD

Professor of Surgery and Chair of the Department from 1964 to 1975

Dr. Dunphy earned his medical degree at Harvard Medical School and completed his surgical residency training at the Peter Bent Brigham Hospital in Boston. He then joined the faculty at Harvard before accepting the position of the Chair for the Department of Surgery at the University of Oregon. In 1964, Dr. Dunphy accepted the position of the Chair for the Department of Surgery at the University of California, San Francisco. Dr. Dunphy was president of the Society of University Surgeons, the American Surgical Association, and the American College of Surgeons. He received honorary fellowships in six foreign colleges of surgeons as recognition of his international stature.

Dr. Dunphy was renowned for excellence in many aspects of surgery, with a special interest in the gastrointestinal tract. He was one of the leading surgical educators of his day, and was greatly admired and respected by his colleagues and residents. Dr. Dunphy conducted research in wound healing at a basic level. Dr. Dunphy strongly believed that prospective academic surgeons should become grounded in basic science, and he was one of the first surgical leaders in the United States to obtain an NIH training grant supporting residents in the laboratory.

2019 Dunphy Professor

Lillian S. Kao, MD, MS

Dunphy Visiting Professor

Lillian S. Kao, MD, MS is Professor and Division Chief of Acute Care Surgery at McGovern Medical School at the University of Texas Health Science Center at Houston (UTHealth) and Director of the Memorial Hermann Red Duke Texas Trauma Institute. She holds the Jack H. Mayfield, MD, Chair in Surgery, and she is the Vice Chair for Research and Faculty Development and is one of two Vice Chairs for Quality for the Department of Surgery. She is faculty for the Master of Science in Clinical Research degree program at UTHealth, and she is the co-founder and co-director for the Center for Surgical Trials and Evidence-based Practice (C-STEP). Since its initiation, C-STEP has graduated 10 residents with Masters Degrees and has six more in the pipeline.

Dr. Kao is a past president of the Association for Academic Surgery, and she is the current president of the Association for Academic Surgery Foundation. She is on the editorial boards of Surgery, Journal of the American College of Surgeons (JACS), Surgical Infections, and American College of Surgeons General Surgery News; she is the social media editor and a deputy editor for JACS. Dr. Kao's interests include quality improvement, dissemination and implementation research, and development of a learning healthcare system and learning healthcare scholars; she is the director of a 30-hospital statewide surgical collaborative, the Texas Alliance for Surgical Quality.

Past Visiting Professors

Bernard Langer, MD
Professor and Chairman of Surgery, University of Toronto
February 5-6, 1988

William Silen, MD
Professor of Surgery, Harvard Medical School
February 3-4, 1989

James Thompson, MD
Professor and Chairman of Surgery, University of Texas, Galveston
February 2-3, 1990

Murray Brennan, MD
Professor and Chair of Surgery, Memorial Sloan-Kettering Cancer Center
February 3-4, 1991

Richard Simmons, MD
Professor and Chairman of Surgery, University of Pittsburgh
January 31-February 1, 1992

Stephen F. Lowry, MD
Professor of Surgery, Cornell University Medical College
February 4-5, 1993

Jared Diamond, Ph.D.
Professor of Physiology, UCLA School of Medicine
February 4, 1994

Samuel A. Wells, Jr., MD
Professor and Chairman of Surgery, Washington University
February 17, 1995

Jonathon E. Rhoads, MD
Chief of Surgical Oncology, University of Pennsylvania, Philadelphia
February 16, 1996

Patricia K. Donahoe, MD
Chief, Pediatric Surgical Services, Massachusetts General Hospital
February 27, 1997

David L. Dunn, MD, Ph.D.
Professor and Chairman of Surgery, University of Minnesota
February 27, 1998

Ori D. Rotstein, MD
Professor of Surgery, Toronto Hospital
February 26, 1999

Olga Jonasson, MD
Director of Education and Surgical Services Department
American College of Surgeons
March 17, 2000

Glenn Steele, Jr., MD Ph.D.
Dean, School of Medicine, University of Chicago
March 9, 2001

Alexander W. Clowes, MD
Professor of Surgery and Chairman, University of Michigan
March 7, 2002

Michael Mulholland, MD, PhD
Professor of Surgery and Chairman, University of Michigan
March 7, 2003

Christian Larsen, MD, PhD
Professor of Surgery, Emory University
March 19, 2004

Danny O. Jacobs, MD, MPH
Chair, Department of Surgery, Duke University Medical Center
March 4, 2005

Steven D. Leach, MD
Chief of Surgical Oncology, Johns Hopkins University
March 3, 2006

M. Judah Folkman, MD
Professor of Pediatric Surgery & Cell Biology, Harvard Medical School
Director, Vascular Biology Program, Children's Hospital, Boston
February 15-16, 2007

Sir Peter Morris, AC, FRS, FRCS
Director, Centre for Evidence in Transplantation
Royal College of Surgeons of England
April 4, 2008

George K. Gittes, MD
Chair of Pediatric Surgery, University of Pittsburgh
April 3, 2009

Joseph P. Vacanti, MD
Chief, Pediatric Surgery, Massachusetts General Hospital
March 12, 2010

Maria Bertagnolli, MD
Professor of Surgery, Harvard
Chief, Surgical Oncology, Brigham and Women's Hospital
April 1, 2011

Michael Harrison, MD
Director Emeritus, Fetal Treatment Center, Professor of Pediatric Surgery,
University of California, San Francisco
April 13, 2012

Martin Elliott, MD
Professor of Cardiothoracic Surgery, University College London
April 5, 2013

Clifford Ko, MD
Director, Division of Research and Optimal Patient Care
Director, National Surgical Quality Improvement Program (ACS NSQIP)
American College of Surgeons
April 25, 2014

Jennifer Grandis, MD
Associate Vice Chancellor, Clinical and Translational Research
Director, Clinical and Translational Science Institute (CTSI)
Professor of Otolaryngology, University of California San Francisco
March 11, 2015

Robert C. Robbins, MD
President and Chief Executive Officer, Texas Medical Center
April 27, 2016

Samuel RG Finlayson, MD, MPH
Professor and Chair, Department of Surgery
University of Utah
April 12, 2017

Carla Pugh, MD
Professor of Surgery, Stanford University
April 25, 2018

2018 Symposium Winners

FRONT ROW (left to right): Julie Ann Sosa (Department Chair), Carla Pugh (Dunphy Visiting Professor), Michael Zobel, Greg Haro, Kelly Mahuron
BACK ROW (left to right): Jhoanne Bautista, James Ross, Casey Ward, Nicole Conkling, Simon Chu, Peter Stock (Chair of Research Committee), Ariane Christie, Lauren Eyler

BEST ABSTRACT PRESENTATION – Casey Ward, MD

Preservation of Pancreatic Islet Grafts and Reversal of Diabetes Using Novel Parathyroid Cotransplantation

BEST QUICK-SHOT PRESENTATION – James Ross, MD

The Ex Vivo Perfused Human Lung as a Model of Acute Lung Injury in Sepsis

OUTSTANDING ABSTRACT PRESENTATION – S. Ariane Christie, MD

Machine Learning Without Borders? An Adaptable Tool to Optimize Mortality Prediction in Diverse Clinical Settings

OUTSTANDING ABSTRACT PRESENTATION – Jhoanne Bautista, MD, PhD

Definitive Characterization of Extrathymic AIRE-expressing cells (eTACS)

OUTSTANDING ABSTRACT PRESENTATION - Simon Chu, MS

Comparative Analysis of Alloantigen-Stimulated Gene Expression in HIV+ Transplant Rejectors versus Non-Rejectors

HONORABLE MENTIONS

Tumor Infiltrating Lymphocyte Directed Neoadjuvant Therapy in Operable Stage III Melanoma
Kelly Mahuron, MD

An Improved Method of Minimally Invasive Pulmonary Metastasectomy that Allows Full Palpation of the Lung without Chest Wall Disruption
Greg Haro, MD

Optimization and Validation of the Economic Clusters Model for Facilitating Health Disparities Research in Low-Resource Settings
Lauren Eyler, MD

Quick-shot: Developing a Murine Model to Study Novel Therapies for Tolerance in Vascular Composite Tissue Allotransplantation
Nicole Conkling, MD

Quick-shot: Hyponatremia and Complex Biliary Disease
Michael Zobel, MD

To view the 2018 program and abstracts, scan QR code on this page.

8:30 AM Welcome Remarks
Julie Ann Sosa, MD, MA, FACS, Leon Goldman, MD Distinguished Professor & Chair, Department of Surgery

Introduction
Peter Stock, MD, PhD, Professor of Surgery & Chair, Research Committee

Quick-Shot Presentation (5 min) | Standard Presentation (10 min)

SESSION 1
Moderator: Johannes Kratz, MD

8:35 AM Predicting Acute Respiratory Distress Syndrome in Severe Blunt Trauma: The Utility of Interleukin-18
Genna Beattie, MD, UCSF East Bay Program, 1st Year Research Fellow

8:40 AM Repeat CT Head Scan is Not Indicated in Trauma Patients Taking Novel Anticoagulation: A Multi-Institutional Study
Caitlin Cohan MD, UCSF East Bay Program, 1st Year Research Fellow

8:45 AM Building Artificial Intelligence Systems for Surgery in Extreme Environments
Danyal Mehmet Fer, MD, UCSF East Bay Program, 1st Year Research Fellow

8:50 AM Bundle Payment for Care Improvement Advanced (BPCI-A) for Major Bowel Surgery: Initial Insights into How to Succeed in the Era of Payment Reform
Caitlin Collins, MD, UCSF Surgery, 1st Year Research Fellow

8:55 AM A Bioreactor Containing Primary Human Renal Epithelial Cells Supported in Vivo Without Immunosuppression
Rebecca Gologorsky MD, UCSF East Bay Program, 1st Year Research Fellow

9:00 AM Deep Immune Profiling of High- vs. Low-Risk Lung Adenocarcinoma
Iris Liu, UCSF Medical Student

9:05 AM Novel Microtubule and PARP Inhibitor Drug Attenuates Neointimal Hyperplasia in a Rat Model of Arterial Injury
Greg Haro, MD, UCSF Surgery, 2nd Year Research Fellow

9:15 AM Pancreatic Islet and Parathyroid Co-Transplantation for Treatment of Diabetes in Intra-Muscular Site: "PARADIGM"
Casey Ward, MD, UCSF Surgery, 2nd Year Research Fellow

9:25 AM Layilin is enriched in tumor-infiltrating CD8+ T cells in human cancer and contributes to anti-tumor immunity
Kelly Mahuron, MD, UCSF Surgery, 2nd Year Research Fellow

9:35 AM Extrathymic AIRE-expressing Cells are a Class of Migratory Dendritic Cells that can Mediate Tolerance to both Endogenous and Exogenous Antigens
Jhoanne Bautista, MD, PhD, UCSF Surgery, 2nd Year Research Fellow

9:45 AM A Role for Aire-Expressing Cells in Maternal-Fetal Tolerance
Eva Mae Gillis-Buck, MPhil, UCSF Medical Student

9:55 AM ***Break***

SESSION 2
Moderator: Tammy T. Chang, MD, PhD

10:15 AM Residents as Key Effectors of Change in Improving Opioid Prescribing Practices
Elizabeth Lancaster, MD, UCSF Surgery, 1st Year Research Fellow

10:20 AM Surgical correction of breast animation deformity with implant pocket conversion to a neo-prepectoral plane
Michael Holland, MD, UCSF Plastic Surgery, 1st Year Research Fellow

10:25 AM Post-Operative Opioid Requirements in Living Kidney and Liver Donors
Hillary Braun, MD, UCSF Surgery, PGY3 Resident

10:30 AM Human ARF tumor suppressor suppresses zebrafish cardiac regeneration
Solomon Lee, MD, MS, UCSF Plastic Surgery, PGY1 Resident

10:35 AM	A systematic Review of Delays In Diagnosis and Barriers To The Care Of Colorectal Cancer In Low- And Middle-Income Countries *Nathan Brand, MD, MSc, UCSF Surgery, PGY1 Resident*
10:40 AM	Patient Complexity Varies by Surgical Specialty and Does Not Strongly Correlate with Work Relative Value Units *Joel Ramirez, BS, UCSF Medical Student*
10:45 AM	The Ex Vivo Perfused Human Lung Is Resistant To Alveolar Epithelial Injury From High-Dose S. Pneumoniae Bacteremia *James Ross, MD, MS, UCSF Surgery, 2nd Year Research Fellow*
10:55 AM	Value and feasibility of phone follow up in Ethiopian surgical patients / Comprehensive improvement of infection prevention practices in Ethiopian Operating Rooms using a quality improvement framework *Nichole Starr, MD, MPH, UCSF Surgery, 1st Year Research Fellow*
11:05 AM	Silicon Nanopore Membrane-Based Implantable Hemodialysis: A Preclinical Proof-of-Concept Study *Jarrett Moyer, MD, UCSF Surgery, 3rd Year Research Fellow*
11:15 AM	Chimeric antigen receptor (CAR) T-regulatory cells as a tolerance strategy in a murine surgical transplantation model *Nicole Conkling, MD, UCSF Surgery, 3rd Year Research Fellow*
11:30 AM	***Lunch Break Boxed lunches provided***

SESSION 3

Moderator: Lucy Kornblith, MD

12:45 PM	Evaluating long-term patient-reported quality of life after masculinizing mastectomy using the GENDER-Q; a novel quality of life instrument *Andre Alcon, MD, UCSF Plastic Surgery, 1st Year Research Fellow*
12:50 PM	An Analysis of Differentially Hydroxymethylated Genes in HIV-Infected Kidney Transplants Undergoing Acute Rejection *Arya Zarinsefat, MD, UCSF Surgery, 1st Year Research Fellow*
12:55 PM	Importance of Operative Team Expertise - The Presence of a Dedicated Specialty Operating Room Nurse is Associated with Fewer Delays *Whitney Goering, MD, VAMC, Patient Safety & Quality Fellow*
1:00 PM	Biomechanics of ascending thoracic aortic aneurysm: Computational modeling and pursuit of wall stress based rupture risk *Andrew Wisneski, MD, UCSF Surgery, 1st Year Research Fellow*
1:05 PM	Fetal molecular therapies to treat neurological disease in mice with lysosomal storage disorders *Quoc-Hung Nguyen, MD, UCSF Surgery, 2nd Year Research Fellow*
1:15 PM	Parathyroid CD34+ cells induce neovascularization from donor and recipient leading to chimeric vessel formation and improved engraftment of co-transplanted pancreatic islets *Yvonne Kelly, MD, UCSF Surgery, 1st Year Research Fellow*
1:25 PM	The Role of C-C Motif Chemokine Ligand 2 (CCL2) in Metastatic Neuroblastoma *Michael Zobel, MD, UCSF Surgery, 1st Year Research Fellow*
n/a	Successful enhancements of CRISPR knockout screens to study gene level regulation in primary human lymphocytes *Oren Shaked, MD, UCSF Surgery, 2nd Year Research Fellow* *(Not participating in symposium, as he is at his own wedding. Abstract included as a courtesy)*
1:35 PM	***Break***
2:15 PM	Keynote Lecture: Academic Surgery and the Art of the Pivot *Lillian S. Kao, MD, MS, Dunphy Visiting Professor*
3:30 PM	Closing Remarks & Awards *Peter Stock, MD, PhD, Research Committee Chair*

Predicting Acute Respiratory Distress Syndrome in Severe Blunt Trauma: The Utility of Interleukin-18

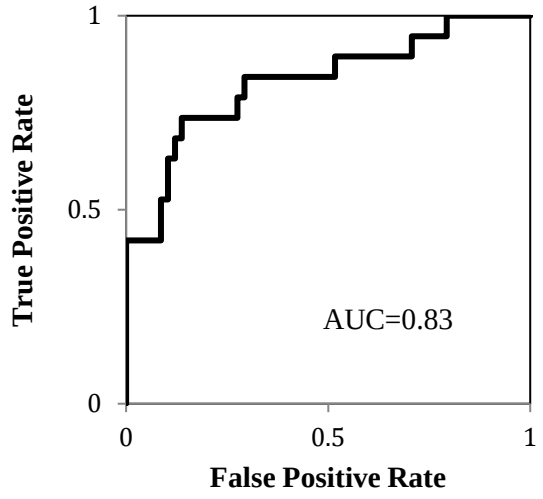
Genna Beattie MD[1], Caitlin Cohan MD[1], Gregory P. Victorino MD FACS[1]
[1]Department of Surgery, University of California San Francisco, East Bay, Oakland, CA

Introduction: Direct pulmonary injury and innate immune response activation primes the lungs for acute respiratory distress syndrome (ARDS) in trauma. Recently identified as a key mediator in ARDS pathogenesis is the inflammasome dependent release of Interleukin-18 (IL-18). As such, we hypothesized plasma IL-18 is a diagnostic predictor of ARDS in severe blunt trauma.

Methods: Secondary analysis of the Inflammation and the Host Response to Injury database was performed on plasma cytokines collected within 12 hours of severe blunt trauma. Trauma related cytokines, including IL-18, were compared between ARDS and non-ARDS patients, and were evaluated for association to ARDS using logistic regression. Threshold cytokine concentrations predictive of ARDS were then determined using receiver-operating curve (ROC) analysis.

Results: Cytokine analysis of ARDS (n=19) compared to non-ARDS patients (n=61) demonstrated elevated plasma IL-18 in ARDS and IL-18 strongly correlated with ARDS on logistic regression after confounder adjustment (p=0.008). Additionally, ROC analysis revealed IL-18 as a strong ARDS predictor (AUC=0.83, figure 1), with a threshold IL-18 value of 170 pg/ml (Youden Index: 0.3). IL-18 remained elevated in ARDS compared to non-ARDS patients during the acute injury phase ($p \leq 0.02$). Other trauma related cytokines did not correlate with ARDS.

Figure 1: Initial IL-18 Level and ARDS Development



Conclusion: In severe blunt trauma, IL-18 is a robust predictor of ARDS and remains elevated throughout the acute injury phase. These data support IL-18 as a key ARDS biomarker, promoting early identification of trauma patients at greater risk of developing ARDS. Timely recognition of ARDS and implementation of advantageous supportive care practices may reduce trauma related ARDS morbidity and costs.

Repeat CT Head Scan is Not Indicated in Trauma Patients Taking Novel Anticoagulation: A Multi-Institutional Study

Caitlin Cohan MD, Genna Beattie MD, Jessica Cox MD, Joseph Galante MD, Amy M. Kwok MD, MPH, Rachel C. Dirks PhD, & Gregory Victorino MD

Introduction: Guidelines for imaging anticoagulated patients following traumatic injury are unclear. An interval CT head (CTH) is commonly performed after an initial negative CTH to assess for delayed intracranial hemorrhage (ICH-d). The rate of ICH-d is largely unknown for those taking novel anticoagulants (NOACs), which appear to have a better safety profile than warfarin in the non-traumatic setting. We hypothesized that patients taking NOACs would have a lower rate of ICH-d than those on warfarin and more favorable clinical outcomes when ICH-d occurred.

Methods: Anticoagulated patients presenting with blunt trauma to multiple level I trauma centers between 2016 and 2018 were evaluated. Patients with intracranial hemorrhage on initial CTH and those taking non-oral anticoagulation or antiplatelet agents alone (without warfarin or NOAC) were excluded. Outcomes included: ICH-d, administration of reversal agents, neurosurgical intervention, readmission, and death. Multivariable regression was performed to evaluate for patient factors associated with development of ICH-d.

Results: A total of 739 patients met inclusion criteria. Patients were divided into a warfarin only group (n=409) and NOAC only group (n=330). The average age was 76 years old with 49% males. Repeat CTH was performed in 52% of cases. The incidence of ICH-d identified by repeat CTH in the NOAC group was 2.5% (4/159) vs. 4% (9/224) in the warfarin group, p=0.42. For all patients taking NOACs, including those without a repeat CTH, there were no neurosurgical interventions or deaths related to head injury. In the entire warfarin group, there was one neurosurgical intervention and 2 deaths due to head injury. Reversal agents were administered in 1.8% (6/330) of patients in the NOAC group versus 13.7% (56/409) in the warfarin group, p<0.01. On multivariate regression analysis of both groups, male sex (OR 18.6, p=0.03) and AIS head ≥2 (OR 25.4, p=0.04) were strongly associated with development of ICH-d.

Conclusion: In the NOAC group, ICH-d occurred only 2.5% of the time when CTH was routinely repeated. Regardless of routine repeat CTH, none of the patients taking NOACs required neuro-intervention or died as a result of their head injury. Our findings suggest NOACs may have a better safety profile following trauma compared to warfarin and challenge the use of repeat imaging in this patient population

	NOAC	Warfarin	p
Repeat CTH (%)	48	55	0.07
ICH-d (%)	2.5	4	0.42
Reversal Agent Administered (%)	1.8	13.7	<0.01
Neurosurgical Intervention (n)	0	1	N/A
Readmission (%)	4.2	1	0.06
Deaths from Head Injury (n)	0	2	N/A

Building Artificial Intelligence Systems for Surgery in Extreme Environments

Danyal Fer[1,2,3], Brijen Thananjeyan[2] ,Ye Tien[3], Ken Goldberg[2], Pablo Garcia Kilroy[3]-
UCSF East Bay[1] UC Berkeley[2], Verb Surgical[3]

- **INTRODUCTION**:
  A critical issue for surgery in extreme environments is separation from surgical expertise. This deficit can potentially be addressed through artificial intelligence (AI), bypassing the challenges faced with limited resources in unique environments. We describe the performance of semiautonomous stay suture placement in a mesh overlying a phantom hernia and (benefits or advantages) associated with encoding surgical tasks with computer vision.

- **METHODS**:
  At the UC Berkeley Autolab we calculated stay suture placement trajectories to optimize for dexterity and maneuverability while maintaining a distance of two centimeters from the outermost edge of the hernia. This was reinforced utilizing a reward function which avoided joint singularities determined by 16 physically performed stay suture tasks. We then developed a graphical user interface (GUI) to initiate the suture placement.

  At Verb Surgical we annotated surgical videos identifying constituent phases of operations, surgical tasks, and surgical tools. We then trained convolutional neural networks on the recognition of these characteristics for various outputs.

- **RESULTS OR PROGRESS:**
  At UCB we developed a GUI to identify the center of the simulated hernia and subsequently allowed the surgeon to initiate automated placement of stay sutures. These suture throws were optimized to ensure that each throw was as far from joint limits as possible to facilitate ease of needle recovery by the surgeon.

  At Verb Surgical, we created algorithms to recognize the surgical steps of complex bypass and colorectal surgeries with sufficient accuracy to provide clinical insights. We have also developed algorithms to recognize tools and surgical maneuvers. Lastly, we produced models which are able to anticipate surgical actions and subsequent environmental effects based upon prompting frames of surgical videos.

- **CONCLUSIONS:**
  We demonstrate that AI models have the capacity to assist with complex surgical procedures. While these models are far from being able to provide robust surgical support in extreme environments, they may have utility in local support for surgical education and subtask automation.

Bundle Payment for Care Improvement Advanced (BPCI-A) for Major Bowel Surgery: Initial Insights into How to Succeed in the Era of Payment Reform

Authors: Caitlin Collins, MD, Elizabeth Wick, MD

Introduction

There is a national imperative to curb healthcare spending and shift to value-based reimbursement. The Center for Medicare and Medicaid Services's (CMS) "Bundled Payment for Care Improvement Advanced (BPCI)," an alternative payment model that pays a fixed price for "care episode"(acute care surgical hospitalization and ANY post-discharge care for 90 days). Primarily adopted in medicine and orthopedics, we are one the few hospitals participating in bowel surgery.

Methods

Our bowel surgery bundled payment initiative includes a team of residents, faculty, nurses, social work, and Epic analysts. This transdisciplinary group is sub-divided into pre-operative, inpatient, and post discharge working groups to address issues faced within those particular care domains. We have integrated ACS-NSQIP, Epic and CMS to identify opportunities for improvement that will translate into improved quality and value for the entire episode.

Results

With BPCI orthopedics, the main opportunity to improve value was reduction in the use of rehabilitation and nursing facilities after discharge. In contrast, the greatest opportunity for BPCI major bowel is with reduction in 90-day re-admissions to an acute care hospital. Many of the high utilization major bowel patients had multiple re-admissions within the 90-day care episode for management of surgical complications.

Conclusion

The strategy to succeed in BPCI major bowel needs to bring together surgical quality improvement to reduce complications with enhanced post-discharge care management to help patients avoid rehospitalization.

A Bioreactor Containing Primary Human Renal Epithelial Cells Supported in Vivo Without Immunosuppression

Rebecca Gologorsky, Eun Jung Kim, Jarrett Moyer, Ana Santandreu, Charles Blaha, Jimmy Ly, Alonso Torres, Rebecca Shaheen, Deepika Sarode, Nathan Wright, William Fissell, Shuvo Roy

Introduction: The bioartificial kidney is intended to contain human cells in an immunologic sanctuary and provide total renal replacement. In vitro experiments have demonstrated the efficacy of silicon nanopore membranes (SNM) as an immunologic barrier to inflammatory cytokines with selective permeability that supports human renal epithelial cell (HREC) viability. Here, we test an SNM-based bioreactor in vivo to demonstrate HREC viability and functionality without immunosuppression or anticoagulation in a swine model.

Methods: HREC were grown to confluence on semipermeable polycarbonate membranes coated with type IV collagen and separated from blood flow by SNM with 10 nm pores, allowing passage of nutrients but not immunoglobulins or immunocytes. The bioreactor was implanted in the neck of a healthy Yucatan pig for three days and perfused via anastomoses to the external jugular vein and carotid artery. A static control was comprised of HRECs grown on identical membranes in cell culture media at 37˚C.

Results: HREC were evaluated for viability and biomarkers of functionality upon device explantation. Cell viability was over 90% as shown by fluorescence imaging after staining with calcein AM and ethidium homodimer-1. Cells remained confluent with tight junctions expressed by Zona Occludens (ZO1) protein comparable to that of the control. γGlutamyltransferase (γ-GT) activity, which is a renal tubule cell (RTC)-specific marker, and qPCR evaluating expression of RTC surface markers including AQP1 and NHE3 were consistent with healthy static control RTCs. *NAG* activity, a biomarker of RTC damage, was low.

Conclusion: We show immunoprotection of primary human renal cells and preservation of renal tubule cell functional markers in a bioreactor after three-day implantation in a swine. This is a promising model for bioartificial kidney development in humans that may lead to total renal replacement.

Deep Immune Profiling of High- vs. Low-Risk Lung Adenocarcinoma

Iris H. Liu, Gavitt A. Woodard, Arjun Rao, Vincent Chan, Max Krummel, Lawrence Fong, David Jablons, Johannes R. Kratz

Introduction:

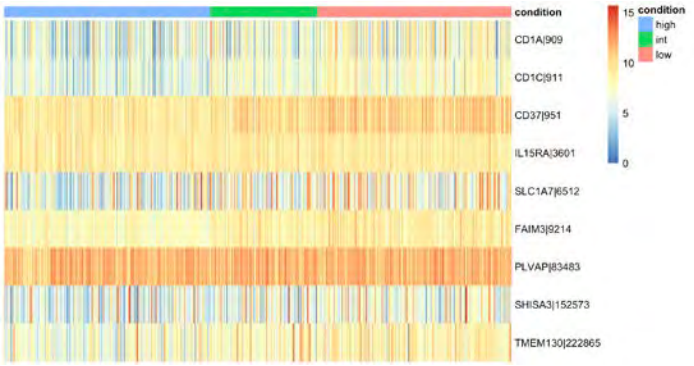
Despite the advances made in diagnosing and treating NSCLC over the past several decades, the mortality of early-stage non-small cell lung cancer (NSCLC) remains high at 40-50% within 5 years of curative-intent surgical resection. Our lab has pioneered a clinically validated molecular assay for the stratification of early-stage, resectable NSCLC by recurrence risk, which has been implemented at UCSF to guide treatment for identified high-risk patients, resulting in survival mirroring that of their low-risk counterparts. We hypothesize that whole transcriptome shotgun sequencing (WTSS) can reveal deeper alterations underlying the novel molecular signature, which (1) may drive the risk of preoperative occult metastasis and post-operative recurrence, and (2) may present targets for improved integration of molecular medicine in the surgical care of patients with high-risk NSCLC.

Methods:

Banked samples of resected early-stage lung adenocarcinoma were dually characterized using the validated molecular risk assay and by whole transcriptome shotgun sequencing (WTSS). RNAseq data was then stratified by compartment (e.g. tumor vs. immune vs. stromal). Differential expression analysis was conducted in R, and candidate pathways were elucidated by gene ontology enrichment analysis.

Results/Progress:

In collaboration with the Fong Lab, we have performed dual WTSS and molecular risk analysis on 12 resected early-stage lung adenocarcinomas. Using DESeq2, 42 genes have emerged as differentially expressed between high- and low-risk tumors. From these, DAVID gene ontology analysis extracted 2 gene clusters, both of which are related to immune regulation. In particular, IL15RA and CD37 have emerged as potential actionable targets in high-risk early stage lung cancers.



Conclusions/Next Steps:

Expression profiles of low- vs. high-risk lung adenocarcinomas reveal that immune regulation may play a important role in outcomes after resection of early-stage lung cancer. Future studies include functional validation of actionable gene targets, which may include RNA interference or CRISPR-mediated modeling in tissue culture models and animal models to study the effects of specific genes on tumor cell growth and invasion.

Novel Microtubule and PARP Inhibitor Drug Attenuates Neointimal Hyperplasia in a Rat Model of Arterial Injury

Greg J. Haro, Evan C. Werlin, Hideo Kagaya, Pei-Yu Lin, Mian Chen, Yi-Wei Yang, Csaba J. Peto, Hassan Lemjabbar-Alaoui, David M. Jablons, and Michael S. Conte

Introduction

Long-term failure following endovascular intervention for peripheral arterial disease can be due to neointimal hyperplasia, an exaggerated healing response of smooth muscle cell proliferation. Cancer therapeutics may have a cross-disciplinary role in preventing cell division and possibly diminishing this pathologic response. The objective of this study was to evaluate the effectiveness of a novel cancer drug (19-26) to attenuate neointimal hyperplasia in a rat model of arterial injury.

Methods

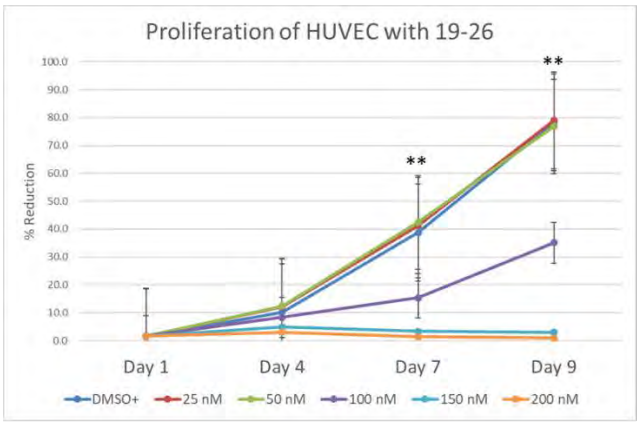
19-26 is a novel cancer drug developed in the UCSF Thoracic Oncology Laboratory that inhibits both microtubules and poly ADP ribose polymerase (PARP), and its unique chemical structure allows for oral administration. The in vitro effects of 19-26 on protein expression, cell cycle, proliferation, migration, and cytotoxicity were studied in smooth muscle and endothelial cell lines. The safety and effectiveness of 19-26 were evaluated in a rat model of carotid angioplasty. 19-26 at 50 mg/kg/dose or 10% TPGS / 0.01 N HCL (Vehicle) were given orally every 12 hours one day prior to and six days following surgery. Morphometric, histologic, and genetic analyses were performed 3 and 14 days after surgery.

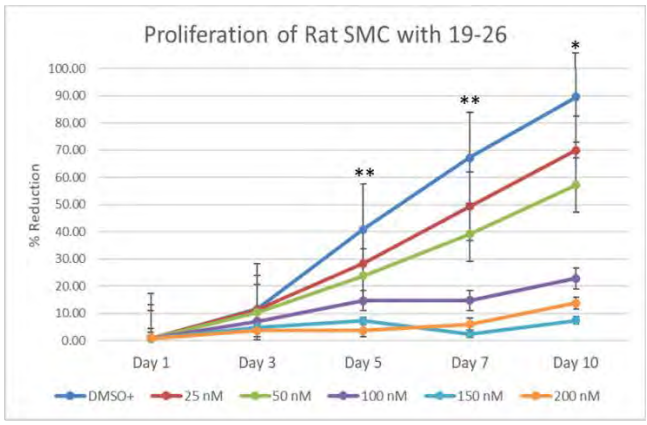
Results

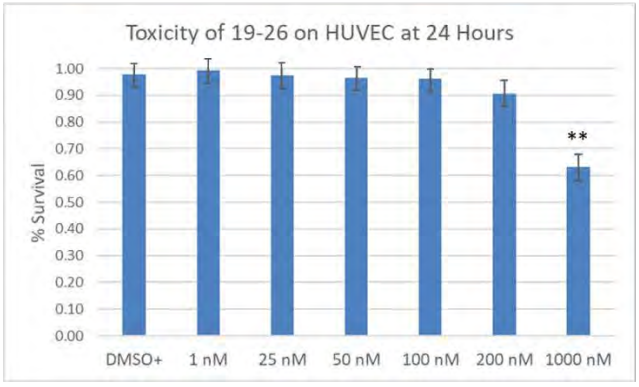
In vitro, 19-26 acted on expected drug targets and reduced tubulin and PAR expression. Reduction in proliferation in smooth muscle and endothelial cell lines was seen in doses as little as 50 nM and was completely halted ≥150 nM (both $P<0.02$). In the presence of growth factor, smooth muscle cell migration up to 48 hours was inhibited at ≥100 nM ($P<0.01$). There was no evidence of in-vitro cytotoxicity at concentrations less than <200 nM ($P=0.21$), though significant cytotoxicity was seen at 1000 nM for both cell types ($P<0.01$). Overall, endothelial cells were more resistant to 19-26 than smooth muscle cells. Following in vivo exposure, all rats appeared healthy without diarrhea or hair loss, and all carotid vessels were patent without evidence of thrombosis. 19-26 reduced neointimal formation after carotid angioplasty by 50% compared to vehicle (0.18 mm^2 Vehicle, 0.09 mm^2 19-26; $P<0.01$). Following steady state at 3 days after surgery, there was no difference in the proportion of apoptotic cells in 19-26 vs vehicle treated rats (13.3% Vehicle, 12.1%; $P=0.83$).

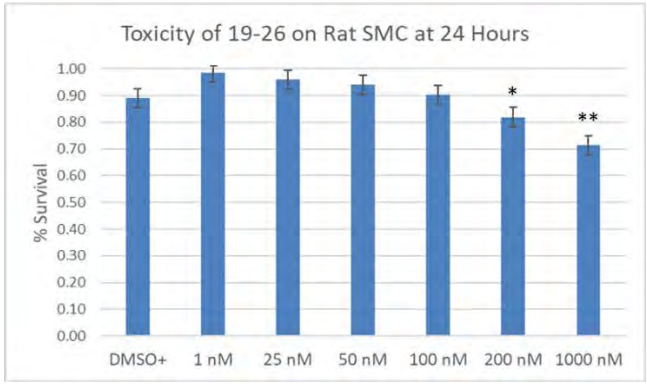
Conclusions

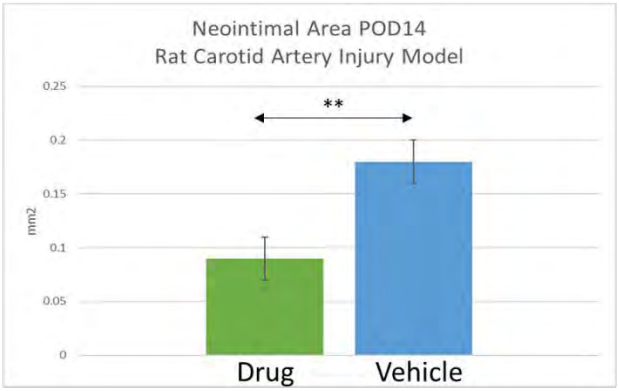
19-26 attenuates neointimal hyperplasia without associated toxicity in a rat model of carotid angioplasty. This effect is likely due to decreased arterial smooth muscle cell proliferation and migration. Further studies are needed to define the optimal dosing and duration of therapy.

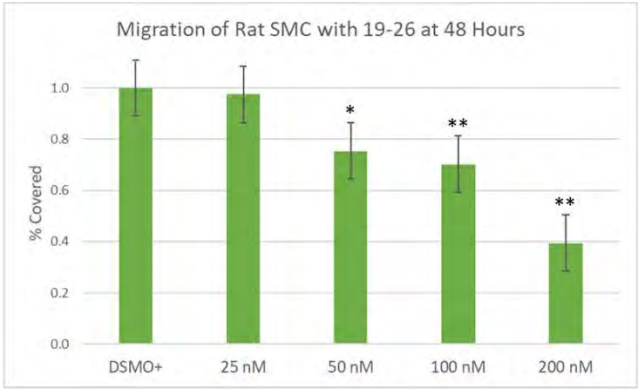


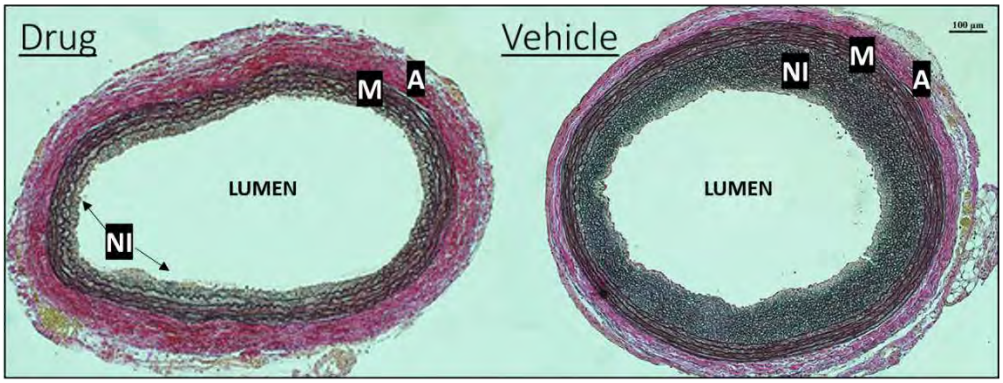












NI=Neointima
M=Media
A=Adventitia

*P<0.05; **P<0.01

Pancreatic Islet and Parathyroid Co-Transplantation for Treatment of Diabetes in Intra-Muscular Site: "PARADIGM".

Casey Ward[1], Yvonne Kelly[1], Gaetano Faleo[1], Greg Szot[1], Zion Congrave-Wilson[1], Linda Vo[2], Eleonora de Klerk[2], Gopika Nair[2], Audrey Parent[2], Vinh Nguyen[1], Charity Juang[2], Mathias Hebrok[2], Wenhan Chang[4], Quan-Yang Duh[3,4], Peter Stock[1,2], Qizhi Tang[1,2]

[1] UCSF Department of Surgery, Division of Transplant Surgery
[2] UCSF Diabetes Research Center
[3] UCSF Department of Surgery, Division of Endocrine Surgery
[4] San Francisco Veteran Affairs Medical Center, Division of Endocrinology and Endocrine Surgery

Introduction: Islet transplantation can cure type 1 diabetes; however, multiple donors are often needed to achieve insulin independence due to extensive perioperative loss of islets from ischemic injury. In comparison, parathyroid gland (PTG) auto and allo-transplantation in the intra-muscular (IM) site is an established successful surgical procedure. In this study, we aim to understand and exploit the properties of PTG engraftment in order to understand how both survival factors and angiogenesis play a synergetic role in preserving islet engraftment.

Materials and Methods: Luciferase-expressing mouse islets or human embryonic stem cell enriched beta clusters (eBCs) were co-transplanted with or without PTG in the IM of syngeneic or immunocompromised mouse recipients, respectively. Human islets from deceased donors with or without human PTG were co-transplanted in IM of streptozotocin induced diabetic immunodeficient NSG mice. Graft mass was quantified using bioluminescence imaging in recipients of luciferase expressing grafts. Human islet and PTG supernatant were analyzed for secreted proteins via Luminex.

Results and Discussion: Co-transplantation of PTG with both mouse islets and human eBC in the IM resulted in near complete preservation of the transplanted grafts in comparison to significant islet loss in mouse islet alone (+PTG-90.3% vs Alone-11.7%, p-value 0.001) or eBC alone grafts (+PTG-86.4% vs Alone-16.6%, p-value 0.02) after 28 days (n=5 per group). Furthermore, PTG co-transplantation with a sub-optimal mass of mature human islets (1000IEQ) achieved diabetes reversal in NSG mice. After 100 days, 67% (6/9) of PTG co-transplanted mice remained diabetes free compared to 0% (0/9) of mice with islets in IM alone (p-value 0.005).

To elucidate the mechanism behind PTG's protection of islets in co-transplantation, we developed a theoretical framework for islet transplant survival highlighting the role of survival factors in the immediate peri-transplant period followed by accelerated angiogenesis in less than 5 days. Comparing secreted proteins from PTG and human islet supernatant, we show the presence of multiple known pro-islet survival factors (PTHrP, HGF, IL-6, Osteopontin) and pro-angiogenic factors (Ang2, FGF-2, PLGF) secreted by PTG that are absent in purified human islets. Additionally, together with PTG's rich vascular endothelial progenitor cell population of CD34+45- cells, PTG secretes G-CSF and CXCL12 which mobilize additional supportive cells to the intra-muscular transplant site.

Conclusion: We show for the first time that co-transplantation of PTG with mature islets and stem-cell-derived beta cells leads to increased survival and diabetes reversal in the IM site. Furthermore, we developed and proved a theoretical framework for islet survival that necessitates both early survival factors and accelerated angiogenesis. The culmination of this work has led to the opening of a Phase I/IIa FDA clinical trial in transplant patients for the treatment of Type 1 diabetes.

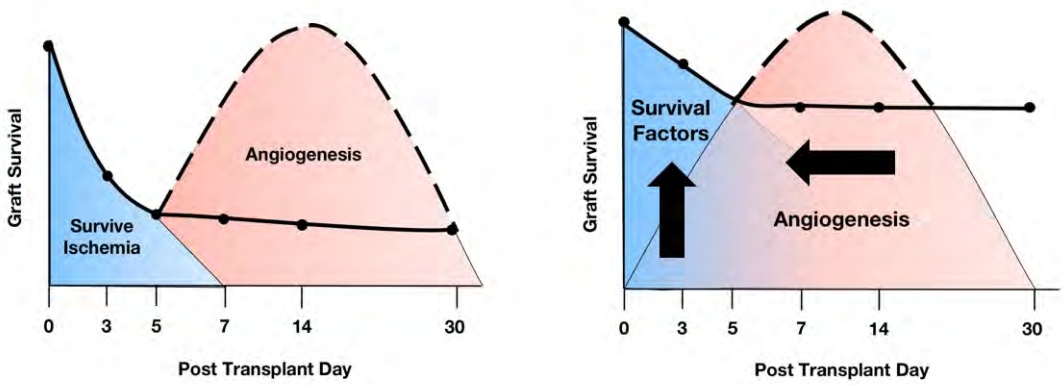


Figure 1: ***Theoretical islet transplant survival curve. Left)*** *Current and* ***Right)*** *therapeutic model for islet transplant survival in the extra-hepatic site at present. The requirement for survival factors in the first five days post-transplant is a critical component of any therapeutic model to maintain optimum graft mass until revascularization occurs. Accelerating angiogenesis from >14 days to less than 5 days is additionally paramount to preserve greatest graft mass post-transplant.*

Layilin is Enriched in Tumor-Infiltrating CD8$^+$ T Cells in Human Cancer and Contributes to Anti-Tumor Immunity by Enhancing LFA-1-Mediated Adhesion

Kelly M. Mahuron, Joshua M. Moreau, Devi P. Boda, Adil Daud, Michael Alvarado, and Michael Rosenblum

INTRODUCTION:
Desirable clinical responses to anti-PD-1 immunotherapy depend upon tumor infiltration of PD-1 expressing CD8$^+$ T cells. Our laboratory has recently demonstrated that the frequency of PD-1^{hi}CTLA-4^{hi} CD8$^+$ tumor-infiltrating lymphocytes (TILs) in metastatic melanoma biopsies predicts treatment efficacy. Deeper understanding of the nature of these cells may provide additional avenues to enhance their accumulation and cytotoxic capability.

RESULTS:
In the current study, we employed bulk and single cell whole transcriptome sequencing of melanoma TILs as a discovery strategy to identify candidate genes of functional significance. Among highly enriched genes in PD-1^{hi} cells, we identified LAYN, which codes for the poorly characterized C-type lectin layilin. Layilin has recently been associated with TILs in hepatocellular carcinoma and non-small-cell lung cancer and has been hypothesized to function as an inhibitory receptor. To determine the functional role of layilin on CD8$^+$ T cells, we developed conditional knockout mice and a CRISPR-Cas9 protocol to delete LAYN in human PBMCs. Contrary to recent reports that suggest layilin is an inhibitory receptor, both B16F10 and MC38 cancer models had increased growth in mice where CD8$^+$ T cells were specifically layilin deficient compared to wild type controls. Correspondingly, experiments using human CD8$^+$ T cells and CRISPR-mediated deletion of LAYN produced a defect in antigen specific cancer cell killing *in vitro*. However, both mouse and human LAYN deficit CD8$^+$ T cells exhibited normal proliferation and cytokine production. Given layilin's known molecular association with the cytoskeletal protein talin, we hypothesized a role for layilin in regulating integrin function. *In vitro* adhesion assays revealed a defect in the ability of LAYN deficit CD8$^+$ T cells to adhere to ICAM-1 coated substrates. Blocking antibodies confirmed this interaction to be LFA-1 integrin dependent.

CONCLUSIONS:
Taken together, we definitively demonstrate that layilin is not an inhibitory receptor but rather augments cytotoxic T cell effector function through integrin regulation. The mechanism of layilin function may be amenable to therapeutic targeting in human disease, but careful consideration should be applied in the context of cancer immunotherapy.

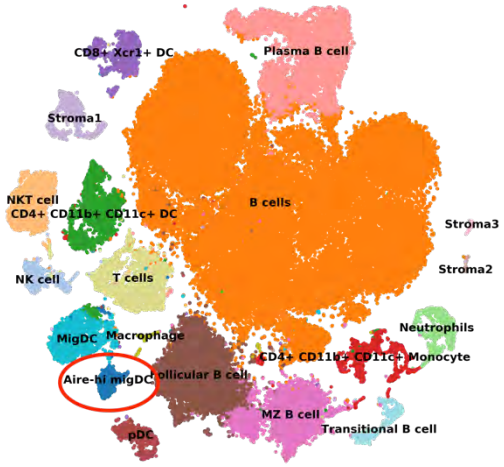
Extrathymic AIRE-expressing Cells are a Class of Migratory Dendritic Cells That Can Mediate Tolerance to Both Endogenous and Exogenous Antigens

Jhoanne Bautista MD PhD, Nathan Cramer, Jennifer Liu PhD,
Benjamin Yuen PhD, James Gardner MD PhD, Mark Anderson MD PhD

INTRODUCTION: Extrathymic AIRE-expressing cells (eTACs) are tolerogenic dendritic cells (DCs) that express the Autoimmune Regulator gene and are found in secondary lymphoid organs in both mice and humans. They generate a panel of tissue restricted antigens (TRAs) distinct from the array presented in the thymus, and they delete autoreactive CD8+ killer T cells and render pathogenic CD4+ helper T cells anergic. Here, we report on the definitive characterization of these cells and their surprising versatility in acquiring exogenous antigens that they can also present to mediate tolerance.

METHODS: The characterization of eTACs is based on single cell RNA sequencing (scRNA seq) of mouse lymph node cells with the assignment of cell identity predicted by machine learning algorithms, and validated using flow cytometry and lineage tracing in reporter mice. Tolerance assays include a diabetes induction model and a hapten-based skin tolerance test.

RESULTS: Unsupervised clustering of scRNA seq data identified a unique cell cluster that expresses moderately high levels of AIRE. This population was mapped using reference data from ImmGen via 3 independent machine learning algorithms to a class of migratory dendritic cells. We confirmed this identification by performing flow cytometry on lineage tracing mice that follow the development of classical DCs from the expression of Zbtb46 through CD11c.



Functionally, we re-demonstrated the eTACs that have been genetically engineered to express a pancreas-specific autoantigen can prevent diabetes. Conversely, deletion of eTACs results in an exaggerated hypersensitivity reaction in a skin tolerance assay that requires migratory dendritic cells taking up antigen in one location and suppressing inflammation in another.

CONCLUSIONS: Extrathymic AIRE-expressing cells comprise a class of migratory dendritic cells that can mediate durable tolerance not only to endogenous self-antigens but also to exogenous antigens that have been introduced in a non-inflammatory context. This versatility hints at a great potential for use of eTACs to mediate tolerance for a wide variety of applications including solid organ transplantation and autoimmunity.

A Role for Aire-Expressing Cells in Maternal-Fetal Tolerance

Eva M. Gillis-Buck, James M. Gardner, Mark S. Anderson, and Tippi C. MacKenzie

Introduction: Healthy pregnancy relies on maternal tolerance to both paternal alloantigens and a unique set of maternal pregnancy-associated self-antigens, such as those arising from the decidua and developing placenta. The Autoimmune Regulator gene (Aire) prevents autoimmunity by promoting the expression of self-antigens, leading to clonal deletion of self-reactive T cells. We hypothesize that maternal Aire-mediated expression of pregnancy-associated self-antigens promotes maternal tolerance to the developing fetus, and that dysfunction of Aire-expressing cells may play a role in pregnancy complications such as recurrent miscarriage and implantation failure, which are often associated with autoimmunity.

Methods: Aire-diphtheria toxin receptor (AireDTR) transgenic females were bred to wildtype (WT) males, and plugged females were treated with DT during the first nine days of pregnancy to ablate maternal Aire-expressing cells ("AireDTR+DT dams"). WT females were bred to WT males and given identical DT treatment ("WT+DT dams"). Other WT females were bred to AireDTR males and given DT. Maternal blood and tissues were collected at mid-pregnancy for progesterone ELISA, histology, and immune profiling by flow cytometry.

Results: Ablation of Aire-expressing cells during the first half of pregnancy increased the risk of complete embryo resorption (abortion of the entire litter). AireDTR+DT dams (N=32) were 5.7 times as likely to show complete embryo resorption and half as likely to have healthy embryos at mid-pregnancy, compared to WT+DT dams (N=36). When live embryos were present in the AireDTR+DT uterus, average embryo weight was significantly less than those of WT+DT dams ($p<0.001$), suggesting intrauterine growth restriction or early stage resorption. AireDTR+DT dams had significantly fewer $FoxP3^+$ Tregs ($p<0.0001$) and more $CD4^+$ T cells ($p<0.0001$) in the maternal thymus, and increased frequency of CD3+ CD4- CD8- T cells in the uterus ($p<0.05$). AireKO mice are known to experience ovarian autoimmunity and subsequent progesterone deficiency; however, we observed no significant difference in serum progesterone between WT+DT and AireDTR+DT dams ($p>0.05$), and progesterone supplementation failed to prevent resorption in AireDTR+DT dams. To investigate whether the observed resorptions were secondary to developmental failure of AireDTR+ embryos, we bred AireDTR males to WT females and treated the plugged females with DT (N=22), so that approximately half of developing embryos would be transgenic and in an environment without maternal Aire-deficiency. There was no significant difference in the number of WT and AireDTR+ embryos that survived to E9.5, and resorption rate was comparable to pregnancies with all wildtype embryos. All p-values were calculated using Student's t test. Relative risk of each pregnancy outcome is shown in the table.

Pregnancy outcome at E9.5	Relative risk (AireDTR vs WT+DT)	95% confidence interval	P value
Resorbing embryos	5.78	1.37 – 24.5	0.0171
No implantation	1.41	0.67 – 2.97	0.3617
Live embryos	0.49	0.29 – 0.83	0.0073

Conclusions: Ablation of Aire-expressing cells during early pregnancy leads to maternal T cell imbalance and increased risk of fetal loss, which cannot be explained by maternal progesterone deficiency nor DT-mediated ablation of transgenic embryos. These data suggest a potential role for Aire-expressing cells in supporting maternal-fetal tolerance. Next steps include searching for maternal autoantibodies in AireDTR+DT dams and differentiating the roles of thymic versus extrathymic Aire by performing reciprocal thymic transplants in WT and AireDTR females.

Title: Residents as Key Effectors of Change in Improving Opioid Prescribing Practices

Authors: Elizabeth Lancaster, Tasce Bongiovanni, Joseph Lin, Rhiannon Croci, Kenzo Hirose, Elizabeth Wick

Introduction:
There is a national imperative to curb the flow of opioids into our communities. In academic medical centers, the majority of discharge opioid prescriptions are written by residents who receive predominantly ad hoc, peer-to-peer education on perioperative analgesia. We aimed to understand baseline knowledge and practice around discharge opioid prescribing and to develop an optimal perioperative analgesia program focused on providing appropriate opioid quantities and increasing non opioid pain adjuncts.

Methods:
Surgical residents led a transdisciplinary team of faculty, anesthesiologists, pharmacists, advanced practice providers, and health informaticians to identify how baseline prescribing habits differed from best practices, and to understand the educational needs to bridge this gap. Based on the needs assessment, we developed multifaceted educational interventions including grand rounds, didactic and case-based conferences, and pocket cards. Residents' attitudes toward opioid prescribing were assessed using an anonymous survey before and after the educational component. We developed automated reports from the electronic health record to evaluate prescribing practices for the select procedures.

Results:
After our educational interventions, residents' beliefs as to the opioid quantity necessary after common general surgical operations decreased significantly. Prior to the educational intervention, there was significant discrepancy between the resident beliefs as compared to their practice. This gap narrowed with education (Table 1). There was a significant decrease in the quantity of opioids prescribed (oral morphine equivalents, OMEs) on discharge for most included operations. Additionally, there was a marked spillover effect, leading to a decrease in the OMEs prescribed for all general surgery operations, even when excluding those that were the primary focus of our intervention (Table 1).

Operation	**Mean Resident Response to Survey (OME per patient)**			**Mean Opioid Prescribed (OME per patient)**		
	Pre-Intervention	*Post-Intervention*	*p-value*	*Pre-Intervention*	*Post-Intervention*	*p-value*
Lap Chole	84	62	0.002	162	94	<0.001
Lap Appy	69	51	0.003	121	82	0.04
Inguinal Hernia	85	58	0.002	154	104	0.003
Endocrine	62	30	<0.001	90	24	<0.001
Anorectal Procedure	72	37	<0.001	133	98	0.19
General Surgery	n/a	n/a	n/a	294	163	<0.001

Table 1. Results of survey of surgical residents: how many Norco pills one should a patient be prescribed after laparoscopic cholecystectomy, laparoscopic appendectomy, inguinal hernia repair, cervical endocrine surgery, and anorectal procedures? This is compared to the mean number of pills actually prescribed for these procedures. Data is converted to milligrams of oral morphine equivalent (OME).

Conclusions:
Residents can effectively lead and drive rapid change in perioperative discharge opioid prescribing. We saw significant change in residents' beliefs and in opioid prescribing patterns over only a three month period. Ongoing education and feedback is essential to sustaining this improvement given the ongoing gap between resident beliefs and practice.

Surgical Correction of Breast Animation Deformity with Implant Pocket Conversion to a Neo-Prepectoral Plane

Michael C. Holland, MD, Rachel Lentz, MD, Hani Sbitany, MD

Introduction

Animation deformity is an undesirable outcome of subpectoral breast reconstruction that results in abnormal breast contraction with activity, breast pain, and increased implant visibility. Surgical correction requires implant removal and conversion of the reconstruction to a neo-prepectoral plane. We present our institutional experience with our preferred surgical technique to treat this challenging problem, and outline solutions for increased success in these patients.

Patients and Methods

A retrospective review was performed of all patients undergoing conversion of their subpectoral breast reconstruction to a neo-prepectoral plane at our institution. Patient demographics and surgical details were analyzed, and post-operative outcomes and morbidity were assessed. The effects of changing operative strategies on enhanced success are also reported.

Results

A total of 80 breast conversions were performed over a 2.5 year period. All patients demonstrated resolution of animation deformity on mean follow up of 15.2 months. Two reconstructions (2.5%) required an unplanned return to operating room, while 11 reconstructions (13.8%) were treated for infection. Pre-conversion fat grafting and the utilization of acellular dermal matrix (ADM) were both associated with reduced incidence of post-operative asymmetry and capsular contracture ($p < 0.05$). There were no reconstructive failures associated with conversion to a neo-prepectoral pocket.

Conclusion

Treatment of animation deformity in the reconstructed patient can be safely performed by surgical conversion to a neo-prepectoral plane. The use of ADM, and pre-conversion fat grafting, in appropriate patients can improve results. We promote this operative algorithm for all reconstructive patients experiencing symptomatic animation deformity with subpectoral breast reconstruction.

Post-Operative Opioid Requirements in Living Kidney and Liver Donors

Hillary J. Braun MD, Dieter Adelmann MD, Trevor Grace MD, Marisa E. Pulcrano MD, John P. Roberts MD, Claus U. Niemann MD, Nancy L. Ascher MD, PhD

Introduction:
Approximately 6% of opioid-naïve surgical patients develop opioid dependence post-operatively, and the CDC found that patients prescribed more than 50 morphine equivalents (MEQ) per day have twice the risk of opioid overdose compared with patients prescribed less than 20 MEQ/day. Living kidney and liver donors represent a unique population of patients who undergo surgery with no medical indication. Pain control in the post-operative setting is typically multimodal, however nearly all patients receive inpatient opioids and outpatient opioid prescriptions at discharge, placing them at risk for opioid dependence. The purpose of this study was to examine and describe opioid requirements in living kidney and liver donors prior to and following discharge at our single, high volume transplant center.

Methods:
We conducted a retrospective review of all living kidney and liver donors at our institution between 2012-2018 and performed descriptive analyses on donor demographics and opioid requirements. Morphine equivalents prescribed at discharge were compared with recommendations from comparable surgical procedures; laparoscopic donor nephrectomies were compared with laparoscopic colectomy (recommended 150 morphine equivalents at discharge), and living donor hepatectomy was compared with open small bowel resection (recommended 150 morphine equivalents at discharge).

Results:
A total of 623 kidney donors and 156 liver donors were included in the analysis. All kidney donors underwent laparoscopic donor nephrectomy; all liver donors underwent open donor hepatectomy.

Kidney donors were predominantly female (65.7%), with a median age of 42 years, and were related to their recipient (54.7%). 137 patients (22%) had an opioid medication on their medication list at the time of admission, and 18 (2.9%) had a history of anxiety or depression. Median length of hospital stay was three days. On the day prior to discharge, donors had a median of 40 morphine equivalents (IQR 22.5-72.5). Kidney donors were discharged with a median of 400 morphine equivalents (IQR 225-400) for a total of 5 days post-discharge with a median of 60 maximum morphine equivalents per day (IQR 40-80). No patients were discharged on ibuprofen and 125 (20.1%) were discharged on acetaminophen. Eleven patients (1.8%) were readmitted within 30 days.

Liver donors were predominantly female (53.8%), with a median age of 46 years, and were most often related to their recipient (67.9%). 13 patients (8.3%) were taking an opioid medication at the time of admission, and 16 (10.2%) had a history of anxiety or depression. Median length of hospital stay was five days. On the day prior to discharge, patients required a median of 40 morphine equivalents (IQR 20-61.25). Patients were discharged with a median of 375 morphine equivalents (IQR 300-500) for a total of 6.67 days with a median of 50 morphine equivalents per day (IQR 45-90). Two patients (1.3%) were discharged with ibuprofen, 56 patients with acetaminophen (35.9%), and 19 patients with gabapentin (12.2%). Nine patients (5.8%) were readmitted within 30 days.

Conclusion:
Kidney donors were discharged with 60 maximum morphine equivalents per day, which is greater than the CDC recommendation of 50 morphine equivalents, while liver donors were discharged with 40 morphine equivalents per day. Implementation of aggressive post-operative multimodal pain control regimens and education on opioid prescribing patterns is needed to help decrease the amount of opioids prescribed to this patient population.

Human ARF Tumor Suppressor Suppresses Zebrafish Cardiac Regeneration

Solomon Lee, Stanley Tamaki, Jason Pomerantz

Introduction: This study explores how the human ARF tumor suppressor, while importantly preventing oncogenesis, may simultaneously inhibit mammalian epimorphic regeneration. The Alternative Reading Frame (ARF) protein is a tumor suppressor encoded by the Cdkn2a gene to maintain the postmitotic state with no orthologs represented in highly regenerative species, suggesting that ARF may suppress regeneration. We aim to characterize the impact and target of ARF during the more complex and clinically translatable processes of heart regeneration after massive myocardial infarction.

Methods: Transgenic zebrafish lines expressing ARF under control of the heat shock promoter (hs:ARF) and natural human ARF promoter (ARF:ARF) were created and breed. Cryoinjury was performed on transgenic fish and wild type (WT) controls by applying a liquid nitrogen probe to their hearts under anesthesia. Hearts were collected on 0, 1, 4, 7, 11, 15, 30, and 45 days post-injury (dpi) for analysis. Regenerative progress was analyzed using histology, immunofluorescence, and qPCR of tissue-specific regenerative markers.

Results: ARF expression was upregulated during the cardiac regenerative process and slowed the rate of morphological recovery. In hs:ARF fish, AFOG and troponin staining revealed a 48.7% ($p<0.01$) reduction in myocardial recovery compared to WT fish. In ARF:ARF fish, myocardial recovery was reduced by 2.3% ($p=0.96$), 20.4% ($p=0.47$), 41.3% ($p<0.01$), 36.1% ($p=0.05$), and 24.3% ($p<0.01$) at 1, 4, 7, 15, and 30 dpi respectively. A cardiomyocyte proliferation index generated by MEF2/PCNA staining confirmed cardiomyocyte-specific suppression in ARF:ARF heart regeneration by 46.6% ($p=0.01$) at 11 dpi. Tissue-specific gene expression was tracked by qPCR in ARF:ARF and WT fish to further elucidate the cell types and processes affected by the ARF protein. Fgf17b, vegfaa, and Twist1b were reduced by 42% ($p<0.01$), 43% ($p<0.01$), and 55% in ARF:ARF hearts at 11 dpi, reflective of decreases in myocardial regeneration, vascular regeneration, and epithelial-to-mesenchymal (EMT) transition respectively. There was no significant difference in fgfr2c expression ($p=0.44$), a marker of epicardial regeneration.

Conclusions: ARF's selective impact on myocardial regeneration, vascular regeneration, and EMT, while not affecting epicardial regeneration, elucidates the specific regenerative mechanisms that ARF suppresses. ARF targets dedifferentiation and transdifferentiation processes while not affecting simple proliferation, activating as a barrier to mammalian epimorphic regeneration by being unable to separate regeneration from tumorigenesis. Our findings show that ARF will require alteration in conjunction with other genes to permit regeneration.

A Systematic Review of Delays in Diagnosis and Barriers to the Care of Colorectal Cancer in Low- and Middle-Income Countries

Nathan Brand, Lian Qu, Ann Chao, Andre Ilbawi

Background

Of the 746,000 colorectal cancer (CRC) diagnoses made each year, the majority occur in high-income countries (HIC), while over 50% of deaths occur in low- and middle-income countries (LMIC). Stage of disease at diagnosis is a significant prognosticator of survival and the higher rates of advanced stage diagnoses made in LMIC may contribute to the difference in death rates between HIC and LMIC. This review focuses on delays and barriers to CRC diagnoses of patients in LMIC, where CRC incidence is increasing.

Methods

We conducted a systematic review of peer reviewed literature published on these topics in LMIC. Inclusion criteria for our systematic review was any full text article that addressed barriers to care or delays in early diagnosis of CRC that was conducted in LMIC. Studies were required to contain any of the following: (i) defined or reported delay intervals in the diagnosis of symptomatic CRC or (ii) reported predictive factors or barriers that delayed early diagnosis of symptomatic CRC.

Results

Of the 10,193 abstracts screened, 9 studies met inclusion criteria. All 9 studies were conducted in middle-income countries. Five studies assessed the intervals along the pathway from symptom onset to cancer treatment, and significant delays were identified along all stages of the cancer care continuum. All 5 studies identified that the greatest delay occurred prior to disease diagnosis. Of the 4 studies that assessed the individual intervals of CRC diagnosis; 2 (50%) found the greatest delay occurred during the interval between symptom onset and presentation to the healthcare system, and 2 (50%) found the greatest delay occurred between first presentation to the healthcare system and cancer diagnosis. Six studies assessed barriers to cancer care, and 4 studies assessed knowledge of CRC. All studies found low levels of knowledge of CRC as a disease, its risk factors, or how it is diagnosed, in both the general population and among healthcare workers.

Conclusion:

Despite the increasing burden of CRC in LMIC, there is little published research on delays to CRC diagnosis and treatment or the barriers that cause them in resource-limited settings. Our review demonstrates significant delays throughout the entire process of cancer diagnosis and treatment and identifies the period prior to CRC diagnosis as the most vulnerable to delays. In addition, we have identified low levels of knowledge about CRC in both the general population and healthcare workers. Our study highlights the tremendous need for research and action to reduce CRC morbidity and mortality in LMIC.

Patient Complexity Varies by Surgical Specialty and Does Not Strongly Correlate with Work Relative Value Units

Joel L. Ramirez, Warren J. Gasper, Carolyn D. Seib, Emily Finlayson, Michael S. Conte, Julie Ann Sosa, James C. Iannuzzi

Introduction: There is little data on patient complexity variation across surgical specialties. Understanding surgical population differences can inform policy decisions about resource allocation and reimbursement. This study identified variation in patient complexity across surgical specialties and assessed correlation between complexity and work relative value units (RVU).

Methods: The 2017 ACS-NSQIP was queried for cases involving the specialties of general, neurological, vascular, urology, orthopedic, cardiac, thoracic, plastics, and ENT. Ten markers of patient complexity were measured, including: ASA class ≥4, number of major comorbidities, emergent operation, major complications, concurrent procedures, additional procedures, length of hospital stay (LOS), non-home discharge, readmission, and mortality. Specialties were ranked by individual markers of complexity and then summed, creating an overall complexity score and rank that was then compared with general surgery as the referent.

Results: Overall, 936,496 patients were identified. When markers of complexity were considered individually, cardiac had the longest median operative time (225 minutes; IQR=165-292) and their patients were most complex across four individual markers: ASA class ≥4 (78.5%; 95%CI=77.2-79.8%), 30-day mortality (3.39%; 95%CI=2.85-4.00%), major complications (56.9%; 95%CI=55.3-58.4%), and mean LOS (9.79 days; 95%CI=9.52-10.1). Vascular patients were most complex by number of major comorbidities (2.73; 95%CI=2.72-2.74) and 30-day readmissions (10.1%; 95%CI=9.82-10.3%). Compared to general, cardiac patients were most complex with 40% increased complexity and ENT was least with 60% decreased complexity (Table). RVUs were very weakly correlated with overall complexity score (Spearman's ρ=0.07; p<0.01).

Conclusions: There were substantial differences between patient complexity across surgical specialties, which very weakly correlated with RVUs. This suggests that RVUs are inadequate in capturing surgical complexity.

Table. Surgical Specialty Overall Complexity and Primary Work Relative Value Units (RVU)

	Overall Complexity Score	Ratio of Complexity Score	Median RVUs (IQR)
1. Cardiac	75	1.42	33.8 (33.1 - 43.3)
2. Vascular	71	1.34	20.5 (10.2 - 22.5)
3. Thoracic	66	1.25	23.5 (14.5 - 25.8)
4. Neurological	57	1.08	23.53 (15.4 - 27.5)
5. General	53	1.00 (Referent)	11.9 (9.5 - 20.8)
6. Orthopedic	40	0.70	20.7 (12.4 - 20.7)
7. Urology	37	0.75	15.3 (8.0 - 26.8)
8. Plastics	30	0.57	15.9 (10.4 - 17.1)
9. ENT	21	0.40	11.2 (4.4 - 15.6)

THE *EX VIVO* PERFUSED HUMAN LUNG IS RESISTANT TO ALVEOLAR EPITHELIAL INJURY FROM HIGH-DOSE *S. PNEUMONIAE* BACTEREMIA

James T. Ross, Nicolas Nesseler, Jeffrey E. Gotts, Aleksandra Leligdowicz, Michael A. Matthay

Introduction: ARDS is an important complication of sepsis, but develops less frequently in non-pulmonary than in pulmonary sepsis. We used an *ex vivo* perfused human lung model to study non-pulmonary sepsis by instilling high dose *S. pneumoniae* into the perfusate and measuring endothelial and epithelial permeability, alveolar fluid clearance, and bacterial kinetics. Based on our prior studies in sheep, we hypothesized there may be a differential susceptibility of the pulmonary endothelium and epithelium to bacterial-induced injury.

Methods: We modified our *ex vivo* perfused human lung preparation (Lee et al, PNAS, 2009) for these studies. One lung was selected and the pulmonary artery and main bronchus was cannulated. A 3-Fr catheter was passed via the bronchus into the distal airspaces. The lung was perfused with DME-H21 and 5% bovine serum albumin at 37°C to maintain a pulmonary arterial pressure of 10 mmHg. Perfusate drained passively from the pulmonary veins into a reservoir for recirculation. Lungs were inflated with room air at CPAP of 8 cmH_2O. 100 ml of fresh human blood was added to the perfusate. 10^{10} cfu *S. pneumoniae* (serotype 19F) was instilled either into the perfusate or into the distal airspaces. Alveolar fluid clearance (AFC) was measured at 0 and 5 hours by instilling 100ml of 5% albumin into the distal airspaces and comparing the protein concentration at 5 and 35 minutes. Lung fluid balance was measured by weight gain over 6 hours and epithelial protein permeability by accumulation of IgM (MW 900kD) from the perfusate into the airspaces.

Results: Lungs in the control group had minimal weight gain (74 ± 48% of starting weight), preserved AFC (normal AFC at 1 and 5h in 93%) and minimal accumulation of IgM in the airspaces (median 1mg, IQR 0.5-3mg). Addition of *S. pneumoniae* into the perfusate was associated with significant weight gain (153 vs 74% in controls, p = 0.006); however, there was minimal IgM accumulation in the airspaces and AFC was preserved. In contrast, addition of S. pneumoniae into the airspaces was associated with significant IgM accumulation (median 6mg, IQR 2-9mg, p = 0.02) and AFC compromise (p<0.001). Following addition of *S. pneumoniae* to the perfusate, cultures demonstrated a rapid drop in bacterial cfu (**Figure 1A**). This change was independent of the addition of blood to the circuit (**1B**), and did not occur in the perfusion circuit with blood but without a lung (**1C**).

Conclusions: While the human lung endothelium is susceptible to injury from *S. pneumoniae* bacteremia, the alveolar epithelium is very resistant to injury, with no increase in permeability to protein and preserved alveolar fluid clearance. In addition, the ex vivo perfused human lung has an endogenous capacity to kill large numbers of circulating bacteria. These findings provide insights into the basic mechanisms that may protect the human lung during bacteremia.

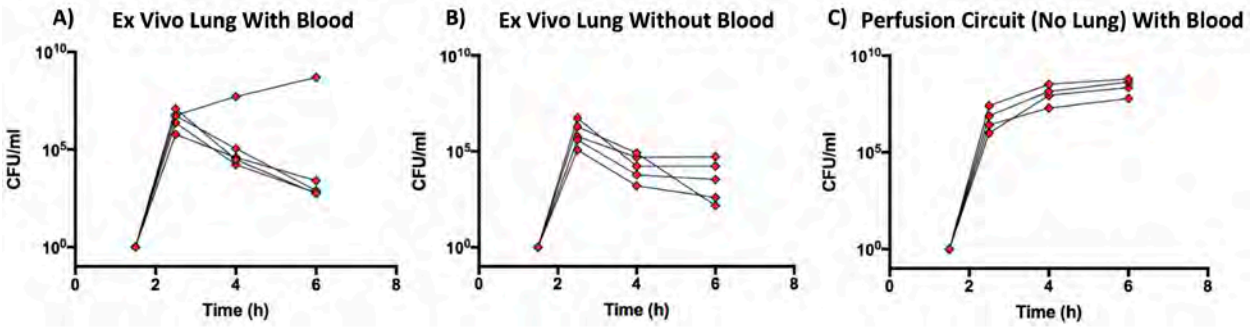


Figure 1. Microbiology of Ex Vivo Perfused Human Lung Perfusate with Addition of *S. pneumoniae* into the Perfusate

Value and Feasibility of Phone Follow Up in Ethiopian Surgical Patients

Authors: Nichole Starr, MD, MPH[1,2], Natnael Gebeyhu, MD[3], Assefa Tesfaye, MD[3], Jared Forrester, MD[2,4], Abebe Bekele, MD, MPH[5,6], Senait Bitew, MPH[2], Thomas Weiser, MD, MPH[2,4,7], Tihitena Negussie Mammo, MD[5]

[1]Department of Surgery, University of California San Francisco, California, USA
[2]Lifebox Foundation, Boston, MA, USA
[3]St. Peter's Specialized Hospital, Addis Ababa, Ethiopia
[4]Department of Surgery, Stanford University, Stanford, CA, USA
[5]Department of Surgery, Black Lion Hospital, Addis Ababa University, Addis Ababa, Ethiopia [6]Department of Surgery, University of Global Health Equity, Kigali, Rwanda
[7]Department of Clinical Surgery, Royal Infirmary of Edinburgh, University of Edinburgh, Edinburgh, UK

Introduction:
Surgical site infections (SSI) represent a major cause of morbidity and mortality in Ethiopia. Lack of post-discharge follow up, including identification of SSIs, is a barrier to continued patient care, often due to financial and travel constraints. Phone call follow up is cost effective in low-resource settings and has been successfully used to follow Ethiopian trauma patients. As part of a surgical quality improvement initiative, we aimed to assess patient outcomes at 30 days post-surgery with a phone call.

Materials & Methods:
We conducted mobile phone follow up as part of a surgical quality improvement program, called Clean Cut, aimed at improving compliance with intra-operative infection prevention standards. One urban tertiary referral hospital and one rural district general hospital in Ethiopia were included in the study and outcomes, including SSI, were tracked in-hospital and 30 days post-surgery. In-hospital, patients were assessed daily by direct observation on rounds by ward nurses. Hospital nursing staff called patients at 30 days post-surgery assessing outcomes using a brief questionnaire inquiring about signs of SSI, healthcare-seeking behavior, and treatments provided if patients had any health care encounters since discharge. All data were analyzed in STATA using chi-squared tests for significance.

Results:
A total of 661 patients were included in these two Clean Cut sites; overall 81.4% of patients were reached by 30-day follow up phone call after discharge. The rural study site was able to reach 87% of patients by phone; the urban site reached 71.5% of patients ($p<0.0001$). Regarding surgical infections, 48% of SSIs were captured as outpatient during the phone follow up ($p<0.0001$), and 34% of all complications were captured as outpatient ($p<0.0001$). Phone follow up improved from 65-78% in the first half of project implementation to 90-97% in second half of project implementation. Patients with signs of SSI were referred for treatment at their local healthcare facility.

Conclusion:
Phone follow-up after surgery in Ethiopia is feasible and valuable, and can be accomplished for most surgical patients. Follow up improved over the course of the program, likely indicating a learning curve that, once overcome, is a more accurate marker of its practicability. Phone or other outpatient follow up identified nearly half of all SSI and a third of total complications. Given the increasing use of mobile phones in Ethiopia and ease of implementation, this model could be practical in other low-resource surgical settings.

Factor	Rural Setting	Urban Setting	Overall	p-value

N	415	246	661	
Patient reached by phone	362 (87.2%)	176 (71.5%)	538 (81.4%)	<0.0001
Overall SSI	17 (4.1%)	22 (7.7%)	39 (5.6%)	0.04
Inpatient	7 (1.7%)	13 (4.6%)	20 (2.9%)	0.03
Outpatient	10 (2.4%)	9 (3.2%)	19 (2.7%)	0.55
Overall complications	33 (8.0%)	31 (10.8%)	64 (9.1%)	0.19
Inpatient	22 (5.3%)	20 (7.0%)	42 (6.0%)	0.35
Outpatient	11 (2.7%)	11 (3.6%)	22 (3.1%)	0.37

Comprehensive improvement of infection prevention practices in Ethiopian Operating Rooms using a quality improvement framework: Further refining the Lifebox Clean Cut program

Authors: Nichole Starr[1,2], Assefa Tesfaye[3], Natnael Gebeyhu[3], Jared Forrester[2,4], Thomas Weiser[2,4,5], Tihitena Negussie[6]

[1]Department of Surgery, University of California San Francisco, California, USA
[2]Lifebox Foundation, Boston, MA, USA
[3]St. Peter's Specialized Hospital
[4]Department of Surgery, Stanford University, Stanford, CA, USA
[5]Department of Clinical Surgery, Royal Infirmary of Edinburgh, University of Edinburgh, Edinburgh, UK
[6]Department of Surgery, Black Lion Hospital, Addis Ababa University, Addis Ababa, Ethiopia

Background:
Surgical infections are a major cause of perioperative morbidity and mortality, particularly in low resource settings. Clean Cut is a six-month quality improvement program focused on reducing postoperative infectious complications through strengthening adherence to infection prevention standards embedded in the WHO Surgical Safety Checklist.

Methods:
Clean Cut, co-developed by Lifebox and Ethiopian champions, was piloted in three hospitals in Ethiopia over 2 years. Adherence to Clean Cut standards were assessed during a month baseline period and continuously monitored afterwards, with 30-day outcomes obtained for all enrolled patients. The implementation strategy was then refined and modified after individual interviews and facility-level group meetings with all involved perioperative staff to identify common strengths and weaknesses. Subsequently the program was introduced over six months in two additional Ethiopian hospitals.

Results:
Modifications included 1) creating a local mentor relationship between a tertiary referral hospital and primary hospital, 2) emphasizing active hospital administration participation and engagement of entire operating room (OR) staff in discussions, 3) establishing a platform for shared learning, and 4) instituting supplementary educational trainings reinforcing critical infection prevention standards. Compared to baseline (n=92), adherence to standards improved significantly post-program implementation (n=609). Appropriate use of the WHO Surgical Safety Checklist, proper hand decontamination, sterility indicator use with instruments and surgical linen all improved (Table 1). Additionally, prophylactic antibiotic administration in the OR (rather than prior to entering) increased from 11% to 34% ($p<0.001$). Inpatient surgical site infections (SSI) significantly decreased (6.5% to 2.3%, $p=0.02$). There was a nonsignificant decrease in all inpatient infectious complications (9.8% to 5.4%, $p=0.10$) and overall complications (11% to 7.7%, $p=0.30$), although the study was not powered to detect such changes.

Discussion:
A modified implementation strategy for the Clean Cut program focused on local mentorship and larger team discussions improved communication allowing for more rapid uptake through multidisciplinary process change. Adherence to recognized infection prevention standards improved with an associated SSI reduction. Larger scale implementation with further refinement highlighting established mentor hospitals could improve infection prevention practices in Ethiopian ORs and reduce postoperative infections.

Table 1: Infection prevention adherence and patient outcomes at two Ethiopian hospitals

		BASELINE	FOLLOW-UP	
Category	**Checklist Item**	**Total (%) n=92**	**Total (%) n=609**	**p-value**
Patient Factors	Age – mean (SD)	29.7 (11.4)	29.5 (12.2)	0.88
	Gender (% Female)	75 (81.5%)	515 (84.6%)	0.46
	Hypertension	1 (1.1%)	4 (0.7%)	0.65
	Diabetes	0 (0.0%)	2 (0.3%)	0.58
	Operating Room (% Main OR)	39 (42.4%)	274 (45.0%)	0.64
Skin Prep	Surgeon entered OR with wet hands/alcohol solution before gowning	60 (65.2%)	606 (99.5%)	<0.001
Gown and Drape Integrity	Sterility indicator in gown/drape pack	8 (8.7%)	249 (40.9%)	<0.001
Instruments	Sterility indicator present inside the instrument tray	10 (10.9%)	239 (39.2%)	<0.001
Antibiotics	Antibiotics given prior to incision, if not previously on scheduled antibiotics (includes inpatient ward + OR)	85 (92.4%)	600 (98.5%)	<0.001
	Antibiotics given in operating room, if not on scheduled antibiotics	10 (11.6%)	206 (34.0%)	<0.001
Gauze	Gauze count prior to incision	91 (98.9%)	594 (97.5%)	0.41
	Gauze count at end of operation	92 (100.0%)	604 (99.2%)	0.38
Checklist	Sign In read aloud	56 (60.9%)	457 (75.0%)	0.004
	Sign In performed before anesthesia induction	54 (93.1%)	453 (98.1%)	0.023
	Time out performed	61 (66.3%)	452 (74.2%)	0.11
	Sign Out read aloud	40 (43.5%)	365 (59.9%)	0.003
Patient Outcomes	Inpatient SSI	6 (6.5%)	14 (2.3%)	0.023
	All inpatient infectious complications	9 (9.8%)	33 (5.4%)	0.10
	Overall complications	10 (10.9%)	47 (7.7%)	0.30

Silicon Nanopore Membrane-Based Implantable Hemodialysis: A Preclinical Proof-of-Concept Study

Jarrett Moyer, Jimmy Ly, Nathan Wright, Charles Blaha, William H. Fissell, Shuvo Roy

Background:
Silicon nanopore membranes (SNM) are highly efficient biomimetic and blood-compatible slit-pore membranes. The high efficiency of the membrane enables hemofiltration and hemodialysis by utilizing cardiovascular perfusion pressure to circulate blood over the filters, enabling the prospect of a fully implanted hemodialysis cartridge. This alternative access strategy could offer a solution for patients with complicated conventional access, and lowers barriers to self-care home hemodialysis.

Methods:
A 115 x 57 x 18 mm SNM-based parallel-plate hemodialyzer (SNMHD) was prototyped from polycarbonate and stainless steel. The device was implanted subcutaneously in the neck of a healthy Yucatan mini-pig and anastomosed to carotid artery and jugular vein via ePTFE vascular grafts. Catheters to supply dialysate were tunneled subcutaneously and attached to the SNMHD. The animal was allowed to recover, and 3-hour hemodialysis sessions were performed on the day of surgery, and post-operative days 1, 2, and 3. Dialysate circulated through the SNMHD at 10-15 mL/min, and blood flow through the SNMHD was between 1.0-1.5 L/min, assessed by pulse wave Doppler ultrasound. Blood samples were collected at the initiation of dialysis, and dialysate sampled every hour to assess solute clearance over time. The animal was treated with daily aspirin and clopidogrel beginning three days before the implant, and continuing throughout the post-operative period.

Results:
The animal tolerated surgical implantation and subsequent dialysis sessions without complication. Blood flow through the dialyzer remained brisk throughout the three-day study. All dialysis sessions were completed as planned, via circulation of normal saline through the dialysate catheters. Over the course of the study, creatinine clearance ranged from 11-42 mL/min/m^2. Urea clearance ranged from 26-74 mL/min/m^2. Albumin concentration in the dialysate remained below the detection limit throughout the study.

Conclusion
We demonstrated pre-clinical feasibility of an implantable, pumpless SNM-based hemodialyzer. Further development and refinement of the SNMHD could provide an alternative method for hemodialysis access, and facilitate frequent in-home dialysis.

Chimeric Antigen Receptor (CAR) T-Regulatory Cells as a Tolerance Strategy in a Murine Surgical Transplantation Model

Conkling, Nicole; Kaul, Anupurna; Ferreira, Leonardo; Tang, Qizhi

Introduction: Graft survival in solid organ transplant (SOT) and more recently composite tissue allotransplantation (CTA) has advanced tremendously in the last few decades, with development of effective immunosuppressant medications. Despite the improved toxicity profile of newer drugs, patients are not free from risk nor the morbidity of rejection. Thus, there is significant scientific interest in exploring new cellular strategies to induce tolerance. One promising avenue of research is the adoptive transfer of T-regulatory cells (T-regs), including those that have been genetically modified to express a chimeric antigen receptor (CAR) that could potentially improve the cells' specificity and efficacy in vivo. By selecting a common human antigen, HLA-A2, as our target, we hope to produce a reproducible cellular therapy that could be used in many potential SOT or CTA recipients. We plan to introduce these cells into a humanized mouse model of heterotopic heart transplant to prevent or mitigate rejection.

Methods: Human Tregs were transduced with the A2 CAR construct using a lentivirus. Transduction success was measured by CAR expression using flow cytometry. *In vitro* cell function was measured by exposing the CAR T-regs to A2-positive cells, and subsequently assaying markers of activation and retention of T-reg phenotype.

For the experimental surgical model, an A2-positive donor heart is harvested and transplanted into the abdomen of an A2-negative NSG mouse. The aorta and pulmonary arteries are anastomosed to the descending aorta and IVC, respectively (Figure 1). Once circulation is restored, the allograft will begin to beat, and the abdomen is closed. The recipient mouse is monitored carefully for recovery, and graft viability can be assessed by gently palpating the abdomen. Once the graft is healed, the mice can then be sensitized using A2-negative human PBMCs, and the experimental groups will simultaneously receive either polyclonal or A2 CAR T-regs.

Results: Extracellular expression of the A2 CAR was verified using a tetramer (Figure 2). Activation markers and FOXp3 expression were measured after exposure to A2-positive K562 cells. CAR T-regs exposed to A2 had higher levels of CD71 compared to controls, and they retained their FoxP3 expression after activation (Figure 3). *In vivo* results in the surgical model are pending.

Conclusion: CAR T-regs represent a promising cellular therapy that can open doors to new strategies for tolerance. Human T-regs can be effectively transduced to express the A2 CAR, which can subsequently effect activation without altering their regulatory phenotype. Application into a heart transplant model, as well as other surgical models, can help prove their efficacy *in vivo* and advance this technology toward clinical use.

Figures:

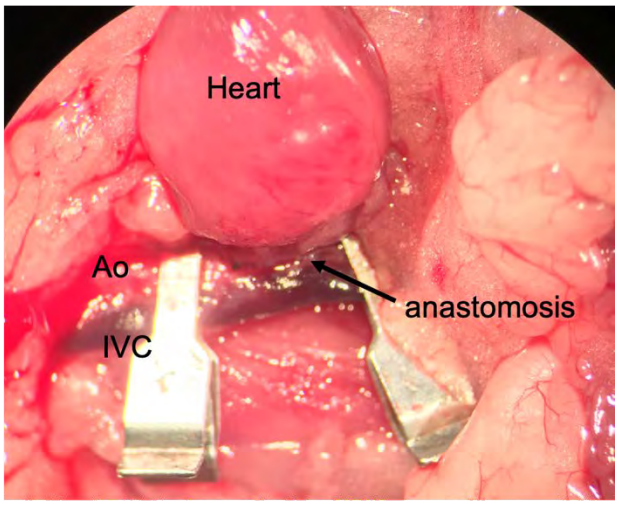


Figure 1. Heterotopic heart transplant with anastomosis of the ascending aorta and pulmonary artery to the abdominal aorta (Ao) and inferior vena cava (IVC), respectively, prior to releasing vessel clamps. The venous anastomosis is visible here.

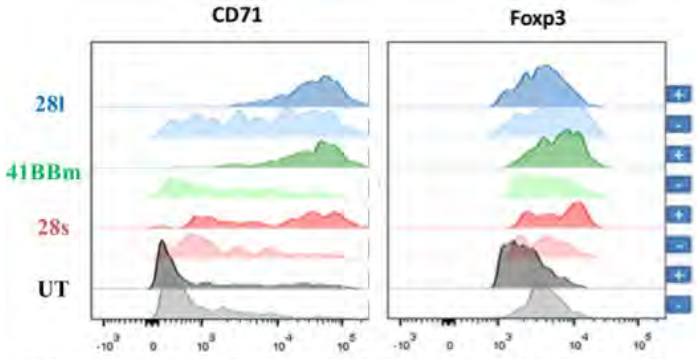


Figure 3. In-vitro activation of A2-CAR-expressing Tregs. A2-CAR-expressing Tregs responded to A2-K562 (+) by upregulation of CD71 but not to A2-negative K562 (-). 28s and 41BBm also upregulated FOXP3,whereas 28l showed high constitutive FOXP3 expression that was maintained after stimulation. *Courtesy of Anu Kaul.*

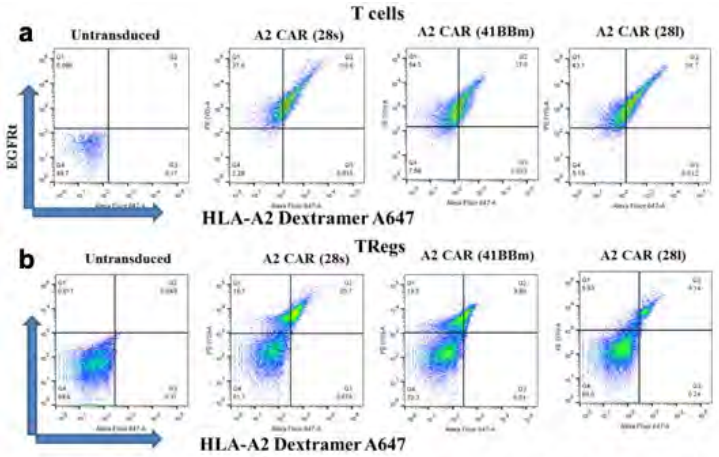


Figure 2. Expression of an HLA-A2-specific CAR. (**a**) a-HLA-A2-scFV-h28z CARs are expressed on the surface of human T cells. (**b**) a-HLA-A2-scFV-h28z CARs are expressed on the surface of human Tregs. The anti HLA-A2 CAR expression was detected by HLA-A2 dextramer. *Courtesy of Anu Kaul.*

Evaluating long-term patient-reported quality of life after masculinizing mastectomy using the GENDER-Q; a novel quality of life instrument for gender-affirming surgery

Andre Alcon, MD; Adrienne Kennedy, MS; Eric Wang, MD; Kelsey Loeliger, MD, PhD; Rachel Lentz, MD; Esther Kim, MD.

Background: There are few investigations into the effects of gender affirming surgery (GAS) on quality of life (QoL) in transgender populations. Furthermore, the psychometric instruments that have been validated for measuring patient reported QoL were developed using cis-gendered populations and thus do not capture the unique obstacles confronting transgender patients. We developed a novel patient-reported instrument to evaluate the QoL of trans-men undergoing chest reconstruction called the GENDER-Q

Methods: Qualitative methods consisting of one-on-one and focus group interviews were conducted to construct a QoL instrument pertinent to masculinizing chest surgery. The GENDER-Q consists of three QoL domains addressing feelings of gender identity, physical appearance, and psychosocial well-being. The surveys were then administered prospectively to trans-men undergoing masculinizing inframammary mastectomy with free nipple grafting before surgery, six weeks after surgery, and one year or more after surgery. Wilcoxon signed-rank tests were used to test for significant differences between the median pre- and post-operative quality of life scores at 6 weeks and one year after surgery. Cronbach's alpha was calculated to measure internal validity while the brief version of the WHO QoL survey was given simultaneously for external validity.

Results: Fifty-one trans-men underwent inframammary mastectomy with free nipple grafting and completed pre-operative surveys. Thirty-six patients completed surveys 6 weeks after surgery (71% response rate) and 22 patients completed surveys one year or more after surgery (43% response rate). The GENDER-Q detected a statistically significant improvement in median quality of life up to two years after surgery with large effect size (0.57-0.62). More modest improvements and effect sizes were found using the brief version of the WHO Quality of Life survey ($p<0.05$). There were no patients that reported regret after surgery, even 2 years after surgery, and all 51 patients reported that surgery changed their lives for the better. Calculation of Cronbach's alpha (0.67-0.81) revealed excellent internal validity in all three domains of the GENDER-Q.

Conclusions: The GENDER-Q is the first quality of life instrument validated for trans-men undergoing chest reconstruction and was significantly more sensitive than the brief version of the WHO quality of life survey. Developing additional GENDER-Q sections applicable to facial, chest, and urogenital reconstructive surgery can provide a more comprehensive assessment of the effects of GAS. Large-scale, longitudinal studies using this new instrument to evaluate the effects of GAS are needed to help better establish the many benefits of GAS to influence public policy and broaden access to GAS nationwide.

An analysis of differentially hydroxymethylated genes in HIV-infected kidney transplants undergoing acute rejection

Arya Zarinsefat, Dmitry Rychkov, Ji Nie, Xiaolong Cui, Chuan He, Peter Stock, Minnie Sarwal

Introduction: Kidney transplantation (KT) is now the standard care for HIV-infected patients with end-stage renal disease. However, despite a depressed immune system, HIV-infected patients experience much higher rates of acute rejection (AR) at 31 and 41% at 1 and 3 years post-KT, respectively. The etiologies of this increased incidence of rejection are not fully understood, and previous work looking at the biologic/genetic mechanism of these increased rejection rates has been limited. 5hmC-Seal is a novel next generation sequencing methodology that utilizes DNA hydroxymethylation patterns, which act as epigenetic markers, as its sequencing target. In our study, we leverage 5hmC-Seal data of plasma cell-free DNA (cfDNA) from kidney transplants of HIV-infected recipients to identify specific hydroxymethylated DNA sites and gene signatures that uniquely characterize AR events.

Methods: Plasma samples from a previous clinical trial studying outcomes of KT in HIV-infected patients were utilized. A pilot study was performed with plasma samples from 14 rejectors and 4 non-rejectors. Samples from pre-KT, post-KT, and near rejection time were selected. 5hmC-Seal analysis was performed in collaboration with the He lab (University of Chicago). Plasma cfDNA samples were sequenced using the Illumina NextSeq 500. Raw sequencing data was aligned using Bowtie 2 with default parameters. The peak calling of 5hmC-enriched regions was performed using MACS2. HOMER was used to combine peaks and calculate tag counts. rlog-transformation was used for data normalization. R package DESeq2 was used to perform analysis and visualize 5hmC modification levels on differentially hydroxymethylated genes. Comparison of pre-/post-KT samples was performed. A p-value cutoff of 0.05 and log-fold change of 0.5 was used to select genes of interest.

Results: We found 261 up-regulated and 141 down-regulated genes. Functional analysis of up/down-regulated genes was performed, which revealed up-regulation of multiple genes in post-KT AR, including *NCF1* (involved in antigen processing pathways), *CXCL2* (involved in immunoregulatory and inflammatory processes), and *CTSG* (encodes cathepsin, which has been implicated in various inflammatory and infectious diseases). Heatmaps were created to visualize 5hmC modification levels on differentially hydroxymethylated genes, comparing pre-KT and post-KT 5hmC modification levels among patients who experienced AR **(Fig. 1)**.

Conclusion: Understanding the biological pathways and genes involved in acute rejection of KTs in HIV-infected patients is an unmet need. Our preliminary analysis of the pilot data shows that there are differentially hydroxymethylated genes between pre- and post-KT within the rejector group of HIV-infected patients. Future work will carry through analysis of epigenetic changes and gene expression, along with validation of possible biomarkers as identified from sequenced genes of interest. We plan to include additional patient plasma cfDNA samples to the existing data to increase future power for gene detection and pathway analysis.

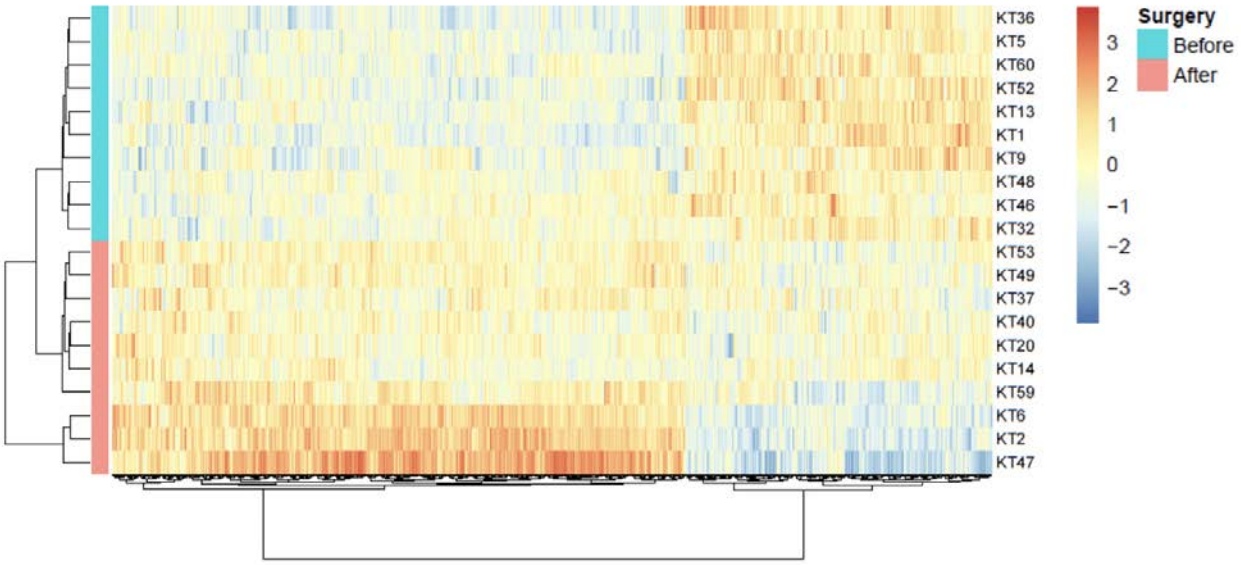


Figure 1. The heatmap of 5hmC modification levels on differentially hydroxymethylated genes (FDR p-value < 0.05) comparing patients with acute rejection at pre-KT and 3-6 months post-KT.

Importance of Operative Team Expertise – the Presence of a Dedicated Specialty Operating Room Nurse is Associated with Fewer Delays and Improved Team Function – an Analysis of 11,203 Cases

Whitney A. Goering MD, Elizabeth A. Devine MSN RN, Lygia Stewart MD

IMPORTANCE: Surgery across all specialties has become more complex, and this demands the need for operating room (OR) team expertise. High reliability OR teams exhibit consistency, coordination and expertise – all of which should improve outcomes. We aim to define the value of nursing expertise as it influences OR team function.

METHODS: We prospectively collected 11,203 OR Briefing/Debriefing Data Forms at the San Francisco VA Medical Center (SFVAMC) from 2006-2011. Case information recorded by the OR team included personnel, delays and issues, anchored-case score, handoff issues and teamwork annotation for all operations. Operations reviewed were completed by 12 surgical specialties, or "sections". In our hospital, each section has a designated circulating nurse (SectRN) who understands the needs and preferences for an operation to run smoothly and is the liaison for section-specific issues. We studied the impact of the SectRN participation on OR team function and analyzed whether SectRN presence improved outcomes.

RESULTS: When the SectRN was present (66% of cases; Figure 1), the overall delay rates were significantly lower (6/100 vs. 12.6/100 cases, P=0.0001; Figure 2), delays decreased in every specialty, fewer cases had any delay (5.5% vs. 10.6%, P=0.0001), good teamwork was more commonly reported (7.1% vs. 5.5%, P=0.0001), handoff issues were less common (0.6% vs. 1%, P=0.018), and the anchored-case score was higher (4.89 vs. 4.82, P=0.0001). Additionally, with a SectRN present there were significantly fewer delays reported for equipment, anesthesia, surgery, nursing, consent and scheduling.

CONCLUSIONS: The presence of a designated SectRN was associated with a 52% relative reduction in delay rates, increased delay-free cases, improved anchored-case scores and increased recognition of good teamwork. The expertise of a SectRN influenced many facets of overall OR team function and surgical outcomes. This is the first study to show the importance of a dedicated specialty nurse as a critical component in creating a consistent and high reliability operating team.

Figure 1. Presence of Section RN by Surgical Specialty. Percentage of the total cases by surgical specialty with a SectRN present depicted in black.

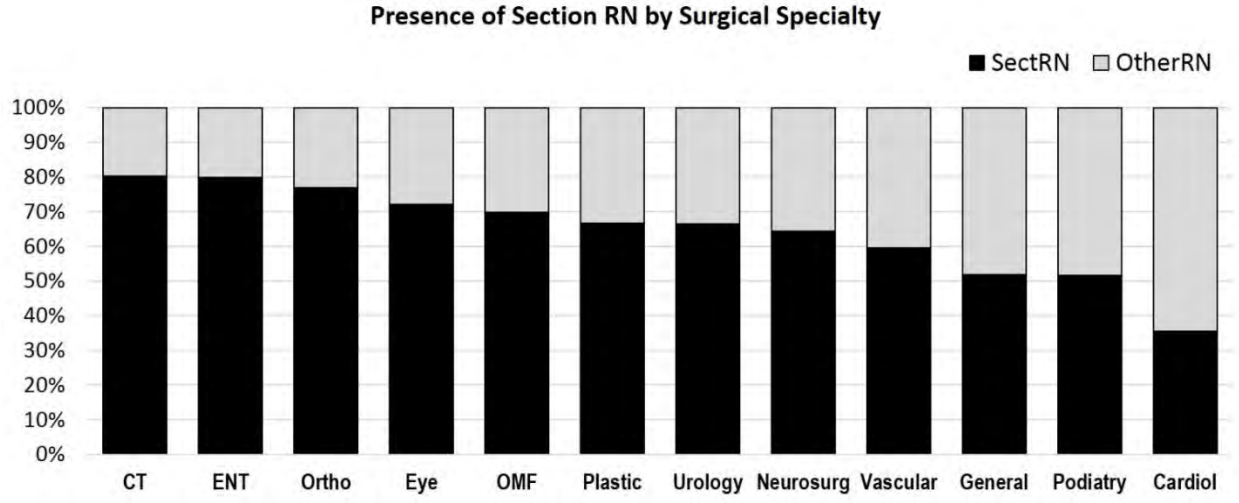
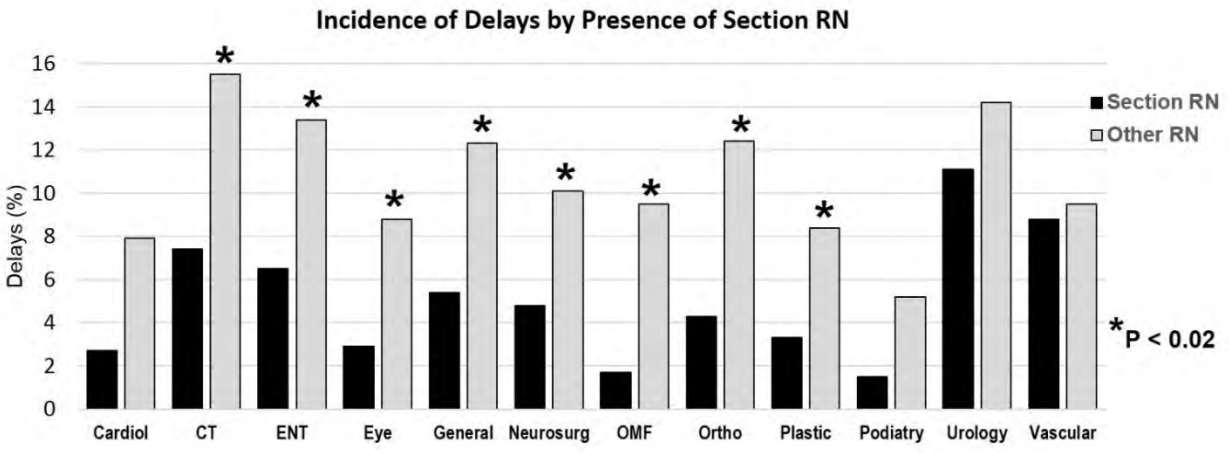


Figure 2: Incidence of Delays by Presence of Section RN. Bar graph of the percentage of total delays in cases with the SectRN versus OtherRN. Significantly fewer delays (denoted by the asterixis) were reported when the SectRN

was present in CT, ENT, Eye, General Surgery, Neurosurgery, OMFS, Orthopedic and Plastic Surgery. Statistical significance of P<0.02 using Chi-Squared.



Title: Biomechanics of Ascending Thoracic Aortic Aneurysm: Computational Modeling and Pursuit of Wall Stress Based Rupture Risk.

Authors: Andrew D. Wisneski, Zhongjie Wang, Yue Xuan, Michael Hope, Julius Guccione, Liang Ge, Elaine E. Tseng.

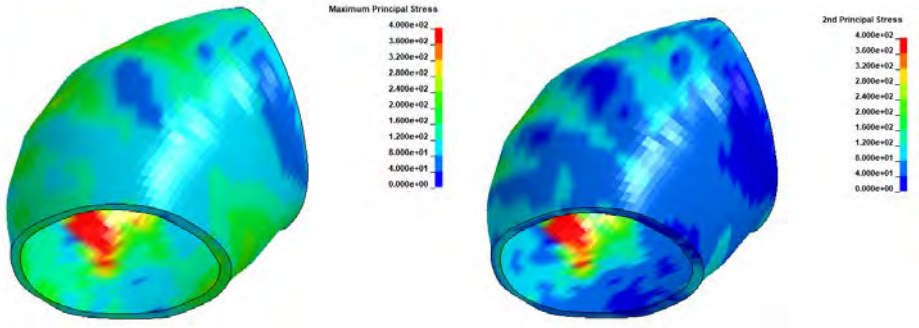
Objective: Ascending aortic dissection frequently occurs at diameters under size guidelines for repair. Rupture/dissection is a mechanical event where wall stress exceeds strength. Aneurysm wall stress may better predict rupture/dissection risk than diameter. We hypothesize wall stress correlates poorly with aneurysm diameter, and smaller aneurysms with elevated stress may have higher risk for rupture.

Methods: 27 males (age 67.6±8.0 years; 12 BAV, 15 TAV) underwent aTAA resection. Mean pre-operative aTAA diameter: 51.5±4.4mm. Excised ATAA specimens underwent micro-CT for wall thickness/geometry. A hyperelastic material model using patient-averaged properties was assigned to aTAA models per valve phenotype. LSDYNA software ran pressure simulations to 120/80mmHg with finite element analysis.

Results: For all aTAA models, 99th-percentile first principal wall stress (FPS) in the circumferential direction and second principal wall stress (SPS) in the longitudinal direction were 229.54±124.8kPa and 146.5.9±74.3kPa, respectively. Results stratifying aTAA by size ≥5.5cm (n=9) vs <5.5cm (n=18) did not yield statistically significant differences in wall stress: peak FPS 202.3±73.7 vs 215.2±103.5 kPa (p=0.74), peak SPS 130.1±34.9 vs 133.1±50.6 (p=0.87). Six aTAA <5.5cm had peak wall stress exceed mean peak wall stress of the ≥5.5cm group by >25%. Correlation was weak between peak stress and diameter (R^2=0.21 BAV, R^2=0.38 TAV).

Conclusions: Computational models with aTAA patient-specific material properties and geometry were created. No significant difference in wall stress between aTAA <5.5cm and ≥5.5cm was measured; peak stress correlated poorly with aTAA diameter demonstrating limitations of diameter-based criteria. These data will lay the foundation for wall stress thresholds to guide aTAA rupture/dissection risk.

Figure: Representative bicuspid aortic valve associated aTAA at systole (120mmHg), demonstrating FPS (left) and SPS (right). Color bar in kPa.



Fetal molecular therapies to treat neurological disease in mice with lysosomal storage disorders

Quoc-Hung L. Nguyen, MD, Bowen Wang, Carlo Eikani, Lucas Smith, Jeremy Shea, PhD, Russell G. Witt, MD, Saul Villeda, Tippi C. MacKenzie, MD

Introduction

Sly Syndrome, or mucopolysaccharidosis type 7 (MPS7), is a lysosomal storage disorder that causes multi-organ dysfunction and in utero fatality in the majority of cases. Although postnatal enzyme replacement therapy (ERT) to replace missing glucuronidase (GUS) enzyme is available, this approach does not penetrate the blood-brain barrier (BBB), and thus does not improve the neurologic phenotype. We have previously shown that in utero ERT (IUERT) can improve neurologic outcomes. Here, we determine whether IUERT specifically targets the brain microglia, as they are natural storehouses of GUS and the key mediators of brain inflammation, and whether in utero hematopoietic stem cell transplantation (IUHSCT) results in microglial engraftment as a strategy for permanent correction.

Methods

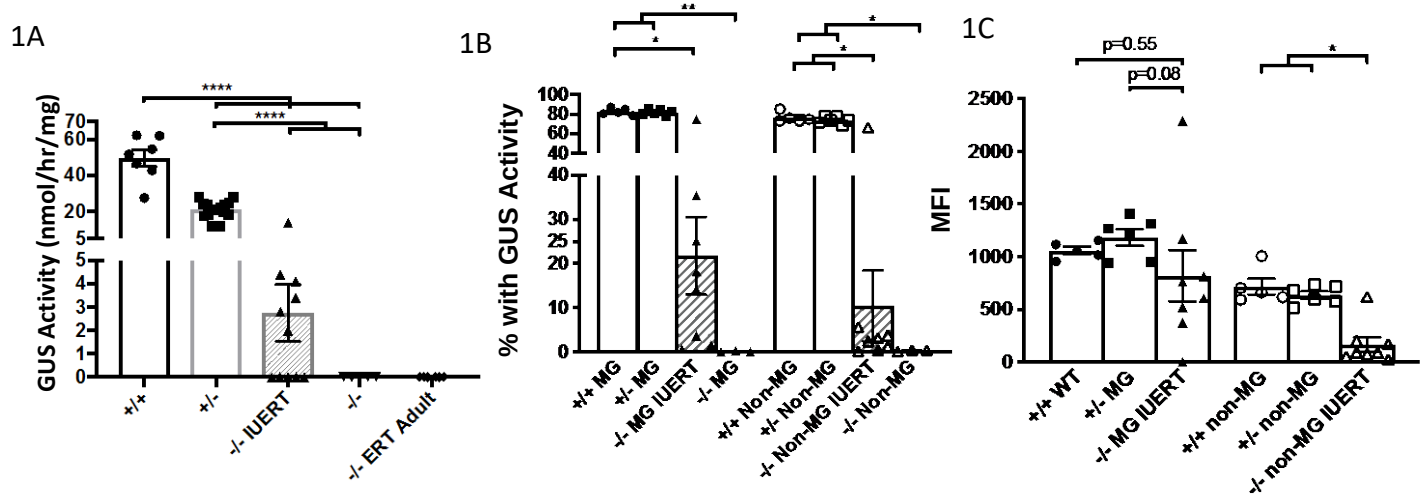
We performed IUERT by injecting GUS into MPS7 fetuses at E14.5 (or adult controls) and analyzed both tissue homogenates (via colorimetric substrate) and brain microglia (via flow cytometry) for enzyme activity after 4-7 days. We performed IUHSCT by transplanting HSCs from CX3CR1-GFP donor mice (such that donor-derived microglia are green) into E14.5 fetal recipients. We examined engraftment in blood and bone marrow and stained brains to detect donor-derived microglia. We assessed the degree of inflammation in MPS7 brains after both IUERT and IUHSCT by staining for CD68.

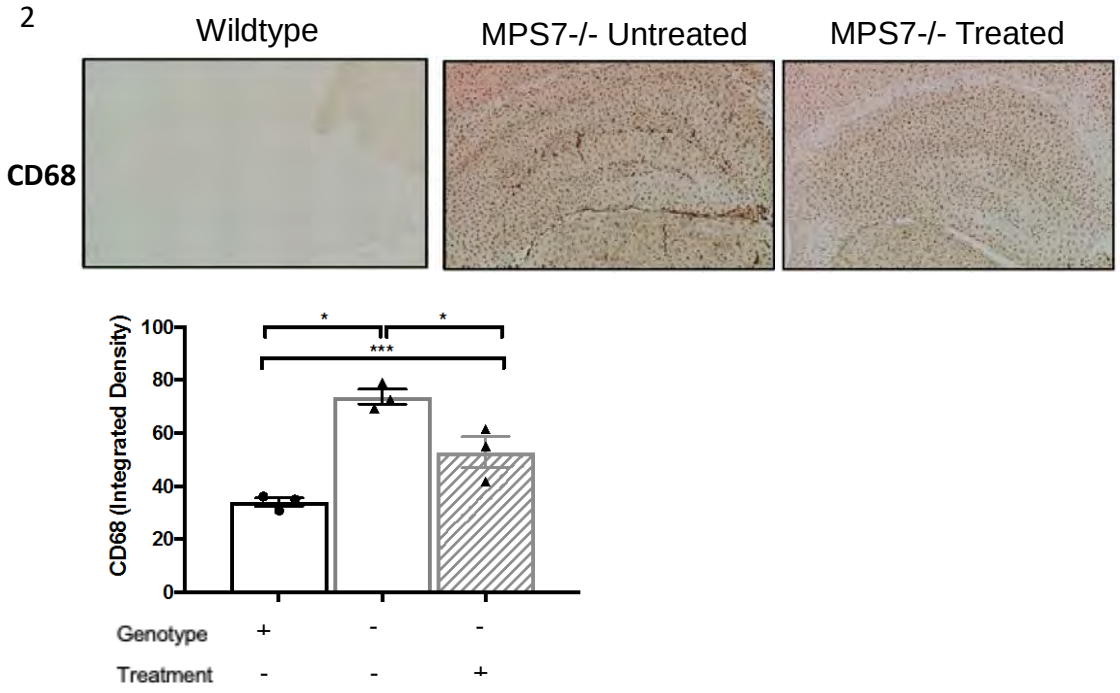
Results

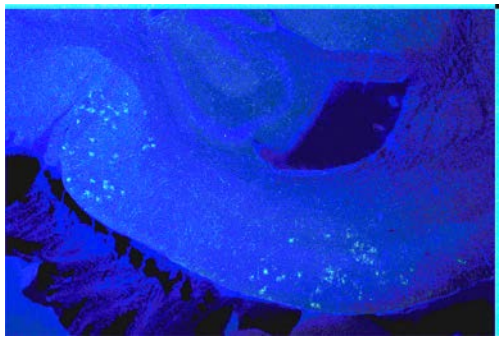
IUERT resulted in detectable biochemical activity of enzyme in the brain 4-7 days after injection while adult treatment did not (Fig. 1A). Flow cytometry revealed that that 21.7 ± 8.7% of microglia produced GUS after IUERT, and levels of GUS in individual cells (calculated as the mean florescence intensity, MFI) were similar to those seen in healthy mice (Fig 1B-C). In one cohort treated with IUERT and harvested as adults, CD68 staining revealed decreased brain inflammation (Fig. 2). After IUHSCT, we detected multilineage engraftment of CD45+ hematopoietic cells in both blood and bone marrow (12.5±2.8% blood, n≥12). Confocal imaging of brains in chimeras revealed several foci of donor-derived microglia, indicated by co-staining for GFP and a second microglial marker, Iba1 (Fig. 3A). MPS7 chimeras had evidence of reduced brain inflammation near donor microglia (Fig. 3B).

Conclusion

IUERT can penetrate the BBB and decrease brain inflammation in mice with MPS7. IUHSCT results in microglial engraftment and may also reduce brain inflammation. These findings support the strategy of in utero molecular therapies for patients with MPS7 and other lysosomal storage disorders, for which we are currently developing a clinical protocol.







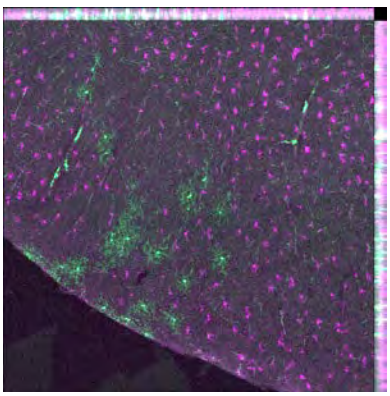


Figure (1A) Biochemical activity of enzyme in the brain 4-7 days after in utero injection or 4-7 days after adult postnatal injection. N≥5 per group. (1B-C) Microglia (MG) and non-microglia (non-MG) percentage of cells that are positive for GUS (1B) and MFI of GUS (1C) in individual cells. N≥5 per group (Kruskal-Wallis with Dunn multiple comparison) (2) Integrated density graph of CD68 staining in mice harvested at 8-10 weeks. N=3 per group (ANOVA with Tukey's multiple comparison). (3A) Confocal image showing multiple foci of engraftment after IUHCT (11% peripheral blood chimera, donor cells green). (3B) Representative image of MPS7 chimeric brain staining for brain inflammation (CD68 in purple).

Title: Parathyroid CD34+ cells induce neovascularization from donor and recipient leading to chimeric vessel formation and improved engraftment of co-transplanted pancreatic islets

Authors: Yvonne Kelly, MD*, Casey Ward, MD*, Quan-Yang Duh, MD, Wenhan Chang, PhD, Peter Stock, MD, PhD, Qizhi Tang, PhD

Abstract:

***Introduction:** Our group recently showed improved engraftment and diabetes reversal when mature islets or stem-cell-derived beta cells (SCIPC) were co-transplanted with parathyroid gland (PTG). The CD34+ vascular endothelial progenitor cells, comprising 3-5% of PTG, partially recapitulated this effect whereas the CD34- cells were less effective. This study aims to test the hypothesis that PTG, particularly the CD34+ cells, protect islets by accelerating and enhancing neovascularization.

***Methods:** Human PTG was dissociated into single cells using enzymatic digestion. CD34+ and CD34- cells were purified using magnetic-activated cell sorting. One fourth of a PTG, 1,000 IEQ human islets or 150,000 PTG CD34+ or CD34- cells were transplanted in the subcutaneous space (SQ) of immunodeficient NSG mice. Sham controls received incisions without transplants. Skin flaps surrounding the transplant sites were created to reveal the vasculature at day 5 post-transplant. Vessel area percentage and number of vascular junctions were quantified using AngioTool (NIH). Skin tissue was prepared for histology by fixing in 4% PFA and embedding in OCT. Cryosections were stained with anti-human von Willebrand factor (vWF) antibody conjugated to Alexa 488, anti-mouse CD31 conjugated to Alexa 647 and DAPI and imaged with confocal microscopy.

***Results:** At 5 days post-transplant, skin flaps with ¼ PTG had 32.9% vessel area with 116 junctions and those with transplanted PTG CD34+ cells had 29.1% vessel area with 103 junctions. In comparison, PTG CD34- flaps had 22.9% vessel area and 62 junctions, human islet flabs had 17.2% vessel area and 84 junctions and sham flaps had 19.5% vessel area and 54 junctions. Histologic assessment of human PTG and CD34+ cell transplants at 14 days showed an abundance of human vWF staining, demonstrating human-derived vessels. These human vessels were surrounded by mouse vessels identified by mouse CD31 staining. Human vWF and mouse CD31 signals were observed in same vessels, suggesting human/mouse chimeric vessel formation. In contrast, the sham and human islet transplants had no human blood vessels and the islet and CD34- transplants were dominated by mouse vessels (**Figure 1**).

***Conclusions:** PTG, and in particular the CD34+ cells, induced early and sustained neovascularization as evidenced by increased vessel area percentage and vascular junctions, compared with sham, islet and CD34- transplants. While the vascular network of PTG CD34- and islet transplants is almost exclusively recipient derived, that of PTG and PTG CD34+ transplants is comprised of both recipient and donor tissue. This rich chimeric network may protect islets from ischemic injury and may one mechanism by which co-transplantation of PTG or its CD34+ component improves engraftment.

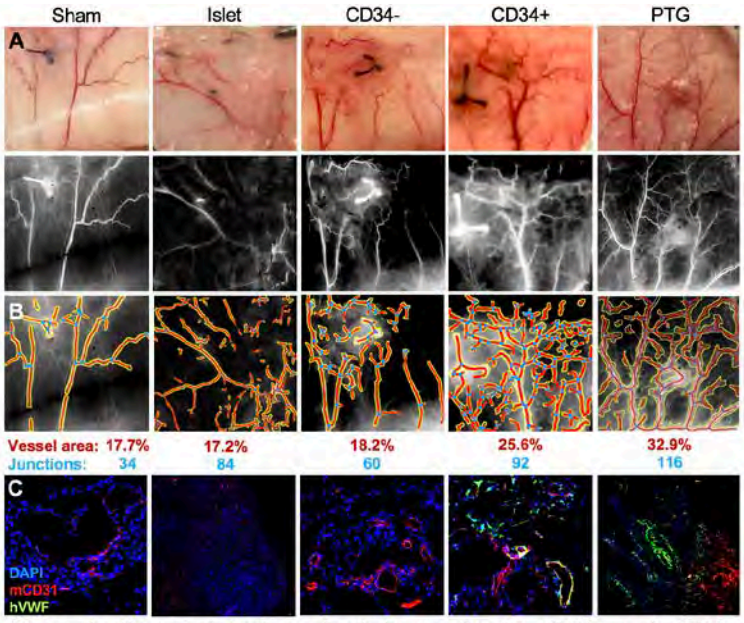


Figure 1. Neovascularization of whole parathyroid, CD34+ and CD34- parathyroid cells and human islets. A. Mice received sham operation, SQ islet transplant, SQ injection of MACS purified CD34- or CD34+ PTG cells, or SQ transplant of intact human parathyroid. **B.** Vessel area and junctions on skin flaps on day 5 were quantified using AngioTool. **C.** Immunofluorescent analysis of skin tissue on day 14 stained with human vWF and mouse CD31 antibodies.

The Role of C-C Motif Chemokine Ligand 2 (CCL2) in Metastatic Neuroblastoma

Michael J. Zobel, MD[1]; Abigail Zamora, MD[1]; Grace Asuelime, MA[1]; Shahab Asgharzadeh, MD[2]; and Eugene S. Kim, MD, FACS, FAAP[1,2]

[1]Division of Pediatric Surgery, Children's Hospital Los Angeles, CA, USA

[2]Division of Hematology, Oncology, and Blood and Marrow Transplantation, Children's Hospital Los Angeles, CA, USA

Introduction:
Approximately 80% of children with high-risk neuroblastoma experience remission after initial therapy, however the majority succumb to relapsed, incurable metastatic disease. The chemoattractant CCL2 has been found to promote metastasis in other cancers by recruiting tumor cells and monocytes to premetastatic sites. We sought to characterize the role of CCL2 in metastatic neuroblastoma.

Methods:
Metastatic human neuroblastoma (CHLA-255) cell lines were generated by orthotopic xenograft implantation in immunodeficient mice followed by harvesting and culturing metastatic cells from the liver and bone marrow. Primary tumor and metastatic cells were analyzed by PCR microarray and proteome array to determine differences in CCL2 mRNA and protein expression, respectively. Matrigel invasion assay was performed using CHLA-255 cells in varying concentrations of recombinant CCL2. CCL2 gene expression was examined across a large neuroblastoma patient dataset. Student's t-test was used; $p<0.05$ was deemed significant.

Results:
PCR microarray and proteome array of metastatic neuroblastoma cells reveal increased mRNA and protein expression of CCL2 compared to primary tumor cells. Increasing concentrations of recombinant CCL2 leads to a statistically significant, dose-dependent increase in tumor cell invasiveness ($p<0.05$) (*Figure 1*). This effect was abrogated by anti-CCL2 antibody ($p<0.05$). Microarray dataset analysis demonstrates elevated CCL2 expression in patients with high-risk disease versus low-risk disease ($p<0.05$) (*Figure 2*).

Conclusion:
CCL2 is expressed by metastatic neuroblastoma cells and is associated with high-risk disease. Recombinant CCL2 leads to increased invasiveness, which is attenuated by anti-CCL2 antibody. CCL2 may represent a therapeutic target in patients with high-risk neuroblastoma.

Figure 1. Matrigel Invasion Assay Data

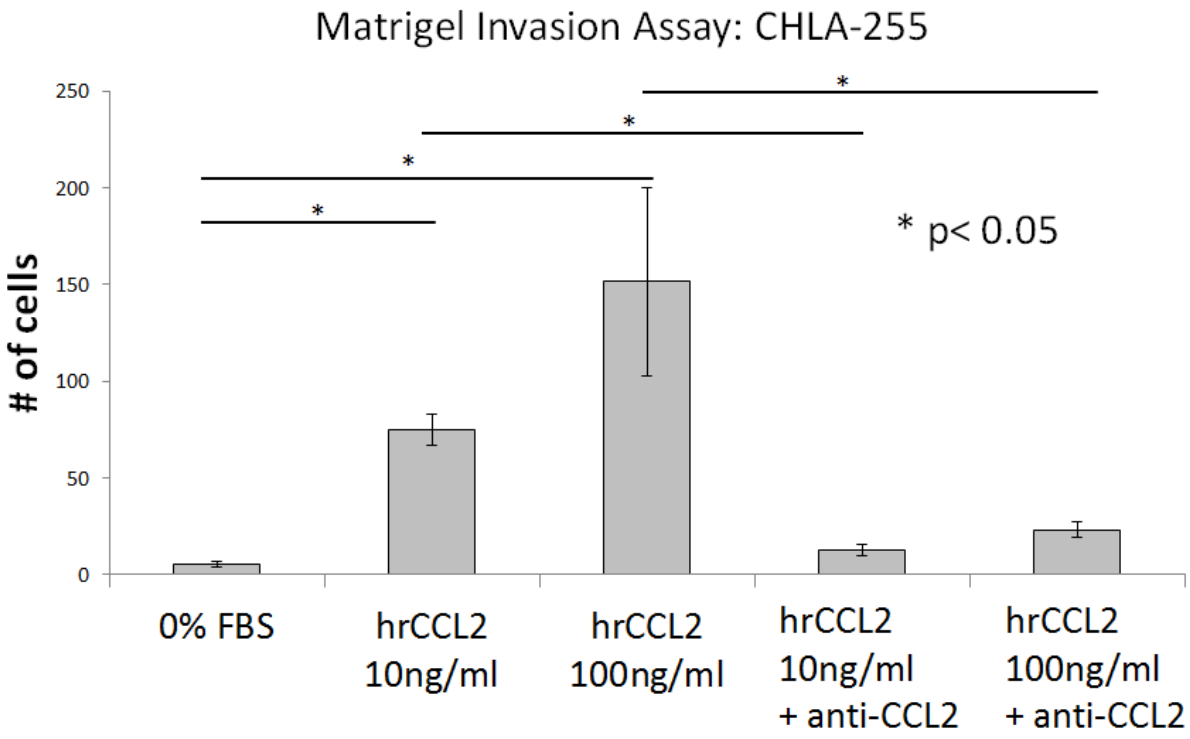
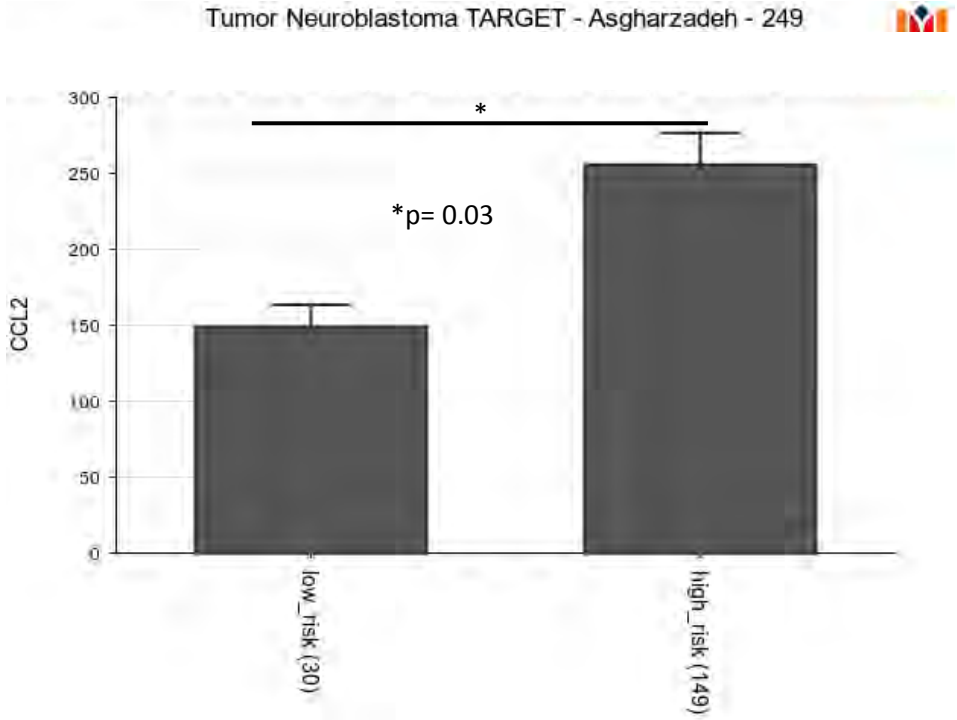


Figure 2. Patient Microarray Dataset Analysis



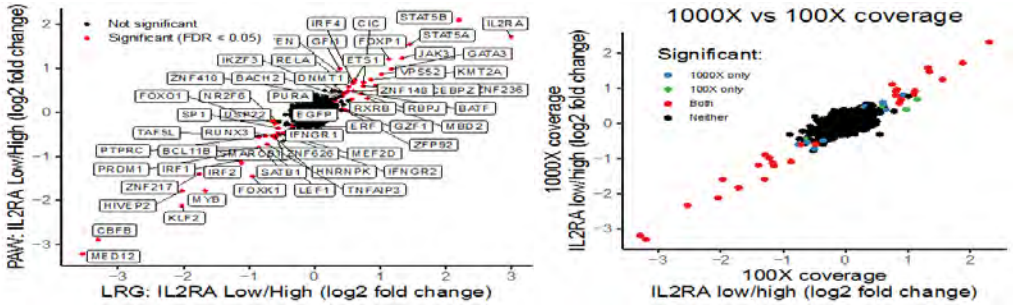
Successful Enhancements of CRISPR Knockout Screens to Study Gene Level Regulation in Primary Human Lymphocytes

Oren Shaked, Jake Freimer, Jessica Cortez, Alexander Marson

INTRODUCTION: Our lab has adapted a technique using CRISPR knockouts in a pooled approach to study macro-level phenotypic changes in primary human lymphocytes. Here, we report our experience adapting this technology to establish regulatory networks of gene level phenotypes. Establishing a detailed framework of cell specific regulatory networks will allow for more directed identification of precision targets for future therapies.

METHODS: We designed a targeted CRISPR knockout library targeting well-annotated transcription factors as well as a host of positive and negative controls. Using a lentiguide vector to deliver the guide-RNA, human CD4+ T cells were infected at specific ratios to ensure only a single virus per cell. Cas9 was electroporated into the cell 48 hours after infection, effecting targeted gene knockout. CD25, a cell surface marker encoded by the IL2RA gene, was selected as the target for our screen. Cells were sorted for high versus low expression of CD25, and sequences to determine which gene knockouts were associated with high versus low expression of our target phenotype (CD25).

RESULTS/In Progress: We were able to achieve 1000x coverage of our targeted library within each sorted population. 60 genes were identified that met statistical significance (FDR < 0.05) in regulating CD25 expression in activated human CD4+ cells. All known genes involved in the regulation and signaling through the IL2RA pathway were identified by our screen as statistically significant including IL2RA, STAT5a, STAT5b, and JAK3, further lending confidence to the success of the screen. Many novel regulators of this pathway were identified. Significant hits are currently being validated. The list of significant hits was marginally affected by dropping coverage from 1000x to 100x.



CONCLUSIONS: The SLICE technology can be successfully adapted to study gene specific regulatory networks via phenotyping of their gene products. Having established that our enhanced protocol works and defining the parameters of the coverage needed to successfully complete this screen, we will be able to study several different phenotypes in parallel within and across many cell types in primary human lymphocytes. This technology will help identify new targets for cell directed therapies, as well as be a platform to study the gene level effects of these therapies.