2024
Margie & Robert E. Petersen
NEUROENDOCRINE TUMOR
RESEARCH SYMPOSIUM

October 28-30, 2024
Boston, Massachusetts

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WELCOME TO THE 2024 MARGIE AND ROBERT E. PETERSEN NEUROENDOCRINE TUMOR RESEARCH SYMPOSIUM

Thank you for joining us at this year's NETRF Research Symposium. NETRF is proud to once again convene the largest global gathering of scientists dedicated to advancing neuroendocrine cancer research. Our symposium is unique in its focus and format to showcase basic and translational research in an interactive and collaborative environment.

During the next three days, you will hear from NETRF grantees, other experts in the field, and early-career NET scientists who will share new discoveries and discuss progress and challenges. This year, we are responding to your feedback by providing more time for informal networking and incorporating the patient voice into our program. Our hope is that you engage with fellow researchers in the spirit of building community and sharing information.

NETRF is the largest global funder of neuroendocrine cancer research. Since 2005, we have invested more than $37 million to fuel research in 71 institutions in 16 countries. NETRF has funded two and a half times more neuroendocrine cancer investigators than the NIH. We are proud of our impact on the field and our success in recruiting new researchers to focus their talents on this formidable disease.

Thank you to NETRF's grantees, past and present, who have dedicated their careers to seeking greater understanding of neuroendocrine cancer, discovering new treatment targets, and working toward cures for hundreds of thousands of patients and their families. Your willingness to share your work and data is truly appreciated. For those of you who are new to the field and our symposium, we welcome you to our diverse and thriving scientific community.

Most importantly, on behalf of the Board of Directors, Board of Scientific Advisors, and the NETRF staff, we thank our loyal donors, whose continued support sustains our ambitious research portfolio. Our amazing staff has worked hard to make this meeting a productive experience for all, so we hope that you enjoy your time in Boston.

Elyse Gellerman
CEO

Todd Gilman
President, NETRF Board of Directors

NEUROENDOCRINE TUMOR RESEARCH FOUNDATION
DEDICATED TO CURING NEUROENDOCRINE CANCER

NETRF Distinguished Service Award

Ramesh Shivdasani, MD, PhD

NETRF is proud to recognize Ramesh Shivdasani, MD, PhD, with the Distinguished Service Award. Dr. Shivdasani served as the Chair of NETRF's Board of Scientific Advisors from 2007 to 2016. He has been active on the Board of Scientific Advisors since its inception and just recently was named as an Emeritus Member. During his impressive tenure, his expertise, leadership, and unwavering commitment have been instrumental in NETRF's growth and advancement of our mission to accelerate research and improve outcomes for those affected by neuroendocrine cancer.

Dr. Shivdasani is known for his research on the cell of origin in intestinal neuroendocrine tumors, as well as his collaboration with other NETRF-funded researchers which yielded the discovery of non-functional PanNET subtypes characterized by different risks of recurrence.

Dr. Shivdasani is Professor of Medicine at Harvard Medical School; Professor of Medicine, Medical Oncology at the Dana-Farber Cancer Institute; and Deputy Director of the Dana-Farber/Harvard Cancer Center, Executive Committee. Until 2017, Dr. Shivdasani maintained a part-time practice in gastrointestinal oncology.

We express our heartfelt gratitude to Dr. Shivdasani for his dedication and profound impact on our community.

NEUROENDOCRINE TUMOR RESEARCH FOUNDATION

DEDICATED TO CURING NEUROENDOCRINE CANCER

AGENDA

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DAY 1 – Monday, October 28, 5:00-8:00 p.m.

- **5:00–6:00 p.m. – Registration, Fenway Foyer, Hotel Commonwealth**
- **6:00–8:00 p.m. – Welcome Reception & Poster Session**

DAY 2 – Tuesday, October 29, 8:00 a.m.-5:30 p.m.

- **8:00–9:00 a.m. – Breakfast & Registration**
- **9:00–9:05 a.m. – Welcome**
- **9:05-9:35 a.m. – Keynote and Distinguished Service Award Presentation – Viewing Intestinal NETs Through the Lens of Normal Human Enteroendocrine Cell Differentiation**
  Ramesh Shivdasani, MD, PhD, Dana-Farber Cancer Institute and Harvard Medical School, with introduction by Chrissie Thirlwell, MBBS, PhD
- **9:35–9:45 a.m. – Patient Voice**
  Elaine Nord

SESSION 1: NEN SCREENS & OMICS, 9:45 a.m.-1:45 p.m.

Session Chairs: Chrissie Thirlwell, MBBS, PhD, University of Bristol Medical School and Sharon Gorski, PhD, BC Cancer

- **9:45–10:00 a.m. – Comprehensive Genomic Profiling of an International Patient Cohort Reveals Diagnostic and Prognostic Signatures for Pancreatic Neuroendocrine Neoplasms**
  Aatur Singhi, MD, PhD, University of Pittsburgh
- **10:00–10:15 a.m. – Assessment of the Current and Emerging Criteria for the Histopathological Classification of Lung Neuroendocrine Tumours in the Lungnenomics Project**
  Matthieu Foll, PhD, International Agency for Research on Cancer
- **10:15–10:30 a.m. – Defining the Transcriptomic Profile of Enterochromaffin Cells in SI-NET Patients**
  Netta Mäkinen, PhD, Dana-Farber Cancer Institute
- **10:30–10:45 a.m. – Discussion**
- **10:45–11:15 a.m. – Coffee Break**
- **11:15-11:30 a.m. – Targeting the Surfaceome of Drug Tolerant Persister Cell Populations in Extrapulmonary High-grade Neuroendocrine Carcinomas**
  C. Allison Stewart, PhD, University of Texas MD Anderson Cancer Center
- **11:30-11:45 a.m. – Simplifying NEN Tissue and Liquid Diagnostics Using Novel and Existing General Neuroendocrine Cell Markers**
  Neil Renwick, MD, PhD, Queen's University

- **11:45 a.m.-12:00 p.m. – A Systematic NEN Spheroid Drug Screen Reveals a Novel Drug Resistance Mechanism in Small Bowel NETs**
  Po Hien Ear, PhD, University of Iowa

12:00–12:15 p.m. – Discussion

12:15–1:45 p.m. – Lunch & Group Photo

SESSION 2: NEN TUMOR MICROENVIRONMENT, 1:45–3:45 p.m.

Session Chairs: James Bibb, PhD, University of Arizona Medical School - Phoenix and Iacovos Michael, PhD, Sunnybrook Research Institute

- **1:45–2:00 p.m. – Estrogen Receptor Alpha Inhibition Increases 177Lu-DOTATATE Efficacy in a Pre-clinical Neuroendocrine Tumor Model**
  Xavier Keutgen, MD, University of Chicago Medicine
- **2:00–2:15 p.m. – Spatial and temporal intratumor heterogeneity of Pancreatic neuroendocrine tumors**
  Jérôme Cros, MD, PhD, INSERM, Université Paris Cité
- **2:15–2:30 p.m. – TILs from Pannet Liver Metastasis: In Search of Novel Adoptive Transfer Strategies for the Treatment of Nets**
  Mauro Cives, MD, University of Bari "Aldo Moro"
- **2:30–2:45 p.m. – Immunosuppressive Myeloid Signaling in Pancreatic Neuroendocrine Tumors Revealed by Single-nucleus RNA-seq**
  Jeanna Qiu, AB, Harvard Medical School
- **2:45–3:00 p.m. – Spatial Profiling of the Pancreatic Neuroendocrine Tumor Microenvironment**
  Christopher Heaphy, PhD, Boston University School of Medicine
- **3:00–3:15 p.m. – Discussion**
- **3:15–3:45 p.m. – Coffee Break**

SESSION 3: EARLY CAREER LIGHTNING TALKS, 3:45–5:00 p.m.

Session Chairs: Talya Dayton, PhD, EMBL Barcelona, and Dawn Quelle, PhD, University of Iowa

3:45–4:15 p.m. – Lightning Talks:

1. **Preliminary Analysis of the Whole Exome Sequencing Data of 16 cases with Neuroendocrine Cell Hyperplasia for Classification as Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH)**
   Hui Yu, MD, PhD, University of Colorado Anschutz Medical Campus
2. **Does Sexual Dimorphism Play a Role in SI-NET and Mesenteric Disease Development?**
   Maria Martins, MSc, University College London
3. **Epigenetic Regulation of Tumor Metastasis in SI-NETs**
   Elham Barazeghi, PhD, Uppsala University
4. **Reconstructing the Evolutionary History of Neuroendocrine Tumor Subtypes**
   Nicolas Alcala, PhD, International Agency for Research on Cancer

5. **Preliminary Results of the COPPER PET in NET Trial: A Randomized, Crossover, Readers Blind, Phase 0/I Study Comparing 61Cu-NODAGA-LM3 and 68Ga-DOTATOC for the Detection of Neuroendocrine Tumors**
   Guillaume Nicolas, MD, University Hospital Basel
6. **Identifying Regulators of GEP-NET Metastasis with in vivo CRISPR Screen**
   William You, HBSc, Sunnybrook Research Institute

- **4:15–4:30 p.m. – Discussion**
- **4:30–4:35 p.m. – Pheo Para Alliance Travel Award Presentation**
- **4:35–5:30 p.m. – Extended Poster Viewing & Happy Hour**
- **5:30 p.m. – End of Day**

DAY 3 – Wednesday October 30, 8:00 a.m.–1:45 p.m.

- **8:00–9:00 a.m. – Breakfast**
- **9:00–9:05 a.m. – Welcome**
- **9:05-9:15 a.m. – Patient Voice, "Get Busy Living"**
  Carrie Camino, NETRF Board of Directors

SESSION 4: NEN THERAPIES & RESISTANCE MECHANISMS, 9:15–10:30 a.m.

Session Chairs: Mauro Cives, MD, University of Bari "Aldo Moro" and Amanda Wasylishen, PhD, University of Cincinnati

- **9:15–9:30 a.m. – Chimeric Antigen Receptor (CAR) Trogocytosis from CDH17CAR T Cells to Tumor Cells is Crucial for Assessing CAR T Antitumor Activity**
  Xianxin Hua, MD, PhD, University of Pennsylvania
- **9:30–9:45 a.m. – A Novel Hormone Based Anti-SSTR bispecific T-cell Engager for the Treatment of Neuroendocrine Tumors**
  Eleonora Pellé, MD, Moffitt Cancer Center
- **9:45–10:00 a.m. – Deciphering the Responses and Resistance to Anti-angiogenic Therapies in PanNET Liver Metastases**
  Minah Kim, PhD, Columbia University
- **10:00–10:15 a.m. – Elucidating the Role of HMGB3 During the Progression and Response to Radiation Therapy of Pancreatic Neuroendocrine Tumors**
  Iacovos Michael, PhD, Sunnybrook Research Institute
- **10:15 a.m.–10:30 a.m. – Expression of Bromodomain and Extra-terminal (BET) Proteins in Pancreatic Neuroendocrine Tumors and Mechanistic Implications of BET BD1- and BD2-selective Inhibition**
  Omair Shariq, MD, PhD, University of Oxford
- **10:30–10:45 a.m. – What Drives Radiation Response to Peptide Receptor Radionuclide Therapy? Insights from Whole Genome and Exome Sequencing of Pancreatic Neuroendocrine Tumors**
  Emma Boehm, MD, Peter MacCallum Cancer Centre

- **10:45-11:00 a.m. – Proteogenomic Characterization of Pancreatic Neuroendocrine Tumors Uncovers Hypoxia and Immune Signature Enrichment in Clinically Aggressive Subtypes**
  Michael Roehrl, MD, PhD, Beth Israel Deaconess Medical Center, Harvard Medical School
- **11:00-11:15 a.m. – Discussion**
- **11:15-11:45 a.m. – Break & Grab Lunch**

SESSION 5: NEN MODELS & PROGRESSION MECHANISMS, 11:45 a.m.–1:45 p.m.

Session Chairs: Christopher Heaphy, PhD, Boston University School of Medicine and Eleonora Pellé, MD, Moffitt Cancer Center

- **11:45 a.m.–12:00 p.m. – DNA Methylation Alterations in Small Intestinal Neuroendocrine Tumours Reveal Candidate Drivers and Independent Epigenetic Evolution**
  Chrissie Thirlwell, MBBS, PhD, University of Bristol Medical School
- **12:00–12:15 p.m. – Using Patient-derived Tumor Organoids to Uncover Mechanisms of Pulmonary Net Progression**
  Talya Dayton, PhD, European Molecular Biology Lab (EMBL), Barcelona
- **12:15–12:30 p.m. – Cellular and Molecular Diversity of Human Pulmonary Neuroendocrine Cells**
  Christin Kuo, MD, Stanford University
- **12:30–12:45 p.m. – The Daxx/Atrx/H3.3 Epigenetic Regulatory Axis in Cell State Regulation and Pancreatic Neuroendocrine Tumor Suppression**
  Amanda Wasylishen, PhD, University of Cincinnati
- **12:45–1:00 p.m. – Exploring the Role of a Novel Adrenomedullary Stem Cell Population in Pheochromocytoma/Paraganglioma Tumourigenesis**
  Yasmine Kemkem, PhD, King's College London
- **1:00–1:15 p.m. – Development of Innovative in vitro and in vivo Patient-derived Cancer Models for Translational Studies in G1/G2 Gastroenteropancreatic Neuroendocrine Tumors**
  Yi Liu, PhD, University of Texas MD Anderson Cancer Center
- **1:15–1:30 p.m. – Discussion**
- **1:30–1:45 p.m. – Close of Meeting**

Research Changes Lives

NETRF works boldly and relentlessly to make an impact today through our research funding while laying the groundwork for tomorrow's breakthroughs. NETRF is the leading global funder of neuroendocrine cancer research, supporting innovative projects across 71 institutions in 16 countries.

We drive scientific advancements by convening global researchers and fostering collaboration.

- Development of CAR T for NETs, now in clinical trial
- Identification of DAXX/ATRX genes implicated in cancer
- Establishment of NET organoids
- Discovery of PanNET and LungNET subtypes

NETRF has grown the neuroendocrine neoplasm (NEN) research community. 100% of NETRF-funded early career grantees remain in research, 90% in NEN research.

"NETRF funding has had an enormous effect on my career as a young scientist by providing me with support to pursue a potentially transformative treatment for NETs using cutting-edge engineering tools."
– Kevin McHugh, PhD, grantee

"Receiving NETRF funding has played a significant role in my research career, especially considering the challenges in securing financial support within the rare cancers field. NETRF's backing has provided stability for my work in this area, while also connecting me with an incredible network of fellow researchers... enhancing the scope and influence of our research."
– Matthieu Foll, PhD, grantee

"NETRF is often the first or only funding source for NEN scientists. We are proud to empower researchers with resources to push the boundaries of neuroendocrine cancer discovery."
– Elyse Gellerman, NETRF CEO

Thank you for being a part of the search for cures for neuroendocrine cancer!

Donate today to shape the future of NEN research NETRF.org/donate or scan this QR code.

NEUROENDOCRINE TUMOR RESEARCH FOUNDATION
DEDICATED TO CURING NEUROENDOCRINE CANCER

22
Clinical Research Projects Including Clinical Trials Since 2006

8
Patents Granted or Pending for New Technology or Discoveries*

5
Industry Partnerships or Spin-Off Companies to Bring New Treatments to Patients*

88
New Collaborations Between Researchers as a Result of NETRF Funding*

100%
of Early-Career Grantees Remain in Research...90% of Them in NET Research*

*Since 2017

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The Genetic Evolution of Pancreatic Neuroendocrine Tumor Progression

Sarah E. Umetsu[1], Sanjay Kakar[1], Stephanie Wang[2], Grace E. Kim[1], Farhana Moon[2], Emily Bergsland[2,3], Grace E. Kim[1], and Nancy M. Joseph[*,1]

1. Department of Pathology, University of California San Francisco, SF, CA, USA. 2. Helen Diller Family Cancer Center, University of California San Francisco, SF, CA, USA. 3. Department of Medicine, Division of Hematology/Oncology, University of California San Francisco, SF, CA, USA.

Presenting author: Nancy Joseph, MD, PhD

Background:

Pancreatic neuroendocrine tumors (PanNETs) are a heterogenous group of tumors with an increasing incidence and now include an aggressive grade 3 (G3 PanNET) category introduced in the 2017 WHO classification. Challenges in pathologic diagnosis and changes in terminology have limited our understanding of the G3 PanNET category and how to optimally manage patients with G3 PanNET. Estimates of the propensity for PanNETs to progress to G3 vary considerably, and in most cases we lack biomarkers that predict which lesions will progress and which will not. Pancreatic NETs have been shown to harbor frequent mutations in MEN1, DAXX, ATRX, mTOR genes (TSC1/2, PTEN), SETD2, and CDKN2A, but the sequence of alterations acquired during tumor progression remains poorly understood. This study aims to gain a deeper understanding of the sequence of genomic changes that occur over time in PanNETs that progress to G3, those that do not progress to G3, and those that present as G3 at diagnosis.

Methods:

We searched the UCSF Pathology database as well as an IRB-approved outcomes database of patients seen at the UCSF Center for Neuroendocrine Tumors and identified 75 patients with PanNET who had multiple serial archival pathology specimens. Sufficient tumor tissue from at least two serial samples was available for 40 patients. Capture-based DNA sequencing of >500 cancer genes is currently being performed on all serial samples with sufficient tumor tissue. Sequencing analysis has been performed on an initial subset of cases, as we await completion of the sequencing

Results:

Figure 1 summarizes the key putative driver mutations in 17 longitudinal tumor samples from 7 patients showing grade progression over time. As expected, the most common driver alterations in this cohort were in MEN1 (82%), DAXX (41%), ATRX (35%), and TSC1/TSC2 (35%). Progression from low-grade (G1/G2) to high-grade (G3) involved acquisition of additional genomic alterations not seen in the prior G1/G2 samples in all cases, most commonly in CDKN2A or TP53. Acquisition of both TP53 & RB1 alterations was seen in two patients. The transition from G1/G2 to G3 involved acquisition of TSC1/2 in 3 cases and CDKN1B in 2 cases.

Conclusions:

Sequencing of PanNET samples from an initial cohort of patients who have undergone serial biopsies over time demonstrates acquisition of genetic alterations during PanNET grade progression. The transition to G3 most commonly involved acquisition of CDKN2A or TP53. A larger cohort of samples will be examined to delineate the genetic landscape of PanNET grade progression and will be compared to the genetic landscape of PanNETs that do not show grade progression over time, which may uncover biomarkers to help risk-stratify patients and predict response to treatment as well as understand treatment resistance.

Proteogenomic Characterization of Pancreatic Neuroendocrine Tumors Uncovers Hypoxia and Immune Signature Enrichment in Clinically Aggressive Subtypes

Atsushi Tanaka[1,2], Makiko Ogawa[1,2], Yihua Zhou[3], Yusuke Otani[1,2], Ronald C. Hendrickson[4], David S. Klimstra[5], Julia Y. Wang[6], and Michael H. Roehrl[1,2]

1. Department of Pathology, Beth Israel Deaconess Medical Center, Boston, MA, USA. 2. Harvard Medical School, Boston, MA, USA. 3. ICU Department, Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, China. 4. Former address: Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, NY, USA, 5 Paige. AI, New York, NY, USA. 6. Curandis, New York, NY, USA.

Presenting author: Michael H. Roehrl, MD, PhD

Background:

Pancreatic neuroendocrine tumors (PanNETs) represent well-differentiated endocrine neoplasms with variable clinical outcomes. Predicting patient outcomes using the current tumor grading system is challenging. In addition, traditional systemic treatment options for PanNETs, such as somatostatin analogs or cytotoxic chemotherapies, are very limited.

Methods:

To address these issues, we characterized PanNETs using integrated proteogenomics and identified four subtypes.

Results:

Two proteomic subtypes showed high recurrence rates, suggesting clinical aggressiveness that was missed by current classification. Hypoxia and inflammatory pathways were significantly enriched in the clinically aggressive subtypes. Detailed analyses revealed metabolic adaptation via glycolysis upregulation and oxidative phosphorylation downregulation under hypoxic conditions. Inflammatory signature analysis revealed that immunosuppressive molecules were enriched in immune hot tumors and might be immunotherapy targets.

Conclusions:

In this study, we characterized clinically aggressive proteomic subtypes of well-differentiated PanNETs and identified candidate therapeutic targets.

Description and Preclinical Safety Assessment of [18F]FluoFAPI for Clinical Translation: Automated Radiosynthesis, Dosimetry and Single Acute Dose Toxicological Evaluation

Jason A. Witek[1], Allen F. Brooks[1], Sahil M. Kapila[2], Wade P. Winton[1], Jenelle R. Stauff[1], Peter J. H. Scott[1,3], Benjamin L. Viglianti[1]

1. Department of Radiology, University of Michigan Medical School, Ann Arbor, MI 48109, United States. 2. Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, United States. 3. The Interdepartmental Program in Medicinal Chemistry, University of Michigan College of Pharmacy, Ann Arbor, MI 48109, United States.

Presenting author: Jason Witek, MD, PhD

Background:

Cancer associated fibroblast have become a new target for therapy. Fibroblast present within malignancies express the Fibroblast Activation Protein (FAP). Inhibitors to FAP (FAPI), are small molecules recently developed as a theranostic agent for imaging and radiotherapy. All currently used FAPI rely on a linker chelator complex attached to the 'inhibitor.' We describe a new automated method of direct attachment of the radioisotope to the inhibitor resulting in >50% MW reduction with the hope of improved tumor to background ratio and tumor uptake.

Methods:

[18F]FluroFAPI was developed from a Sn precursor. This allowed subsequent automated radioflourination. We obtained the biodistribution of [18F]FluroFAPI in rats, performed estimated human radiation dosimetry, and performed a 100x expected single dose toxicology analysis for eventual first in human experiments.

Results:

Synthesis of the Sn precursor for FluorFAPI and automated synthesis of [18F]FluroFAPI was demonstrated. [18F]FluroFAPI had favorable estimated human radiation dosimetry, and demonstrated no adverse effects when injected at a dose 100x that planned for [18F]FluroFAPI.

Conclusions:

With successful development of an automated synthesis of [18F]FluroFAPI, first in human testing can be planned with the hope of improved tumor to background performance compared to other FAPI agents.

Defining the Transcriptomic Profile of Enterochromaffin Cells in Si-Net Patients

Netta Mäkinen[1,2], Yosuke Kasai[3], Grace E. Kim[4], Chrissie Thirlwell[5,6], Eric Nakakura[3], Matthew Meyerson[1,2,7]

1. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. 2. Cancer Program, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. 3. Department of Surgery, University of California, San Francisco, California, USA. 4. Department of Pathology, University of California, San Francisco, California, USA. 5. Bristol Medical School, University of Bristol, Bristol, UK. 6. Department of Oncology, UCL Cancer Institute, London, UK. 7. Departments of Genetics and Medicine, Harvard Medical School, Boston, Massachusetts, USA.

Presenting author: Netta Mäkinen, PhD

Background:

Small intestinal neuroendocrine tumors (SI-NETs) represent one of the major cancer subtypes of the small bowel, accounting for ~40% of all small intestinal malignancies. SI-NETs are thought to originate from enterochromaffin cells, which constitute less than 1% of the epithelial cells of the gastrointestinal tract. Enterochromaffin cells are a specialized type of enteroendocrine cells that synthesize, store and secrete ~90% of the serotonin (5-hydroxytryptamine or 5-HT) in the human body. The absence of recurrent genomic driver alterations in SI-NETs has motivated the search for other potential causes of SI-NET pathogenesis, including transcriptomic and epigenomic profiling of these lesions. These studies have been limited, however, by the lack of a reference for enterochromaffin cells. The goal of this project has been to characterize the gene expression landscape of enterochromaffin cells in the ileum of SI-NET patients and to form a reference for cancer-to-normal cell comparisons.

Methods:

Our sample cohort consisted of 19 fresh-frozen normal ileum specimens from ten multi- and nine unifocal SI-NET patients. We performed single-nucleus RNA sequencing to identify subpopulations of enterochromaffin cells within each sample. We have used Seurat (v5) and harmony for the data analysis and integration of the samples, respectively. Four cell markers were used for the identification of enterochromaffin cells in our data set: SLC18A1, TPH1, CHGA and CHGB.

Results:

A total of 132,751 high-quality nuclei were available for our analysis, the number varying from 1,164 to 15,009 nuclei per sample. After the integration of single-nucleus RNA sequencing data from all 19 normal ileum samples, five most variable genes identified in the data set were DEFA5, CNTNAP2, CTNNA2, SYT1 and NRXN1. For example, DEFA5 is a known cell marker for Paneth cells. We have successfully detected an enteroendocrine cell cluster within each sample. We are currently counting the total number of enterochromaffin cells within our integrated data set, and subsequently, we will assess their transcriptomic profile.

Conclusions:

A better understanding of the cellular and molecular mechanisms that underlie SI-NETs is essential for the non-invasive management, early detection and prevention of these tumors. Next, we will use the transcriptomic profile of enterochromaffin cells as a reference for cancer-to-normal cell comparisons in a cohort of 10 SI-NETs.

Spatial and Temporal Intratumor Heterogeneity of Pancreatic Neuroendocrine Tumors

Pauline Bourdeleau1*, Zaima Afzal-Awan[2]*, Johanna Pokossy-Epée[1,2], Juliette Palle1, Matthieu Tihy[3], Olivia Hentic[1], Anne Couvelard[2,3], Maxime Ronot[2,4], Vinciane Rebours[1,2], Philippe Ruszniewski[1,2], Louis de Mestier[1,2]*, Jérôme Cros[2,5]*

*Contributed equally. 1. Université Paris-Cité, Department of Pancreatology and Digestive Oncology, Beaujon Hospital (APHP.Nord), Clichy, France. 2. Center of Research on Inflammation, INSERM UMR1149, Paris, France. 3. Université Paris-Cité, Department of Pathology, Bichat Hospital (APHP.Nord), Clichy, France. 4. Université Paris-Cité, Department of Radiology, Beaujon Hospital (APHP.Nord), Clichy, France. 5. Université Paris-Cité, Department of Pathology, Beaujon Hospital (APHP.Nord), Clichy, France.

Presenting author: Jérôme Cros, MD, PhD

Background:

PanNETs show intra-tumour heterogeneity, notably regarding Ki67 index which is a major prognostic biomarker. An evolution model derived from intertumor heterogeneity data was proposed starting either from beta cells leading to good prognosis PanNET or from alpha cells through the sequential alteration of MEN1 followed by DAXX or ATRX leading to more aggressive PanNETs. Spatial and temporal intratumor heterogeneity and its prognostic impact have been poorly explored so far. We aimed to describe the temporal evolution of grade/Ki67 and ADM (ATRX-DAXX-MENIN) status in patients with PanNET, and to explore their molecular intratumor heterogeneity to better understand the biological mechanisms involved in their progression.

Patients and methods:

We retrospectively studied 109 patients with sporadic PanNETs and serial tumour samples over time (n=286) treated in one expert institution (1993-2021). PanNET morphology, Ki67 and ADM status (IHC) were centrally re-assessed on each sample. Prognostic factors (overall survival) and factors associated with Ki67 increase were explored using multivariable Cox proportional hazard models or logistic regression models, respectively. 12 PanNET with a massive intratumor heterogeneity (Intratumor delta Ki67 >15%) were selected for multiregion sampling, DNAseq (Panel 571 gene panel) and RNAseq.

Results:

The median time interval between samples was 49.6 months. Increase in PanNET grade (36% of patients) was associated with poorer prognosis (adjusted HR = 1.96, [1.04-3.69], p=0.037). Increased Ki67 (61% of patients) was also associated with poorer prognosis with an optimal threshold of > +7% (adjusted HR 3.05, [1.56-6.12], p=0.001). Increase in Ki67 was time-dependent but an increase ≥ 2%/year remained prognostic (adjusted HR 1.96, [1.02-3.73], p=0.041). The risk of increase in Ki67 ≥ 2%/year was higher in patients who received alkylating agents (adjusted OR 13.4, [2.63-107.5], p=0.005) and was lower in those with disease control under somatostatin analogues (adjusted OR 0.17, [0.03-0.78], p=0.005). ADM switch was exceptional during progression. DNAseq revealed very little genomic intratumor heterogeneity, most mutations occurring very early and were already present in the low Ki67 areas. PanNET with ADM were underrepresented in these aggressive PanNET (25%) while driving sporadic VHL (17%) and CDKN1A (17%) alterations were more frequent than expected. There was no ADM status spatial heterogeneity and no cases with a MEN1 only mutation. RNAseq and methylome studies are ongoing.

Conclusion:

Increase in tumour grade and Ki67 is frequent in PanNETs, associated with significantly poorer prognosis and favoured by alkylating agents. Genomic intratumor heterogeneity in PanNET was low with ADM mutations occurring very early, questioning whether alpha-like-MEN1 only PanNET are just an intermediate stage or a true evolutionary path. ADM does not appear to be the main driver in highly heterogeneous and aggressive PanNET.

Deciphering the Responses and Resistance to Anti-Angiogenic Therapies in Pannet Liver Metastases

Yeonghwan Kim[1], Sophie O'Keefe[1], Nicola James[3], Helen Remotti[1], Tito Fojo[2], Minah Kim[1]

1. Department of Pathology and Cell Biology. 2. Department of Medicine, Columbia University Irving Medical Center, New York, New York, USA. 3. Regeneron Pharmaceuticals Inc., Tarrytown, New York, USA.

Presenting author: Minah Kim, PhD

Approximately 40% of patients with pancreatic neuroendocrine tumors (PanNET) present with liver metastasis at diagnosis. PanNET is characterized by extreme vascularity, suggesting that angiogenesis inhibitors may represent beneficial treatments for patients with PanNET. Drugs targeting VEGF signaling pathway have shown efficacy in patients with well-differentiated, metastatic, or non-resectable PanNET. However, metastatic recurrence has limited therapeutic success in patients. From recent and ongoing investigations, we sought to decipher the responses and resistance to anti-angiogenic therapies, specifically targeting angiopoietin-2 (ANGPT2) or VEGF, in PanNET. Our recent study demonstrated that ANGPT2, a vascular destabilizing factor, promotes immune suppression by impairing T cell recruitment into the tumors. This study emphasized the importance of targeting the tumor vasculature for anti-tumor immunity and tumor control in advanced PanNET. At the same time, our current study also focuses on response and resistance to anti-VEGF therapy. Using RT2;AB6F1 mice, which spontaneously develop primary and liver metastases of PanNET, we found that short-term VEGF inhibition (1 week) decreased metastatic burden in the liver, whereas prolonged inhibition (5 weeks) no longer exhibited anti-metastatic effects, with increased vascular leakage. Notably, in our experimental metastasis model, which develops PanNET liver metastasis in the absence of primary tumors, prolonged treatment with anti-VEGF showed a durable response, implying the role of primary tumors in therapeutic resistance in the liver. With the goal of identifying the molecular determinants of anti-VEGF resistance in the metastatic progression of PanNET, we're currently analyzing single-nucleus RNA sequencing on livers treated with IgG or anti-VEGF. This transcriptomic analysis will identify potential genes and pathways involved in anti-VEGF resistance, allowing us to conduct functional studies on candidate molecules to elucidate their roles in anti-VEGF resistance in advanced PanNETs. This project has the potential to identify novel therapeutic targets to overcome anti-VEGF resistance in PanNET patients.

The Balancing Act of Implementing the "Best" Biochemical Testing to Diagnose Pheochromocytoma

Rachelle M. Muschett

Comprehensive Cancer Center, University of New Mexico, University of New Mexico Health Sciences Center, Albuquerque, NM, 87131, USA.

Presenting author: Rachelle M. Muschett

Background:

This transdisciplinary implementation science project is inspecting the nuances of reducing the time gap between science and practice for diagnosing pheochromocytomas and paragangliomas (PPGLs) with biochemical testing. This project evaluates provider selection of biochemical tests, preanalytical process in commercial labs, and reference limits. Errors or miscommunication at any these steps decrease the chance of an accurate diagnosis of PPGL. PPGLs are a rare and possibly deadly diseases if not caught soon enough.

As the speed of science is increasing, so is the need to ground research in practice to ensure breakthroughs are not lost in the pipeline of dissemination. Part of the reluctance and increased time in this situation to implement the science has to do with the fact medicine in the U.S. is commercial and therefore has a fiduciary responsibility to either make money or minimize financial loss. For example, recent research in this field has illustrated the plasma metanephrines test is best done with a prechilled tube and indwelling catheter after the patient has laid supine for 20-30 minutes. However, this complicates an assay which is only reimbursed $16.86 from Medicare. The commercial labs must grapple with the multiplicity of science and providing the highest quality of care to patients, while weighing out the cost to their organization.

Methods:

This project is engaging with commercial laboratories, integrated health clinics, and non-profits to hash through the complexities of implementing the science in a comprehensive approach. All CMS claims data for catecholamines and metanephrines tests has been collated from 2013-2022, to evaluate practice trends and how they compare to society guidelines, emerging research, and trends in Australia, the UK, Germany and Sweden. Specifically, this project has partnered with Mayo Clinic and the two largest commercial labs (representing 72% of the volume of these biochemical tests) in the U.S. to possibly update reference limits and preanalytical process. Thought leaders also include Johns Hopkins who are considering adding methoxytyramine, a dopamine metabolite, to the existing plasma metanephrines panel. The predominant U.S. practice is to test for dopamine in the catecholamines panel, which has proven to be significantly lower specificity and sensitivity for most PPGL's.

We are simultaneously collaborating to construct a patient guide for the Pheo Para Alliance non-profit organization, as one method of disseminating best practice. Of the approximately 100,000 PPGL biochemical tests completed annually in the U.S., most are ordered by Clinicians who are not PPGL experts and have not kept up with best practice in the literature. To complicate matters, the Endocrine Society's 2014 PPGL Clinical Guidelines was "retired" with a new Expert Consensus Report yet to be initiated. Putting information in the hands of the patients can help with this knowledge gap.

Results:

This project is ongoing, with results forthcoming.

Conclusion:

This project is attempting to expedite dissemination by creating dialogue between patients, researchers, laboratory leaders, and clinicians who will hopefully come to some level of consensus as to quality and scope of care.

Differential Gene Expression in Patients of African Ancestry with Pancreatic Neuroendocrine Tumors

Elquis Castillo[1], Brendon Herring[1], Rachael Guenter[1], Isra Elhussin[2], Bassel F. El-Rayes[3], Asfar Azmi[4], Herbert Chen[1], Po Hien Ear[5], Clayton C Yates[2], J. Bart Rose[1]

1. Department of Surgery, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA. 2. Department of Pathology, Oncology, and Urology, Johns Hopkins School of Medicine, Baltimore, Maryland, USA. 3. Department of Hematology and Oncology, Heersink School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA. 4. Barbara Ann Karmanos Cancer Institute, Department of Oncology, Wayne State University School of Medicine, Detroit, Michigan. 5. Department of Surgery, University of Iowa, Iowa City, Iowa, USA.

Presenting author: Elquis Castillo II, MS

Background:

Pancreatic neuroendocrine tumors (pNETs) in Black patients have been shown to have a more aggressive phenotype. Transcriptomic analyses of these tumors are historically lacking in this patient population. Furthermore, drivers of disparate outcomes between Black and White patients are unknown. Genetic ancestry has been shown to be a more informative variable than self-reported race when interrogating biological mechanisms. Thus, we hypothesized differential gene expression would be observed based on genetic ancestry of patients with pNETs.

Methods:

Sixty-eight low grade patient tumors (30 male and 38 female) from two different institutions were surgically resected and underwent transcriptomic sequencing with an average coverage of 200X using the Illumina Novaseq 6000 platform. Genetic ancestry was determined by ADMIXTURE using samples from the 1000 Genome Project as a reference panel. Differentially expressed genes (DEGs) were determined by the R package DESeq2. Gene set enrichment analysis (GSEA) was performed using the R package cluster Profiler. Significance was defined as $p < 0.05$ for GSEA analysis and p adjusted < 0.05 for DEGs.

Results:

Admixture determined 46 patients had >50% European ancestry (EUR) while 27 had >50% African ancestry (AFR). There were 44 grade 1 tumors (26 EUR, 18 AFR) and 24 grade 2 tumors (16 EUR, 8 AFR) included. Principal component analysis showed 17% and 10% genetic variance between Ancestral and Grade cohorts respectively. We identified 50 statistically significant DEGs between the ancestral cohorts. GSEA found many of the genes activated in patients with majority African ancestry were involved in heterocyclic and aromatic compound biosynthesis and metabolic processes. Notably, patients with majority African ancestry had increases in gene expression of Tyrosine Hydroxylase (TH) and Tryptophan Hydroxylase 1 (TPH1), quantified as log2 fold changes of 3.9 and 3.3.

Conclusions:

TPH1 is the rate limiting enzyme in serotonin synthesis, a biogenic amine found to be elevated in pNETs and other cancers. TPH1 and other DEGs in pNET patients with majority African ancestry may be contributing to disparate outcomes. Validation in larger datasets is required before widespread clinical use can be recommended.

Chimeric Antigen Receptor (CAR) Trogocytosis from CDH17CAR T Cells to Tumor Cells is Crucial for Assessing CAR T Antitumor Activity

Xianxin Hua, Azin Aghamajidi, Jinglu M. Wu, and Eva Q. Wu

Department of Cancer Biology, Abramson Cancer Research Institute, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104.

Presenting author: Xianxin Hua, MD, PhD

Background and significance to NETs:

Neuroendocrine tumors (NETs) pose significant challenges to therapy due to their propensity to develop resistance to various treatment modalities. Patients with NETs, particularly those with multiple metastatic sites, often face poor prognosis. Cancer immunotherapies, including the use of adoptive T cells engineered with chimeric antigen receptors (CAR) and checkpoint blockade inhibitors, have demonstrated remarkable efficacy in treating certain leukemias and some solid tumors. However, CAR T cell therapy for NETs remains underdeveloped. In our previous research, we developed novel nanobody-directed CDH17 CAR T cells to treat NETs in preclinical models, and it is imperative to further increase the functionality of CDH17CAR T cells to suppress neuroendocrine tumors.

Materials and Methods/Experimental Approach:

Two types of CDH17CARs derived from distinct CDH17 nanobodies were generated and used for comparing their in vitro and in vivo antitumor activities. In addition, transfer of CAR/VHH from T cells to tumor cells in the co-culture was examined by flow cytometry analysis. In vitro cytotoxicity and the cytokine releases were evaluated using LDH release assay and ELISA assays, respectively. The tumor xenograft models were used to assay the in vivo CAR T antitumor activity.

Results:

While investigating ways to improve CDH17 CAR T cells, we discovered that the CAR protein on CAR T cells could be transferred to the target tumor cell surface, a process known as trogocytosis. This transfer of CAR protein from T cells to tumor cells can diminish the CAR T cell-mediated killing of target cells in vivo, though not necessarily in vitro. Furthermore, we observed that different CDH17 binder-derived CAR T cells vary in their capacity to transfer CAR protein to target tumor cells. Some CAR T cells exhibit strong cytotoxicity to tumor cells in vitro but only moderate antitumor activity in vivo, likely due to CAR protein transfer via trogocytosis.

Conclusions/next steps:

Our findings suggest that the ability of the different binder-derived CAR T cells to transfer CAR protein to target cells may differ. Furthermore, trogocytosis may not cause obvious impact on in vitro CAR T cytotoxicity, but substantially diminish the CAR T antitumor activity in vivo. These results underscore the importance of measuring CAR trogocytosis activity and comparing a CAR's in vitro and in vivo tumor activities.

Impact statement:

These studies provide new insights into how to further augment the CDH17CAR T cell's anti-NE tumor function via modulating CAR trogocytosis.

Elucidating the Role of HMGB3 During the Progression and Response to Radiation Therapy of Pancreatic Neuroendocrine Tumors

Nilakshi Kulathunga[1], Zoey Wang[1,2], Areeba Qureshi[1], Benjamin Lok[3], Kathrin Tyryshkin[4], Anne-Claude Gingras[5], Iacovos Michael[1,2]

1. Sunnybrook Research Institute, Toronto, ON, Canada. 2. Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada. 3. Princess Margaret Cancer Centre, Toronto, ON, Canada. 4. Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada. 5. Pathology and Molecular Medicine, Kingston, ON, Canada.

Presenting author: Iacovos Michael, PhD

Introduction:

We have recently demonstrated that Pancreatic Neuroendocrine tumors (PanNETs) undergo a transition from a relatively benign molecular subtype to an aggressive and highly metastatic molecular subtype via dedifferentiation. This process leads to the reactivation of progenitor-like gene regulatory networks organized by master regulators. Through computational approaches, we have identified the High Mobility Group Box 3 (HMGB3) as one of these master regulators, typically expressed in pancreatic endocrine progenitors and re-expressed in the aggressive dedifferentiated PanNETs. This study aims to delve into its role during the PanNET dedifferentiation and tumor progression, a research area that is yet to be fully explored.

Methods:

To dissect the HMGB3 protein interactome, we used the proximity-dependent biotin identification (BioID) assay and analyzed our data using computational approaches. We used gain-of-function and loss-of-function approaches to assess the functional role of HMGB3 in vitro and in vivo orthotopic tumor assays.

Results:

Strikingly, the analysis of the HMGB3 protein interactome unveiled that it is implicated in chromatin remodeling and organization by interacting with various complexes, such as the Polycomb complexes and the SWI/SNF complexes, as well as in DNA damage response and repair by interacting with a different set of proteins, such as the BRCA1/BARD1 complex and PARPs. Consistently, Hmgb3 knock-down in PanNET cells sensitizes them to radiation, as assessed by colony formation assays. Finally, we found that HMGB3 knock-down hinders the ability of high-grade PanNET cells to form tumors in mice.

Conclusions:

Overall, our work demonstrates how the re-activation of embryonic signaling pathways contributes to the intrinsic characteristics of aggressive high-grade PanNETs. This study provides mechanistic insights into HMGB3's role during tumor progression and radiation therapy response by controlling chromatin organization and DNA damage response. Our current efforts focus on further characterizing the HMGB3 protein interactions and mechanism of action and elucidating its role during radioligand therapy and other types of treatments for PanNETs.

Glycomic Characterization of Pancreatic Neuroendocrine Neoplasms

Sharon M. Gorski[1], Suganthi Chittaranjan[1], Emma Titmuss[2], Nicholas M. Twells[3], Steve E. Kalloger[4], Christine Chow[5], David F. Schaeffer[4], Jonathan M. Loree[6], Lara K. Mahal[3]

1. Genome Sciences, BC Cancer Research Institute, Vancouver, BC, Canada. 2. Medical Oncology, BC Cancer, Vancouver, BC, Canada. 3. Glycomics Institute of Alberta, University of Alberta, Edmonton, Alberta, Canada. 4. Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada. 5. Molecular and Advanced Pathology Core, University of British Columbia, Vancouver, BC, Canada. 6. Medical Oncology, University of British Columbia, Vancouver, BC, Canada.

Presenting author: Sharon Gorski, PhD

Background:

Pancreatic neuroendocrine neoplasms (PNENs) are characterized by substantial molecular and clinical heterogeneity which poses challenges for effective disease management. While PNENs often harbour distinctive sets of molecular alterations, the roles of molecular features in the management of this disease have been limited, and responses to therapies are often insubstantial and vary between tumours of the same class. There remains a challenge of how to reliably classify PNENs and effectively treat PNEN patients.

The glycome is the repertoire of glycan structures (carbohydrates) produced by cells. Glycans can be bound covalently to proteins or lipids in an enzymatic process termed glycosylation that responds dynamically to both intracellular and extracellular conditions. Glycans can form distinct patterns on cells in normal development and diseases. For example, changes in the glycome were associated with malignancy and metastasis, making them valuable biomarkers for disease states. Dysregulation of protein glycosylation was also shown to contribute to disease pathogenesis in other cancer types but has not been well studied in the context of PNENs. To address this, the overall objective of this study is to characterize human PNEN glycomes to identify new candidate biomarkers and therapeutic targets for high-risk PNEN patients.

Methods:

We analyzed transcriptome data derived from a retrospective cohort of >80 PNEN specimens for alterations in glycosyltransferases and their known acceptor proteins. Changes in glycosyltransferases/acceptors were evaluated relative to four previously defined PNEN molecular subgroups. To directly identify glycosylation-related alterations, FFPE samples from the retrospective PNEN cohort, metastatic PNEN cases, PNEN cell lines and normal human islets are being processed for analyses on a lectin microarray. Glycomic signatures will be investigated for associations with clinicopathological features and molecular subgroups.

Results:

Statistically significant PNEN sub-group specific changes in glycosyltransferase gene expression were detected, along with alterations in levels of genes encoding acceptor proteins, consistent with potential dysregulation of glycoproteins. Several glycosyltranserases that regulate cell proliferation and metastasis (FUT9, GALNTs, MGAT5B and sialyltransferases) were significantly increased in the Proliferative molecular subgroup which is associated with an inferior overall survival ($p=0.0024$; logRank test). GCNT4 transcripts were found to be significantly decreased in the Proliferative subgroup and correlated with an increase in the expression of cell proliferation-related acceptor proteins. Transcripts corresponding to sialytransferase ST6GAL2 and its acceptor proteins were increased in a distinct molecular subgroup, Stromal-Mesenchymal, which was associated previously with induction of epithelial-to-mesenchymal transition.

Conclusions:

Our analyses indicate candidate glycosylation-related alterations that suggest new disease classifiers and also drivers of disease for further investigation in the context of PNENs.

A Systematic NEN Spheroid Drug Screen Reveals a Novel Drug Resistance Mechanism in Small Bowel NETs

Catherine G. Tran[1], Carlos H.F. Chan[1], Anand Rajan[2], Luis C. Borbon[1], Jonathan Shilyansky[1], Guiying Li[1], Scott K. Sherman[1], James Egan[1], Ellen Abusada[2], Courtney A. Kaemmer[3], Terry A. Braun[4], Jacqueline Mudd[5], Marie Migaud[6], Jing Tang[7], Ramaswamy Govindan[8], Ryan Fields[5], Chandrikha Chandrasekharan[9], Douglas Spitz[10], Joseph S. Dillon[9], Dawn E. Quelle[3], Andrew M. Bellizzi[2], James R. Howe[1], and Po Hien Ear[1]

1. Department of Surgery, University of Iowa Carver College of Medicine, Iowa City, IA. 2. Department of Pathology, University of Iowa Carver College of Medicine, Iowa City, IA. 3. Department of Pharmacology and Neuroscience, University of Iowa Carver College of Medicine, Iowa City, IA. 4. Center for Bioinformatics and Computational Biology, College of Engineering, University of Iowa, Iowa City, IA. 5. Department of Surgery, Washington University School of Medicine, St. Louis, MO. 6. Department of Pharmacology, University of South Alabama, Mobile, AL. 7. Institute for Molecular Medicine Finland (FIMM), University of Helsinki, FI-00290 Helsinki, Finland. 8. Department of Medicine, Washington University School of Medicine, St. Louis, MO. 9. Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, IA. 10. Department of Radiation Oncology, University of Iowa Carver College of Medicine, Iowa City, IA.

Presenting author: Po Hien Ear, PhD

Neuroendocrine neoplasms (NENs) are rare cancers that arise from neuroendocrine cells. NENs are classified as well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs). Small bowel NETs (SBNETs) and pancreatic NETs (PNETs) are generally slow growing but they commonly metastasize to the liver and can become aggressive cancers. NECs are rapidly growing, and patients have poor prognosis. Little is known about the drug sensitivity profile of SBNETs, PNETs and NECs due to a paucity of cellular and animal models of these malignancies.

We have successfully cultured NEN cells from clinical samples as patient-derived spheroids (PDS) and showed that they express appropriate tumor markers. We systematically screened 20 NEN (12 SBNET, 5 PNET, and 3 NEC) spheroid cultures against a library of 175 compounds (147 FDA-approved anti-cancer drugs, 8 lab selected compounds, and 20 structurally diverse molecules) and compared their drug sensitivity profiles to identify the most effective drug classes and to better understand the biology of each NEN subtype. Top drug hits were validated for their anti-tumor properties in NEN PDS and patient-derived xenograft (PDX) mouse models.

Our NEN PDS cultures identified common and unique drug sensitivity profiles for each type of NEN. SBNET spheroids were more resistant to many classes of anti-cancer drugs, which was due to overexpression of cytochrome P450 genes. Consistent with clinical findings, PNET spheroids showed increased sensitivity to tyrosine kinase and mTOR/PI3K inhibitors compared to SBNET & NEC spheroids. NEC spheroids showed the broadest sensitivity to many anti-neoplastic compounds. The top candidate drug identified from our screen was romidepsin, a histone deacetylase inhibitor. Romidepsin displayed anti-tumor properties in vitro and in vivo for all 3 NEN models and was highly synergistic with rapamycin, an mTOR inhibitor similar to the SBNET approved drug everolimus. Excitingly, low-dose romidepsin effectively inhibited tumor growth when combined with low-dose rapamycin in an SBNET PDX mouse model.

These NEN PDS drug screens enabled direct drug testing in primary tumor cultures to identify promising drugs that could be used alone or in combination with currently approved-NEN therapies. Histone deacetylase inhibitors, such as romidepsin, may be effective against SBNETs. NEN PDS models also serve as a valuable resource for understanding the unique biology and mechanisms of drug resistance for specific NEN subtypes.

Using Patient-derived Tumor Organoids to Uncover Mechanisms of Pulmonary Net Progression

Laura Salamó Palau, Andrés Marco Gimenez, Rossana Passannante, and Talya L. Dayton

European Molecular Biology Laboratory (EMBL), Tissue Biology and Disease Modelling Unit, Barcelona, Spain.

Presenting author: Talya L. Dayton, PhD

Background:

Pulmonary neuroendocrine tumors (NETs) show a range of clinical behaviors, from a slowly progressing disease to metastatic and recurrent cancers with poor prognosis. This heterogeneity in clinical outcomes for patients with pulmonary NETs presents a major clinical challenge and means all patients require lengthy follow-up, underscoring the need for biomarkers predictive of aggressive disease and effective therapeutic strategies for the treatment of all pulmonary NETs.

Methods:

We have developed the first described patient-derived tumor organoids (PDTOs) from low-grade pulmonary NE tumors, including a newly defined, clinically aggressive subtype of NE tumor, a supra-carcinoid. Notably, we found that a subset of pulmonary NETs expresses high levels of EGFR and depends on EGF for growth in culture thereby uncovering a targetable growth dependency in a subset of pulmonary NETs. Building on these findings, we hypothesize that a subset of pulmonary NETs progresses to aggressive cancer states through mutations activating pathways downstream of EGFR, particularly MAPK and PI3K.

We propose to use CRISPR/Cas9-mediated gene editing to introduce MAPK- and PI3K-activating mutations in these pulmonary NET PDTOs to investigate progression mechanisms via phenotypic and molecular characterization, including proliferation assays, RNA sequencing, ATAC sequencing, and DNA methylation studies.

Results:

We have established optimized protocols for nucleofection of NET PDTOs to introduce gene editing components into NET cells and generated the necessary molecular tools for generating gene edited NET PDTOs.

Conclusions:

We have developed molecular tools and protocols that enable the generation of NET PDTOs with MAPK-activating or PI3K-activating mutations. The results of these experiments are expected to identify potential vulnerabilities of pulmonary NETs that can be exploited for therapeutic purposes. Our work aims to facilitate the development of strategies to stratify and treat patients with pulmonary NETs and to prevent the transition of their tumors to aggressive disease, ultimately improving patient outcomes.

MEN1/DAXX Alterations are Associated with Improved Overall Survival and Treatment Response in Patients with Pancreatic Neuroendocrine Tumors

Rushabh Gujarathi[1], Sara Abou Azar[2], Joseph Tobias[2], Xavier M. Keutgen[2], and Chih-Yi Liao[1]

1. Section of Hematology and Oncology, Department of Medicine, University of Chicago, Chicago, Illinois, USA.
2. Section of Endocrine Surgery, Department of Surgery, University of Chicago, Chicago, Illinois, USA.

Presenting author: Rushabh Gujarathi, PhD

Background:

Alterations in the MEN1 and DAXX genes are common in pancreatic neuroendocrine tumors (PNETs). Previous data have revealed that MEN1- and DAXX/ATRX-altered tumors show longer overall survival (OS). Pre-clinical data suggests that alterations in the MEN1 and DAXX genes may increase radiation efficacy in tumor cells by influencing DNA damage repair. We explored the associations of these alterations with patient prognosis and response to surgical and systemic therapies used in metastatic PNETs.

Methods:

A retrospective chart review was conducted. Patients with well-differentiated PNETs seen at University of Chicago between 2013 and 2023 with available tumor NGS results were included. Patients with MEN1 syndrome were excluded. Cases with deleterious alterations (truncating mutations, missense mutations considered pathogenic, and/or copy number losses) in the MEN1 and DAXX genes were considered as MEN1/DAXX altered (MEN1/DAXXat). Two patients with a variant of uncertain significance only in MEN1 and DAXX respectively were not considered as "altered". The primary outcome was OS. The secondary outcome was progression free survival (PFS), after peptide receptor radionuclide therapy (PRRT), capecitabine/temozolomide (CAPTEM), and surgical debulking for metastatic disease. Baseline clinicopathological features were compared using Fischer's exact test. Kaplan-Meier estimations and Cox proportional hazards regression analysis were conducted.

Results:

62 patients were included. Median follow-up was 42.13 months (IQR, 28 – 67.6). 28 (45.2%) patients had at least one deleterious alteration reported in the MEN1/DAXXat. At diagnosis, the MEN1/DAXXwt (wild type) and MEN1/DAXXat groups were similar in terms of median age (55.5 years vs. 54.2 years; p = 0.99), presence of metastatic disease (26/34, 76.5%; vs. 22/28, 78.6% p = 0.99), extrahepatic metastases (12/34, 35.3%; vs. 11/28, 39.3% p = 0.8), or bone metastases (7/34, 20.6% vs. 2/28, 7.1%; p = 0.17). At diagnosis, the MEN1/DAXXwt group showed a higher proportion of patients with grade 3 disease (vs. grade 1/2; 12/34, 64.7% vs. 2/28, 7.1%; p = 0.01). OS after diagnosis (19 deaths recorded) was longer in the MEN1/DAXXat group (median NR vs. 53.8 months; HR, 0.39; 95% CI, 0.15 – 1.03; log-rank p = 0.049). In patients with metastatic disease at any point (N = 61), OS was longer after diagnosis of metastases in the MEN1/DAXXat group (median NR vs. 53.5 months; HR, 0.33; 95% CI, 0.12 – 0.89; p = 0.03). Among patients who received PRRT, PFS was longer in the MEN1/DAXXat group (N = 27; 26.5 months vs. 12.9 months; HR, 0.30; 95% CI, 0.12 – 0.77; p = 0.01). PFS did not vary significantly among patients who received CAPTEM (N = 30; 17.8 months vs. 12.4 months; HR, 0.65; 95% CI, 0.28 – 1.53; p = 0.32) or underwent surgical debulking for metastatic disease (N = 38; 14.5 months vs. 11.3 months; HR, 0.77, 95% CI, 0.38 – 1.55; p = 0.46).

Conclusions:

PNETs with MEN1/DAXX alterations may represent a subtype with favorable prognosis. MEN1/DAXXat cases showed favorable response to PRRT. The role of these alterations to inform therapy choice and treatment sequencing in PNETs warrants further exploration.

Towards a Uniform Grading System for Mesenteric Fibrosis in Small Intestinal Neuroendocrine Tumors

Eva van der Slik[1], Richard A. Feelders[1], Andrew R. Hall[2], Anand M. Iyer[1], Martyn E. Caplin[3], Krista Rombouts[2], Maria C. Martins[2], Harry Hodgetts[2], Peter van Koetsveld[1], Johannes Hofland[1], Wouter W. de Herder[1], Leo J. Hofland[1], TuVinh Luong[3], Marie-Louise F. van Velthuysen[4]

1. Department of Internal Medicine, sector Endocrinology, ENETS Centre of Excellence, Erasmus MC Cancer Institute, University Medical Center Rotterdam, The Netherlands. 2. Regenerative Medicine and Fibrosis Group, Institute for Liver and Digestive Health, Royal Free Hospital, University College London, London, UK. 3. Neuroendocrine Tumour Unit, ENETS Centre of Excellence, Royal Free London NHS Foundation Trust, London, UK. 4. Department of Pathology, ENETS Centre of Excellence, Erasmus MC Cancer Institute, University Medical Center Rotterdam, The Netherlands.

Presenting author: Eva van der Slik, MD

Background:

Mesenteric fibrosis (MF) frequently develops in patients with a small intestinal neuroendocrine tumor (SI-NET) and is localized around metastases in the mesentery. With CT imaging MF can be observed as strands radiating from the mesenteric mass. However, a recent study indicated that in approximately 40% MF is not detected using CT imaging. Histopathological examination can therefore be considered as the golden standard for detecting MF. However, to date there is no uniform histopathological scoring system for diagnosing or grading MF, which can explain variations in MF assessment between different institutions. Both for clinical as well as for research purposes it would be beneficial to have a reproducible and precise scoring system for MF. Our aim is to retrospectively validate a new MF scoring method in order to develop a more standardized scoring system.

Methods:

We collected unstained slides derived from FFPE blocks of mesenteric metastases from 52 patients that underwent surgery for SI-NET in the past 20 years. To visualize MF, collagen was stained with picrosirius red. Subsequently, the width of the fibrotic capsule surrounding the tumor and the width of the thickest intratumoral fibrotic band were measured. In addition, the collagen proportional area (CPA in %) of the tumor mass with and without including the surrounding fibrotic capsule was digitally analyzed. To investigate the reproducibility of this scoring system that includes the different measurements, as well as the CPA, the picrosirius red staining and digital analysis of the samples were performed separately at two institutions. Complementary, a radiological scoring will be executed by two independent radiologists, using a CT scan performed <6 months pre-operatively. Interobserver variation for both the histopathological scoring system and the radiological scoring system will be calculated using the Spearman's correlation coefficient. The histopathological scoring data will be compared to the radiological scoring data and linked to clinical patient characteristics.

Results:

Preliminary analysis showed that the Spearman's correlation r for the CPA measurement of the tumor including the capsule was 0.743 [95% CI 0.588, 0.846, $p < 0.001$]. CPA analysis of the tumor tissue without the capsule resulted in a lower correlation value of 0.498 [95% CI 0.261, 0.679, $p < 0.001$]. The measurement of the width of the capsule surrounding the tumor and of the thickest intratumoral fibrosis band showed a higher variability than accepted (Spearman's correlation r of resp. 0.640 and 0.395). Radiological scoring is ongoing.

Conclusions:

In our study on the histopathological assessment of MF in SI-NET we found that CPA measurement of the entire mass including the fibrotic capsule has an acceptable interobserver variability and is more reproducible in comparison to the other measurements. However, the data indicate opportunities for improvement. Especially in the measurements regarding intratumoral tissue significant variation is observed, which can be partially attributed to systematic errors, but also clearly points to the necessity for more precise definitions. The histological scoring will be compared to the radiological scoring data and the clinical characteristics of the patients.

This research into mesenteric fibrosis is funded by NETRF Accelerator Grant 702627 (UCL/Exeter/Erasmus)

The Daxx/Atrx/H3.3 Epigenetic Regulatory Axis in Cell State Regulation and Pancreatic Neuroendocrine Tumor Suppression

Amanda R. Wasylishen[1], Chang Sun[2], Sajad Wani[1], Samantha N. Zumwalde[1], Yuan Qi[3], Patrick M. Krause[1], Gilda P. Chau[2], Xiaoping Su[3], and Guillermina Lozano[2]

1. Department of Cancer Biology, University of Cincinnati College of Medicine, Cincinnati, OH, USA. 2. Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX, USA. 3. Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Presenting author: Amanda R. Wasylishen, PhD

Background:

Homeostasis represents an essential balance between adjusting to changing conditions and maintaining overall stability, with perturbations contributing to diseases including diabetes, pancreatitis, and cancers. Epigenetic mechanisms are central to homeostasis, including histone variants and the chaperone complexes that mediate their deposition. Histone 3.3 (H3.3) is a replacement variant for canonical histone H3 and is deposited in heterochromatin by a complex containing DAXX and ATRX. The importance of this epigenetic regulatory axis is emphasized by the early embryonic lethality of mice when any component is deleted, along with recurrent somatic mutations in human cancers. This includes mutually exclusive loss-of-function mutations in DAXX or ATRX in 43% of pancreatic neuroendocrine tumors (PanNETs). The understanding of the physiologic functions of this regulatory complex and its component parts remains in its infancy.

Methods:

We have generated and characterized new germline Daxx mouse models to effectively dissect this epigenetic regulatory complex in vivo, including mutants the impair the interactions between Daxx:Atrx (Daxx-Y130A) and Daxx:H3.3 (Daxx-S226A), and a third mutant that causes Daxx mislocalization (Daxx-dSIM).

Results:

Remarkably, these mice do not phenocopy each other and clearly demonstrate that Daxx has both Atrx-dependent and independent functions in vivo. Daxx-Y130A mice are viable and fertile while Daxx-S226A and Daxx-dSIM mice are early postnatal lethal. Comprehensive transcriptome analysis demonstrates i) consistent de-repression of endogenous retroviruses (ERVs) with associated transcriptional changes in nearby single-copy genes across the mutant panel and ii) profound and specific changes in immune cell populations in Daxx-S226A and Daxx-dSIM mice.

Conclusions:

Collectively these data implicate ERV dysregulation in the pathogenesis of PanNETs and provide new insights into the physiologically relevant functions of this epigenetic regulatory complex. Our current studies are focused on further dissecting how dysregulation downstream of perturbations in this axis contribute to tumorigenesis and using this knowledge to identify opportunities for therapeutic intervention.

Identifying Compounds with Preferential Toxicity Towards Sdhb-Deficient Cells Via Synthetic Lethality Chemical Screening

Qianjin Guo[1], Sooyeon Lee[1], Neali Armstrong[1], Brian Lim[1], Rebecca C. Schugar[1], Haixia Xu[1], Leor Needleman[1], Electron Kebebew[2], Justin P. Annes[1,3,4]

1. Department of Medicine, Division of Endocrinology, Stanford University, Stanford, CA, USA. 2. Department of Surgery and Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA. 3. Endocrine Oncology Program, Stanford University, Stanford, CA, USA. 4. Chemistry, Engineering, and Medicine for Human Health (ChEM-H) Institute, Stanford University, Stanford, CA, USA.

Presenting author: Qianjin Guo, PhD

Background:

Pheochromocytomas and Paragangliomas (PPGLs) are rare neuroendocrine tumors arising from the adrenal medulla and extra-adrenal paraganglia, respectively. About 40% of PPGLs are hereditary, and nearly half of these cases are caused by germline mutations in the succinate dehydrogenase (SDH) subunits. PPGLs can secrete harmful levels of catecholamines, cause mass effects, and harbor malignant potential. Although most PPGLs are curable by surgery, up to 20% of PPGLs are metastatic. Unfortunately, treatments for metastatic PPGL remain palliative. Succinate dehydrogenase subunit B (SDHB) deficiency confers a greatly increased risk for metastasis. As a result, SDHB deficiency accounts for almost half of the metastatic PPGLs. Hence, discovering novel therapeutic avenues that improve the prognosis for metastatic SDHB-PPGL patients, is an urgent and unmet need.

Methods:

To explore novel treatment for SDHB-PPGLs, we deployed quantitative high-throughput chemical screening (qHTS) with the NIH Clinical Compound Collection, which contains more than 700 compounds used in clinical trials. Given the limited availability of human PPGL cell line models, we utilized SDHB-deficient UOK269 and SDHB reconstituted UOK269 (UOK269WT) cells for proof-of-concept screening. Briefly, UOK269 and UOK269WT cells were stably transduced to express H2B-GFP and H2B-mCherry, respectively. 96-Well plates were seeded with a 1:1 mixture of H2B-GFP-UOK269 and 5,000 H2B-mCherry-UOK269WT cells and screened at four treatment concentrations (10 nM, 100 nM, 1,000 nM, and 10,000 nM) in duplicate. After 72 hours, relative cell viability was determined using an Operetta CLS High-Content Analysis System. To overcome the human PPGL cell line limitation and validate the hit compounds, we are developing (validating) a newly generated human PPGL cell line.

Results:

Chemical screening identified several drugs with preferential cytotoxicity towards UOK269 cells. To confirm selectivity, we retested hit drugs on UOK269WT cells with or without 3-NPA treatment, a mitochondrial complex II inhibitor used to mimic SDHB-deficiency. We found a single compound, drug Y, that exhibited cooperativity with 3-NPA treatment; suggesting that the cytotoxicity of drug Y is modulated by mitochondrial complex II inhibition and SDHB deficiency. Additionally, recognizing the limitations of tumor cell-line screening, we evaluated the cytotoxic activity of drug Y validation using a unique Sdhb-deficient mouse primary cell model. To verify the drug Y effect in the PPGL context, we are developing a new human PPGL cell line model. Currently, we have established a primary pheochromocytoma culture which retains chromaffin cell markers including synaptophysin, tyrosine hydroxylase (TH), and phenylethanolamine N-methyltransferase (PNMT), suggesting their chromaffin cell identity. These cells propagate in 2D culture as a mixture of monolayer and spheroid-like formations.

Conclusions:

Using high-throughput screening, we identified a compound with preferential cytotoxicity towards SDHB-deficient cells. This activity was validated using Sdhb-deficient mouse primary cells, indicating potential applicability in animal model systems and beyond. We also developed a human PPGL cell line which, once fully validated. Will be leveraged for hit compound verification and future screening efforts.

TILs from panNET Liver Metastasis: In Search of Novel Adoptive Transfer Strategies for the Treatment of Nets

Nada Chaoul[1], Gabriella D'Angelo[1], Andrea Giglio[1], Eleonora Pelle'[2], Alessandro Silvestris[3], Eleonora Lauricella[1], Barbara Mandriani[1], Raffaele Palmirotta[1], Maria Laura Matrella[4], Gaetano Villani[4], Ernesto Picardi[3], Graziano Pesole[3], Giuseppe Ingravallo[4], Jaydira Del Rivero[5], Jerome Cros[6], Anguraj Sadanandam[7], Ilaria Marinoni[8], Camillo Porta[1], Jonathan Strosberg[2], Mauro Cives[1]

1. Interdisciplinary Department of Medicine, University of Bari "Aldo Moro", Bari, Italy. 2. Department of GI Oncology, Moffitt Cancer Center & Research Institute, Tampa, FL, USA. 3. Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari "Aldo Moro", Bari, Italy. 4. Department of Basic Medical Sciences, Neurosciences and Sense Organs, "Aldo Moro" University of Bari, 70124, Bari, Italy. 5. Developmental Therapeutics Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 94158, USA. 6. Université Paris Cité, Department of Pathology, Beaujon/Bichat University Hospital (APHP), Clichy/Paris, France. 7. Division of Molecular Pathology, The Institute of Cancer Research, London, UK; Centre for Translational Immunotherapy, Division of Radiotherapy and Imaging, The Institute of Cancer Research, London, UK; Centre for Global Oncology, Division of Molecular Pathology, The Institute of Cancer Research. 8. Institute of Pathology, University of Bern, Bern, Switzerland.

Presenting author: Mauro Cives, MD

Background:

The anti-tumor activity of tumor infiltrating lymphocytes (TILs) in NETs is currently unknown.

Methods:

We collected matched blood, FFPE and cryopreserved or fresh samples of liver metastases from 27 patients with well-differentiated panNETs (7 G1;17 G2; 3 G3). FFPE samples were subjected to WES and RNAseq to predict number and quality of tumor neoantigens. IHC was used to assess HLA-I and HLA-II expression as well as to digitally quantify CD3+ cell infiltration. Expression of HLA molecules is being validated in an independent cohort by TMA. Multi-region analysis of individual tumor samples was carried out to evaluate the spatial heterogeneity of TIL distribution. In vitro-mapped TIL outgrowth was compared with the intratumor regional characteristics, and spatial biology experiments are ongoing to shed light on possible regional differences in the transcriptomic profile of T cells. TILs were expanded for up to 105 days and weekly enumerated and phenotyped by flow cytometry. TCR sequencing was performed to assess over time TCR skewing. The Seahorse technology was used to evaluate TILs' metabolism. TILs deriving from different tumor regions were co-cultured with autologous tumoroids to assess their antitumor reactivity. Secretion of IFN-g, Granzyme B and TNF-a was measured by ELISA. Confocal microscopy was used to determine tumoroid infiltration by TILs.

Results:

PanNET liver metastases exhibited a relatively low mutational burden, with a median of 12 pathogenetic variants per sample. MEN1 and DAXX were the most frequently mutated genes (38% and 25% of samples, respectively). Neoantigen prediction revealed a median of 3 HLA-I strong binders (the analysis for HLA-II is ongoing). HLA-I was expressed in 26/27 samples, whereas HLA-II was expressed in 0/27 samples. TMA validation is underway. TILs were successfully grown from 17/27 patients and preREP-sufficient numbers were reached in 59% of samples (71% and 40% from fresh and cryopreserved tumors respectively). TILs' outgrowth was independent of clinical parameters, whereas TILs yield was significantly correlated with T cell density by IHC ($p<0.05$) and TLS presence ($p<0.01$). Wide differences were observed in T cell yield according to the different tumor regions analyzed. T cells were the predominant population to grow in the TIL cultures at the time of cryopreservation with CD4+ T cells /CD8+ T cells ratio of 5:1. Tregs accumulated within the tumors, and their depletion boosted TIL proliferation. We observed a switch in CD8+ T cell differentiation (from TE to TEM) after 2 weeks of culture. Such a switch was accompanied by a metabolic reprogramming, with reduced efficiency of OXPHOS overtime. CD39 and TIGIT were the most expressed exhaustion markers by CD4+ and CD8+ TILs. When cocultured with autologous tumorods, TILs deriving from different tumor regions exhibited heterogeneous antitumor activity, spanning from no tumor recognition to massive production of pro-inflammatory cytokines and up-regulation of activation markers such as CD69/CD39 as well as exhaustion markers. TILs showing anti-tumor reactivity were able to infiltrate co-cultured tumoroids and displayed a significantly higher respiratory capacity and glycolytic capacity. TILs not showing anti-tumor reactivity showed a higher presence of CD8+ Tregs.

Conclusions:

PreREP sufficient TIL numbers were reached in approximately 60% of cases. TILs comprise both anti-tumor reactive clones and bystander lymphocytes. Isolation and expansion of tumor-reactive TILs may enhance the efficacy of TILs adoptive transfer.

Radiomics Model to Predict Symptoms & Complications from Small Intestinal NET Mesenteric Metastases

Conrad Von Stempel[1], Anela Blazevic[2], Martijn Starmans[2], Wouter W. de Herder[2], Richard A. Feelders[2], Martyn Caplin[2], Christos Toumpanakis[2]

1. Neuroendocrine Tumour Unit, ENETS Centre of Excellence, Royal Free London NHS Foundation Trust, London, UK. 2. Erasmus University Medical Center (Erasmus MC), Rotterdam, Netherlands.

Presenting author: Conrad Von Stempel, MD

Background:

The development of mesenteric metastases and associated mesenteric fibrosis (MF) in small intestinal neuroendocrine tumours (SI-NET) can cause significant complications and have implications in patients' survival. Conventional computed tomography (CT) seems suboptimal for accurate assessment of the degree of fibrosis and prediction of complications. A better model is needed, therefore, in order to select patients who may benefit from a prophylactic surgical approach in the mesentery. Blazevic et al from "Erasmus NET Unit" demonstrated (Endoc Relat Cancer 2021) the promising role of a "radiomics model," as a predictive "tool" for development of complications of mesenteric metastases and fibrosis in 68 of their patients. The aim of this study is to validate that "radiomics model" through a different patients' cohort from Royal Free, NET Unit

Methods:

Twenty patients with SI-NET were included in this preliminary study. Ten of them were asymptomatic and did not proceed to surgery (Group A), whilst the remaining had symptoms of intestinal ischaemia and/or obstruction (Group B). Demographic parameters (age, gender), clinical parameters (tumour grade and tumour stage at diagnosis), biomarkers' (CgA and 5-HIAA) levels, CT findings for all patients, as well as surgical indication (obstruction, pain) were collected. The mesenteric cuff around the metastatic node has been segmented (including desmoplasia), using a semi-automated segmentation program ITK-SNAP.

Results:

Patients who were symptomatic and underwent surgery were younger, less-syndromic and with lower mesenteric tumour burden. Mean performance parameters (95% confidence intervals) : "AUC": 0.45, "Accuracy": 0.45, "BCA": 0.44, "F1-score": 0.43, "NPV": 0.42, "Precision": "0.46, "Sensitivity": "0.6, "Specificity": 0.3.

Conclusion:

The "radiomics model" was not validated, at his point, through this different patients' cohort. Reasons for this include differences in segmentation, patients' selection and acquisition protocol. A combined dataset and another external validation dataset are needed to obtain robust conclusions. Currently CT scans of the Rotterdam cohort are analysed using semi-automated segmentation. The results of this study will determine the segmentation method for the validation study.

Application of an OTP/ASCL1/HNF1a Immunohistochemistry Panel in Metastatic Pulmonary Carcinoid

F. Heijboer*[1], T. van Weert*[2], W. Buikhuisen[3], T. Walter[4], P. Robelin[4], J. von der Thusen[5], L. Hillen[2], L. Moonen[2], J Blandine[6], M. Brandao[7], T. Vandamme[8,9], S. Chhajlani[8,9], V. Hervie[10], A. Koumarianou[11], L. Kodach[12], B. Hiddinga[13], EJ Speel[2], AM. Dingemans[1], JL Derks[1, 14]

*Shared first author. 1. Department of Respiratory Medicine, Erasmus MC Cancer Institute, University Medical Center, Rotterdam, The Netherlands. 2. Department of Pathology, GROW School for Oncology & Reproduction, Maastricht University Medical Center, Maastricht, The Netherlands. 3. Department of Thorax Oncology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. 4. Hospices Civils de Lyon, Hôpital Edouard Herriot, Department d'Oncologie Médicale, Lyon, France. 5. Department of Pathology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands. 6. Department of Pulmonology, Hôpital Universitaire de Bruxelles (HUB), Brussels, Belgium. 7. Clinic of Thoracic Oncology, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium. 8. Center for Oncological Research (CORE), Integrated Personalized and Precision Oncology Network (IPPON), University of Antwerp, Antwerp, Belgium. 9. Oncology, NETwerk Antwerpen-Waasland CoE, Edegem, Belgium. 10 Hospices Civils de Lyon, Hôpital Edouard Herriot, Department pathology, Lyon, France. 11. Department medical oncology, Attikon University Hospital, Athens, Greece. 12. Department of Pathology, The Netherlands Cancer Institute, Amsterdam, the Netherlands. 12. Department of Pulmonology, University Medical Center Groningen, Groningen, the Netherlands. 13. GROW School for Oncology & Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands.

Presenting author: J.L. Derks, MD

Background:

Multi-omic studies have identified three lung carcinoid subtypes (A1, A2, B) with unique expression of the OTP, ASCL1, and HNF1a genes. We developed an immunohistochemical (IHC) panel for lung carcinoid subtype identification and clinical-pathological correlation. In this study, we investigated the relationship between the sub types of metastatic lung carcinoid (MLC) with clinical characteristics and therapy outcome.

Methods:

A European multicenter retrospective cohort was established of patients with MLC who received treatment with a somatostatin analog (SSA), everolimus, chemotherapy (temozolomide & capecitabine or oxaliplatin based), or peptide receptor radionucleotide therapy (PRRT) in any subsequent line with a maximum of three. Metastatic or primary tumors of MLC were revised for pathological diagnosis and evaluated for expression of the IHC markers OTP, ASCL1, HNF1a, and Ki-67 to determine the subtype.

Results:

Clinical data collection is ongoing; for n = 125 patients, data collection has been completed. In an additional 85, the data are still being collected. Tumor formalin fixed paraffin embedded tissues are collected from 94 MLC tumors, and staining was performed in 35. A national inquiry has been established to collect tumor samples in cases in which tumor tissue is currently lacking. An additional 30 international cases are committed to the project and are awaiting clinical data/tissue sample retrieval. Provisional evaluation of treatment lines (multiple per patient possible) includes >100 SSA, >60 everolimus, >40 capecitabine-temozolomide, >30 PRRT, and >30 other chemotherapy regimens.

Conclusion:

The project has been hampered by a slower than expected retrieval of clinical data and tumor samples, mostly because of initial legal hurdles. Data and clinical sample retrieval is ongoing and shows that the project can provisionally meet its designated target for most treatments except PRRT. In the coming months, we aim to finalize the clinical data and proceed with the IHC evaluation subtyping and pathology revision of all identified cases.

The Crosstalk Between Cancer-Associated Fibroblasts and Small Intestinal Neuroendocrine Tumor Cells in a 3D Spheroid Model

Eva van der Slik[1], Richard A. Feelders[1], Peter van Koetsveld[1], Anand M. Iyer[1], Johannes Hofland[1], Rosanna van den Dungen[1], Martyn E. Caplin[3], Krista Rombouts[2], Christina Thirlwell[4], Maria C. Martins[2], Harry Hodgetts[2], Marie-Louise F. van Velthuysen[1], Wouter W. de Herder[1], Leo J. Hofland[1]

1. Department of Internal Medicine, sector Endocrinology, ENETS Centre of Excellence, Erasmus MC Cancer Institute, Rotterdam, The Netherlands. 2. Regenerative Medicine and Fibrosis Group, Institute for Liver and Digestive Health, Royal Free Hospital, University College London, London, UK. 3. Neuroendocrine Tumour Unit, ENETS Centre of Excellence, Royal Free London NHS Foundation Trust, London, UK. 4. University of Exeter - College of Medicine and Health, Exeter, United Kingdom.

Presenting author: Eva van der Slik, MD

Background:

At diagnosis more than 50% of small intestinal neuroendocrine tumors (SI-NETs) have metastasized to the mesentery where they often induce an extensive fibrotic reaction. The pathogenesis of this mesenteric fibrosis (MF) is believed to involve overproduction of serotonin and stimulatory effects of certain growth factors. Recent studies also show the importance of the tumor microenvironment, especially the interaction of SI-NET cells with cancer-associated fibroblasts (CAFs), in the pathogenesis of MF. The aim of this study is to generate an appropriate preclinical model, representing the tumor microenvironment, to examine this interaction between CAFs and SI-NET cells.

Methods:

Fibroblasts from SI-NET tissue originating from the primary tumor, the mesenteric metastasis or adjacent mesentery were isolated and cultured. The isolated CAFs were characterized using inclusion (vimentin, αSMA) and exclusion markers (CD31, E-cadherin). RNA isolated from the different CAF cultures was analyzed for expression of a panel of genes associated with different CAF subsets. Mono- and co-cultured spheroids were generated with isolated CAFs and GOT1 cells, a cell line originating from a SI-NET metastasis. Furthermore, spheroids were cultured without or with 1% added matrigel. The spheroids were immunohistochemically characterized for proliferation (Ki-67), activation (αSMA, COL1A1) and identification (vimentin). Proliferation of 3D-cultured spheroids was further quantified using flow cytometry. Complementary, time-dependent CAF and SI-NET cell proliferation was studied using real time visualization of GFP-expressing GOT1 cells in an IncuCyte® imaging system.

Results:

Identification of CAF and NET cells in the co-cultured spheroids with vimentin and synaptophysin showed that patient-derived CAFs and GOT1 cells mainly formed clusters instead of randomly mixing. When matrigel was added, this altered into a cellular distribution pattern more resembling SI-NETs in situ. Immunohistochemical staining showed an increased expression of the fibroblast activation marker αSMA (3 out of 4) and of the matrix synthesis marker COL1A1 (2 out of 4) in the co-cultured spheroids compared to the separate spheroids, especially located at sites where GOT1 cells and CAFs interact. In spheroid co-cultures, an inhibitory effect of SI-NET cells on CAF proliferation was observed (>20% decrease in 3 out of 3 cultures). Contrary, a strong stimulatory effect of CAFs on SI-NET cell proliferation was found (>25% increase in 2 out of 3). Concerning CAF characterization preliminary qPCR sequencing data showed high expression of genes associated with CAFs in the isolated cultures. Interestingly, clear differences were observed between different CAF cultures. These expression signatures will be linked to clinical data of the patients.

Conclusions:

Our data show potent interactions between SI-NET cells and CAFs in a novel preclinical spheroid model. Within the co-cultured spheroids, the increase in activation markers indicates that SI-NET cells are able to activate CAFs, while CAF proliferation was inhibited. Conversely, a strong stimulatory effect of co-culturing with CAFs on the proliferation of SI-NET cells was observed. Next steps include analysis of factors secreted by GOT1 and CAFs using proteomic analysis and investigating the effects of anti-fibrotic drugs in this spheroid model.

This research into mesenteric fibrosis is funded by NETRF Accelerator Grant 702627 (UCL/Exeter/Erasmus)

Immunosuppressive Myeloid Signaling in Pancreatic Neuroendocrine Tumors Revealed by Single-nucleus RNA-seq

Jeanna M. Qiu[1, 2, 3, 4], Jae-Won Cho[3], Carina Shiau[1, 2], Steven Wang[1,2], Jennifer Su[1, 2], Jimmy A. Guo[1, 2], Martin Hemberg[2,3], William L. Hwang[1,2]

1. Center for Systems Biology, Department of Radiation Oncology and Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, MA. 2. Broad Institute of MIT and Harvard, Cambridge, MA. 3. The Gene Lay Institute of Immunology and Inflammation, Brigham and Women's Hospital and Harvard Medical School, Boston, MA. 4. Harvard Medical School, Harvard-MIT MD-PhD Program, Boston, MA.

Presenting author: Jeanna M. Qiu, MD/PhD candidate

Background:

There is an urgent need to understand the tumor immune microenvironment (TIME) of pancreatic neuroendocrine tumors (PNETs). Although response to immunotherapies among PNETs in recent clinical trials has been unsatisfactory, previous studies have highlighted the importance of myeloid cells in clinical outcomes. In particular, tumor-associated macrophage infiltration is associated with worse prognosis and myeloid cells in gastroenteropancreatic neuroendocrine tumors express immunosuppressive ligands. However, how this immunosuppressive phenotype in myeloid cells is maintained has yet to be fully elucidated. Therefore, understanding signaling in the TIME is important to identify potential modulators of immune response that can be targeted to sensitize PNETs to immunotherapy.

Methods:

We applied single-nucleus RNA-seq (snRNA-seq) to 20 surgically-resected, untreated, primary PNETs of different grades and stages from our biobank. Following quality control, doublet identification and removal, we separated cells into malignant and non-malignant subsets and annotated different cell types. We focused our downstream analyses on the 14 nonfunctional PNETs that passed quality control. We used consensus non-negative matrix factorization (cNMF) on the malignant compartment to identify cell states shared across multiple patients. To understand how malignant cell state impacts interactions in the tumor microenvironment, we inferred cell-cell interactions between different cell types and cell states from the snRNA-seq data with CellChat. Finally, we inferred metabolic activity within single cells from the snRNA-seq data using Compass.

Results:

We recovered 9,472 non-malignant and 82,267 malignant single-nucleus profiles. Using cNMF, we identified a spectrum of cell states in malignant cells from nonfunctional PNET specimens, including two novel ones: Program 1, composed of genes related to synaptic signaling and axon guidance (NRXN3, NTNG1, GRIA1) and Program 2, composed of genes related to the cytoskeletal remodeling (MYO1E, EZR, VCL) and VEGFA signaling (NR4A1/3, ITGAV, EGR3). Notably, cell-cell interaction analysis predicted that macrophages engage in glutamate signaling with malignant cells via ionotropic and metabotropic glutamate receptors, with the highest expression of glutamate receptors in Program 2-malignant cells. Signaling through ionotropic glutamate receptors activates MAPK/ERK signaling in other malignancies under hypoxic or VHL-mutated conditions, and we similarly found that the expression of MAPK/ERK pathway and HIF-inducible genes was highest in Program 2-malignant cells. Next, we found that the expression of glutaminase was higher in macrophages from samples with a high proportion of Program 19. As glutamate in macrophages can be converted to alpha-ketoglutarate and used in the citric acid (TCA) cycle to drive M2-macrophage polarization, we explored this in our data and found that macrophages from Program 19-high samples indeed exhibited higher levels of M2 marker expression, glutamate metabolism, and TCA cycle activity.

Conclusions:

Given the poor response of PNETs to immunotherapies in clinical trials, there is an urgent need to understand signaling in the TIME to identify potential therapeutic targets that may potentiate the response to immunotherapy. Our results identify previously unknown crosstalk between macrophages and malignant cells, suggesting that glutamate metabolism and signaling both maintains an immunosuppressive environment and promotes proliferation of malignant cells. Validation studies using an independent cohort are ongoing.

Simplifying NEN Tissue and Liquid Diagnostics Using Novel and Existing General Neuroendocrine Cell Markers

Neil M. Renwick[1], Harrison R. Mayotte[1], Simona G. Damiani[1], Tashifa Imtiaz[1], Xiaojing Yang[1], Geneviève C. Digby[2], Nicole C. Panarelli[3], Kathrin Tyryshkin[1,4]

1. Department of Pathology and Molecular Medicine, Queen's University, Kingston, ON, Canada. 2. Department of Medicine, Queen's University, Kingston, ON, Canada. 3. Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, New York City, NY, USA. 4. School of Computing, Queen's University, Kingston, ON, Canada.

Presenting author: Neil Renwick, MD, PhD

Background and Significance:

Neuroendocrine neoplasms (NENs) are clinically diverse tumors and cancers that are challenging to diagnose. Due to this delay, many NENs are metastatic at diagnosis and treatment effectiveness is reduced. microRNAs (miRNAs) are small regulatory RNA molecules that are also excellent biomarkers due to their abundance, specificity, and stability in tissues and biofluids. Because miRNAs can be used to classify cancer, we examined their classificatory utility in 14 NEN pathological types and site-matched control tissues (Nanayakkara et al., NAR Cancer 2020). Based on this study, we hypothesized that miRs-375 and -7 are general neuroendocrine markers like Chromogranin A (CGA), Synaptophysin (SYP), Insulinoma-Associated Protein 1 (INSM1), and Yes-associated protein (YAP1). Identifying novel general neuroendocrine markers could simplify NEN tissue and liquid diagnostics.

Methods:

To assess the diagnostic utility of miRs-375 and -7 in tissue and plasma, we (i) constructed a tissue microarray (TMA), comprising 122 non-diseased, 62 NEN, and 33 non-NEN cancer control tissues from 25 different anatomic sites, and examined the distribution of miRs-375, -7, CGA, SYP, INSM1, and YAP1 using chromogenic in situ hybridization and immunohistochemical staining, and (ii) assessed the abundance of miRs-375 and -7 in platelet-depleted plasma from 84 individuals with lung NENs, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (SCC), and non-neoplastic lung disease (n=21 per diagnostic group) using miRNA real-time PCR. Differences in normalized miRs-375 and miR-7 levels between diagnostic groups were determined using the Kruskal-Wallis test followed by post-hoc Dunn testing; results were expressed as mean ± standard deviation; p-value <0.05 was considered significant.

Results:

In our TMA study, we found miR-375 and miR-7 positive staining cells in 51 (42%) and 27 (23%) of 121 non-diseased tissues, and 48 (77%) and 38 (61%) of 62 NEN tissues, respectively; staining was absent in all 33 non-NEN cancer control tissues. We are currently determining the number and distribution of CGA, SYP, INSM1, and YAP1 staining cells and their overlap with miR-375 and -7 stained cells. In our plasma study, we found that plasma miR-375 levels were significantly higher in lung NEN patients (-7.8±2.7) compared to patients with lung SCC (-11.9±1.4, p=3.8 x 10-6), LAD (-12.0±1.7, p=3.6x 10-6), or NNLD (-12.0±1.5, p=4.6 x 10-6). Plasma miR-7 levels trended higher in lung NEN patients (-14.3±0.7) compared to patients with LUAD (-15.0±0.9, p=0.041), LUSC (-14.6±0.8, p=0.67), or NNLD (-15.0±0.9, p=0.034).

Conclusions:

Our findings indicate that miRs-375 and -7 are general neuroendocrine markers that can be used to detect neuroendocrine cells and neoplasms in tissue and to accelerate lung NEN diagnosis using plasma. We are currently evaluating these miRNA markers in a wide range of other NEN and non-NEN tissues and plasma samples, including from persons with gastrointestinal and pancreatic NENs. We are aiming to establish rapid, simple, and inexpensive NEN diagnostic tests that enable accurate diagnosis and accelerate time-to-treatment.

Engaging the Endocannabinoid System in Neuroendocrine Neoplasms Potentiates Treatment Outcomes and Null Drug Resistance

Shani Avniel-Polak[1], David Polak[2], Simona Glasberg[2]

1. Neuroendocrine Tumor unit, Department of Endocrinology, Hadassah Hebrew University Medical Center, Jerusalem, Israel. 2. Department of Periodontology, Hadassah Hebrew University Medical Center, Jerusalem, Israel. 3. Neuroendocrine Tumor unit, Department of Endocrinology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

Presenting author: Shani Avniel-Polak, PhD

Background:

Neuroendocrine neoplasms (NENs) diagnosis and treatment are complex because of their heterogeneous biological behavior. Surgical repair is limited to a small group of patients, while unresectable NENs are treated with a variety of non-curable agents with limited efficacy and development of resistance over time. Recent data suggest anticancer abilities by the endocannabinoid system (ECS), impairing cancer development as seen in glioblastoma multiforme, breast-, prostate- and pancreatic cancers however the role of ECS in NENs is unclear. The current study aims to elucidate the ECS role in NENs and its engagement with state-of-the-art treatment to augment treatment efficacy and overcome resistance.

Methods:

ECS expression on NEN cells and biopsies were profiled using FACS/immunofluorescence staining and RNA-Seq. The impact of ESC blocking on cell viability was examined by XTT and apoptosis was examined using Annexin/PI staining. Cell cycle analysis was determined by PI or CFSE labeling using flow cytometry. In vivo, the anti-tumor effect was tested by combining Everolimus with ECS antagonists in NENs xenograft mice model. Gene expression and pathway analysis were evaluated by RNA Seq. Metabolomics was used to characterize cells' metabolite profile.

Results:

The expression of ECS on NENs cells and primary tumors exhibits a unique expression that differentiates cancerous from healthy cells. Furthermore, NENs cell viability was gravely impaired when ECS was blocked with a robust effect in combination with Everolimus which also prevented drug resistance. Interestingly, in the murine NENs xenograft model, the combination therapy synergistically reduced tumor size. Gene expression shows that the combination therapy affects genes associated with changes in cells metabolic state. Indeed, mitochondrial respiration measurement exhibits an impair functional metabolic state of NEN cells.

Conclusions:

Increasing reports indicate that manipulation of the ECS can impair cancer development and possibly have antitumorigenic traits. However, no data on an ECS-specific role in NENs exist, neither on NENs development nor as a new anti-tumor option for NENs patients. Our results show a promising approach to NEN therapy based on the engagement of ECS with a state-of-the-art NEN therapy that leads to robust efficacy and reduced resistance.

Cellular and Molecular Diversity of Human Pulmonary Neuroendocrine Cells

Christin S. Kuo[1], Parth Arora[1], Daniel Chambliss[1], Bailey Wallen[2]

1. Department of Pediatrics, Stanford University School of Medicine, Stanford, CA. 2. Department of Surgery, Stanford University School of Medicine, Stanford, CA.

Presenting author: Christin Kuo, MD

Background:

Neuroendocrine cells are one of the most rigorously studied of the rare cell types in the lung using mouse models. Single cell lineage studies and molecular profiling have led to a rich understanding of their distribution, development, diversity, and a growing understanding of their physiologic functions in airway regeneration following injury and as specialized sensory cells that modulate respiratory behavior. In contrast, little is known about the functions, normal locations and diversity of human pulmonary neuroendocrine cells (PNECs). Diseases associated with abnormal PNECs in human lung are remarkably diverse and the clinical presentation and histology of neuroendocrine lesions in proximal airways differ from those in the distal regions. Classical understanding of normal and pathologic human PNECs were largely derived from non-systematic sampling of human lung tissue without precise anatomic localization. Thus, we sought to systematically determine the underlying normal diversity of human PNECs along the bronchial to alveolar branches.

Methods:

We systematically analyzed entire bilateral lungs from human donors without any known chronic lung, but whose lungs were not successfully matched for transplant. We prepared precision cut lung slices from representative airway regions. By flexible bronchoscopic navigation, we identified distinct distal airway regions and inflated lung tissues. The cellular complexity within the most distal airways and alveoli in human lung are challenging to study in situ, but our methods of tissue preparation and visualization achieve cellular and sub-cellular resolution. Using a combination of high-resolution imaging and 3D reconstruction we visualized and quantified the cellular and molecular features of over 8,000 human NECs from the most proximal bronchi to the most distal bronchioles and alveolar regions.

Results:

Based on our study of the anatomic distribution of PNECs, we classified the entire intrapulmonary airways into 5 distinct regions mapped onto a classic representation of human airway branching generation. Distinguishing anatomic and histologic features of each region will be discussed in detail. We identified remarkable cellular diversity along the bronchial and bronchiolar airways as well as differences in the abundance of solitary PNECs compared to clustered PNECs by anatomic region. In proximal bronchi, solitary PNECs had multiple cellular extensions and many cells had additional cellular extensions that terminated on non-PNECs, resembling classic cell structures of cells that communicate by contact-mediated paracrine signaling. We present an extensive collection of PNECs in proximal airways and describe their cellular and molecular differences from those within the distal regions and a comparison to two patients with carcinoid tumors. To molecularly identify PNECs in each region, we systematically quantified expression of both classic or human-specific PNEC neuropeptides across each lung region.

Conclusion:

We found anatomic, cellular, and molecular characteristics of PNECs that were found in the human lung, but not in mouse. On-going and future research goals include comparing the molecular profiles of pulmonary carcinoids which arise in distinct regions. This work establishes an anatomic, cellular, and molecular framework for studying the diversity of human pulmonary neuroendocrine cells with implications for understanding the cell origin for the entire spectrum of lung neuroendocrine disorders.

Identification of Pathogenic Structural Variants Using Machine Learning in Neuroendocrine Tumors

Amy MiHyun Jang[1], Daniel Ackerman[1,2,] Elise R. Seyferth[1,2,3,] Wuyan Li1[,2], Daniel DePietro[1,2,3], Abashai Woodard[1,2,3], Michael Soulen[1,2,3], Terence P. F. Gade[1,2,3]

1. Penn Image-Guided Interventions Laboratory, University of Pennsylvania, Philadelphia, PA, USA.
2. Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA. 3. Division of Interventional Radiology, University of Pennsylvania, Philadelphia, PA, USA.

Presenting author: Amy MiHyun Jang, BS, current MD student

Background:

Our research seeks to advance our understanding of the molecular changes underlying neuroendocrine neoplasms (NENs) through the validation of structural variants (SVs) identified using optical genome mapping (OGM). Many SVs function as oncogenic drivers, but short-read sequencing is limited in SV detection. We leverage OGM, which utilizes ultra-high molecular weight (UHMW) DNA for enhanced sensitivity in detecting SVs. Our dataset of neuroendocrine tumor (NET) samples is unique in our use of OGM and our protocol for extracting tumor growth measures and transarterial chemoembolization (TACE) response from CT/MRI imaging studies within the same cohort. We posit that the application of machine-learning models specifically trained to determine pathogenicity of SVs on our NET dataset will unveil novel pathogenic biomarkers with potential for better subtyping of TACE treatment response.

Methods:

We conducted OGM and next-generation sequencing (NGS) on a comprehensive cohort consisting of 30 metastatic NET samples alongside matched control samples of normal white blood cells when available. Following Bionano's solid tumor DNA extraction guidelines, we used the Bionano Saphyr platform for UHMW DNA analysis. Our dataset was integrated with StrVCTVRE, a random forest classifier, and PhenoSV, a transformer-based deep learning model, to define pathogenicity scores for identified SVs.

Results:

Our analysis of our cohort has identified SVs overlapping with known tumor suppressor regions such as ARID1A, PTPRD, PTEN, and NF1. While these and other tumorigenic genes showed high pathogenicity scores, notably, ARID1A, PTEN, and NF1 displayed significantly elevated scores in both StrvCTVRE and PhenoSV analyses. This finding is reassuring, as these genes are known to be affected by larger structural mutations: ARID1A, a component of the SWI/SNF complex, is frequently altered by SVs; PTEN is commonly associated with deletions; and NF1 exhibits similar patterns. Employing both a random forest classifier and a deep learning model enables us to uncover linear and nonlinear relationships within our dataset. By isolating SVs with high pathogenicity scores from both models and ensuring their absence in matched normal control samples, we can define a subset with higher pathogenicity potential.

Conclusions:

We have identified thousands of SVs in metastatic NET samples, many of which have unknown disease relevance. The high pathogenicity scores indicated by StrvCTVRE and PhenoSV for known oncogenic drivers highlight the potential of these tools in deciphering our unknown SVs. To refine our analysis, we will use Kendall's Tau rank coefficient to compare the ranked lists from the two models. A new ranked list will be generated, giving more weight to SVs that are consistently ranked higher in both StrVCTVRE and PhenoSV, thus prioritizing the most consistently significant findings. Although these machine-learning models may not directly translate to biological interpretability, they provide a strong starting point for identifying areas for further exploration and narrowing the focus of future analyses. Our results indicate that employing machine-learning models trained to assess the pathogenicity of SVs on our NET dataset holds significant potential for identifying novel pathogenic biomarkers, which, in turn, could guide more effective therapies.

Deciphering the Microenvironments That Shape the Resistance to Anti-VEGF Therapy in PanNET

Yeonghwan Kim[1], Sophie O'Keefe[1], Helen Remotti[1], Tito Fojo[2], Minah Kim[1]

1. Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA.
2. Department of Medicine, Columbia University Irving Medical Center, New York, NY, USA.

Presenting author: Yeonghwan Kim, PhD

Approximately 40% of patients with pancreatic neuroendocrine tumors (PanNET) present with liver metastasis at diagnosis, which correlates with poor prognosis. Among the limited therapeutic options available, anti-angiogenic therapy has been considered due to the increased vascularity and high levels of vascular endothelial growth factor (VEGF). Sunitinib, one of the few FDA-approved drugs for progressive PanNET treatment, has shown meaningful results for advanced PanNET patients by targeting the VEGF signaling pathway. Nevertheless, resistance to anti-VEGF therapy and metastatic recurrence have limited therapeutic success. Therefore, understanding the mechanisms of resistance to VEGF inhibitor during metastatic progression is essential for developing novel therapeutic strategies for advanced PanNET treatment. Using RT2;AB6F1 mice, which spontaneously develop primary and liver metastases of PanNET, we found that inhibition of VEGF signaling for 1 week decreased metastatic burden in the liver, whereas prolonged inhibition over 5 weeks resulted in similar metastatic burden to the control group, accompanied by comparable vascular density and increased vascular leakage. To identify molecular determinants driving anti-VEGF resistance in liver metastases, we recently performed Single-nucleus RNA sequencing analysis on livers treated with IgG or anti-VEGF. Following the ongoing transcriptomic analysis, we will validate and conduct functional studies on candidate molecules to elucidate their roles in anti-VEGF resistance in advanced PanNETs. Our study will potentially identify novel therapeutic targets to overcome anti-VEGF resistance in PanNET patients.

Unveiling the Heterogeneous Landscape of Non-Canonical Neoantigens in Pancreatic Neuroendocrine Tumours

Adrian Larykeryd, Ashwanth Harish Mahalingham, Yuta Ikami, Anguraj Sadanandam

Division of Molecular Pathology, Institute of Cancer Research, London.

Presenting author: Anguraj Sadanandam, PhD

Background:

Recent advancements have significantly improved our understanding of the heterogeneity and immunological landscapes of pancreatic neuroendocrine tumours (PanNETs), especially in metastases-like primary (MLP-1/2), intermediate, and insulinoma subtypes. Although immunotherapies provide modest therapeutic benefits to patients, these benefits may be restricted by the low mutational neoantigen loads observed in PanNETs. Thus, it is crucial to gain a comprehensive understanding of the neoantigen landscape derived from non-canonical genomic regions, including RNA splicing, across these subtypes.

Methods:

RNAseq gene expression analysis was performed using our published PanNETassigner signature and correlation method for subtyping. The rnavar tool was utilized for RNAseq variant calling, IsoformSwitchAnalyzeR for subtype-specific isoform switch analysis, gene enrichment analysis for examining immune and gene-related features, and NetMHCpan for neoantigen peptide-major histocompatibility complex (MHC) analysis.

Results:

Single-end RNAseq data from 113 PanNET samples from publicly available cohorts were used to identify four subtypes. The intermediate subtype was the most frequent. We compared PanNETs to 88 potential normal pancreas samples from a publicly available cohort. We identified 403 isoforms associated with 261 genes. Isoform switching was most prevalent between intermediate and MLP-1/MLP-2, insulinoma and normal pancreas, and insulinoma and intermediate subtypes. Among the different isoform switch types, we observed increased open reading frame (ORF) modifications, followed by domain loss/gain and altered coding potential. Non-sense mediated decay and intron retention were less frequent. Gene enrichment analysis revealed increased representation of neurotypes and islet cell types, with notable isoforms and genes associated with hypoxia, mTOR signalling and the MLP-1 subtype. Increased neoantigen load (defined as more than 75 peptides per transcript binding to MHC) was associated with a subset of splice variants, and this was also associated with cohort-specific HLA typing, which is a work in progress.

Conclusion:

Our findings highlight the complexity of isoform switching and its impact on neoantigen presentation in PanNET subtypes. This deeper understanding of the neoantigen landscape could inform more effective immunotherapeutic strategies for PanNET patients. This work is ongoing, and we plan to expand our analysis to include different grades of PanNETs.

Estrogen Receptor Alpha Inhibition Increases 177Lu-DOTATATE Efficacy in a Pre-clinical Neuroendocrine Tumor Model

Xavier M. Keutgen, Olga Lakiza, Campbell Herring, Nicolas Lopez, Jason Schwarz, Jelani Williams

University of Chicago Medicine, Chicago, IL.

Presenting author: Xavier M. Keutgen, MD

Background:

Patients with metastatic neuroendocrine tumor (NETs) suffer from a diminished lifespan. Newer therapies such as Peptide Receptor Radionuclide Therapy (PRRT), using 177Lu-DOTATATE, have aimed to improve survival in metastatic NETs, but result in palliation rather than cure. Combining radioligand therapies (RLT) with radiosensitizers has not been widely explored so far. Herein we propose to use estrogen receptor alpha (ERα) antagonists to improve PRRT effectiveness in pre-clinical NET models.

Methods:

ESR1 gene and ERα protein expression were analyzed in the pancreatic- (QGP1, QGP1.SSTR2), small bowel- (GOT1) and lung- (NCI-H727) NET cell lines. QGP1 cells were exposed to estrogen (E2)-starvation to assess the role of E2 on cell proliferation. ESR1-inhibition using siRNA was used to assess its role in transcribing DNA repair genes. To determine the effect of Fulvestrant, an ERα-antagonist, in combination with external beam radiation (IR), clonogenic assays were performed in all cell lines (using 100nM of Fulvestrant and IR doses of 1Gy, 2Gy, 3Gy, 4Gy, 6 Gy and 8Gy respectively). Next-Generation-RNA sequencing was performed in QGP1 cells exposed to IR (4Gy) +/- Fulvestrant (100nm). In-vivo experiments using mouse xenografts with QGP1 and QGP1.SSTR2 tumors were generated. Mice were treated with Fulvestrant (5mg s.c. every q2 days), 20Gy IR or 0.8mCi of 177Lu-DOTATATE, or both; tumor volume and survival metrics were calculated.

Results:

ESR1 mRNA was consistently expressed in all NET cell lines, but significantly lower than the breast cancer cell line MCF-7 ($P<0.001$). Cell proliferation increased in QGP1 ($p<0.01$), GOT1 ($p<0.01$) and NCI-H727 ($p<0.01$) cells when exposed to E2 (0.5nM). Transient ESR1 knockdown (siESR1, 30nM) and Fulvestrant (100nM) in combination with IR (4Gy) decreased cell viability in QGP1 cells when compared to IR alone ($p<0.001$ and $p< 0.01$). siESR1 also decreased Rad51m BRCA and BRCA2 mRNA expression in QGP1 cells ($p<0.01$). Clonogenic assays showed that combining Fulvestrant with IR decreased number of colonies for QGP1.SSTR2 ($p<0.01$), BON1 ($p<0.05$) and NCI-H727 ($p<0.05$). In-vivo, mean tumor volume and median survival was significantly different when comparing mice xenografts in the Fulvestrant+ IR and IR groups (242mL vs. 575mL at day 30, $p<0.05$ and 56 vs. 34 days $p<0.01$). Similar results were observed when combining Fulvestrant with 177Lu-DOTATATE in QGP1.SSTR2 xenografts. ERα 66kDa, the most common isoform, was not detected in NET cells. The ERα 36kDa isoform was present but did not decrease after Fulvestrant exposure ($p>0.05$). gH2AX protein expression, a marker for DNA double strand breaks, did not increase when combining Fulvestrant with IR ($p>0.05$), suggesting that the observed radiosensitizing effect is not through ERα-induced DNA repair inhibition. Preliminary NGS RNA sequencing and KEGG pathway analysis suggests that apoptotic markers are increased in Fulvestrant-treated cells.

Conclusions:

ERα-inhibition with Fulvestrant radiosensitizes NET cells in pre-clinical models using IR and PRRT, although the exact mechanisms are still being investigated. A Phase I study to evaluate the safety and preliminary efficacy of Fulvestrant in combination with 177Lu-DOTATATE for advanced metastatic pNETs will be opening soon at our institution.

A Novel Hormone Based Anti-SSTR Bispecific T-cell Engager for the Treatment of Neuroendocrine Tumors

Eleonora Pelle[1], Mauro Cives[2], Elliot Medina[3], Charlotte C. Mason[3], Sebastian A. Snedal[4], Xiomar E. Bustos-Perez[4], Gabriele Maiorano[5], Vincent Luca[3], Patrick Hwu[6], Daniel Abate-Daga[7], Jonathan Strosberg[1]

1. Department of GI Oncology, Moffitt Cancer Center, Tampa, Florida, USA. 2. Department of Interdisciplinary Medicine, University of Bari Aldo Moro, Bari, Italy. 3. Department of Drug Discovery, Moffitt Cancer Center, Tampa, Florida, USA. 4. Departments of Immunology, Moffitt Cancer Center, Tampa, Florida, USA. 5. Institute of Nanotechnology NANOTEC, National Research Council, Lecce, Italy. 6. Moffitt Cancer Center, Tampa, Florida, USA. 7. Departments of Immunology and Cutaneous Oncology, Moffitt Cancer Center, Tampa, Florida, USA.

Presenting author: Eleonora Pellé, MD

Background:

Somatostatin receptor 2 (SSTR2) is overexpressed in well-differentiated NETs. We designed a novel bispecific T-cell engager targeting SSTR2 via Somatostatin-14 (SST14), the hormone that physiologically binds the SSTR2, linked with a scFV-based anti-CD3.

Methods:

The optimized sequence of the engager was subcloned into a vector (pAcGP67a) designed for protein expression in insect cells using Baculovirus, and the recombinant protein was subsequently expressed in Trichoplusia-ni (High Five) cells, isolated and characterized by chromatography. Flow cytometry and Image Stream flow cytometry were used to determine the interaction of the molecule with CD3 and SSTR2. Effector CD3+ T cells were isolated from the peripheral blood of healthy donors and target 293T cells were stably transduced to concurrently express SSTR2 and GFP. Effector and target cells were co-incubated in the absence or presence of the engager at different concentrations. The SSTR2-parental 293T cell line was used as negative control, while anti-CD3/CD28 beads were added in positive control preparations. The engager-induced T cell activation was evaluated measuring the cytokine secretion by ELISA, and the engager-induced cytotoxicity was assessed by real-time quantitative live-cell imaging.

Results:

The T-cell engager was detected by flow cytometry on approximately 85% of T cells at a concentration of 100nM. The engager interaction with SSTR2+ and its subsequent internalization was detected by image stream between 100nM and 20nM. IFN-γ, TNF-alpha and Granzyme-B secretion was significantly higher when the T cells were co-cultured with SSTR+ 293T cells in the presence the engager at 100nM as compared with conditions using SSTR- 293T cells or in absence of the molecule. Similar results were observed at a lower engager concentration (20nM). Additionally, the 100nM engager exhibited antiproliferative activity when added to SSTR+ 293T cell cultures in the presence of T cells. Furthermore, a dose-dependent cytotoxic activity was observed when the cells were incubated with the 20nM engager.

Conclusion:

To our knowledge, this is the first T-cell engager to incorporate a hormone in one binding site, exerting a dose-dependent antiproliferative activity against SSTR2-expressing cells.

SiFA x NAMB: Automated Synthesis of Net-Targeting 18f-Peptide Receptor Ligands for Pet Imaging via a Combination "Silicon-fluoride Acceptor" and "Non-anhydrous, Minimally Basic" Approaches

Kevina Chavda[1], Shereecia M. Bangura[2], Cory P. Zaparaniuk[3], Derek R. Morim[2], Yinglan Pu[4], Eric W. Price[3] and James A.H. Inkster[1]

1. Department of Chemistry & Chemical Biology, McMaster University, Hamilton, Ontario, Canada. 2. Nuclear Operations & Facilities, McMaster University, Hamilton, Ontario. Canada. 3. Department of Chemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. 4. Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

Presenting author: Kevina Chavda, B.Sc

The technical complexities of synthesizing 18F-peptide PET agents in automated synthesis units (ASUs)- as required for human use- hinders the development of these radiotracers for clinical cancer imaging. Three associated bottlenecks are: a) peptide sensitivity to the high temperatures and basic mixtures employed in canonical 18F-fluorination chemistry; b) the t½ of 18F (109.8 min), which often necessitates the need for simple radio-protocols; c) limits to the number and minimum volume of fluid transfers (typically 0.3 mL) possible using an ASU.

"SiFA" technology involves the direct (i.e. one chemical step) 19F/18F isotopic exchange labelling of di-tert-butylphenylfluorosilane-modified peptides. Reactions proceed at RT and require only nmol of precursor. [18F]SiTATE is a SiFA-bearing Tyr3-octreotate derivative that has been found superior to [68Ga]DOTATOC for imaging of liver, bone and lymph node metastases. In addition, the longer t1/2 of 18F vs. 68Ga (68 min) allows for the treatment of multiple patients with a single batch of [18F]SiTATE. Widespread clinical adoption of [18F] SiTATE has been hampered by a difficult-to-optimize automated synthesis which requires a) the use of a highly basic and unstable K222-OH complex to elute [18F]F-; b) careful titration of the eluate with oxalic acid and c) introduction of a small volume of 19F-peptide solution via cannula.

"NAMB" chemistry is an innovative means to prepare reactive [18F]F- that avoids time-consuming azeotropic drying and basic reactions by eluting [18F]F- from small QMA columns with non-basic anions. After addition of precursor in organic solvent, nucleophilic fluorination occurs with heating, despite ≤6% water present. We discovered that SiFA reactions proceed under NAMB conditions at RT, and have thus developed a general protocol for the automated synthesis of [18F]SiFAlyated peptides, using [18F]SiTATE as a model.

Manual experiments resulted in RCYs ≤49% (all yields NDC) and informed the straightforward translation of [18F]SiTATE onto a GE Fastlab™ ASU. [18F]F- in [18O]H2O water was trapped on 12 mg QMA cartridges, then efficiently released directly into a reactor containing 50 nmol [18F]SiTATE using tetraethylammonium tosylate (25 µmol) in MeCN:H2O (8:2; 0.3 mL). Final reaction mixtures (1 mL) contained MeCN with 20% DMSO and 6% water. After 5-30 min at RT with N2 sparging, [18F]SiTATE was isolated by C18 SPE.

Initial ASU runs afforded [18F]SiTATE in 10-18% (n=3) after 30 min with >98% radiochemical purity, starting from 138±44 mCi of [18F]F-. Decreasing reaction times did not significantly impact RCYs (18%@5 min and 24%@20 min respectively). Interestingly, increasing temperature (37°C) or introducing manual stirring lowered RCYs (6%, both cases), which prompted suspicions that 18F was being lost as volatile [18F]HF. Indeed, when sparging was omitted, RCY increased to 33%.

[18F]SiTATE was prepared using a simple automated protocol that requires no azeotropic evaporation, pH titration, or small volume transfers. Next steps will focus on reducing water content to increase RCC. We will also report our efforts to prepare novel SiFAylated GLPr1-targeting agents based on exendin-4. Radiometalated exendins evaluated as insulinoma imaging agents exhibit significant kidney accumulation that can obscure imaging of the pancreas. [18F]SiFA-peptides should exhibit altered biodistribution patterns relative to those labeled with metal chelators.

Comprehensive Genomic Profiling of an International Patient Cohort Reveals Diagnostic and Prognostic Signatures for Pancreatic Neuroendocrine Neoplasms

Mark S. Etherington, MD[1], Azfar Neyaz, MD[2], Katelyn Smith, BA[2], Brian Theisen, MD[3], Patrick Henn, MD[4], Ethan S. Sokol, PhD[5], Smruthy Sivakumar, PhD[5], Anna Vera D. Verschuur, MD[6], Wenzel M. Hackeng, MD[6], Roderick J. O'Sullivan, PhD[7], Ta-Chiang Liu, MD PhD[8], Amer H. Zureikat, MD[1], Alessandro Paniccia, MD[1], Randall E. Brand, MD[9], Kevin McGrath, MD[9], Adam Slivka, MD PhD[9], Herbert J. Zeh, MD[10], Melissa E. Hogg, MD[11], Brian A. Boone, MD[12], Anne Marie O'Broin-Lennon, MD[13], Walter G. Park, MD[14], Patricio M. Polanco, MD[10], Jin He, MD[15], Diane M. Simeone, MD[16], Cristina R. Ferrone, MD[17], Marco Del Chiaro, MD[18], Rebecca M. Minter, MD[19], Michael J. Pishvaian, MD[20], Andrew Hendifar, MD[21], Thorvandur R. Halfdanarson, MD[22], Janie Zhang, MD[23], Dennis Hsu, MD[23], Anwaar Saeed, MD[23], Samuel J. Klempner, MD[24], Ben George, MD[25], Matthew H. Kulke, MD[26], C. Benedikt Westphalen, MD[27], Liron Pantanowitz, MD PhD[2], James M. Mitchell, MD[28], Anthony J. Gill, MD[29], Elizabeth A. Montgomery, MD[30], Chanjuan Shi, MD[31], Kevin M. Waters, MD PhD[32], Danielle A. Hutchings, MD[32], Elizabeth D. Thompson, MD[33], Ralph H. Hruban, MD[33], Anirban Maitra, MBBS[34], Anju H. Singhi[2], Abigail I. Wald, PhD[2], Marina N. Nikiforova, MD[2], Claudio Luchini, MD PhD[35,36], Seung-Mo Hong, MD PhD[37], Lodewijk A.A. Brosens, MD PhD[6], Christopher M. Heaphy, PhD[26], Aatur D. Singhi, MD PhD[2]

1. Department of Surgery, University of Pittsburgh Medical Center, Pittsburgh, PA, USA. 2. Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA. 3. Department of Pathology and Laboratory Medicine, Henry Ford Health, Detroit, MI, USA. 4. Department of Pathology, University of Colorado, Denver, CO, USA. 5. Foundation Medicine, Inc. Cambridge, MA, USA. 6. Department of Pathology, University Medical Center Utrecht, Utrecht University, The Netherlands. 7. Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA, USA. 8. Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO, USA. 9. Department of Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA. 10. Department of Surgery, University of Texas Southwestern, Dallas, TX, USA. 11. Department of Surgery, NorthShore University Health System, Evanston, IL, USA. 12. Department of Surgery, West Virginia University Health Sciences Center, Morgantown, WV, USA. 13. Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD, USA. 14. Department of Medicine, Stanford University, Stanford, CA, USA. 15. Department of Surgery, Johns Hopkins Medical Institutions, Baltimore, MD, USA. 16. Department of Surgery, New York University Langone Health, New York, NY, USA. 17. Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA. 18. Department of Surgery, University of Colorado Anschutz Medical Campus, Aurora, CO, USA. 19. Department of Surgery, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA. 20. Department of Oncology, Johns Hopkins Medical Institutions, Baltimore, MD, USA 21. Department of Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA. 22. Division of Medical Oncology, Mayo Clinic Cancer Center, Rochester, MN, USA. 23. Department of Medicine, Division of Hematology-Oncology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA. 24. Department of Medicine, Division of Hematology-Oncology, Massachusetts General Hospital Cancer Center, Boston, MA, USA. 25. Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA. 26. Section of Hematology and Medical Oncology, Boston University and Bostin Medical Center, Boston, MA, USA. 27. Department of Medicine III and Comprehensive Cancer Center, University Hospital, Klinikum Grosshadern, Ludwig-Maximilians-University of Munich, Marchioninistr. 15, 81377, Munich, Germany. 28. Department of Pathology, University of Texas Southwestern, Dallas, TX, USA. 29. NSW Health Pathology, Department of Anatomical Pathology, Royal North Shore Hospital, NSW Health Pathology, St Leonards, NSW 2065, Australia and University of Sydney, Sydney NSW 2006, Australia. 30. Department of Pathology, University of Miami Miller School of Medicine, Miami, FL, USA. 31. Department of Pathology, Duke University Medical Center, Durham, NC, USA. 32. Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, USA. 33. Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, USA. 34. Departments of Pathology and Translational Molecular Pathology, Sheikh Ahmed Center for Pancreatic Cancer Research, University of Texas MD Anderson Cancer Center, Houston, TX, USA. 35. Department of Diagnostics and Public Health, Section of Pathology, University of Verona, Verona, Italy. 36. ENETS Center of Excellence, University and Hospital Trust of Verona, Verona, Italy. 37. Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea.

Presenting author: Aatur D. Singhi, MD, PhD

Background:

Pancreatic neuroendocrine neoplasms (PanNENs) are a heterogeneous group of diseases and primarily consist of well-differentiated pancreatic neuroendocrine tumors (NF-PanNETs) and poorly-differentiated pancreatic neuroendocrine carcinomas (PanNECs). There currently represents two distinct challenges with PanNENs: (1) defining high-risk prognostic groups for NF-PanNETs and (2) distinguishing between WHO grade 3 NF-PanNETs and PanNECs. Therefore, comprehensive genomic profiling (CGP) and orthogonal immunohistochemical testing was performed on a large series of PanNENs to identify distinct diagnostic and prognostic signatures for these increasingly encountered neoplasms.

Methods:

Comprehensive genomic profiling from an international patient cohort was performed for 931 locally advanced and/or metastatic PanNENs (649 NF-PanNETs and 282 PanNECs). Prevalent alterations for NF-PanNETs were confirmed through orthogonal immunohistochemical testing, which evaluated a separate, international cohort of 603 NF-PanNET patients. Immunohistochemical findings were correlated with clinicopathologic features to include relapse-free survival (RFS) and disease-specific survival (DSS).

Results:

A comparative genomic analysis between NF-PanNETs and PanNECs identified differences in prevalence for MEN1, DAXX, ATRX, TSC2, PTEN, SETD2, ARID1A, and VHL ($p<0.032$). Interestingly, TP53 and CDKN2A alterations were found in both neoplasms, but within specific clinical, pathologic, and molecular contexts. Focusing on mutually exclusive alterations for NF-PanNETs, orthogonal immunohistochemical testing for DAXX, ATRX, SETD2/H3K36me3, ARID1A, PTEN, and VHL/GLUT1 revealed aberrant expression to correlate with larger tumor size, higher WHO grade, and distant metastases ($p<0.004$). Further, aberrant expression for at least 1 biomarker correlated with shorter RFS and DSS rates ($p<0.001$) and was an independent, adverse prognostic factor for RFS ($p<0.001$), especially among small (≤2.0 cm) NF-PanNETs ($p=0.033$), and DSS ($p=0.020$).

Conclusion:

The molecular and immunohistochemical findings identified within this study provide crucial insights into PanNENs, underscoring the importance of CGP and immunohistochemical testing, for precise diagnosis and improved prognostication, respectively.

Modelling Mesenteric Fibrosis in Small Intestine Neuroendocrine Tumours (Si-Nets): Patient-derived Matrix as a Platform for in vitro Studies

Maria C. Martins*[1], Harry Hodgetts*[1], TuVinh Luong[1,2], Andrew R. Hall[1,2], Eva van der Slik[3], Richard A. Feelders[3], Amy Webster[4], Christos Toumpanakis[5], Dalvinder Mandair[5], Peter van Koetsveld[3], Leo J. Hofland[3], Christina Thirlwell[6], Martyn E. Caplin[5] and Krista Rombouts[1]

1. Regenerative Medicine and Fibrosis Group, Institute for Liver and Digestive Health, Royal Free Hospital, University College London, London, UK. 2. Sheila Sherlock Liver Centre, Royal Free London NHS Foundation Trust, London, UK. 3. Department of Internal Medicine, sector Endocrinology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands. 4. University of Exeter - College of Medicine and Health, Exeter, United Kingdom. 5. Neuroendocrine Tumour Unit, ENETS Centre of Excellence, Royal Free London NHS Foundation Trust, London, UK, 6. University of Bristol Medical School, University of Bristol, Bristol, UK.

Presenting author: Maria Castanho Martins, MSc

Background:

Mesenteric fibrosis (MF) affects up to 50% of small intestine neuroendocrine tumours (SI-NETs) patients, causing significant morbidity and mortality. MF pathophysiology is poorly understood, limiting the development of treatments and identification of biomarkers. A significant challenge in producing translational data for SI-NETs is a lack of robust methods to model the disease. This project aims to improve the understanding of MF and identify useful diagnostic and predictive molecular markers, whilst developing a new 3D model to study SI-NETs and MF in vitro.

Methods:

Fresh tissue collected from SI-NET patients was used to isolate primary fibroblasts from normal intestine tissue and normal mesentery, whilst cancer-associated fibroblasts (CAF) were isolated from primary tumour, and mesenteric metastases of the same patients. Spheroid cultures of GOT1 cells (an SI-NET cell line) were optimised and grown for 14 days or 21 days.

SI-NET patient tissue from normal intestine and mesenteric mass was decellularized and lyophilized to obtain an extracellular matrix (ECM) powder. Decellularization quality control was performed using histological analysis (H&E and Sirius Red) and DNA quantification. ECM powders were solubilised, mixed with nanocellulose and a cell suspension to form a 3D hydrogel. The ECM hydrogels were used to culture normal intestine, normal mesentery fibroblasts, or SI-NET CAFs in monoculture and co-cultures with GOT1 spheroids. Cells were cultured for 14 days in ECM hydrogels and assessed by PrestoBlue assay, immunohistochemistry (IHC), and RT-qPCR. 3D hydrogel cultures were further subjected to TGFB treatment for 6 days and gene expression was assessed using RT-qPCR.

Results:

DNA analysis and H&E showed efficient decellularization of normal intestine tissue and mesenteric mass tissue from SI-NET patients, while Sirius Red showed preservation of ECM.

Spheroids, CAFs and primary fibroblasts showed stable viability in mono- and co-culture over 14 days in bioengineered 3D ECM hydrogels. H&E staining and IHC of cell specific markers synaptophysin and alpha smooth muscle actin for GOT1 and fibroblasts, respectively, showed the distribution of the co-cultured cells throughout the hydrogel. The expression of fibrogenic markers was significantly increased in co-cultured hydrogels when treated with TGFB compared to untreated hydrogels, with alpha smooth muscle actin, TIMP1 and interleukin-6 expression increasing over 2-fold. This effect was not seen in the monocultures.

Fibroblasts from normal intestine showed no change in viability between monocultures or co-cultures with GOT1 spheroids either in normal intestine or mesenteric mass ECM hydrogels, while fibroblasts from normal mesentery and CAFs from primary tumour and mesenteric metastases showed a significant increase in viability in co-cultures with GOT1 spheroids compared to monocultures.

Conclusions:

SI-NET cells and SI-NET primary fibroblasts can be cultured in a 3D model using human ECM hydrogels generated from SI-NET patient tissue. Cells in co-culture with GOT1 spheroids showed a higher sensitivity to pro-fibrogenic cues, suggesting an important crosstalk in the setting of mesenteric fibrosis. This model will be used as a platform to better understand MF pathophysiology in SI-NET patients and to test candidate anti-fibrotic drugs targeting MF.

This research into mesenteric fibrosis is funded by NETRF Accelerator Grant 720627 (UCL/Exeter/Erasmus).

Does Sexual Dimorphism Play a Role in Si-Net and Mesenteric Disease Development?

Daniah Hassan[1], Dalton Bally[2], Maria C Martins[1], Harry Hodgetts[1], Krista Rombouts[1], Christos Toumpanakis[2], Martyn E Caplin[2], Dalvinder Mandair[2]

1. Regenerative Medicine and Fibrosis Group, Institute for Liver and Digestive Health, Royal Free Hospital, University College London, London, UK. 2. Neuroendocrine Tumour Unit, ENETS Centre of Excellence, Royal Free London NHS Foundation Trust, London, UK.

Presenting author: Maria Castanho Martins, MSc

Background:

Small-intestinal neuroendocrine tumours (SI-NET) are rising in incidence and over 50% of patients present with metastatic disease at the time of diagnosis. Mesenteric and hepatic metastases are among the most common and are associated with increased morbidity and mortality. Up to 50% of patients also develop mesenteric fibrosis leading to severe complications such as intestinal oedema and obstruction. Furthermore, it has recently been suggested that mesenteric fibrosis risk increases in women around menopause. The aim of this study was to analyse sexual dimorphism in a large cohort of patients with SI-NET.

Methods:

Data collected from patient records for 849 patients (recruited 2009-2021, Royal Free Hospital, London) was analysed for the presence of sexual dimorphism in key characteristics such as age, sex, grade, stage, presence of mesenteric metastases and size, presence of fibrosis and urinary 5HIAA. Survival analysis was conducted for male and female patients, mesenteric metastases, mesenteric fibrosis and tumour multifocality.

Results:

Of 849 patients identified, 54% were male. Mesenteric metastases (57% male vs 42% female, $p < .001$) and mesenteric fibrosis (49 % male vs 39% female, $p < .01$) were more prevalent in males. Male sex [OR; 1.727 95% CI 1.093-2.728, ($p = .048$)] and age of diagnosis [OR; 1.020 95% CI 1-1.040, ($p = .048$)] were statistically significant predictors of mesenteric metastases. Median survival time did not differ significantly based on sex. Mesenteric metastases presence increased with age for males and females being statistically significant for females only. Presence of mesenteric fibrosis was significantly different in the 51-57 age groups (57% male vs 33% female, $p = .008$). Male patients in the >70 age group had the highest incidence of mesenteric fibrosis.

Conclusions:

Male sex is an independent factor for mesenteric metastases. Elderly females have higher prevalence of mesenteric metastases that may be linked to the effect of sex hormones post-menopausal. Future work investigating sex hormone level in patients and expression of receptors in tissue will elucidate further on the role of pre-menopausal status in mesenteric fibrosis protection.

This research into mesenteric fibrosis is funded by NETRF Accelerator Grant 720627 (UCL/Exeter/Erasmus).

Targeting the Surfaceome of Drug Tolerant Persister Cell Populations in Extrapulmonary High-Grade Neuroendocrine Carcinomas

C. Allison Stewart[1], Kavya Ramkumar[1], Alberto Duarte[1], Runsheng Wang[1], Jody Vykoukal[2], Ali Ibrahim[1], Yuanxin Xi[3], Lixia Diao[3], Alexa Halliday[1], Samir Hanash[2], Jing Wang[3], Lauren A. Byers[1], Carl M. Gay[1]

1. Department of Thoracic/Head & Neck Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA. 2. McCombs Institute for the Early Detection and Treatment of Cancer, University of Texas MD Anderson Cancer Center, Houston, TX, USA. 3. Department of Bioinformatics and Computational Biology, University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Presenting author: C. Allison Stewart, PhD

Background:

High-grade neuroendocrine carcinomas (hgNECs) are aggressive malignancies that arise, most commonly, from the aerodigestive tracts, though, more rarely from other sites. Frustratingly, hgNECs are, initially, exquisitely sensitive to chemotherapy and/or radiation, but these responses are short-lived and inevitable relapses occur. While targeting the surfaceome in hgNECs with antibody drug conjugates (ADCs) and bi/tri-specific T-cell engagers (BiTEs/TriTEs) demonstrate promising results, the most advanced of these targets (DLL3, SEZ6) are not present on the surface of YAP1-positive, drug tolerant persister cells (DTPCs). Therefore, we hypothesize that hgNEC relapse is driven, at least in part, by the emergence and expansion of DTPCs and that surfaceome-targeting to specifically eradicate these populations will improve patient response.

Methods:

In order to characterize DTPCs, established hgNEC cell lines and models were infected with the Watermelon doxycycline-inducible proliferation tracking experiments with and without high dose chemotherapy for 14 days followed by transcriptomic, methylomic, and proteomic profiling (i.e., mass spectrometry, flow cytometry). A cohort of tissue, plasma samples, and patient derived xenograft models (PDX) were assembled from more than 80 patients with rare hgNECs (i.e. those other than SCLC) treated at MD Anderson Cancer Center. Methylomic analysis of circulating tumor (ct)DNA, biopsies, and/or PDX models from 41 hgNEC patients at multiple timepoints has been performed. For many of these patients, additional analyses have been performed to assemble a molecular atlas of both treatment-naïve and, especially, relapsed disease for validating surface targets.

Results:

Long term chemotherapy-treated viable cells were sorted by high or low mCherry levels to capture both cycling (low mCherry) or non-cycling (high mCherry) DTPCs. Non-cycling DTPCs were rare and made up less than 3% of viable cells. Consistent with being DTPCs, neither population was sensitive to chemotherapy treatment and exhibited a reduced cisplatin response signature score compared to DMSO-treated controls ($P<0.002$). Non-cycling DTPCs were enriched for senescence associated secretory phenotype genes. Transcriptionally and proteomically, the two DTPC populations were remarkably similar to one another, but distinct from controls. Both DTPC populations demonstrated an enrichment in YAP/TAZ target score ($P=0.006$) and cancer stem cell gene expression and a reduction in replication stress signature ($P<0.001$). Neuroendocrine (NE) score was increased in DTPCs ($P=0.03$), which was consistent with increased gene expression of NE gene, NCAM1 ($P<0.001$), and cell surface levels of CD56 ($P<0.001$) by flow cytometry. In addition to CD56, other known (i.e., AXL) and novel proteins (i.e., ALCAM), that are actionable as targets, were enriched on the cell surface of DTPCs.

Conclusions:

Chemotherapy tolerant hgNEC DTPCs are distinct from cells without therapeutic pressure and demonstrate unique mechanisms of resistance, drug response, and perhaps, most importantly, a discrete cell surface proteome. Next steps include testing efficacy of DTPC-specific surface-targeting methods (e.g., ADC, BiTEs, or chimeric antigen receptor T-cells) in hgNEC PDX models. Identifying DTPC-specific surface proteins has the potential to refocus hgNEC clinical efforts toward cell surface-targeting strategies, including ADCs and BiTEs, to provide more durable progression-free intervals for patients plagued by striking, but all-too-transient responses with current therapies.

Preliminary Results of the Copper Pet in NET Trial: A Randomized, Crossover, Readers Blind, Phase 0/I Study Comparing 61cu-Nodaga-Lm3 and 68ga-Dotatoc for the Detection of Neuroendocrine Tumors

Guillaume P. Nicolas[1], Damian Wild[1], Wolfgang A. Weber[2], Leila Jaafar[3], Melpomeni Fani[1]

1. Department of Theragnostics, University Hospital Basel, Switzerland. 2. Department of Nuclear Medicine, Technical University Munich, Germany. 3. Nuclidium AG, Basel, Switzerland.

Presenting author: Guillaume Nicolas, MD

Background:

Currently approved Gallium-68 (68Ga) or copper-64 (64Cu) labeled somatostatin receptor (SST) agonists (NETSPOT®, SOMAKIT® and Detecnet™) for positron emission tomography (PET) imaging of gastroenteropancreatic NET patients have significant limitations. The high costs, limited production capacity and unfavorable physical properties of the radioisotopes have motivated us to introduce Copper-61 (t1/2 = 3.33 h, 61% β+-fraction, Emax = 1.2 MeV), a still unexplored radioisotope with better physical properties compared to 64Cu (t1/2 = 12.7 h, low β+-fraction (18%)), longer half-life and lower energy compared to 68Ga (t1/2 = 68 min, Emax = 1.9 MeV), and more cost-effective production and scale up capacity. Given the superior imaging properties showed by 68Ga-labeled SST antagonists over the agonists, we combine 61Cu with the SST antagonist NODAGA-LM3. During the first year of this 2-year project, we have established and optimized i) 61Cu production at 2 sites (Zurich, Switzerland) and Munich, Germany) using enriched solid targets (Ni-61), ii) delivery to a clinical site (Basel, Switzerland) and showed feasibility of satellite distribution, iii) set up the GMP production of 61Cu-NODAGA-LM3 for human use at the University Hospital Basel and iv) submitted the clinical trial application to the Ethics Committee and Swiss regulatory authorities. During the 2nd year we aim to conduct the clinical study to show safety, biodistribution, dosimetry and diagnostic efficacy of 61Cu-NODAGA-LM3 PET/CT in NET patients.

Methods:

This is a first-in-human, prospective, open-label, randomized, crossover controlled, readers blind, phase 0/I, single center PET/CT study with 61Cu-NODAGA-LM3. Our aim is to investigate safety, pharmacokinetics, the best time point for imaging, dosimetry and preliminary diagnostic efficacy (vs 68Ga-DOTA-TOC PET/CT) in 8 patients with well-differentiated gastroenteropancreatic and bronchopulmonary NET. Frequency of adverse events will be reported, and severity will be scored using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Organ dosimetry will be calculated based on images acquired 1, 3, and 18 h post injection. Scans will be reviewed by 2 independent blinded readers. The gold standard for adjudicating true and false positive findings will be based on either biopsy or 2- to 7-month follow-up with the best composite imaging for a given patient (including liver MRI, contrast-enhanced CT and/or SST PET/CT).

Results:

So far, 61Cu production and Good Manufacturing Practice (GMP) production of 61Cu-NODAGA-LM3 has been established and study documentation has been submitted. The study has received approval from the Ethics Committee of Northwestern Switzerland and is registered on Clinicaltrials.gov (NCT ID not yet assigned). National regulatory authorities have issued the first review and final approval is pending. We are expecting our first-in-patient in the 3rd quartal of 2024 and completion of the first 8 patients by the end of 2024, as planned.

Conclusion:

Since the beginning of the study (Q1 2023) we have set up the production for human use of the investigational medicinal product 61Cu-NODAGA-LM3 and successfully applied for a first-in-human prospective clinical trial. Ethics approval is in place while regulatory approval is expected during the 3rd quartal 2024. Preliminary safety, dosimetry, biodistribution, pharmacokinetics and efficacy data will be presented.

Customizing Chelators for Targeted Radionuclide Therapy: Insights from DFT Modeling

Dongyoul Lee[1], Jeongyun Kim[2], Ku Kang[3], Kihyun Shin[4], Won Bo Lee[2], and Michael K. Schultz[5]

1. Department of Physics and Chemistry, Korea Military Academy, Seoul, Republic of Korea. 2. School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea. 3. Agency for Defense Development, Daejon, Republic of Korea. 4. Department of Materials Science and Engineering, Hanbat National University, Daejeon, Republic of Korea. 5. Perspective Therapeutics, Coralville, Iowa, USA.

Presenting author: Dongyoul Lee, PhD

Background:

Targeted radionuclide therapy (TRT) has demonstrated significant potential in the treatment of cancers by targeting highly expressed biomolecules of cancer cells using radiolabeled peptides, small molecules, or antibodies. One of the key factors leading to the success of TRT is the sophisticated matching of the chelator to the specific radionuclide, ensuring stability and targeted delivery.

Methods:

In this study, ab initio Density Functional Theory (DFT) modeling techniques were utilized to analyze the interaction energies between radionuclides and chelators and to determine the chelation configuration of the complexes. Multiple chelators extensively used in TRT (i.e., DOTA, NOTA, and TETA), were investigated with alpha emitters (i.e., 225Ac, 212Pb, 149Tb), beta emitters (i.e., 177Lu, 90Y), and imaging tracers (i.e., 68Ga, 64Cu) respectively.

Results:

The computational analyses elucidated that the physicochemical properties, particularly size and charge compatibility between chelators and radionuclides, influence the stability of the complexes. NOTA-Ga, DOTA-Y, and TETA-Cu complexes showed the highest levels of stability as suggested previously. The configuration analysis suggested that the coordination number varied depending on the ionic radius. Small ions (e.g., Ga3+, Cu2+) tend to prefer 6-coordinated complex (octahedron), whereas large ions (lanthanide ions) are mostly found in 8-coordinated complexes (square antiprism). Bader charge analysis indicated that the stability of the chelator-radionuclide complex increased when minimal alterations occurred in the total charge before and after chelation.

Conclusions:

The results validated our computational strategy as an effective tool for analyzing chelator-radionuclide interactions and potentially customizing chelators for TRT. Ongoing studies are expected to suggest novel chelator compositions for improved stability and specificity with respect to the radionuclides of interest.

Exploring the Role of a Novel Adrenomedullary Stem Cell Population in Pheochromocytoma/Paraganglioma Tumourigenesis

Yasmine Kemkem[1], Alice Santambrogio[1,2], James Kaufman-Cook[1,2], Thea L. Willis[2], Emily J. Lodge[2], Val Yianni[2], Abdullah F. Albash[1], Miriam Vazquez-Segoviano[1,2], Olivia Sherwin[1,2], Rebecca J. Oakey[1], Cynthia L. Andoniadou[1,2]

1. King's College London, London, United Kingdom. 2. Dresden University of Technology, Dresden, Germany.

Presenting author: Yasmine Kemkem, PhD

Background:

Pheochromocytomas/paragangliomas (PPGLs) are rare neuroendocrine tumours, which respectively arise in the neural crest (NC)-derived adrenal medulla and the paraganglia. Around one third of PPGLs are associated with inherited cancer susceptibility genes, the highest rate among all tumour types. Germline SDHB mutations, encoding succinate dehydrogenase B, are responsible for the most aggressive form of PPGLs with up to 70% of mutation carriers developing metastasis. Understanding the pathogenesis of PPGLs, and the development of new therapies, is hindered by the lack of validated disease models. In many tumours, cells with stem-like properties are at the root of tumour initiation/maintenance but a stem cell population of the adrenal medulla has not been identified.

Methods & Results:

Using genetic lineage tracing in vivo and transcriptomic data from the mouse adrenal medulla, we show that neural crest-derived cells expressing Sox2 expand to self-renew and give rise to new chromaffin cells in both neonates and adults, supporting their function as a stem cell population. SOX2+ cells are therefore ideal to target for expression of tumour-inducing mutations. We establish a system to isolate and culture pure murine and human populations of adrenomedullary SOX2+ stem cells in vitro and demonstrate that these cells can be expanded and gene-edited, to express mutant Sdhb. Normal and mutant SOX2+ adrenomedullary stem cells (ASCs) were implanted in ovo onto the chick chorioallantoic membrane (CAM), where they were assayed for expansion, contribution of chromaffin cells and invasion/metastasis, demonstrating that mutant cells were able to metastasise to the host lung of the developing chick. To determine the consequences of Sdhb deletion in progenitor/stem cells, we targeted Wnt1-expressing neural crest, Sox10-expressing Schwan Cell Precursors (SCPs) and Sox2-expressing stem cells to conditionally delete Sdhb in mice.

Conclusions:

In this study, we show that a subset of sustentacular cells, known to have a support role within the adrenal medulla, are in fact Sox2/SOX2+ postnatal adrenomedullary stem cells (ASCs). Targeting the developing neural crest, SCPs, and the newly identified ASCs, with an Sdhb mutation in mouse models, we found that SDHB is critical for embryonic neural-crest migration and that postnatal deletion results in abnormal adrenal and extra-adrenal tissue growths

Increasing the Therapeutic Window in Peptide Receptor Radionuclide Therapy with Long-acting Somatostatin Analogues

Else A. Aalbersberg[1], Chayenne Veerman[1], Michelle Versleijen[1], Jeroen J.M.A. Hendrikx[1,2], Marcel P.M. Stokkel[1], Margot E.T. Tesselaar[3]

1. Department of Nuclear Medicine, Netherlands Cancer Institute, Amsterdam, the Netherlands. 2. Department of Pharmacy and Pharmacology, Netherlands Cancer Institute, Amsterdam, the Netherlands. 3. Department of Medical and Gastrointestinal Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands.

Presenting author: Else Aalbersberg, PhD

Background:

Both peptide receptor radionuclide therapy (PRRT) and long-acting somatostatin analogues (LA-SSA) make use of the somatostatin receptor. To avoid competitive binding by either treatment, guidelines recommend withdrawing LA-SSA 4-6 weeks before PRRT. Previously, we have retrospectively shown that LA-SSAs administration within this 4-6 week window prior to PRRT, does not negatively affect tumor uptake of [177Lu]Lu-HA-DOTATATE but decreases the uptake in healthy liver and spleen. We therefore hypothesize that continuous use of LA-SSAs during PRRT does not negatively affect the absorbed dose in tumor lesions.

Methods:

The main objective of the study is to determine the effect of LA-SSAs (i.e. octreotide LAR and lanreotide) on the absorbed dose in tumor lesions during PRRT with [177Lu]Lu-HA-DOTATATE. Secondary objectives include the effect of LA-SSAs on the absorbed dose in normal tissues, tumor-to-background ratio, and pharmacokinetic parameters of [177Lu]Lu-HA-DOTATATE. Also exploratory objective health-related quality of life assessments (HRQoL) are performed.

34 patients using LA-SSAs and referred for PRRT are randomized into two groups: (1) receiving LA-SSA 1-8 days prior to PRRT cycle 1 and receiving LA-SSA 4-6 weeks prior to PRRT cycle 2; (2) receiving LA-SSA 4-6 weeks prior to PRRT cycle 1 and receiving LA-SSA 1-8 days prior to PRRT cycle 2. This allows for intra-patient comparisons. Following the administration of [177Lu]Lu-HA-DOTATATE, SPECT/CT imaging of the thorax and abdomen is performed after 4h, 24h, and 5-7d to visualize the biodistribution and to enable dosimetric analysis. Blood samples will be drawn after 2h and after each SPECT/CT for pharmacokinetic analysis and to estimate the dose to the bone marrow. HRQoL questionnaires are provided prior to PRRT and after 3 weeks.

Results:

The study was approved by the medical ethics committee in May 2024 and is now actively recruiting. At the NETRF meeting we hope to present the results from the first patients.

Conclusion:

This prospective clinical trial has been designed to study the effect of LA-SSAs on the uptake of [177Lu]Lu-HA-DOTATATE in tumors and normal tissues. We hypothesize that LA-SSAs do not negatively affect the tumor or healthy tissue absorbed dose when continued during PRRT (i.e. administered less than 4 weeks prior to PRRT). If confirmed, this would negate the need for patients to discontinue LA-SSAs multiple times during PRRT and to switch from LA-SSAs to short-acting SSAs. Furthermore, potentially this approach could increase the therapeutic window, i.e. increase the delivered dose in tumor lesions without increasing toxicity.

Insights into Intestinal Neuroendocrine Tumors in Relation to Normal Human Eec Differentiation

Pratik N. P. Singh[1,2,3], Paloma Cejas[2], James Howe[4], Matthew Kulke[1,3], Jennifer Chan[1,3], Qiao Zhou[5] and Ramesh A. Shivdasani[1,2,3,*]

1. Department of Medical Oncology and 2. Center for Functional Cancer Epigenetics, Dana-Farber Cancer Institute, Boston, MA 02215, USA. 3. Department of Medicine, Harvard Medical School, Boston, MA 02115, USA. 4. Division of Surgical Oncology and Endocrine Surgery, University of Iowa Carver College of Medicine, Iowa City, IA, USA. 5. Hartman Institute for Therapeutic Organ Regeneration, Department of Medicine, Weill Cornell Medicine, New York, NY 10065, USA. *Corresponding author: ramesh_shivdasani@dfci.harvard.edu

Presenting author: Pratik Singh, PhD

Background:

Molecular understanding of small intestinal neuroendocrine tumors (SI-NETs) is limited; a low mutational burden is characterized by few recurrent alterations, such as those that inactivate the cyclin-dependent kinase (CDK) inhibitor CDKN1B in <10% of cases. Native enteroendocrine cells (EECs), which these tumors resemble, are scarce; the lack of reference data from normal EECs limits identification of gene and cis-element dysregulation in SI-NETs.

Methods:

We established transcriptional landscapes (RNA-seq) in bulk cultures and at single-cell (sc) resolution during the trajectory of normal human EEC differentiation from ileal stem cells in vitro (PMID 38733993). We then profiled 18 SI-NET transcriptomes and considered them together with 78 previously reported SI-NET transcriptomes and published scRNA-seq data from 3 SI-NETs. To identify underlying cis-regulatory elements, we profiled chromatin accessibility by ATAC-seq (n=3 SI-NETs) and active (H3K27ac, n =10 SI-NETs) and repressive (H3K27me3, n=5 SI-NETs) histone marks using ChIP-seq. We compared the findings in SI-NETs against the novel reference profiles of normal human EEC differentiation and maturation.

Results:

SI-NET transcriptomes correlated best with those of mature enterochromaffin (EC) rather than stem or progenitor cells or other EEC types. Tumors express up to 90% of genes enriched in mature EECs alongside certain genes normally restricted to undifferentiated states. Unlike normal mature EECs, which lack genes of early precursor states, SI-NETs co-expressed selected stem/progenitor state markers and some genes normally restricted to non-EC cells in the same tumor cells. Four types of SI-NET enhancers underlie this aberrant expression pattern: those normally accessible and marked with H3K27ac only in normal stem/progenitor cells (type 1); those that remain accessible but never marked with H3K27ac during normal EEC differentiation (type 2); those ordinarily inactive in EEC differentiation (type 3 – de novo SI-NET enhancers); and those ordinarily active in both EC and non-EC cells (type 4). EECs normally differentiate through an intermediate HES6hi/ASCL1+ oscillatory state before NEUROD1+ pre-terminal cells emerge; ASCL1 expression then persists only in mature EC cells. In contrast, although SI-NETs express NEUROD1, they strictly lack ASCL1 and other genes normally co-expressed with ASCL1. Notably, ASCL1 and loci encoding transcription factors that define non-EC cell types (ISL1, ARX, PAX6, PDX1) are marked with H3K27me3, signifying epigenetic silencing. SI-NETs express CDKN1B but other CDK inhibitors, e.g., CDKN2A and CDKN1C, are epigenetically silenced, suggesting that tumor cells may especially depend on wild-type CDKN1B function.

Conclusions:

SI-NETs correspond principally to mature EC cells, with discernible features of non-EC and progenitor cells; persistent expression of the latter genes likely contributes to malignant properties. Like NETs in other tissues, where ASCL1 and NEUROD1 expression is mutually exclusive, SI-NETs express NEUROD1 but lack ASCL1, a molecular phenotype that is distinct from that of normal EC cells. Chromatin and transcriptional features of SI-NETs, revealed for the first time with respect to normal EEC differentiation, will help identify tumorigenic pathways and candidate therapeutic targets.

Delineating the Molecular Heterogeneity of Pancreatic Neuroendocrine Tumours

Zoey Wang[1,2], Areeba Qureshi[1], Nilakshi Kulathunga[1], William You[1,2], Lina Chen[3,4], Weei-Yuarn Huang[3,4], Carol Schuurmans[1,4], Housheng H. He[2,5], Iacovos P. Michael[1,2]

1. Sunnybrook Research Institute, Toronto, ON, Canada. 2. Department of Medical Biophysics, University of Toronto, Toronto, ON, Canada. 3. Sunnybrook Health Sciences Centre, Toronto, ON, Canada. 4. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada. 5. Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada.

Presenting author: Zoey Wang, MSc

Background:

Pancreatic neuroendocrine tumours (PanNETs) originate from pancreatic islets and are characterised by frequent metastasis, clinical recurrence, and high mortality rate. Although targeted therapies are available, they have limited response rates and rarely lead to complete remission. As a result, most patients eventually succumb to advanced, metastatic disease with a dismal 5-year survival rate of only 25%. There is thus an urgent need to understand the mechanisms of PanNET progression to identify novel therapeutic strategies. PanNET classification in the clinic is based on proliferative index, tumour size, presence of metastasis, and hormone-producing capacity (i.e., functional/non-functional). At the molecular level, significant heterogeneity exists in tumour mutation burden and tumorigenic pathways, which can contribute to varied response to therapies and disease prognosis. Here, we dissect the intra- and inter-tumour heterogeneity of PanNET with single-cell transcriptomic profiling.

Methods:

Single-cell RNA sequencing was performed with formalin-fixed, paraffin-embedded PanNET surgical specimens (n=24) using the Flex technology by 10x Genomics. Notably, Flex is probe-based and compatible with fixed samples. Archived samples were fixed between 2015–2021 and comprised a total of 14 primary tumours and 10 metastases (4 lymph node, 6 liver) from 14 patients (9 female, 5 male), including 3 with matched primary and metastatic tumours. The primary tumours comprised 2 insulinomas, 1 glucagonoma, 2 gastrinomas, and 9 non-functional tumours. Data analysis was performed with Seurat (R package), and included quality control, normalisation, scaling, dimensionality reduction, clustering, and cell type annotation. Differential gene expression analysis was performed on the cancer cell fraction between liver metastases and primary tumours.

Results:

A total of 151,056 single cells were captured, with 137,971 cells (91%) passing quality control. Gene signatures for different cell types (e.g., endocrine and exocrine pancreas, immune cells, fibroblasts, hepatocytes) were established based on published transcriptomic data, and used to annotate cell clusters. Significant intra- and inter-tumour heterogeneity was observed in PanNETs. On average, primary tumours demonstrated higher proportions of immune and stromal cells than liver metastases. Within the cancer cell population, heterogeneity is also seen in the expression of pancreatic endocrine markers (e.g., CHGA). Differential expression analysis identified 317 upregulated and 207 downregulated genes in liver metastases compared to primary PanNET; gene set enrichment analysis of the upregulated genes resulted in signatures related to hypoxia, cell adhesion, and cytoskeletal organisation. Interestingly, neurogenesis-related genes were also enriched, suggesting a switch in cell fate at the transcriptomic level in metastases.

Conclusions:

Here, we demonstrate Flex as a reliable and efficient technology that potentiates the use of archived, fixed samples in single-cell transcriptomics. Using this approach, we probed the intra- and inter-tumour heterogeneity of PanNET. We further identified differentially expressed genes in metastases compared to primary tumours, whose potential as prognostic markers will be examined with a PanNET tissue microarray recently established by our group. Our results also implicate hypoxia in PanNET metastases, in addition to the activation of neuronal features by cancer cells. Future work will delineate the trajectory and gene regulatory networks underlying PanNET progression and explore potential sex differences. This may illuminate novel therapeutic vulnerabilities of aggressive PanNET.

What Drives Radiation Response to Peptide Receptor Radionuclide Therapy? Insights from Whole Genome and Exome Sequencing of Pancreatic Neuroendocrine Tumors

Emma Boehm[1,2], Aidan Flynn[1], Athena Hatzimihalis[3], Annette Hogg[2], Grace Kong[2], Kelly Waldeck[3], Ben Lawrence[4,5], Christin G. Print[5,6], Joep H. A. Vissers[1], Sean M. Grimmond[1], Richard W. Tothill[1], Rodney J. Hicks[7]

1. Department of Clinical Pathology and University of Melbourne Centre for Cancer Research, The University of Melbourne, Parkville, Victoria, Australia. 2. Department of Cancer Imaging, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Victoria, Australia. 3. Models of Cancer Translational Research Centre, Research Division, Peter MacCallum Cancer Centre, 305 Grattan St, Melbourne, Victoria, Australia. 4. Department of Oncology, Faculty of Medicine and Health Sciences, University of Auckland, Auckland, New Zealand. 5. Maurice Wilkins Centre for Biodiscovery hosted by the University of Auckland, Auckland, New Zealand. 6. Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand. 7. St Vincent's Hospital Department of Medicine, The University of Melbourne, Parkville, Victoria, Australia.

Presenting author: Emma Boehm, MD

Background:

Despite ostensibly similar baseline clinical characteristics including WHO grade and degree of somatostatin receptor (SSTR) expression, response to peptide receptor radionuclide therapy (PRRT) varies widely between patients, suggesting radiation response may be governed by underlying genomic mechanisms including DNA-damage recognition and repair (DDR). Indeed, DDR inhibitors are an emerging class of agents for PRRT radiosensitization with encouraging preclinical data from our group and others. Since pancreatic neuroendocrine tumours (pNETs) have a well-defined set of mutations and are generally responsive to PRRT, this study aimed to evaluate the mutational landscape of pNET patients characterized by response to PRRT.

Methods:

PNET patients treated with PRRT who had pretreatment +/- post-treatment biopsy material suitable for genomic analysis were identified. Depending on tissue quality, whole genome or whole exome sequencing was performed and correlated with clinical response to PRRT at 3-month follow-up using RECIST1.1 and previously established molecular imaging response criteria, and progression-free survival (PFS).

Results:

45 samples from 39 pNET patients were analysed (56% female; grade at diagnosis: G1 n=7;G2 n=23; G3 n=7; unknown n=2), including PRRT-naïve samples (n=29) and post-PRRT (median 36.3GBq, range 13.4-91.6) samples (n=16). PRRT responses varied from resistant (n=5, 13%), sensitive-nondurable (PFS >3-12months, n=10), to sensitive-durable (PFS >12 months, n=21). 3 patients achieved complete molecular imaging responses and did not progress during follow-up (87, 106, 208 months respectively, exceptional responders). The somatic mutational landscape included known pNET drivers: MEN1 (n=14), ATRX (n=10), DAXX (n=11), TSC2 (n=4), KMT2C (n=7) and TP53 (n=6). There were no differences in mutational landscapes or TMB between the resistant and sensitive groups, acknowledging small comparative numbers.

Interestingly, 13/39 patients had undergone a grade change compared to initial diagnosis: G1 to G3 n=3; G2 to G3 n=9; G3 to NEC n=1 ID:B29. Eight of these patients had prior PRRT exposure (2 with concurrent temozolomide), 4 had prior temozolomide exposure as part of chemotherapy. B29 received PRRT between the well-differentiated G3NET and NEC study samples, both samples had the same TP53 and RB1 mutation, and RB1 loss was confirmed by IHC in the pre-PRRT G3NET diagnostic sample. No predominant mutational signature, including SBS11, nor MLH1/MSH6 mutation, was identified in the transformed samples.

Eight patients had paired pre- and post-PRRT samples: 6 of these patients had subsequent PRRT with median PFS declining from 9.5 to 6 months. Following PRRT no recurrent emergent mutations were identified. Nevertheless, across the cohort, compared with PRRT-naïve samples, post-PRRT samples were enriched for mutational signature ID8 (p =0.0038), which is potentially associated with double-stranded DNA break repair by non-homologous end joining (NHEJ).

Conclusions:

While we did not observe emergence of DNA mutations to explain high-grade transformation or resistant clinical behaviour following PRRT, surviving cells post-PRRT seemed to be enriched for clones with NHEJ. This may support our recent NETRF-funded demonstration that CRISPR knockdown of key genes regulating NHEJ, including DNA-PK and Artemis, renders cells more sensitive to PRRT. A multi-omic approach including gene expression and methylation analysis will be undertaken in future to address outstanding questions regarding PRRT response in NET.

Identifying Regulators of GEP-NET Metastasis with in vivo CRISPR Screen

William You[1,2], Zoey Wang[1,2], Nilakshi Kulathunga[2], Lina Chen[2,3], Weei-Yuarn Huang[2,3], Simron Singh[2], Chris Lowden[4] and Daniel Schramek[4], Iacovos P Michael[1,2]

1. Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada. 2. Sunnybrook Research Institute, Toronto, Ontario, Canada. 3. Department of Laboratory Medicine and Pathology, University of Toronto, Toronto, Ontario, Canada. 4. Lunenfeld-Tanenbaum Research Institute, Sinai Health, Toronto, Ontario, Canada.

Presenting author: William You, HBSc

Background:

Gastroenteropancreatic neuroendocrine tumours (GEP-NETs) are rare, heterogeneous, and aggressive malignancies, with many patients diagnosed in advanced stages with liver metastasis. While we and others have previously implicated various pathways in NETs metastasis, such as the miR-23b cluster/ALK7 axis, the mechanisms underlying GEP-NET metastasis remain poorly understood. Herein, we used an unbiased and systematic approach to identify pathways involved in metastasis. Specifically, we used GEP-NET patient samples available at the Sunnybrook Research Institute to identify differentially expressed genes (DEGs) in liver metastases compared to primary tumours. We hypothesize that these genes are functionally implicated in GEP-NET liver metastasis.

Methods:

To identify DEGs, we performed single-cell RNA sequencing on clinically annotated formalin-fixed paraffin-embedded samples from patients with small intestine NETs (SINETs) and pancreatic NETs (PanNETs). To validate the potential regulators of GEP-NET liver metastasis, we will use in vivo CRISPR knockout and activation (CRISPRko and CRISPRa, respectively) screens targeting these candidate DEGs. First, we will use CRISPRko to switch off the potential repressors of GEP-NET metastasis (downregulated genes in metastasis) and use CRISPRa to switch on the potential drivers of GEP-NET metastasis (upregulated genes in metastasis) in metastatic GEP-NET model cell lines. Then, we will develop experimental metastasis mouse models via intrasplenic injections of the genetically modified cells and assess the gRNA library representation in the liver metastases at the endpoint. Compared to the original library representation in the cell pool, gRNAs enriched in CRISPRko and CRISPRa screens will respectively represent potential anti-metastatic and pro-metastatic genes. Finally, we will validate the role of the most prominent pro-metastatic gene and elucidate its mechanism of action by both in vivo and in vitro loss-of-function assays.

Results:

Our single-cell RNA sequencing data have identified 413 DEGs in SINET metastases and 524 DEGs in PanNET metastases compared to primary tumors, with 29 DEGs common to both. Many of these genes are associated with metastasis and poor prognosis in NET patients. To prepare for in vivo CRISPR screens, we have established stable Cas9-expressing GEP-NET model cell lines, verified their Cas9 functionality, and confirmed the metastatic capabilities of these cell lines in SCID mice through intrasplenic injection. For the next step, we will infect these cells with the respective gRNA libraries and perform intrasplenic injections to conduct the CRISPRko and CRISPRa enrichment screens in SCID mice. Following the identification of hits from the CRISPR screens, we will verify the role of the most prominent pro-metastatic gene by loss-of-function assays.

Conclusion:

In conclusion, by characterizing the regulators of GEP-NET metastasis using in vivo CRISPR knockout and activation screens, we aim to unravel the mechanisms underlying GEP-NET progression and facilitate the development of novel therapeutics, ultimately improving patient outcomes and quality of life.

Spatial Transcriptomics of Multifocal Ileal Neuroendocrine Tumors Reveals Tumor Heterogeneity based on Tumor Microenvironment and New Biomarkers

Akitada Yogo[1,2], Naoki Akanuma[3], Grace E. Kim[3], Chrissie Thirwell4, Netta Mäkinen[5,6], Matthew Meyerson[5,6,7] and Eric K. Nakakura[1,2]

1. Division of Surgical Oncology, Section of Hepatopancreaticobiliary Surgery, Department of Surgery, University of California, San Francisco, San Francisco, California, USA. 2. Helen Diller Family Comprehensive Cancer Center, San Francisco, California, USA. 3. Department of Pathology, University of California, San Francisco, CA, USA. 4. Bristol Medical School, University of Bristol, Bristol, UK. 5. Department of Medical Oncology, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA, USA. 6. Cancer Program, Broad Institute of Harvard and MIT, Cambridge, MA, USA. 7. Departments of Genetics and Medicine, Harvard Medical School, Boston, MA, USA.

Presenting author: Akitada Yogo, MD, PhD

Background:

Ileal neuroendocrine tumors (i-NETs) are characterized by a high incidence of multiple primary tumors (>30-40%) and production of serotonin/other hormones. Recent whole genome sequencing analyses revealed an absence of shared somatic variations among synchronous primary tumors, so the mechanisms underlying multifocal tumor development are not known. In this study, we evaluated gene expression patterns of multifocal i-NETs with spatial resolution in order to develop new hypotheses about tumorigenesis focusing on the tumor microenvironment. b. Methods – FFPE blocks of surgically resected specimens from 4 patients with multifocal i-NETs were used. Tissue microarrays were constructed from 72 cores (18 one-mm cores per patient). Spatial gene expression libraries were constructed using Visium v1 (10x Genomics). A total of 8,295 spots were analyzed: a median of 3,102 genes, 5,944 UMI counts per spot, and a total of 16,778 genes were detected in each capture area. R packages including Seurat, clusterProfiler, and monocle3 were used for data analysis. c. Results – Spatial transcriptomics analysis reliably captured spatial information of malignant and non-malignant cells in distinct tissue compartments within the ileum and regional lymph node/mesenteric masses. Unsupervised clustering demonstrated differences of the i-NET in various microenvironments--mucosa, submucosa, muscularis propria, and intranodal/perinodal regions of the lymph node/mesenteric masses. In all 4 patients, gene expressions of tumor cells in the mucosa were similar among multifocal tumors while tumor cells in other microenvironments clustered separately. Trajectory analysis was consistent with the supposition that tumors in the mucosa are likely the origin of i-NETs found in the other microenvironments. Tumor cells in all microenvironments exhibited characteristic gene expression patterns of serotonin receptors (HTR1B, HTR1D, and HTR7), ghrelin receptor (GHSR), GIP receptor (GIPR), and several gene sets found by over-representation or enrichment analysis. d.Conclusions – This is the first spatial transriptomics analysis of multifocal i-NETs revealing similarities among tumors located in the mucosa and distinct clustering of tumors situated in other microenvironments. The finding that tumor heterogeneity in multifocal i-NETs varies depending on the microenvironment has not been previously described. The spatial data also reveal known, as well as, new biomarkers of i-NETs. In particular, we identified serotonin and other hormone receptors—some of which may be tumor specific--suggesting possible endocrine/paracrine signaling in i-NETs.

Expression of Bromodomain and Extra-Terminal (Bet) Proteins in Pancreatic Neuroendocrine Tumors and Mechanistic Implications of Bet Bd1- and Bd2-Selective Inhibition

Omair A. Shariq[1,2], Kate E. Lines[1], Katherine A. English[1], Inmaculada Rioja[3], Andreas Selberherr[4], Ann Oberg[5], Rabinder Prinjha[3], Duncan Richards[6] and Rajesh V. Thakker[1]

1. Academic Endocrine Unit, Radcliffe Department of Medicine, University of Oxford, OCDEM, Churchill Hospital, Oxford, UK. 2. Department of Surgery, Mayo Clinic, Rochester, MN. 3. Epigenetics Discovery Performance Unit, GlaxoSmithKline, Stevenage, UK. 4. Department of Surgery, Medical University of Vienna, Vienna, Austria. 5. Department of Quantitative Health Sciences, Mayo Clinic College of Medicine, Rochester, MN. 6. Botnar Research Centre, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Medicine, University of Oxford, UK.

Presenting author: Omair Shariq, MD, PhD

Background:

Effective treatments for metastatic pancreatic neuroendocrine tumors (PNETs) are scarce. We previously showed that inhibiting bromodomain and extra-terminal (BET) epigenetic reader proteins via non-selective targeting of their two bromodomains (BD1 and BD2) reduces proliferation in preclinical PNET models. However, the clinical relevance of BET proteins in PNETs and the functional differences between BD1 and BD2 are unclear. Here, we characterized BET expression in human PNETs and performed efficacy and mechanistic studies using next-generation BD1- (BD1i) and BD2-selective (BD2i) BET inhibitors.

Methods:

BET protein expression was evaluated by immunohistochemistry in functioning and non-functioning human PNETs (n=52) versus normal adjacent islets (n=18). RNA sequencing (RNA-seq) and gene set enrichment analyses (GSEA) were performed in an independent human PNET cohort (n=46) in order to identify biological pathways upregulated in metastatic versus non-metastatic tumors. Effects of BD1i and BD2i on viability, apoptosis, and cell cycle progression were assessed in three PNET cell lines (BON1, QGP1, MIN6). Effects on cell viability were additionally evaluated in ex vivo neoplastic islets derived from 6-month-old Men1L/L/RIP2-Cre mice, where PNET development is driven by Men1 knockout specifically in pancreatic beta cells. Early (6h) and late (48h) transcriptional responses to BET inhibition were examined in vitro using thiol(SH)-linked alkylation for the metabolic sequencing of RNA (SLAM-seq) and standard RNA-seq, respectively, in BON1 cells. Epigenomic responses were also explored using chromatin immunoprecipitation sequencing (ChIP-seq) for the active transcription histone mark H3K27ac.

Results:

All three BET proteins (BRD2, BRD3, and BRD4) were overexpressed by 1.5-2.5-fold in human PNETs versus normal pancreatic islets ($p<0.01$). GSEA showed enrichment of BET-regulated gene sets in metastatic tumors (normalized enrichment score 1.31, adjusted $p=0.04$). BD2i marginally affected cell viability (half-maximal inhibitory concentration [IC50] 55-90 μM), apoptosis, and cell cycle profile, while BD1i had a moderate impact on all phenotypes (IC50 1-1.5 μM). Synergistic effects were observed when BD1i and BD2i were combined (IC50 0.2-0.5 μM). In ex vivo cultured neoplastic islets, viability was reduced by <30% with BD2i ($p<0.005$), ~40% with BD1i ($p<0.0001$), and ~60% with BD1i+BD2i ($p<0.0001$). RNA-seq and SLAM-seq revealed transcriptional changes that were minimal with BD2i (n=73 and n=10 differentially expressed genes, respectively), modest with BD1i (n=354 and n=60 genes), and greatest with BD1i+BD2i combined (n=1739 and n=375 genes). Integrated transcriptomic and H3K27ac ChIP-seq analyses revealed the cell cycle-related genes E2F2 and CKAP2L as early and late direct BET inhibitor targets, respectively, both of which were only downregulated when BD1i and BD2i were combined. In human PNETs, high E2F2 and CKAP2L mRNA expression were associated with shorter progression-free survival after surgery (22 vs 120 months, $p=0.048$; 23 vs 105 months, $p=0.03$, respectively).

Conclusions:

Our studies confirm BET proteins as clinically relevant targets in human PNETs and show that dual (BD1+BD2) inhibition has a synergistic effect on efficacy. These findings have important implications for the clinical application of next-generation epigenetic modulators. Furthermore, E2F2 and CKAP2L emerge as key BET inhibitor targets with prognostic value, suggesting their importance in PNET pathogenesis and as predictors of treatment response.

Establishment of Patient-Derived Pre-clinical Models for Evaluating Therapy Response in Gastroenteropancreatic Neuroendocrine Tumors (GEP-NETs)

Nilakshi Kulathunga[1], Zoey Wang[1,2], Alberto Lens-Pardo[3], Rusha Chakraborty[1], Areeba Qureshi[1], Emmanuel Cherin[4], Christine E. Demore[2,4], Hubert Tsui[1,5], Julie Hallet[6,7], Calvin Law[6,7], Simron Singh[6,8], David Andrews[1,9], Hon S. Leong[1,2], and Iacovos P. Michael[1,2]

1. Biological Sciences Platform, Sunnybrook Research Institute, Toronto, ON. 2. Department of Medical Biophysics, University of Toronto, Toronto, ON. 3. Centro de Oncología Experimental, Grupo de Investigación en Tumores Gastrointestinales y Neuroendocrinos, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), CNIO, Madrid, Spain. 4. Physical Sciences Platform, Sunnybrook Research Institute, Toronto, ON. 5. Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre, Toronto, ON. 6. Clinical Evaluative Sciences, Sunnybrook Research Institute, Toronto, ON. 7. Department of Surgery, University of Toronto, ON. 8. Department of Medicine, University of Toronto, 9. Department of Biochemistry, University of Toronto, Toronto, ON, Canada.

Presenting author: Nilakshi Kulathunga, PhD

Background:

Neuroendocrine tumors (NETs) are rare tumors originating from hormone-producing cells dispersed throughout the body, mainly in the gastrointestinal (GI) tract and pancreas, termed Gastroenteropancreatic NETs (GEP-NETs). They exhibit significant heterogeneity in etiology, morphology, and behavior. Early diagnosis of GEP-NETs is challenging, as symptoms often emerge at advanced metastatic stages. Late-stage diagnosis and low prevalence complicate clinical trials. Therefore, pre-clinical models are essential to understanding GEP-NET biology and assessing therapies. This study established two human pre-clinical models: patient-derived organoids (PDOs) and patient-derived xenografts ex-ovo (PDXovo), and therapeutic responses were evaluated. Ultimately, these tools facilitate determining patient-specific therapy decisions to improve clinical outcomes.

Methods:

The study was approved by the Research Ethics Board of the Sunnybrook Health Sciences Centre, and written consent was obtained from patients. Fresh tissue samples (blood and tumor) were collected with medical oncologists and surgical oncologists at the Odette Cancer Centre (OCC) at Sunnybrook Hospital. Fresh tissue samples from primary and metastatic NETs were directed to the lab for PDO and PDX establishment, while the remaining tissue was snap-frozen and bio-banked. PDOs (3D cultures) are derived from these samples cultured in hydrogels for drug testing. The drug response was assessed using image-based High-Content Cellular Analysis (HiCCA). In the PDXovo model fresh tumor samples were engrafted onto the chorioallantoic membrane (CAM) of fertilized avian eggs. After seven days of engraftment or drug treatment, tumor volume and vascularity were measured using ultrasound. Tumors were then harvested for immunohistochemistry, western blot, and flow cytometry.

Results:

PDOs and PDXovos were established with a success rate of >95% and a take rate of >90%, respectively. Our data show that PDOs from liver metastasis grow at a higher rate compared to the corresponding primary tumors and respond differently to various treatments. Ultrasound scanning of PDXovos revealed successful tumor growth and increased vascularity. We established over 100 PDXovos from 9 different patients, originating from 19 different primary tumors (including small intestine, pancreas, and cecum) and metastases (lymph node and liver). PDXovos are characterized by immunostaining for tumor-specific neuroendocrine (chromogranin and the somatostatin receptor SSTR2) and other microenvironment markers. Overall, our analysis confirmed that the histology of PDXovos resembles human patient tumors.

Conclusion:

The significance of this study is the establishment of two patient-derived preclinical models for evaluating GEP-NET therapy. PDO model offers a high-throughput approach for drug screening, while PDXovo provides a cost-effective and efficient method for further evaluation. Furthermore, implementing the biobank resulted in the collection of a repository comprising over 100 specimens of fresh frozen tissue sections. PDOs and PDXovos are now being used for further drug testing, including radiation therapy, mainly Protein Receptor Radiotherapy (PRRT);177Lu-DOTATATE, and other types of treatments, such as tyrosine kinase inhibitors and immunotherapy. In conclusion, the established PDO/PDXovos preclinical models will enable us to characterize the response to existing therapies and identify novel combination therapies to improve the patient's overall survival.

Innovative Patient-derived Models of Neuroendocrine Neoplasms: Autologous ECM-composed Scaffolds and Zebrafish Xenografts

Chiara Liverani[1], Chiara Calabrese[1], Sofia Dellavalle[1], Giacomo Miserocchi[1], Chiara Spadazzi[1], Claudia Cocchi[1], Alessandro De Vita[1], Silvia Vanni[1], Sofia Gabellone[1], Oneda Cani[1], Lucas Moron Dalla Tor[2], Federica Pieri[3], Giorgio Ercolani[4], Carlo Alberto Pacilio[4] and Nicoletta Ranallo[5]

1. Preclinic and Osteoncology Unit, Bioscience Laboratory, IRCCS Istituto Romagnolo Per Lo Studio Dei Tumori (IRST) "Dino Amadori", Meldola, Italy. 2. Department of Medicine and Surgery, University of Parma, Parma, Italy, 3. Pathology Unit, Morgagni-Pierantoni Hospital, Forlì, Italy. 4. General and Oncologic Surgery, Morgagni-Pierantoni Hospital, Forlì, Italy. 5. Clinical and Experimental Oncology, Immunotherapy, Rare Cancers and Biological Resource Center, IRCCS Istituto Romagnolo Per Lo Studio Dei Tumori (IRST) "Dino Amadori," Meldola, Italy.

Presenting author: Chiara Liverani, PhD

Background:

Neuroendocrine neoplasms (NENs) are a subgroup of poorly characterized and highly heterogeneous rare tumors with challenging management. High-priority unmet needs include the identification of biomarkers for patient stratification and prediction of clinical outcomes. However, the paucity of reliable NEN preclinical models has represented a barrier for the discovery of driver molecular alterations associated with disease pathogenesis, progression and responsiveness to anticancer agents. This study proposes the use of two innovative near-patient models based on autologous extracellular matrix (aECM)-scaffolds and zebrafish (ZF) xenografts integrated with next generation sequencing analysis for the phenotype- genotype investigation of primary NEN cells. The aim is to elucidate the molecular mechanisms that drive proliferation and aggressiveness of NEN cells.

Method:

Primary tumor cells were isolated from surgical specimens of patients with NEN (all grades and sites of origin) and cultured in aECM scaffolds or injected into ZF embryos. We used the aECM model to characterize tumor cells in terms of proliferation rate, morphology and metabolic features. In ZF we characterized cell engraftment ability, local or distant invasiveness and angiogenesis. The transcriptome profile of each tumor tissue was analyzed by RNA sequencing using healthy matched samples as references. Data from in vitro and in vivo screenings will be used to cluster tumors according to indolent vs aggressive status. Sequencing of the tumor clusters will define a potential signature for tumor aggressiveness.

Results:

Twentythree gastroenteropancreatic-NEN patients and five Merkel cell carcinoma (MCC) patients have been enrolled in the study. Primary cells were used to establish and optimize the proposed models. Specifically, we demonstrated that: i) aECM scaffolds recreate a tissue-like context and preserve NEN cell morphology and differentiation; ii) ZF xenografts efficiently recapitulate the processes of tumor invasiveness and angiogenesis. By deriving cells from both the primitive and the metastatic lesions of an ileal NEN patient, we also demonstrated that ZF xenografting reports NEN metastatic potential. We observed that, when injected into ZF embryos, cells derived from the primitive tumor induce distant localization in the 23% of embryos, while cells derived from the metastatic lesion in the 79%. Moreover, cells isolated from patients with aggressive MCCs showed high invasive rates and angiogenic ability in ZF. RNA-sequencing of these MCC specimens highlighted the involvement of the following pathways: ECM remodeling, epithelial-mesenchymal transition and TNF-□ and PI3K-Akt signaling.

Conclusions:

Here we developed two innovative near-patient NEN models coupled with omics analysis that are expected to generate new knowledge on the disease underlying molecular determinants. This study will be beneficial for the development of NEN prognostic signatures and for the discovery of potential disease vulnerabilities.

Loss of DAXX and ATRX Protein Expression Results in Increased Radiosensitivity and Ischemic Resistance of Bon-1 and QGP-1 Cells

Jessica C. Puzzuoli[1], Caleb Solivio[2], Christopher M. Heaphy[3,4], Mindy K. Graham[4], Anthony J. Rizzo[4], Diane Reidy[1], Eric Chan[1], James Russell[1], Etay Ziv[1]

1. Memorial Sloan Kettering Cancer Center. 2. California University of Science and Medicine. 3. Boston University Chobanian & Avedisian School of Medicine. 4. The Johns Hopkins University School of Medicine.

Presenting author: Jessica C. Puzzuoli, PhD

Background:

Well-differentiated pancreatic neuroendocrine tumors (PNETs) often present late with unresectable liver metastases. These are often treated with liver-directed therapies including ischemia-based trans arterial embolization (TAE) or radioembolization (TARE). DAXX and ATRX mutations are relevant in PNETs. Our previous clinical data has shown poor response to TAE in DAXX-mutated PNETs; however, an association with response to TARE has not been reported. The purpose of this study was to evaluate the effect of loss of DAXX or ATRX protein expression on radiosensitivity and ischemic sensitivity in two PNET cell line models and compare TARE responses in patients with and without DAXX and ATRX mutations.

Methodology:

CRISPR/Cas9-generated knockouts (KOs) were made in two PNET cell lines: DAXX KOs were derived from Bon-1 cells (C16, C45) and ATRX KOs were from QGP-1 cells (QAX12, QAX24). Cells were treated in normal conditions or ischemia and tested for viability at different timepoints. Cells were irradiated with varying doses and colony formation was counted. To assess apoptosis, a Caspase-3 assay was performed for ischemia and radiation. The cohort study was approved by the IRB at MSKCC. Consecutive participants with PNET who underwent TARE were identified retrospectively (n=20) and clinical and treatment covariates were recorded as well as DAXX/ATRX mutation status. Time to local progression was measured and competing risk analysis was performed using Grey test.

Results:

All KOs demonstrated increased viability in comparison to the Bon-1 and QGP-1 cells at days 3 and 5 post-ischemia. Bon-1 demonstrated increased apoptotic activity compared to C16 and C45 cells at days 1, 3, and 5 post-ischemia ($p<0.0001$). All KOs exhibited greater sensitivity to radiation treatment in comparison to the Bon-1 and QGP-1 cells. Bon-1 and QGP-1 cells had increased survival fraction post-radiation ($p<0.0001$). Moreover, C16 and C45 cells demonstrated higher apoptotic activity than Bon-1 at 48 and 72 hours post radiation ($p<0.0001$). There were 11/20 (55%) patients with a DAXX/ATRX mutation. The median ki67 was 19% (IQR: 18%), there were 11/20 (55%) grade 2 and 9/20 (45%) grade 3 tumors, 6/30 (30%) had greater than 50% tumor burden, and most patients had prior treatments with PRRT (60%), TAE (75%), or chemotherapy (65%). We did not detect any differences between wild type and DAXX/ATRX mutant patients in terms of age, grade, tumor burden, prior treatments or dose delivered. Competing risk analysis showed longer time to local progression after TARE in patients with DAXX/ATRX mutations ($p < 0.001$). The median time to local progression after TARE was 8 months in DAXX/ATRX wild type patients compared with 24 months in patients with a DAXX/ATRX mutation.

Conclusion:

In correlation with our clinical data from patients treated with TAE and TARE, loss of DAXX and loss of ATRX protein expression in their respective PNET cell lines resulted in increased ischemic resistance and increased radiosensitivity compared with Bon-1 and QGP-1 wildtype cell lines. Current and future studies include investigation of signaling pathways contributing to these outcomes as well as the cellular mechanisms involved.

mTORC1-ATF4 Signaling Drives Amino Acid Transport in PanNETs

Scott A. Oakes[1,3,4], Greg Malnassy[1], Diane Silva[1], Anjali Kotamarthi[1], Tatsuki Ueda[2], Nicolas Chevrier[2]

1. Department of Pathology, University of Chicago, Chicago, IL, USA, 2. Pritzker School of Molecular Engineering, University of Chicago, Chicago, IL, USA, 3. Committee on Cancer Biology, University of Chicago, Chicago, IL, USA, 4. Committee on Molecular Metabolism and Nutrition, University of Chicago, Chicago, IL, USA.

Presenting author: Scott Oakes, MD

Background:

Based on its efficacy in clinical studies, the mTORC1 inhibitor everolimus (Afinitor®) was FDA approved in 2011 for patients with advanced pancreatic neuroendocrine tumors (PanNETs); however, its benefits are relatively modest with a ~6-month average increase in progression-free survival and no profound impact on disease-free survival. To more effectively utilize this treatment, it is critical to elucidate the key signaling outputs of the mTOR pathway on PanNET tumorigenesis and the escape mechanisms that allow tumor cells to resist mTORC1 inhibition.

We previously discovered that PanNETs often upregulate the transcription factor ATF4, which is best known as the primary effector of the integrated stress response (ISR), but was also recently shown to be downstream of mTORC1 signaling in some cancers. Under conditions where amino acids are limiting, ATF4 transcriptionally upregulates genes that promote amino acid transport and biosynthesis. However, the connection (if any) between mTORC1, ATF4 and amino acid supply has not been extensively studied in PanNETs.

Methods:

In order to elucidate the potential mechanisms behind everolimus resistance, we have extensively analyzed ATF4 expression and target genes in BON-1 and QGP-1 human PanNET cell lines before and after a timecourse of everolimus treatment. In addition, we have generated everolimus resistant BON-1 and QGP-1 cell lines by culturing them in the presence of clinically relevant concentrations of everolimus over several months and have subjected them to RNA-Seq analysis to identify differentially expressed genes and potentially modulated pathways.

Results:

We identified SLC1A5, a glutamine transporter, as one of the most highly upregulated ATF4 targets in PanNETs. We find that the expression of SLC1A5 rapidly falls in both QGP-1 and BON1 cells following mTORC1 inhibition, and that forced overexpression of ATF4 strongly upregulates SLC1A5. We show that shRNA-mediated knockdown of SLC1A5 leads to remarkable decreases in QGP-1 and BON1 cell proliferation when compared to cells expressing a scrambled shRNA control. To confirm the dependency of PanNET cell lines on glutamine intake, we cultured QGP-1 and BON1 in glutamine-depleted media and observed profound decreases in cell proliferation. Additionally, treatment with a selective SLC1A5 inhibitor, V-9302, produces dose-dependent decreases in QGP-1 and BON1 growth as assessed by colony formation and cell proliferation assays. Furthermore, SLC1A5 is significantly upregulated in everolimus-resistant QGP-1 and BON1 cell lines, both of which remain equally sensitive to V-9302 treatment when compared to parental cell lines. Given that our PanNET cell lines are unable to grow in vitro when SLC1A5 is knocked down, we recently designed QGP-1 and BON1 cells containing doxycycline-inducible shRNA constructs against SLC1A5 and are testing their ability to grow as xenografts in immunocompromised mice.

Conclusions:

The TORC1 signaling pathway in PanNETs promotes expression of the ATF4 transcription factor and its downstream target the SLC1A5 glutamine transporter. Everolimus resistant PanNET cell lines demonstrate upregulation of ATF4 in the absence of mTORC1 signaling, likely due to activation of the integrated stress response (ISR). Studies are ongoing to test the role of ATF4 and SLC1A5 in mTORC1 inhibitor resistance in PanNET cell lines and mouse models.

Reconstructing the Evolutionary History of Neuroendocrine Tumor Subtypes

Nicolas Alcala[#,$1], Alexandra Sexton-Oates[#1], Emilie Mathian[#1], Noah Candeli[2], Alex Di Genova[3], Catherine Voegele[1], Zhaozhi Li[4], Maike L. Morrison[5], Jaehee Kim[4], Talya Dayton[2], Matthieu Foll[$1], Lynnette Fernandez-Cuesta[$1]

# These authors contributed equally; $ These authors jointly supervised this work. 1. Rare Cancers Genomics Team, Genomic Epidemiology branch, International Agency for Research on Cancer/World Health Organization, Lyon, France. 2. European Molecular Biology Laboratory (EMBL) Barcelona, Barcelona, Spain. 3. Instituto de Ciencias de la Ingeniería, Universidad de O'Higgins, Rancagua, Chile. 4. Department of Computational Biology, Cornell University, Ithaca, NY, USA. 5. Department of Medicine, Stanford University, Stanford, CA, USA.

Presenting author: Nicolas Alcala, PhD

Background:

We showed that lung neuroendocrine tumors (NETs) are subdivided into subtypes that have strikingly different tumor microenvironments (TMEs) (Alcala et al. 2019). Carcinoids A1 have good prognosis, are biased toward female patients and have the most dendritic cell infiltration; A2 also have good prognosis and are biased toward younger patients; B have worse prognosis, are biased towards male patients and have the most monocyte infiltration; supra-carcinoids have the cell morphology of carcinoids but the molecular profile and abysmal prognosis of large-cell neuroendocrine carcinomas, and high neutrophil and macrophage infiltration. Our reanalyses of 241 published transcriptomes of gastroentoropancreatic and intestinal NETs shows that tumors from distinct body sites form distinct homogeneous groups based on the body site. We hypothesize that the TME of NETs influence their evolution toward the different molecular subtypes.

Methods:

We are combining data from our lungNENomics cohort (whole-genome sequencing data for n=76 lung NETs) and publicly available cohorts of NETs from other organs (gastrointestinal NETs for n=13 from Makinen et al. 2022 and n=11 from Elias et al. 2021, and pancreatic NETs n=102 from Scarpa et al. 2017). We are reconstructing the evolutionary trajectory of driver events events (gene amplifications, SNVs, indels) to find phylogenies that preferentially lead to a given subtype. In parallel, we are using RNA-seq to quantify cell types from the TME through deconvolution, and identify the viral and microbial biota in the TME. Finally, we are assessing the influence of the TME on NET evolution by combining WGS and RNA-seq data to find expressed neoantigens and assess whether they were subject to natural selection using the ratio of nonsynonymous to synonymous mutations.

Results:

We have developed a set of bioinformatic tools to perform comprehensive characterizations of tumors and their TME and applied them to lung NETs. Preliminary results indicate that lung NET molecular group A1 evolve through specific evolutionary trajectories involving unique driver alterations or chromosome losses; groups A2 and B also each have specific driver alterations but also a common trajectory involving chromosome gains suggesting a possible common origin of the groups. Supra-carcinoids seem to be able to evolve through multiple independent trajectories. We found very few neoantigens in lung NETs, as well as very low levels of immune infiltration especially in groups A1, A2, and B. Nevertheless, tumors from all molecular groups show signs of immune edition suggesting that patient's immune systems did recognize and significantly kill tumor cells during tumor development. Supra-carcinoids harbour the greatest mutational and neoantigens burden, and also a specific immune and stromal-rich TME that may provide the immunosuppressive environment necessary for the tumor to escape patients' immune systems in spite of their higher immunogenicity.

Conclusion:

The tools developed for the project are now ready to be used on NETs from all body sites. Results are expected to reveal the role of the immune system in shaping tumor evolution across NETs, hopefully informing our understanding of NET development, and thus prevention and future efforts to develop targeted immunotherapies.

Preliminary Analysis of the Whole Exome Sequencing Data of 16 Cases with Neuroendocrine Cell Hyperplasia for Classification as Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH)

Daniel T. Merrick[1], Hui Yu[1], Thomas Danhorn[1], Nathaniel Pakosz[1], Tami Bang[2] and York E. Miller[1,3]

1. University of Colorado Anschutz Medical Campus, Aurora, CO, USA. 2. National Jewish Health, Denver, CO, USA. 3. Rocky Mountain Regional Veterans Affairs Medical Center, Aurora, CO, USA.

Presenting author: Hui Yu, MD, PhD

Background:

Diffuse Idiopathic Pulmonary Neuroendocrine Cell Hyperplasia (DIPNECH) is a condition characterized by bilateral multiple foci of neuroendocrine cell hyperplasia (NECHs) involving the medium or small airways. The disease is associated with airway obstruction that may lead to respiratory failure. The NECH foci in many cases can progress to invasive carcinoid tumors at multiple sites. In this study, we have employed whole exome sequencing (WES) to explore our hypothesis that DIPNECH results from a somatic mutation affecting pulmonary neuroendocrine cells resulting in proliferation and wide dispersal within the bronchial epithelium.

Methods:

A cohort of 60 patients was identified in the University of Colorado Cancer Center pathology archive. Cases with adequate tissue have been classified as definite DIPNECH, possible DIPNECH and non-DIPNECH based on the clinical chart review, radiographic and histologic review. For all cases, two or more foci of NECH, the associated carcinoid tumor and/or tumorlet, if present, and normal lymph node or lung tissue were micro-dissected from formalin fixed paraffin embedded (FFPE) tissue. WES was performed and two assessments were carried out to identify potential shared variants. Identical variants that were present in more than one specimen were identified and assessed for presence in multiple lesions within a patient to indicate clonal relationships. An additional inter-case comparison across patients identified genes or gene families that showed missense or other coding variants in more than one patient.

Results:

Of the 54 cases with complete histologic and clinic-radiologic review, 31 cases were identified as definite DIPNECH, 15 cases are possible DIPNECH, and 8 cases of non-DIPNECH. An interim analysis of sixteen cases has multiple NECH lesions and/or carcinoid tumorlet, or invasive carcinoid tumor allowing for comparisons of clonal relatedness of lesions within a patient. The WES data of these 16 cases showed two somatic variants that were shared across multiple lesions within a subset of DIPNECH cases providing evidence of clonal relatedness of spatially separate lesions within patients. A promoter region variant in CNPY3 was present in all lesions of three different patients. An intronic variant in TLN2 was seen in three patients including one in which it was present in all tested lesions. For the inter-case common variants analysis, 96 genes showed potentially function altering variants common in two or more cases including 65 genes with variants in ≥ 3 cases. In several instances more than one gene from a gene family demonstrated these types of somatic mutations. Included amongst these were variants in CSMD3 and several NBPF family genes that are involved in neural function and tumorigenesis, DNA repair genes BRCA1 and MSH6, pro-proliferative/anti-apoptotic PRAME family genes and chromatin remodeling SMARC family genes.

Conclusions:

The findings from the initial analysis of 16 cases suggests there may be a common variant present in lesions within some patients and potential drivers of the neuroendocrine lesions in DIPNECH may be present, although a single dominant specific driver is not yet apparent in the preliminary evaluation. The analysis of structural variants of WES data is ongoing. Preparation of the remaining 35 cases for WES analyses is in progress. Twenty-four additional cases of sporadic carcinoid tumor are also being prepared for WES and comparison to DIPNECH associated premalignant and invasive neuroendocrine lesions.

Assessment of the Current and Emerging Criteria for the Histopathological Classification of Lung Neuroendocrine Tumours in the LungNENomics Project

Matthieu Foll[1], Emilie Mathian[1,2], Youenn Drouet[3,4], Alexandra Sexton-Oates[1], Mauro Papotti[5], Giuseppe Pelosi[6], Jean-Michel Vignaud[7,8], Luka Brcic[9], Audrey Mansuet-Lupo[10], Nicolas Alcala[1], Sylvie Lantuejoul[11], Lynnette Fernandez-Cuesta[1]

1. Rare Cancers Genomic Team, Genomic Epidemiology Branch, International Agency for Research on Cancer (IARC-WHO), Lyon, France. 2. Department of Mathematics and Informatics, Ecole Centrale de Lyon, France. 3. UMR CNRS 5558 LBBE, Claude Bernard Lyon 1 University, Villeurbanne, France, 4. Prevention & Public Health. Department, Centre Léon Bérard, Lyon, France. 5. Department of Oncology, University of Turin, Italy. 6. Department of Oncology and Hemato-Oncology, University of Milan, Italy. 7. Department of Biopathology Institut De Cancérologie de Lorraine (CHRU-ICL), France. 8. University Hospital of Nancy (CHRU), France. 9. Diagnostic and Research Institute of Pathology, Medical University of Graz, Austria. 10. Department of Pathology, Hôpital Cochin, AP-HP, Université de Paris, France. 11. Department of Biopathology, Centre Léon Bérard & Pathology Research Platform, Cancer Research Center of Lyon, France.

Presenting author: Matthieu Foll, PhD

Background:

The 2021 WHO classifies Lung neuroendocrine tumours (LNETs) into typical and atypical carcinoids (TC and AC). Accurate diagnosis is critical due to increased risk of metastatic disease and relapse in AC. However, recent analyses have identified clinically and biologically meaningful subgroups that do not directly correspond to this classification. Unsupervised multi-omics analysis has identified three molecular groups (A1, A2, B) and uncovered a new aggressive entity, the supra-carcinoids.

Introduction:

The lungNENomics project analysed 259 lung neuroendocrine tumours (NETs) reviewed by six expert pathologists. Patients were diagnosed based on the 2021 WHO criteria, with atypical carcinoids defined by the presence of focal necrosis and/or two to ten mitoses per 2 mm^2. We investigated two markers of tumour proliferation: the Ki-67 index and phospho-histone H3 (PHH3) proteins expression, quantified by pathologists and automatically via deep learning. Additionally, an unsupervised deep learning algorithm was trained on scanned haematoxylin and eosin whole-slide images to uncover previously unnoticed morphological features with diagnostic value.

Results:

The accuracy in distinguishing typical from atypical carcinoids is hampered by inter-observer variability in mitotic counting and the limitations of morphological criteria in identifying aggressive cases. Our study reveals that different Ki-67 cut-offs can categorize LNETs similarly to current WHO criteria. Counting mitoses in PHH3+ areas does not improve diagnosis, while providing a similar prognostic value to the current criteria. With the advantage of being time-efficient, automated assessment of these markers leads to similar conclusions. Lastly, state-of-the-art deep learning modelling does not uncover undisclosed morphological features with diagnostic value. Surprisingly, the same deep-learning approach can discriminate our previously proposed molecular groups with specific morphological features associated with each group.

Conclusions:

Although the mitotic count criterion could be replaced by manual or automated assessment of Ki-67 or PHH3, these markers do not significantly improve the prognostic value or reproducibility of the current classification. Although the potential of morphological features in the current classification of TC/AC seems exhausted, the unsupervised model suggests features for a more clinically relevant morpho-molecular classification.

Validation of an Organoid Model to Effect Precision Medicine in Pheochromocytoma and Paraganglioma

Hector Gonzalez-Cantu[1,8], Cynthia M Estrada-Zuniga[1], Maite Calucho[2,3], Mehwish Noureen[4], Bethany Landry[1], ZiMing Cheng[1], Huyen Thi-Lam Nguyen[2], Ahmad Al Shihabi[2], Qianjin Guo[1], Nicole Bechmann[5], Graeme Eisenhofer[5], James Powers[6], Art Tischler[6], Yanli Ding[7], Xiaojing Wang[4], Alice Soragni[2,3]* and Patricia L.M. Dahia[1,8]*.

1. Div Hematology and Medical Oncology, Dept. Medicine, University of Texas Health San Antonio (UTHSA), San Antonio, TX. 2. Dept. Orthopaedic Surgery, UCLA. 3. Jonsson Comprehensive Cancer Center. 4. Dept Population Sciences, UTHSA. 5. Institute of Clinical Chemistry and Laboratory Medicine, Universität Dresden, Germany. 6. Tufts University. 7. Dept. Pathology, UTHSA. 8. Mays Cancer Center, UTHSA. *Contributed equally.

Presenting author: Hector Gonzalez-Cantu, PhD

Background:

Pheochromocytomas and paragangliomas are uncommon, slow-growing neural crest-derived tumors that secrete catecholamines and display considerable genetic, clinical, biochemical, and morphological diversity, with frequent disruption of hypoxia pathways. Approximately 60% of PPGLs have an identifiable genetic driver, many of which can be part of clinically complex hereditary syndromes. The surgical removal of tumors is the most effective treatment for PPGLs. However, in some instances, such as metastatic, recurrent, or inoperable PPGLs, other forms of treatment must be employed, and few options are currently available. It becomes essential to have a study model available that is amenable to drug screening. Here we describe a patient-derived PPGLs organoid model. By studying the histologic, biochemical, functional, and molecular characterization, we show that this model resembles the primary tumors and lends itself to high-throughput drug screening.

Methods:

Twenty-two samples of PPGLs were successfully included in the protocol, generating organoids from fresh and/or frozen tissue that uses few cells with no need for expansion. The samples were derived from patients aged 15-83 years carrying SDHB, CSDE1, NF1, VHL, EPAS1, RET, MAML3 mutations or unknown driver event. Organoids were characterized using the following strategies: 1) histology/immunohistochemistry, 2. catecholamine secretion (by LC-MS/MS), 3. sequencing (bulk or single cell RNAseq, 4. drug screening, and 5. growth rate estimation quantified using a machine learning-based pipeline.

Results:

PPGL organoids were cultured both short-term (6 days) and long-term (4 weeks), both under normoxia and 1% hypoxia. Several viable cell types were identified by H&E staining. ChGA and TH neural crest markers staining confirmed the neural crest origin of the organoids both at early and late cultures, S100 and CD34 indicated the presence of sustentacular and endothelial (vascular) cells, respectively. We confirmed that the catecholamines secreted by the organoids matched the pattern of the primary tumor both in short and long cultures (3/3). Sensitivity profiles of 35 drugs (alone or in combination) pinpoint tumor-specific responses. Growth rate and sequencing data analyses are ongoing.

Conclusions:

We successfully established PPGL organoids that closely mimic the heterogeneity of the original tumor. These models will provide the ability to investigate tumor initiation and progression and may reveal novel patterns of drug sensitivity and resistance, which could pave the way for the establishment of precision medicine in inoperable/advanced PPGLs.

Establishing & Characterizing a Pancreatic Neuroendocrine Tumor Mouse Model with Humanized Telomeres

Shifei Wu[1, 2], Jeffery Bruce[3], Geraldine Mbamalu[1], Michael Iacovos[4], Trevor Pugh[3], Daniel Schramek[1,2]

1. Centre for Molecular and Systems Biology, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada. 2. Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada. 3. Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada. 4. Sunnybrook Research Institute, University of Toronto.

Presenting author: Shifei Wu

Pancreatic neuroendocrine tumor (PNET) patients have an overall mortality rate of 60%. Most of them have an unpredictable, highly variable clinical course. Insufficient understanding of PNET prevents the development of effective therapies and doctors from stratifying patients into 1) those with rapidly developing tumors who will benefit from early aggressive therapies and 2) those with indolent tumors who could be spared from unnecessary treatments and their toxic side effects. Recent genomic profiling studies of human PNETs revealed the prevalence and a strong association between MEN1/ATRX/DAXX mutations, alternative lengthening of telomere (ALT), and chromosomal instability (CIN), as well as the prevalence of alterations in the mTOR pathway and HLA locus. However, whether and mechanistically how these alterations affect PNET progression remain largely unknown. Our project aims to develop a novel metastatic PNET mouse model that recapitulates genomic, transcriptomic, molecular, histological, and clinical features seen in human PNETs. To achieve this, we use genetically engineered mouse models and in vivo CIRSPR/Cas9-mediated gene editing to concurrently knockout multiple frequently mutated PNET tumor suppressor genes and model telomere dysfunction using TERT knockout mice. The successful establishment of a metastatic PNET mouse model will shed light into PNET etiology and empower the development of novel therapeutic strategies.

Harnessing Ferroptosis Initiating Drugs to Target GEP-NETs

Stephanie A. Planque[1], Yan Zuo[1], and Jeffrey A. Frost[1]

1. Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, Houston, TX, USA.

Presenting author: Jeffrey A. Frost, MD

Background:

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a heterogenous group of tumors that are generally slow growing and resistant to the majority of cancer therapeutics, which target cell proliferation. We have found that GEP-NET cells are sensitive to oxidative stress, and that combining agents that target distinct oxidative stress detoxifying proteins results in a synergistic killing of GEP-NET cells irrespective of their proliferative state. Cells generally use two mechanisms to protect against cytoplasmic reactive oxygen species (ROS), namely the thioredoxin and glutathione antioxidant systems. Our approach targets both of these systems.

Methods:

BON1, QGP1, and HPNE cells were treated with a clinically approved inhibitor of thioredoxin reductase, auranofin, and inhibitors of the SLCA11 cystine transporter. SLCA11 provides the precursor for cysteine and is an essential amino acid for producing glutathione. Cell proliferation was measured using CyQuant kits (Invitrogen). Apoptosis was measured using fluorescent Annexin-V and propidium iodide (Thermo-Fisher). Reactive oxygen species were measured using CellROX reagents (Invitrogen). Glutathione was measured using GSH-Glo glutathione assay kit (Promega).

Results:

Our data shows that the thioredoxin system inhibitor auranofin synergizes with the cystine transporter inhibitor imidazole ketone erastin (IKE) to kill BON1 GEP-NET cells. The degree of synergy allows the use of doses of each drug that are ineffectual at inhibiting tumor cell proliferation on their own. Annexin V labeling shows that the cells are dying by apoptosis rather than ferroptosis. This is supported by the observation that the class II ferroptosis inducing agent RSL3, which blocks the ferroptosis gatekeeper GPX4, does not synergize with auranofin. Importantly, non-transformed pancreatic epithelial cells are relatively resistant to this treatment. The mechanism for synergy will be explored.

Conclusions:

Our data indicates that simultaneous inhibition of the thioredoxin antioxidant system and the SLCA11 cystine transporter synergistically kills GEP-NET cells while sparing non-transformed pancreatic epithelial cells. As this approach does not specifically rely on cell proliferation, it has the potential to treat GEP-NETs that are resistant to other therapeutic approaches.

The Role of the B7x Signaling Pathway in the Development and Progression of Neuroendocrine Tumors

Ziqiang Yuan[1], Alexandra Adams[1], Svetlana Bagdasarov[1], Fuqian Shi[1], Asha Adem[1], Daniel Slegowski[1], Edmund C. Lattime[1], Chang Chan[1], Xingxing Zang[2], Steven K. Libutti[1]

1. Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey. 2. Albert Einstein College of Medicine, Bronx, New York.

Presenting author: Ziqiang Yuan, MD

Background:

Cancer immunotherapy is rapidly becoming an important component of treatment for patients with a variety of tumor types. A newly characterized member of the B7/CD28 family, B7x, is expressed in a number of tumors and can modulate cancer development and progression by inhibiting T-cell function, thus making it an appealing target for immunotherapy. We demonstrated that B7x is upregulated in human and murine pancreatic neuroendocrine tumors (PNETs). Furthermore, we demonstrated that blockade of B7x with an anti-B7x antibody can inhibit tumor cell proliferation and induce apoptosis, both of which improve the tumor immune microenvironment and result in increased survival in the preclinical setting. In this Accelerator Award, we will investigate: 1. The molecular mechanisms leading to B7x upregulation in PNET tumorigenesis; 2. The role of immune checkpoint B7x activation on PNET tumorigenesis; and 3. Whether loss of MEN1 drives an immunogenic phenotype in islet cells that is suppressed by B7x. During this reporting period, we further investigated the molecular mechanism of B7x regulation in the tumor microenvironment (TME) and the role of B7x activation on PNET tumorigenesis in our in vitro and in vivo models.

Materials and Methods:

We assessed the role of immune checkpoint B7x activation on PNET tumorigenesis by crossing our Men1 KO mice with B7x KO mice and assessed the efficacy of novel and high-affinity antibodies targeting the immune checkpoint B7x on the growth of PNETs using our Men1 KO mice. In addition, we identified the immune cell populations present in the tumor microenvironment as well as underlying immune regulatory mechanisms by complementary FACS and multiparameter IHC as well as single cell RNA-seq of human tumor specimens. Finally, we performed T cell Receptor repertoire (TCR) determinations from human PNET tissues by TCR-seq.

Results:

We demonstrated that Men1/B7x double KO mice exhibited decreased islet β-cell proliferation and tumor transformation accompanied by increased T-cell infiltration compared with Men1 single knockout mice. We have also shown that systemic administration of a B7x mAb to our Men1 KO mice with PNETs promotes an antitumor response mediated by increased T-cell infiltration. Furthermore, we demonstrated through single cell RNA-seq of human PNET specimens that some T cell and NK cell immune regulators (T cell immune checkpoints: PD-1, LAG3, and Tim-3; NK cell immune checkpoints: KIR2DL2, KIR2DL3, and KIR2DL4) were upregulated in the immune populations of TME. Single cell RNA-seq analysis additionally demonstrated that IGF2 and VEGF signaling pathways are upregulated in these tumors. Finally, in our preliminary analysis from TCR-seq data, we found that some novel peptides may drive an immunogenic phenotype and manifest in the immune tumor microenvironment of PNETs.

Conclusion:

Our results suggest that B7x and other immune checkpoints may be the critical mediators of tumor immunity in the tumor microenvironment of NETs. Therefore, targeting B7x and immune related pathways will offer an attractive strategy for the immunotherapy of patients suffering from NETs.

Characterization of the Interactions between TMEM127, the Ubiquitin Pathway, and RET

Hector Gonzalez-Cantu, M.Sc.[1,2], Syeda Yusra Munawar[1], Nadia Reyna[1], Patricia L.M. Dahia, MD, PhD[1,2]

1. Department of Medicine, Div. of Hematology and Oncology, UT Health San Antonio, San Antonio, Texas, USA.
2. Mays Cancer Center, San Antonio, Texas, USA.

Presenting author: Hector Gonzalez-Cantu, PhD

Background:

TMEM127 codes for a transmembrane protein which is linked to susceptibility to adrenal neuroendocrine tumors known as pheochromocytomas through loss-of-function mutations. Recently, we reported that TMEM127 protects against tumorigenesis through its role as an adaptor protein to the HECT ubiquitin ligase NEDD4 and leading the proto-oncogene RET to degradation. However, the specific interactive domains between these proteins, as well as the regulation of this mechanism have not been defined. Besides this model in chromaffin cells, congruent TMEM127-HECT ubiquitin ligase mediated mechanisms of cell surface protein regulation have emerged in models of immune surveillance, both in cancer and bacterial infection, highlighting the biological and translational relevance of characterizing these interactions. In this project, we aimed to map the interactive domains between TMEM127 and NEDD4 and investigate the biological context in which TMEM127 suppresses RET.

Methods:

To map the interactive domains between NEDD4 and TMEM127, we designed recombinant DNA constructs targeting individual and combinations of NEDD4 functional domains and tested TMEM127 interactions with NEDD4 by cell-based co-immunoprecipitation studies. To analyze the biological context in which TMEM127 regulates RET, we generated dysfunctional RET mutant constructs, and tested the impact of TMEM127 on RET abundance, activation and downstream signaling.

Results:

Our co-immunoprecipitation studies suggest that TMEM127 shows selective interaction to NEDD4 WW domains. Our cell-based modeling of RET mutations that disrupt localization and/or glycosylation differentially impact TMEM127- RET interactions.

Conclusions:

Our findings support domain-specific TMEM127 and NEDD4 interactions, suggesting that mechanisms regulating this interaction may be shared with other canonical NEDD4 binding partners. Our findings also point to TMEM127 regulating mature RET stability and signals; however, whether this is specifically due to RET localization or maturation remains to be answered and will be investigated further.

Epigenetic Regulation of Tumor Metastasis in SI-NETs

Elham Barazeghi, Samuel Backman, Fredrik Axling, Olov Norlén, Per Hellman, Peter Stålberg

Department of Surgical Sciences, Endocrine Surgery, Uppsala University, Uppsala, Sweden.

Presenting author: Elham Barazeghi, PhD

Background:

Most patients with small intestinal neuroendocrine tumors (SI-NETs) are diagnosed with distant metastases, but the mechanisms driving metastasis are not yet fully understood. Previous studies have shown that SI-NETs have fewer mutations compared to other cancers, but are epigenetically dysregulated, highlighting the potential for epigenetic-targeted therapies. During metastasis, tumor cells gain the ability to invade, migrate, and adapt quickly to new microenvironments. This global transcriptional reprogramming suggests that reversible epigenetic changes, rather than permanent genetic mutations, may be the key regulators of metastasis. Therefore, this project aims to increase our understanding of the epigenetic mechanisms underlying metastatic progression.

Methods:

Multiple primary tumors and metastases from a single patient will be used to perform Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq) analysis. This method uses an engineered, hyperactive Tn5 transposase that cleaves and adds adopters into open regions of chromatin. Tumor cells will be prepared right after tumor resection, counted and 50,000 cells with 90 % viability will be used per ATAC-reaction. The resulting fragmented DNA from these reactions will be then sequenced. After processing the sequencing data, the open chromatin regions identified by ATAC-seq will be compared between primary tumors and metastases to find differentially accessible regions. Transcription factor (TF) motif analysis will be performed to identify enriched TFs in these regions. The identified potential targets will be further studied experimentally using a validation cohort from our biobank, the neuroendocrine cell lines, and the SI-NET xenograft mouse model. Luciferase assay, CRISPR-cas9 gene editing tool, quantitative RT-PCR, immunostaining and western blotting will be applied.

Results:

So far, primary tumors and metastases have been collected from 3 patients with multifocal SI-NETs at the Endocrine Surgery unit at Uppsala University hospital. After tumor resection, tumor cells were prepared and counted. Some of the collected primary tumors were very small, and the resulting cell suspension included high percentage of dead cells. Therefore, in order to prepare ATAC-reaction we have used these samples to optimize the protocol for cell dispersion from the solid tumor tissues. This optimized method will be used to prepare the samples from upcoming patients with multifocal SI-NETs that are operated at Uppsala University hospital.

Conclusion:

Our analyses will allow us to find metastatic specific TF, their specific binding sites and target genes that have a key role in metastatic progression of SI-NETs. By evaluating the potential targets using in vitro and in vivo models we will be able to ultimately unlock specific therapeutic options and benefit metastasis prevention.

Untargeted Serum Metabolomics Analysis Reveals a Unique Biomarker Signature and Potential Treatment Targets in Non-functional PNETs

Alexandra Adams[1], Matthew J. McBride[1,2], Yanxiang Li[2], Shams G. Shams[2], Svetlana Bagdasarov[1], Ziqiang Yuan[1] Steven K. Libutti[1]

1. Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA. 2. Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, USA.

Presenting author: Alexandra Adams, PhD

Background:

Metabolomic profiling is an exciting area of oncologic research that can be used as a diagnostic tool, to identify novel targets for treatment, or to track treatment response. However, little is known about the metabolism of pancreatic neuroendocrine tumors (PNETs). We previously discovered that total choline levels in the PNETs of our MEN1 knockout mouse model were significantly elevated compared to wild type mice. Thus, we hypothesized that circulating metabolites found in human patient serum would reveal a unique metabolic signature differentiating PNET patients from healthy controls.

Methods:

Water soluble metabolites were methanol extracted from serum samples from patients with non-functional PNETs (n=11) and age and sex matched non-cancer controls (n=7). Samples were separated by liquid chromatography (LC) and analyzed on a Thermo Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (MS). Raw LC-MS data was processed and analyzed using El-Maven to assign metabolite identities and quantify abundance.

Results:

The 130 circulating metabolites detected showed high global variability in PNET patient serum compared to controls, yet with mostly distinct clustering between groups. Notably, metabolite inputs to the citric acid cycle were altered, such that the glutamine/glutamate ratio was significantly decreased in PNET patients (0.5 vs 3.0, $p=0.0007$), as were serum concentrations of lactate (997.1uM vs 2575.2 uM, $p<0.0001$) and pyruvate (9.2 uM vs. 93.3 uM, $p<0.0001$) when compared to non-cancer controls. Furthermore, choline concentration was markedly elevated in PNET patient serum compared to controls (65.3 uM vs 10.8 uM, $p<0.0001$), while taurine (75.6 uM vs 182.3 uM, $p=0.0002$) and methionine (9.7 uM vs 17.2 uM, $p=0.03$) were significantly depleted.

Conclusions:

Our results suggest that serum metabolomics may be a valuable tool to screen patients with non-functional PNETs due to a distinct circulating metabolite signature. Further analysis of metabolomic pathways could provide insight into the mechanisms underlying tumorigenesis and inform future treatment approaches.

Development of Innovative in vitro and in vivo Patient-derived Cancer Models for Translational Studies in G1/G2 Gastroenteropancreatic Neuroendocrine Tumors

Yi Liu[1], Jessica Maxwell[2], Daniel Halperin[1], Arvind Dasari[1], Scott Kopetz[1], Xiangsheng Zuo[1], James C. Yao[1]

1. Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA. 2. Department of Surgical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Presenting author: Yi Liu, PhD

Background:

The diagnosed incidence of gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) has risen five-fold in recent decades. Most GEP-NENs are G1/G2 well-differentiated GEP neuroendocrine tumors (GEP-NETs), which grow slowly but remain nonetheless incurable and lethal when advanced. Despite progress, effective systemic treatments for GEP-NETs remain limited. A key barrier is the scarcity of clinically relevant models that accurately reflect human GEP-NET biology. Although patient-derived organoids (PDOs) and patient-derived xenografts (PDXs) have been widely used for modeling human cancers and predicting drug responses, generating such models for G1/G2 GEP-NETs has been unsuccessful due to the slow growth of these tumors. To address this important gap, we have conducted the following studies.

Methods:

Doxycycline (Dox)-inducible TP53R273H and SV40LT lentiviruses, marked with EGFP, were generated, and characterized. Cells digested from 21 surgically resected primary or metastatic tissues of G1/G2 GEP-NETs [pancreatic NET (pNET, n=10), intestinal NETs (n=10); gastrinoma (n=1)] were transduced with these lentiviruses to produce Dox-inducible genetically modified PDOs (GM PDOs). GM PDOs from pNETs transduced with luciferase lentivirus were injected into pancreata of NSG mice to generate orthotopic GM PDO-derived xenografts (GM PDXs). The genetic and biological signatures of the GM PDOs were examined and compared to their original tumor cells through whole genome sequencing (WGS) and RNA-seq analyses. Cell growth rates of GM PDOs cultured with Dox-on, and Dox-off conditions were quantified by measuring EGFP fluorescence intensity. Tumor growth of GM PDXs was monitored through bioluminescence imaging and density. Expression of NET markers (e.g., CHGA, CD56, SYP, SSTR2, INSM1), Ki67, p53 (R273H), and SV40LT in GM PDOs and GM PDXs, with Dox-on or/and Dox-off conditions, and their original tumors was measured by IHC staining and compared.

Results:

A total of 12 GM PDOs (> 6 passages) were successfully generated, including 6 out of 10 pNETs (60%), 5 out of 10 intestinal NETs (50%), and 1 gastrinoma (100%), achieving an overall success rate of 57%. Comparative analyses of WGS showed that these GM PDOs maintained chromosome copy number variants and arrangements, as well as the tumor mutational burdens, of their original tumors. Cell proliferation of GM PDOs accelerated with Dox treatment; Dox withdrawal stopped TP53R273H and SV40LT expression, slowed cell growth, decreased Ki67 expression, and restored CHGA, SYP, SSTR2, and INSM1 expression, suggesting that the effects of Dox-inducible p53R273H and SV40LT proteins on phenotypic changes in GM PDOs were reversible, demonstrating that the biology of GM PDOs in the Dox-off condition were similar to that of the original tumors. Furthermore, one orthotopic GM PDX model from a G2 pNET was successfully generated. In this model, luminescent density gradually but significantly increased from 2 to 8 weeks post-injection. Histologic examination and IHC staining results confirmed GM PDX lesions with strong expression of CHGA, SYP, CD56 and SSTR2 proteins.

Conclusions:

Innovative in vitro and in vivo patient-derived cancer models that could recapitulate the genomic and phenotypic features of human G1/G2 GEP-NETs were successfully developed for the first time. These models yield unique materials and resources that enable advancements in translational studies for GEP-NETs.

Spatial Profiling of the Pancreatic Neuroendocrine Tumor Microenvironment

Junxiang Xu[1], Joakin O. Mori[1], Kenel Dufort[1], Carlo Lanza[1], Katelyn Smith[2], Aatur D. Singhi[2], Ruben Dries[1], Christopher M. Heaphy[1]

1. Department of Medicine, Boston University Chobanian & Avedisian School of Medicine and Boston Medical Center, Boston, MA, USA. 2. Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

Presenting author: Christopher Heaphy, PhD

Background:

There is an urgent need to identify novel biomarkers that can accurately predict prognosis and potential response to systemic and targeted therapies for patients with pancreatic neuroendocrine tumors (PanNETs), a tumor type rapidly rising in prevalence and incidence. Recent genetic and epigenetic studies have revealed novel PanNET subtypes and several new validated prognostic biomarkers, including the presence of alternative lengthening of telomeres (ALT) by telomere-specific fluorescence in situ hybridization (FISH) and ATRX/DAXX protein loss by immunohistochemistry (IHC). In addition, while genetic driver mutations have been identified in this disease, these alterations are limited to cancer cells and do not provide a systematic assessment of information encoded within the intact tumor microenvironment (TME), including the role of the tumor architecture and the spatially organized immunological processes. Thus, to uncover the underlying processes driving tumor initiation and progression in PanNETs, we used a combination of innovative technologies and computational approaches to correlate tumor and TME spatial profiles with established biomarkers to characterize unique PanNET subtypes.

Methods:

Two tissue microarrays (TMAs) were constructed representing 62 archival, non-functional PanNETs with accompanying clinical and pathological data. Specifically, 2.0 mm cores were sampled from corresponding intratumoral and peritumoral regions for each case. The TMAs were assessed with established biomarkers - ALT via telomere-specific FISH and ATRX, DAXX, ARX, and PDX1 protein expression by IHC.

Results:

Overall, ALT was identified in 40% (25 of 62) of the cohort. With the use of the Lunaphore COMET platform, we optimized a panel to interrogate the protein expression using a multiplex immunofluorescence assay enriched for the detection of immune cell populations at the single-cell level. Visium (10X Genomics) was performed on an adjacent section of the TMA to obtain an associated and unbiased spatial whole transcriptome profile, while preserving the morphological context. Next, a spatial multi-modal and multi-scale dataset was generated using Giotto Suite to perform an integrative analysis. Ongoing work is being performed to identify the exact spatial architecture and composition of the tumor and TME in ALT-positive and ALT-negative cases, and how these alterations are related to differences in biological pathways and processes.

Conclusions:

This work will delineate the spatial characteristics of the tumor and TME and, by coupling new biological insights with biomarker discovery, we envision further improving prognosis and uncovering potential therapeutic targets for the treatment of patients with PanNETs.

Targeting CDK4/6 Pathway to Enhance Response to DNA Damaging Agent in Neuroendocrine Carcinoma of the Uterine Cervix

Shaheen Mahira[1], Allison Stewart[4], Runsheng Wang[4], Carl M Gay[4], Jones H Philip[1], Sibel C Yilmaz[1], Robert P Edwards[1,2], Alexander B Olawaiye[1,2], Haider Mahdi[1,2,3]

1. Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA. 2. Magee Women's Hospital, University of Pittsburgh Medical Center, Pittsburgh, PA, USA. 3. Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA. 4. Department of Thoracic/Head and Neck Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

Presenting author: Shaheen Mahira, PhD

Background:

Small cell neuroendocrine carcinoma of the uterine cervix (SCNECC) is a rare and aggressive subtype of cervical cancer characterized by a highly malignant behavior with lymph node metastasis and vascular invasion. Cyclin-dependent kinase, CDK4/6, is involved in DNA replication and repair pathways by regulating the Rb-E2F pathway and its dysregulation in SCNECC results in uncontrolled cell proliferation and homologous recombination repair (HRR). We hypothesize that Rb-E2F pathway inhibition by CDK4/6i can cause cell cycle arrest and HRR impairment and ultimately cell death thereby potentiating the efficacy of DNA damaging agents by overcoming the treatment resistance mechanisms. So, we evaluated the efficacy of the CDK4/6 inhibitor (CDK4/6i) in combination with DNA damaging agents, cisplatin and PARPi.

Methods:

We evaluated the in vivo efficacy of cisplatin, PARPi and CDK4/6i as single and combination therapy using patient-derived xenograft (PDX) model of SCNECC in NSG mice. We performed RT-PCR, western blot and meso scale analysis for mice tumor tissue to investigate the underlying mechanisms. Statistical significance was determined by a two-tailed Student's t test. $p<0.05$ were considered statistically significant.

Results:

Pre-clinical data from PDX model revealed that the cisplatin+CDK4/6i and PARPi+CDK4/6i combinations significantly slowed down the tumor growth compared to all groups and displayed enhanced anti-tumor efficacy. Overall survival probability inferred that cisplatin+CDK4/6i combination treatment prolonged the survival of tumor-bearing mice compared to control. There was a significant downregulation of cell cycle regulatory genes, CHK1 and CHK2 and DNA damage repair genes, BRCA1 and RAD51 with combination therapy in mice tumor tissue. There was a significant increase in secreted free CXCL10 and IFN-β protein levels with combination therapy evidenced by mesoscale cytokine analysis.

Conclusions:

CDK4/6 inhibition sensitized the DNA damaging agents, cisplatin and PARPi resulting in drastic tumor regression in mice bearing PDX neuroendocrine tumor. Combination therapy led to the inhibition of Rb-E2F target genes involved in DNA replication and DNA repair. Taken together, our investigation has enlightened the importance of CDK4/6 inhibition as one of the potential mechanisms for synthetic lethality by conferring sensitivity to DNA damaging agents for the effective treatment of SCNECC.

DNA Methylation Alterations in Small Intestinal Neuroendocrine Tumours Reveal Candidate Drivers and Independent Epigenetic Evolution

Nana Mensah[1], Carla Castignani[1], Netta Mäkinen[2,3], Elizabeth L Cadieux[1,4], Ramesh Shivdasani[2], Pratik Singh[2], Stephen Henderson[5], Javier Herrero[5], Jonas Demeulemeester[6], Stephan Beck[5], Eric Nakakura[7], Matthew Meyerson[2,3,8], Peter Van Loo[1], Amy P Webster[9,*], Chrissie Thirlwell[4,10,*]

1. The Francis Crick Institute, London, UK. 2. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. 3. Cancer Program, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. 4. Achilles Therapeutics plc, London, UK. 5. UCL Cancer Institute, London, UK. 6. KU Leuven Center for Cancer Biology, Leuven, Belgium. 7. University of California, San Francisco, USA. 8. Departments of Genetics and Medicine, Harvard Medical School, Boston, Massachusetts, USA. 9. University of Exeter Medical School, Exeter, UK. 10. University of Bristol Medical School, Bristol, UK. *Corresponding Authors.

Presenting author: Chrissie Thirlwell, MBBS, PhD

Background:

Small intestinal neuroendocrine tumours (SINETs) are rare cancers which present multifocal lesions harbouring independent genetic origins. The SINET epigenome has not been comprehensively characterised due to a lack of the normal cell of origin as a comparator and the confounding effect of infiltrating normal cells.

Methods:

To investigate the DNA methylation landscape of SINETs, we performed reduced representation bisulfite sequencing (RRBS) on 35 SINETs from 11 patients. We performed in silico deconvolution of the SINET methylomes from bulk data using the 'Copy-number Aware Methylation Deconvolution Analysis of Cancers' (CAMDAC) algorithm, adjusting for tumour purity and ploidy confounding. In addition, we performed RRBS on an Enteroendocrine (EE) cell line and validated its suitability as a proxy for the cell-of-origin using EpiSCORE and TPH1 promoter methylation status.

Results:

We detected differentially methylated regions (DMRs) at promoters of known cancer genes, converging on described hub genes SFN (stratifin) and CREB1. Enhancer DMRs linked global hypomethylation with regions targeted by LIM homeobox and Distal-less homeobox transcription factors, while hypermethylation was associated with GATA transcription factor binding sites. Integrating a cohort of unifocal SINETs with multi-region RRBS, we discovered that pairwise DNA methylation distances support a model of independent epigenetic evolution in multifocal SINETs. We identify promoter DMRs between primary tumours and metastases, demonstrating the selection for dynamic methylation in SINET evolution.

Conclusions:

This work contributes novel candidate epigenetic drivers and a broader understanding of SINET epigenetic evolution through careful analysis of DNA methylomes.

Understanding the Microbial Environment of Small-intestinal Neuroendocrine Tumors

Iris Lee[1,2], Anders Dohlman[1,2], Tong Gao[1], Zhouwei Zhang[1,2], Yosuke Kasai[3], Grace E Kim[4], Chrissie Thirlwell[5,6], Eric Nakakura[3], Matthew Meyerson[1,2,7], Netta Mäkinen[1,2]

1. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA. 2. Cancer Program, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. 3. Department of Surgery, University of California, San Francisco, California, USA. 4. Department of Pathology, University of California, San Francisco, California, USA. 5. Bristol Medical School, University of Bristol, Bristol, UK. 6. Department of Oncology, UCL Cancer Institute, London. UK. 7. Departments of Genetics and Medicine, Harvard Medical School, Boston, Massachusetts, USA.

Presenting author: Iris Lee, BS

Background:

Small intestinal neuroendocrine tumors (SI-NETs) often present as multifocal lesions. Notably, these lesions have been shown to be clonally independent, suggesting that a potential environmental factor contributes to pathogenesis, rather than a sole somatic genetic cause. Microbes, which are abundant in the gastrointestinal tract, are among the environmental factors that may influence SI-NET development. The goal of this project is to characterize the SI-NET-associated microbiome and to investigate the potential role of microbes in SI-NET development.

Materials and Methods:

Our sample cohort consisted of 86 synchronous primary ileal NETs and their matched normal ileal mucosa and/or whole blood specimens from 14 patients with multifocal SI-NETs. For each sample, we had whole genome sequencing (WGS) and/or RNAseq data. PathSeq was used to isolate microbial sequencing reads from these tissues and assign them to microbes. To mitigate false-positive microbial signals, sequence data were decontaminated by excluding species appearing in more than 20% of whole blood samples and species that have a low Jaccard similarity between WGS and RNAseq data of matched samples. PERMANOVA, Fisher Exact Test, and MAASLIN2 analyses were performed to characterize and compare the microbial composition of normal ileum and tumor tissue samples.

Results:

After decontamination, the bulk of non-human reads in SI-NET sequence data aligned to bacteria, with rare reads aligning to fungal and viral genomes. Variation in microbial species abundance was not significantly correlated with the tissue phenotype (e.g. tumor vs normal), but rather varied between individual patients. For example, samples from patient P744 were largely comprised of Haemophilus, while Corynebacterium predominated in samples from patient P760. The microbial composition of P852 samples, which were collected in order from one end of the resected ileum to the other, gradually changed across the samples. MAASLIN2 and the Fisher Exact Test indicated that Streptococcus sp. HMSC072D03 was significantly enriched in normal tissue, but no species were enriched in the tumor tissue.

Conclusion:

These data do not provide evidence that microbes are involved in SI-NET development. As a next step, we will perform metagenomic assembly of heretofore unassigned reads to identify potential novel microorganisms not represented in reference genome databases.

Modeling Cdk5-Driven Medullary Thyroid Carcinoma: Insights into Tumor Progression and Therapeutic Targets

Priyanka Gupta[1], Andres F. Diaz[2], Kuldeep Gupta[2], Brendon Herring[3], Nilesh Kumar[4], Angela M. Carter[3], Bart Rose[3], Renata Jaskula-Sztul[3], James A Bibb[2]

1. Sanford Burnham Prebys, San Diego, CA 92121, USA. 2. Department of Translational Neuroscience, University of Arizona College of Medicine Phoenix, Phoenix, AZ 85004, USA. 3. Department of Surgery, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL 35233, USA. 4. Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

Presenting author: Andres F. Diaz, MD/PhD student

Background:

Neuroendocrine (NE) cancers are a diverse group of malignancies with a broad spectrum of clinical behaviors ranging from indolent to aggressive tumors. To better understand these cancers, we developed two distinct murine models of medullary thyroid carcinoma (MTC), a prototypical NE cancer driven by aberrant cyclin-dependent kinase 5 (Cdk5) function. Our study aimed to understand the mechanisms of Cdk5-driven MTC, inform how these mechanisms may extend into other NE cancers, and develop a clinically relevant murine model to uncover therapeutic targets with potential for personalized treatment.

Methods:

We developed two murine models of MTC by driving Cdk5 overexpression using the neuron-specific enolase (NSE) promoter, which is primarily active in neuroendocrine cells, and the calcitonin gene-related peptide (CGRP) promoter, which is restricted to calcitonin-secreting cells. These transgenic models utilized a doxycycline-regulated system to induce p25, a known pro-neoplastic activator of Cdk5. Tumors were harvested after 10 weeks of induction and characterized through whole exome sequencing and transcriptomic analysis to explore their genetic and molecular landscapes. Additionally, biomarkers of Cdk5 activity were analyzed using recombinant phosphorylation-site-specific monoclonal antibodies against Cdk5 substrates developed in our laboratory.

Results:

Following tumor induction, the CGRP-p25OE model exhibited early onset and more aggressive tumor growth compared to the NSE-p25OE model, which developed slower-growing tumors. Exome sequencing revealed 876 somatic mutations in the NSE-p25OE tumors versus 50 mutations in the CGRP-p25OE tumors, indicating significant differences in mutational burden between the two models. Transcriptomic analysis identified 4920 differentially expressed genes (DEGs) in the NSE-p25OE model and 4348 DEGs in the CGRP-p25OE model. Notably, both models showed upregulation of key components of Cdk5 signaling pathways. Further analysis using our phosphorylation-site-specific monoclonal antibodies demonstrated that Cdk5-driven phosphorylation biomarkers, such as P-RBL1 and P-LARP6, were significantly elevated in 27-44% of human MTC tissues, suggesting that these markers can identify a subset of Cdk5-driven tumors. The differences in tumor phenotype were linked to disruptions in mitotic cell cycle processes in the slower-growing NSE-p25OE tumors and alterations in metabolic pathways, particularly fatty acid metabolism, in the aggressive CGRP-p25OE tumors.

Conclusions:

Our study highlights the pivotal role of aberrant Cdk5 activity in driving the progression of medullary thyroid carcinoma (MTC), with distinct molecular pathways underpinning different tumor behaviors. The identification of unique mutations and transcriptomic profiles between the slow-growing NSE-p25OE model and the aggressive CGRP-p25OE model underscores the complexity of Cdk5's involvement in tumorigenesis. These findings suggest that Cdk5 contributes to both mitotic dysregulation and metabolic reprogramming, offering insights into its broader implications across other neuroendocrine cancers. Furthermore, the validation of our phosphorylation-site-specific monoclonal antibodies targeting Cdk5 substrates provides a novel avenue for identifying and potentially stratifying Cdk5-driven tumors in human patients. These biomarkers hold promise not only for advancing diagnostic precision but also for guiding the development of personalized therapies tailored to the specific molecular characteristics of Cdk5-driven NE tumors. Future work will focus on validating these biomarkers across larger patient cohorts and exploring their utility in targeted therapeutic interventions to improve outcomes for patients with aggressive MTC and other Cdk5-dependent NE cancers.

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