Dear Attendees,

Welcome to the 13th International VHL Medical/Research Symposium in Houston, TX. This meeting is organized by The University of Texas MD Anderson Cancer Center and Baylor College of Medicine and supported by the VHL Alliance.

We are excited to have senior VHL researchers from around the globe joining the meeting to discuss their latest findings. We are also proud to introduce young and promising investigators that are entering the field with enthusiasm and talent. We look forward to listening to them present their findings.

With the ultimate goal of improving patient care, the agenda for this year’s meeting touches on the multiple manifestations of VHL through important topics in basic, translational, and clinical research. We will also hear about new method of data collection and diagnostic technologies. The agenda includes a talk about gene editing, allowing us to grapple with whether or not this technology is ready to tackle a disease as complex as VHL. Interactive clinical debates on the pros and cons of various treatment approaches for CNS hemangioblastomas and renal cell carcinoma are also incorporated into the agenda. Our hope is to develop a consensus on the best treatment methodology. Finally, together we will reassess the VHL surveillance guidelines to determine what changes are needed to better detect lesions in patients of all ages. We plan to document and publish the discussions and their outcomes in order to share them with others who are providing clinical care to VHL patients.

Since we last met for the 2016 International VHL Medical/Research Symposium, there have been numerous new, important discoveries and advances. Many of these will be included in this year’s extremely robust poster session. We strongly encourage you to engage in discussion with the presenters during the various breaks over the course of the symposium.

Lastly, but most importantly, it is the VHL patients themselves that inspire the research efforts, dedication to this meeting, and the work on the biology of VHL. They are here to teach us what the disease is, to share their experiences, priorities, and hopes with everybody in the meeting and to inspire us to continue working on curing VHL!

Welcome and enjoy the meeting!

The Meeting Organizers
Organizing Committee

Ruhee Dere, PhD  
Assistant Professor, Molecular & Cell Biology, Center for Precision Environmental Health, Baylor College of Medicine

Eric Jonasch, MD  
Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center

W. Kimryn Rathmell, MD, PhD  
Director, Division of Hematology and Oncology; Cornelius Abernathy Craig Chair, Department of Medicine; Professor of Medicine and Biochemistry, Vanderbilt University School of Medicine

Cheryl Walker, PhD  
Director, Center for Precision Environmental Health; Professor, Molecular & Cell Biology, Medicine, and Molecular and Human Genetics, Baylor College of Medicine

Symposium Sponsor

Peloton Therapeutics

Educational Grant

Exelixis, Inc.

Exhibitors

Novartis Oncology
Pfizer Oncology
### Program Agenda

#### Day 1: Thursday, October 4, 2018

**BREAKFAST AND INTRODUCTIONS**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>7:00 AM</td>
<td>REGISTRATION/CONTINENTAL BREAKFAST</td>
<td></td>
</tr>
<tr>
<td>7:45 AM</td>
<td>WELCOME</td>
<td>W. Kimryn Rathmell, MD, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eric Jonasch, MD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Course Directors</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phil L.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Patient</td>
</tr>
</tbody>
</table>

**NOVEL INSIGHTS INTO VHL BIOLOGY**

**MODERATOR:** Cheryl Walker, PhD

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>8:00 AM</td>
<td>AXL Signaling in ccRCC: Molecular Mechanisms and Targeted Therapy</td>
<td>Erinn Bruno Rankin, PhD &lt;br&gt;Assistant Professor, Radiation Oncology &lt;br&gt;Assistant Professor, Obstetrics &amp; Gynecology - Gynecologic Oncology, Stanford University</td>
</tr>
<tr>
<td>8:20 AM</td>
<td>Identifying Novel Therapeutic Targets for VHL Disease Downstream of a Unique VHL-AURKA-HDAC6 Signaling Axis</td>
<td>Ruhee Dere, PhD &lt;br&gt;Assistant Professor, Molecular &amp; Cell Biology, Center for Precision Environmental Health, Baylor College of Medicine</td>
</tr>
<tr>
<td>8:40 AM</td>
<td>VHL Substrate Transcription Factor ZHX2 as an Oncogenic Driver in RCC</td>
<td>Qing Zhang, PhD &lt;br&gt;Assistant Professor, Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine</td>
</tr>
<tr>
<td>9:00 AM</td>
<td>BREAK</td>
<td></td>
</tr>
</tbody>
</table>
## NOVEL INSIGHTS INTO VHL BIOLOGY (continued)

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:15 AM</td>
<td>The HIFs in Kidney Cancer – New Insights and Targeting Potential</td>
<td>Mei Koh, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Assistant Professor, Pharmacology and Toxicology, The University of Utah</td>
</tr>
<tr>
<td>9:35 AM</td>
<td>Dysregulated Acyl-CoA Metabolism Drives the Clear Cell Phenotype and Tumor Growth in Renal Cell Carcinoma</td>
<td>Edward LaGory, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Life Sciences Research Associate, Stanford University</td>
</tr>
<tr>
<td>9:55 AM</td>
<td>PROFFERED PAPER: Multiple Tumor Suppressors Regulate a HIF-Dependent Negative Feedback Loop Through ISGF3 in Kidney Cancer</td>
<td>Haifeng Yang, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Assistant Professor, Thomas Jefferson University</td>
</tr>
</tbody>
</table>

## GENE EDITING: FACT OR FICTION FOR VHL DISEASE

**MODERATOR:** Eric Jonasch, MD

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:10 AM</td>
<td>AAV Targeted Therapy for Inherited Retinal Dystrophy: Bench to Bedside*</td>
<td>Daniel Chung, DO, MA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clinical Ophthalmic Lead, Spark Therapeutics</td>
</tr>
<tr>
<td></td>
<td>* CME Credits Not Provided</td>
<td></td>
</tr>
<tr>
<td>10:35 AM</td>
<td>Cas9 Mediated Therapy</td>
<td>Giannicola Genovese, MD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Instructor, Genomic Medicine, The University of Texas MD Anderson Cancer Center</td>
</tr>
</tbody>
</table>

## BIOINFORMATICS AND DATA ACQUISITION

**MODERATOR:** Othon Iliopoulos, MD, PhD

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:55 AM</td>
<td>Proffered Paper: New Lessons from an Old Gene: Complex Splicing and a Novel Cryptic Exon in VHL Gene Cause Erythrocytosis and VHL Disease</td>
<td>Betty Gardie, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Associate Professor, Ecole Pratique des Hautes Etudes</td>
</tr>
<tr>
<td>11:10 AM</td>
<td>VISION: VHL Information Sharing International CoNsortium</td>
<td>Raymond Kim, MD, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staff Geneticist, Clinical and Metabolic Genetics, The Hospital for Sick Children; Project Investigator, Genetics &amp; Genome Biology, Research Institute; Assistant Professor, Department of Medicine, University of Toronto</td>
</tr>
<tr>
<td>11:30 AM</td>
<td>Bioinformatics Tools to Gain Insight into Proteomic and Genomic Data</td>
<td>Christine Peterson, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Assistant Professor, Department of Biostatistics, Division of Basic Science Research, The University of Texas MD Anderson Cancer Center</td>
</tr>
<tr>
<td>11:50 AM</td>
<td>LUNCH AND POSTER VIEWING</td>
<td></td>
</tr>
</tbody>
</table>
# NEW DEVELOPMENTS IN IMAGING TECHNOLOGY

**MODERATOR:** W. Kimryn Rathmell, MD, PhD

<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Presenter(s)</th>
</tr>
</thead>
</table>
| 1:25 PM | Novel Imaging Approaches                                                                         | Ivan Pedrosa, MD, PhD  
Jack Reynolds, M.D., Chair in Radiology; Co-Leader of the Kidney Cancer Program, Simmons Comprehensive Cancer Center, The University of Texas Southwestern Medical Center |
| 1:45 PM | Overview of Imaging Modalities in the Management of VHL Lifecycle                               | W. Kimryn Rathmell, MD, PhD  
Director, Division of Hematology and Oncology; Cornelius Abernathy Craig Chair, Department of Medicine; Professor of Medicine and Biochemistry, Vanderbilt University School of Medicine |
| 2:05 PM | $[^{18}F]FAZA$ PET Imaging Reveals Precise Pharmacodynamics *in vivo* of the Novel Chemotherapeutic IACS-010759 | Seth Gammon, PhD  
Assistant Professor, Department of Cancer Systems Imaging, Division of Diagnostic Imaging, The University of Texas MD Anderson Cancer Center |
| 2:25 PM | Novel Kidney Imaging                                                                             | Emily Chang, MD  
Assistant Professor of Medicine, Division of Nephrology and Hypertension, UNC Kidney Center, University of North Carolina School of Medicine |
| 2:45 PM | PROFFERED PAPER: *In silico* Exploration of von Hippel-Lindau (pVHL) Tumor Suppressor Molecular Functions: Correlations between Disease Mutations, Interactors and Pathways | Giovanni Minervini, PhD  
Postdoctoral Fellow, University of Padova |
| 3:00 PM | BREAK                                                                                           | |
### CNS HEMANGIOBLASTOMAS

**MODERATOR: Ian surena MD**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event Description</th>
<th>Speaker(s)</th>
</tr>
</thead>
</table>
| 3:15 PM| **KEYNOTE:** Targeting Metabolism and Angiogenesis in VHL Related Tumors | Othon Iliopoulos, MD, PhD  
Clinical Director, von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center; Associate Professor of Medicine, Harvard Medical School |
| 4:00 PM| **CLINICAL DEBATE:** Optimal Treatment of Hemangioblastomas | Ashok Asthagiri, MD  
Neurological Surgery, University of Virginia School of Medicine  
Ian McCutcheon, MD  
Professor, Department of Neurosurgery, The University of Texas MD Anderson Cancer Center |
| 4:45 PM| **PROFFERED PAPER:** New Radio-Surgical Technique for Endolymphatic Sac Tumor Treatment: Results for the First Two Patients | Jérôme Nevoux, MD, PhD  
Associate Professor  
Otorhinolaryngology CHU Bicêtre - Hôpitaux Universitaires Paris Sud Université Paris Saclay |
| 5:00 PM| ADJOURN | |

### SYMPOSIUM DINNER

6:30 PM
**THIRD COAST RESTAURANT**
6550 BERTNER AVE, 6TH FLOOR  
HOUSTON, TX
Day 2: Friday, October 5, 2018

**RENAL CELL CARCINOMA**

**MODERATOR:** Surena Matin, MD

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Presenters</th>
</tr>
</thead>
</table>
| 8:00 AM| HIF2 in Renal Cancer                                                    | **James Brugarolas, MD, PhD**  
The Sherry Wigley Crow Cancer Research Endowed Chair in honor of Robert Lewis Kirby, MD; Director of Kidney Cancer Program, The University of Texas Southwestern Medical Center |
| 8:20 AM| **CLINICAL DEBATE:** Partial Nephrectomy or Percutaneous Ablation for VHL-Related RCC  
Brian Shuch, MD  
Associate Professor and Alvin & Carrie Meinhardt Endowed Chair in Kidney Cancer Research; Director of the UCLA Kidney Cancer Program, UCLA Medical Center  
Sharjeel Sabir, MD  
Assistant Professor, Interventional Radiology, The University of Texas MD Anderson Cancer Center |
| 8:45 AM| DNA Damage Signaling and Therapeutic Opportunities in VHL Disease       | **Eric Jonasch, MD**  
Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center |
| 9:05 AM| Clinical and Surgical Management of VHL-Related Cysts and Cystic RCC    | **Mark Ball, MD**  
Assistant Research Physician; Staff Clinician, Urologic Oncology Branch, National Cancer Institute (NIH) |
| 9:25 AM| Evolution of Clinical Studies in VHL Disease                            | **Ramaprasad Srinivasan, MD, PhD**  
Investigator, Urologic Oncology Branch, National Cancer Institute (NIH) |
| 9:45 AM| **PROFFERED PAPER:** VHL Knockdown Kidney Cells Induces Macrophage Extravasation and Polarization toward Tumor-Associated Macrophage (TAM) in Progression of Clear-Cell-Renal Cell Carcinoma  
Thi-Ngoc Nguyen  
PhD Candidate, National Central University, Taiwan |
| 10:00 AM| **CLINICAL DISCUSSION**                                                 |                                                                                                                                           |
| 10:30 AM| BREAK                                                                  |                                                                                                                                           |
# VHL ENDOCRINE MANIFESTATIONS

**Moderator:** Steven Waguespack, MD

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenter</th>
</tr>
</thead>
</table>
| 11:00 AM | Germline Genotype Analysis as a Clinical Tool in the Management of Patients with VHL Associated Pancreatic Neuroendocrine Tumors | Amit Tirosh, MD  
Sackler School of Medicine, Tel Aviv University; Head of the Neuroendocrine Tumors Service & Endocrine Oncology Bioinformatics Lab, Sheba Medical Center |
| 11:20 AM | Surgical Management of VHL Endocrine Manifestations                    | Electron Kebebew, MD  
Chief, Division of General Surgery, Stanford Medicine |
| 11:40 AM | Malignant Pheochromocytomas in VHL                                     | Camilo Jimenez, MD  
Professor of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center |
| 12:00 PM | VHL in the Genomic Landscape of Pheochromocytoma and Paraganglioma     | Anne Paule Gimenez-Roqueplo, MD  
Professor, Department of Genetic, Assistance Publique-Hôpitaux de Paris; Hôpital Européen Georges Pompidou, Paris Descartes University |
| 12:20 PM | **PROFFERED PAPER:** Synonymous but not Silent: A Synonymous VHL Mutation Confers Susceptibility to Pheochromocytomas in a Four-Generation Family | Shahida Flores, MS  
PhD Candidate, The University of Texas Health Science Center at San Antonio |
<p>| 12:35 PM | LUNCH AND POSTER VIEWING                                              |                                         |</p>
<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:00 PM</td>
<td>Current Clinical Standards</td>
<td>Dan Gombos, MD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Professor, Department of Head and Neck Surgery, Division of Surgery; Section Chief, Department of Section of Ophthalmology, The University of Texas MD Anderson Cancer Center; Associate Professor, Department of Pediatrics Patient Care, Division of Cancer Medicine; Associate Professor, Department of Ophthalmology, Baylor College of Medicine; Clinical Professor, Department of Ophthalmology and Visual Sciences, The University of Texas Medical Branch at Galveston</td>
</tr>
<tr>
<td>2:20 PM</td>
<td>Murine Model of VHL Retinal Hemangioblastomas</td>
<td>Herui Wang, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fellow, National Cancer Institute, NIH</td>
</tr>
<tr>
<td>2:40 PM</td>
<td>Vascular Abnormalities with VHL Mutations</td>
<td>John Chappell, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Assistant Professor, Research Institute; Center for Heart and Regenerative Medicine, Virginia Tech Carilion Research Institute; Assistant Professor, Biomedical Science, Virginia Tech Carilion School of Medicine; Assistant Professor, Virginia Tech-Wake Forest University School of Biomedical Engineering and Sciences</td>
</tr>
<tr>
<td>3:00 PM</td>
<td>Repurposing Propranolol for the Treatment of von Hippel-Lindau Disease</td>
<td>Luisa Botella, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Centro de Investigación Biomédica en Red de Enfermedades Raras</td>
</tr>
<tr>
<td>3:20 PM</td>
<td>PROFFERED PAPER: New in vivo Model for VHL Retinal Hemangiomas</td>
<td>Anna Matynia, PhD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Associate Researcher, University of California, Los Angeles</td>
</tr>
<tr>
<td>3:35 PM</td>
<td>BREAK</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
<td>Presenter(s)</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>4:00 PM</td>
<td><strong>PROFFERED PAPER:</strong> Universal Reflex Referral to VHL Comprehensive Clinical Care Center of Patients Presenting to Ophthalmologists Leads to Dramatic Improvement in Guideline-Concordant Screening: Results of a Pilot Study</td>
<td><strong>Anthony Daniels, MD, MSc</strong>&lt;br&gt;Assistant Professor of Ophthalmology &amp; Visual Sciences and Radiation Oncology, Director of Ocular Oncology, Vanderbilt University School of Medicine</td>
</tr>
<tr>
<td>4:15 PM</td>
<td>Recommendations for von Hippel–Lindau Tumor Surveillance in Childhood and Adolescence</td>
<td><strong>Surya Rednam, MD</strong>&lt;br&gt;Assistant Director, Clinical Division (Inpatient), Cancer and Hematology Centers; Assistant Professor, Department of Pediatrics, Section of Hematology-Oncology, Texas Children's Hospital; Assistant Professor, Department of Pediatrics, Section of Hematology-Oncology, Baylor College of Medicine</td>
</tr>
<tr>
<td>4:35 PM</td>
<td><strong>ROUND TABLE DISCUSSION</strong></td>
<td><strong>Othon Iliopoulos, MD, PhD</strong>&lt;br&gt;Clinical Director, von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center; Associate Professor of Medicine, Harvard Medical School&lt;br&gt;&lt;br&gt;<strong>Raymond Kim, MD, PhD</strong>&lt;br&gt;Staff Geneticist, Clinical and Metabolic Genetics, The Hospital for Sick Children; Project Investigator, Genetics &amp; Genome Biology, Research Institute; Assistant Professor, Department of Medicine, University of Toronto&lt;br&gt;&lt;br&gt;<strong>Stacy Lloyd, MPH</strong>&lt;br&gt;American Medical Association&lt;br&gt;&lt;br&gt;<strong>Sarah Nielsen, MS, CGC</strong>&lt;br&gt;Genetic Counselor, Section of Hematology/Oncology at the University of Chicago Medicine&lt;br&gt;&lt;br&gt;<strong>Surya Rednam, MD</strong>&lt;br&gt;Assistant Director, Clinical Division (Inpatient), Cancer and Hematology Centers; Assistant Professor, Department of Pediatrics, Section of Hematology-Oncology, Texas Children's Hospital; Assistant Professor, Department of Pediatrics, Section of Hematology-Oncology, Baylor College of Medicine</td>
</tr>
<tr>
<td>5:15 PM</td>
<td>Adjourn</td>
<td></td>
</tr>
</tbody>
</table>
# Day 3: Saturday, October 6, 2018

## THE PATIENT’S PERSPECTIVE

**MODERATOR: TBD**

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker/Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>9:00 AM</td>
<td>WELCOME</td>
<td>Ilene Sussman, PhD</td>
</tr>
<tr>
<td>9:10 AM</td>
<td>Highlights of Basic Research</td>
<td>Othon Iliopoulos, MD, PhD Clinical Director, von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center; Associate Professor of Medicine, Harvard Medical School</td>
</tr>
<tr>
<td>9:40 AM</td>
<td>Clinical Advances</td>
<td>W. Kimryn Rathmell, MD, PhD Director, Division of Hematology and Oncology; Cornelius Abernathy Craig Chair, Department of Medicine; Professor of Medicine and Biochemistry, Vanderbilt University School of Medicine</td>
</tr>
<tr>
<td>10:10 AM</td>
<td>Clinical Trials: Past and Present</td>
<td>Ramaprasad Srinivasan, MD, PhD Investigator, Urologic Oncology Branch, National Cancer Institute (NIH)</td>
</tr>
<tr>
<td>10:40 AM</td>
<td>BREAK</td>
<td></td>
</tr>
<tr>
<td>10:55 AM</td>
<td>MyVHL: Patients Contributing to VHL Research</td>
<td>Ilene Sussman, PhD Executive Director, VHL Alliance</td>
</tr>
<tr>
<td>11:10 AM</td>
<td>New VHLA Programmatic Initiatives</td>
<td>Joshua Mann, MPH Director of Engagement and Outreach, VHL Alliance</td>
</tr>
<tr>
<td>11:25 AM</td>
<td>Medical Coaching: What Is It All About?</td>
<td>Leona deVinne Accendo Consulting</td>
</tr>
<tr>
<td>12:20 PM</td>
<td><strong>DISCUSSION</strong> Educating the Healthcare Professionals</td>
<td><strong>Moderator Eric Jonasch, MD</strong> Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center</td>
</tr>
<tr>
<td>12:50 PM</td>
<td>CCC Process Improvement</td>
<td>Stacy Lloyd, MPH American Medical Association</td>
</tr>
<tr>
<td>11:10 PM</td>
<td><strong>DISCUSSION</strong>: Next Steps</td>
<td>Eric Jonasch, MD Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center</td>
</tr>
<tr>
<td>1:25 PM</td>
<td>LUNCH</td>
<td></td>
</tr>
</tbody>
</table>
Presentation Abstracts
SESSION TITLE

Novel Insights into VHL Biology
AXL Signaling in ccRCC: Molecular Mechanisms and Targeted Therapy

Yiren Xiao¹, Hongjuan Zhao², Yu Miao¹, Donna Peehl², Amato J. Giaccia¹, Erinn B. Rankin¹

¹ Department of Radiation Oncology, ² Department of Urology, Stanford University, Palo Alto, CA, USA

Kidney cancer remains a leading cause of cancer related deaths in the United States. Because kidney cancer is resistant to traditional chemo and radiation therapies, targeted agents are at the forefront of kidney cancer therapy. The von Hippel-Lindau (VHL)/hypoxia inducible factor (HIF) pathway is a central regulator of RCC tumor initiation and progression. However, the identification of additional molecular targets that drive therapeutic resistance and metastatic disease in RCC are needed to improve overall patient survival.

AXL has emerged as an important therapeutic target in cancer that is associated with both metastatic and TKI resistant phenotypes of advanced tumors. We recently discovered that the receptor tyrosine kinase, AXL, is a critical target of VHL/HIF signaling and is an important factor governing the invasive and metastatic potential of RCC. Moreover, we have developed a potent and selective inhibitor of GAS6/AXL signaling, a soluble AXL FC-fusion decoy receptor (sAXL). Here we identify a role for GAS6/AXL signaling in the regulation of RCC angiogenesis and tumor growth. Genetic and therapeutic inhibition of AXL signaling reduced RCC tumor growth at the kidney and at distant metastatic sites such as the liver. AXL inhibition in RCC tumor cells resulted in decreased vessel formation in vivo and decreased angiogenic potential in vitro. Most importantly, AXL expression in RCC patients correlates with the lethal phenotype, further supporting an important role for AXL in the pathogenesis of RCC. These findings identify AXL as a therapeutic target driving both primary tumor growth and the metastatic potential of renal clear cell carcinoma.
Identifying Novel Therapeutic Targets for VHL Disease Downstream of a Unique VHL-AURKA Signaling Axis.

Pratim Chowdhury¹, Reid T. Powell², Clifford Stephan², Ivan P. Uray³ Tia Berry¹, Durga Nand Tripathi¹, Yong Sung Park², Michael A. Mancini², 4, Peter Davies², Ruhee Dere¹

¹ Center for Precision Environmental Health, Baylor College of Medicine, Houston, TX, USA; ² Center for Translational Cancer Research, Institute of Biosciences and Technology, Texas A&M College of Medicine, Houston, TX, USA; ³ Department of Clinical Oncology, University of Debrecen, Debrecen, Hungary; ⁴ Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA

Loss of heterozygosity on chromosome 3p at the von Hippel-Lindau (VHL) locus, and inactivation of the VHL protein is commonly associated with renal cell carcinoma (RCC) and VHL disease. Most famed for its role as a ubiquitin E3 ligase directly involved in proteasomal degradation of hypoxia inducible factor alpha (HIFα), we have recently discovered Aurora Kinase A (AURKA) as a novel substrate of VHL. Notably, our identification of AURKA as a hypoxia-independent target for VHL ubiquitination, established an important function for VHL distinct from its well-recognized hypoxia-regulated activity. In addition, we were able to identify a pathogenic VHL mutant that was capable of differentiating between VHL's function on HIFα and AURKA. Loss of VHL is commonly associated with loss of primary cilia and is causally linked to elevated AURKA. Having established VHL as an important regulator of AURKA under both normoxia and hypoxia, we have developed an image based high-throughput screening (HTS) assay using a dual-labelling image analysis strategy, with the goal of identifying small molecule compounds for the targeted rescue of cilia defects associated with VHL-deficiency. This strategy revealed bexarotene, a synthetic rexinoid, as a bone fide regulator of the primary cilium in VHL-deficient cells. Importantly, bexarotene driven restoration of primary cilia correlated to a decrease in AURKA expression and activity. Treatment of an RCC 786-O tumor xenograft model with bexarotene showed a decrease in tumor volume in bexarotene treated mice compared to the vehicle controls. These data highlight the potential of bexarotene as an intervention strategy to manage renal cytogenesis associated with VHL-deficiency and elevated AURKA expression.
VHL Substrate Transcription Factor ZHX2 as an Oncogenic Driver
In Clear Cell Renal Cell Carcinoma

Qing Zhang
Department of Pathology & Laboratory Medicine, Lineberger Comprehensive Cancer Center
UNC-Chapel Hill, NC, USA

Inactivation of the von Hippel-Lindau (VHL) E3 ubiquitin ligase protein is a hallmark of clear cell renal cell carcinoma (ccRCC). Identifying how pathways affected by VHL loss contribute to ccRCC remains challenging. We used a genome-wide in vitro expression strategy to identify proteins that bound VHL when hydroxylated. Zinc fingers and homeoboxes 2 (ZHX2) was found as a VHL target and its hydroxylation allowed VHL to regulate its protein stability. Tumor cells from ccRCC patients with VHL loss-of-function mutations usually had increased abundance and nuclear localization of ZHX2. Functionally, depletion of ZHX2 inhibited VHL-deficient ccRCC cell growth in vitro and in vivo. Mechanistically, integrated ChIP-Seq and microarray analysis showed that ZHX2 promoted NF-κB activation. These studies reveal ZHX2 as a potential therapeutic target for ccRCC.
The HIFs in Kidney Cancer – New Insights and Targeting Potential

Daniel Fuja1, Xiande Liu2, Archana Agarwal3, Sheryl Tripp3, Guillermina Garcia4, Guoying Wang3, Jose Karam2, Chris Wood2, Rebecca Slack2, Kanishka Sircar2, Pheroze Tamboli2, Neeraj Agarwal3, Thai Ho5, Eric Jonasch2, Mei Yee Koh1

1 Department of Pharmacology and Toxicology, University of Utah, Salt Lake City UT, USA; 2 Department of GU Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston TX, USA; 3 Huntsman Cancer Institute, University of Utah Salt Lake City UT, USA; 4 Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA; 5 Mayo Clinic, Scottsdale, AZ, USA

Clear cell renal cell carcinoma (ccRCC) is the most common and aggressive form of kidney cancer. ccRCC typically lacks the genetic hallmarks of other solid tumors such as PTEN loss or KRAS mutations, suggesting that standard paradigms of cancer therapy could be largely irrelevant. The etiology of ccRCC is uniquely linked to loss of the von Hippel-Lindau (pVHL) tumor suppressor, resulting in the constitutive expression of the hypoxia inducible factors, HIF-1α and HIF-2α. The HIFs drive the transcription of hundreds of genes associated with tumor progression, such as those promoting angiogenesis and metastasis. The current dogma in ccRCC is that HIF-2α is a central driver of disease progression, whereas HIF-1α plays a tumor suppressor role, and is frequently deleted in high-grade tumors. In this study using tissue from over 400 ccRCC patients, we describe new findings showing HIF-1α upregulation within unique non-neoplastic cell populations within the heterogeneous tumor cell mass, which was significantly associated with poor outcome. By contrast, high levels of HIF-2α and HAF, a coactivator of HIF-2α and ubiquitin ligase for HIF-1α, were significantly associated with better outcome. These findings challenge the current dogma and suggest greater nuance in the HIF-1/HIF-2 paradigm in ccRCC.
Dysregulated Acyl-CoA Metabolism Drives the Clear Cell Phenotype and Tumor Growth in Renal Cell Carcinoma

Edward L. LaGory¹, Timothy D. Klasson¹, Annika Enejder², Marjan Rafat³, Amato J. Giaccia¹

¹ Departments of Radiation Oncology and ² Materials Science and Engineering, Stanford University, San Francisco, CA, USA; ³ Chemical and Biomolecular Engineering, Vanderbilt University, Nashville, TN, USA

Von Hippel-Lindau disease is associated with a high lifetime risk for the development of benign renal cysts and clear cell renal cell carcinomas (ccRCC) which together negatively impact kidney function and present the threat of metastatic dissemination. Like most malignancies, VHL-deficient ccRCC tumors exhibit profound metabolic alterations compared to normal kidney tissue. One predominant metabolic alteration in ccRCC is the characteristic clear cell histology driven by the pronounced accumulation of lipid droplets in the cytoplasm of tumor cells. Despite this widely recognized histopathology, the molecular mechanisms that drive lipid accumulation, and how this aberrant metabolic phenotype contributes to malignancy remains elusive. Our studies provide evidence that uptake of fatty acids predominantly drives lipid droplet formation in ccRCC. Using genetic and pharmacologic approaches, we further describe an essential role for long chain acyl-CoA synthetase (ACSL) in the subsequent metabolism of fatty acids into triglycerides, the major component of lipid droplets. We employ genetic approaches to determine that specific ACSL isoforms are important for maintaining lipid droplet formation and sustaining cellular proliferation in ccRCC. Genetic suppression of individual ACSL isoforms or pharmacologic targeting of ACSL activity attenuates cell growth in in vitro models, suggesting that ACSL-mediated lipid droplet formation is essential for ccRCC growth. In addition to ablating lipid droplet formation, ACSL inhibition results in a pronounced accumulation of lipid peroxidation products, indicating that oxidative stress may underlie cell death upon ACSL inhibition. Our findings provide evidence that uptake of exogenous fatty acids drives lipid droplet formation through an ACSL-dependent mechanism and that inhibition of this metabolic pathway profoundly impairs ccRCC growth. Beyond the implication that targeting lipid metabolism may be a useful therapeutic tool in treating ccRCC, our findings highlight the possibility of lipid-based imaging modalities for detection and monitoring of ccRCC.
Multiple Tumor Suppressors Regulate a HIF-Dependent Negative Feedback Loop through ISGF3 in Kidney Cancer

Lili Liao¹,6#, Zongzhi Z Liu⁶#, Lauren Langbein¹#, Weijia Cai¹#, Eun-Ah Cho¹,2#, Jie Na³, Xiaohua Niu⁴, Wei Jiang¹, Zhijiu Zhong⁵, Yuxin Wang⁶, George Stark⁶, Jianxin Sun⁸, Stephen C. Peiper¹, Yaomin Xu⁷*, Qin Yan⁶*, Haifeng Yang¹

¹ Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA, USA; ² Fox Chase Cancer Center; ³ Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA; ⁴ Department of Gastrointestinal Surgery, The Sixth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China; ⁵ Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic;, Cleveland, OH, USA ⁶ Department of Pathology, Yale University, New Haven, CT, USA; ⁷ Department of Biostatistics, Department of Biomedical Informatics, Vanderbilt University Medical Center, Nashville TN, USA; ⁸ Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA; # Co-first authors; * Co-Corresponding authors

**Objectives:** Identify the key tumor suppressing pathway that is shared by key cancer genes in kidney cancer.

**Introduction/Background:** Whereas VHL inactivation is a primary event in clear cell renal cell carcinoma (ccRCC), the precise mechanism(s) of how this interacts with the secondary mutations in tumor suppressor genes, including PBRM1, KDM5C/JARID1C, SETD2, and/or BAP1, remains unclear.

**Methods:** Gene expression analyses by microarray after manipulations of cancer genes. Bioinformatic analysis and standard molecular biology techniques.

**Results:** Gene expression analyses reveal that VHL, PBRM1, or KDM5C share a common regulation of interferon response expression signature. Loss of HIF2α, PBRM1, or KDM5C in VHL-/− cells reduces the expression of interferon stimulated gene factor 3 (ISGF3), a transcription factor that regulates the interferon signature. Moreover, loss of SETD2 or BAP1 also reduces the ISGF3 level. Finally, ISGF3 is strongly tumor-suppressive in a xenograft model as its loss significantly enhances tumor growth. Conversely, reactivation of ISGF3 retards tumor growth by PBRM1-deficient ccRCC cells.

**Conclusions:** After VHL inactivation, HIF induces ISGF3, which is strongly tumor suppressive. ISGF3 activation is reversed by the loss of any of the key secondary tumor suppressors such as PBRM1, KDM5C, SETD2 or BAP1, suggesting that this is a key negative feedback loop in ccRCC. Boosting ISGF3 activity could be a novel therapeutic strategy against ccRCC.
SESSION TITLE

Gene Editing: Fact or Fiction for VHL Disease
AAV Targeted Therapy for Inherited Retinal Dystrophy: Bench to Bedside

Daniel C. Chung
Spark Therapeutics, Philadelphia, PA, USA

Purpose: Several early-phase human trials provided preliminary evidence of safety and efficacy for adeno-associated virus-mediated human RPE65 gene augmentation for RPE65-biallelic mutation-associated inherited retinal dystrophy. We report the clinical development, of Luxturna (voretigene neparvovec-rzyl) the first FDA approved gene therapy for a genetic disease for the treatment of patients with biallelic mutations in the RPE65 gene associated retinal dystrophy with remaining viable retinal cells.

Methods: Thirty-one subjects with disease-causing biallelic RPE65 mutations were randomized 2:1 to intervention or control. Eligibility criteria included age ≥3 years-old; bilateral visual acuity worse than 20/60 and/or visual field less than 20 degrees in any meridian; evidence of sufficient viable retinal cells by fundus photography and optical coherence tomography; ability to be evaluated on mobility testing; and willingness to provide consent or parental permission and assent, where appropriate. Subjects in the intervention group received subretinal injections of Luxturna sequentially to each eye within an 18-day window. Control subjects did not receive Luxturna for at least 1 year from baseline, but completed the same testing regiment as those in the intervention arm. Using a standardized subretinal delivery procedure and under general anesthesia, 1.5E11 vector genomes/eye were delivered in a total volume of 300 µl. A novel, standardized multi-luminance mobility test was the primary efficacy endpoint, with secondary endpoints including full field light sensitivity testing, assigned first eye mobility change score and visual acuity, and additional prespecified endpoints including visual field.

Results: All subjects completed Year 1 follow-up testing. Phase 3 study results include demographics, safety information, and mobility testing change score (performance at 1 year compared with baseline), and secondary endpoints of full field light sensitivity testing, assigned first eye mobility change score and visual acuity. A separate study analyzing mobility test data in untreated normal and retinal dystrophy cohorts was used to validate the mobility test’s ability to distinguish low vision from normal-sighted populations, differentiate a range of performance in low vision subjects, and confirm changes in functional vision over time. The trial of 31 subjects met with statistical significance its primary endpoint, the bilateral mobility test change score ($p = 0.001$), as well as the first two of three secondary endpoints, specifically full-field light sensitivity threshold testing, or FST ($p < 0.001$), and the assigned first eye mobility test change score ($p = 0.001$). Statistical significance was not achieved for the third secondary endpoint, visual acuity ($p = 0.17$). Serious side effects reported in the US Full Prescribing Information include endophthalmitis, that may lead to blindness, permanent visual acuity loss or retinal changes causing vision loss. Other potential side effects that can be associated with Luxturna treatment include hyperemia, cataracts, increased intraocular pressure, retina tears, epiretinal membrane, corneal dellen, macular hole, subretinal deposits, conjunctival edema, eye irritation or pain.

Conclusions: The clinical development of Luxturna (voretigene neparvovec-rzyl) including the primary endpoint of mobility testing and 2 of the 3 secondary endpoints in the Phase 3 trial provided sufficient evidence of the efficacy and safety of Luxturna, a surgically administered gene therapy, to support its approval in the U.S.
Cas9 Mediated Therapy

Giannicola Genovese
Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston TX, USA

Genome editing methods have revolutionized our approach to basic as well as translational research and enabled unprecedented clinical applications.

My talk will focus on the CRISPR/Cas9 technology and provide an overview of the development, the biological bases and the current applications of Cas9 and Cas9 variants in functional genomics with a focus on modeling cancer in *in vivo* experimental model systems. I will also discuss the recent efforts in translational oncological research aimed at the identification of context-specific vulnerabilities through the interrogation of the cancer genome with CRISPR-based libraries.

I will finally discuss ongoing effort and potential future application of the CRISPR technology in the clinical practice.
SESSION TITLE

Bioinformatics and Data Acquisition
New Lessons from an Old Gene: Complex Splicing and a Novel Cryptic Exon in VHL Gene Cause Erythrocytosis and VHL Disease

Betty Gardie,1,2,3,19* Marion Lenglet,1,2,38 Florence Robriquet,2,38 Klaus Schwarz,4 Carme Camps,5,6 Anne Couturier,7 David Hoogewijs,8 Alexandre Buffet,9,10 Samantha JL. Knight,5,6 Sophie Gad,1,11 Sophie Couvé,1,11 Franck Chesnel,7 Pierre Lindenbaum,3 Thomas Besnard,3,12 Sophie Deveaux,14,15 Sophie Ferlicot,14,16 Fabrice Aïraud,12 Céline Garrec,12 Richard Redon,3 Stéphane Bezieau,3,12 Brigitte Bressac-de Paillerets,13 François Girodon,17,19 Maria-Luigia Randi,20 Vincent Bours,22 Joachim R. Göthert,24 Antonis Kattamis,25 Nicolas Janin,26 Celeste Bento,27 Jenny C. Taylor,5,6 Yannick Arlot-Bonnemains,7 Stéphane Richard,1,11,14,158 Anne-Paule Gimenez-Roqueplo,9,10,16,178, Holger Cario,23*

1 Ecole Pratique des Hautes, EPHE, PSL research University, France; 2 CRCINA, INSERM, Université de Nantes, Université d'Angers, Nantes, France; 3 L'institut du thorax, INSERM, CNRS, UNIV Nantes, Nantes, France; 4 Institute for Transfusion Medicine, University of Ulm and Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service Baden-Württemberg-Hessen, Ulm, Germany; 5 Wellcome Centre for Human Genetics, University of Oxford, Oxford, UK; 6 Oxford NIHR Biomedical Research Centre, Oxford, UK; 7 Univ Rennes, CNRS, IGDR (Institut de génétique et développement de Rennes) - UMR 6290, F- 35000 Rennes, France; 8 Department of Medicine/Physiology, University of Fribourg, 1700 Fribourg, Switzerland; 9 INSERM UMR970, Paris-Cardiovascular Research Center at HEGP, Paris, France; 10 Université Paris Descartes, Faculté de Médecine, Paris, France, Équipe labellisée Ligue contre le Cancer; 11 INSERM UMR 1186, Institut Gustave Roussy, Université Paris-Saclay, Villejuif, France; 12 Service de Génétique Médicale, CHU de Nantes, Nantes, France; 13 Molecular Diagnostics Laboratories, Molecular Haematology Dept, Oxford University Hospitals Trust, Oxford, UK; 14 Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre, France; 15 Réseau Expert National pour Cancers Rares de l'Adulthood National RENCA "Predir" and Réseau d'Oncogénétique National RENCA "Maladie de VHL et prédispositions au cancer du rein," Service d’Urologie, Assistance publique, Hôpitaux de Paris, Hôpital Bicêtre, Le Kremlin-Bicêtre, France; 16 Pathology Department, Hôpitaux Universitaires Paris Sud, Assistance Publique Hôpitaux de Paris, Le Kremlin Bicêtre, France; 17 Service d'hématologie Biologique, Pôle Biologie, CHU Dijon, Dijon, France; 18 Inserm UMR1231 “Lipides Nutrition Cancer” équipe "Protéines de Stress et Cancer”, FCS Bourgogne Franche Comté, LipSTIC Labex, F-21000 Dijon, France; 19 Laboratory of Excellence GR-Ex; 20 First Medical Clinic, Department of Medicine- DIMED, University of Padua, Padua, Italy; 21 Clinic of Pediatric Hemato-Oncology, Department of Woman's and Child's Health, University of Padua, Padua, Italy; 22 Service de génétique humaine du CHU Sart Tilman, B-4000 Liège, Belgium; 23 Department of Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, The Netherlands; 24 Department of Hematology, West German Cancer Center, University Hospital Essen, Essen, Germany; 25 First Department of Pediatrics, National and Kapodistrian University of Athens, Greece; 26 Centre de Génétique Humaine, Cliniques universitaires Saint-Luc, B-1200 Bruxelles, Belgium; 27 Department of Hematology, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal; § ß * These authors contributed equally to this work

Background: Von Hippel-Lindau disease is an autosomal dominant disorder characterized by the development of highly vascularized tumors. In 2002, an additional phenotype, the Chuvash polycythemia, has been associated with a particular homozygous VHL mutation (VHL-R200W). Chuvash polycythemia is an autosomal recessive form of congenital erythrocytosis without tumors. Since then, other missense VHL mutations have been described in patients with congenital erythrocytosis, at homozygous or compound heterozygous status.

Intriguingly, some patients present with VHL disease in the absence of identified mutations or deletions in VHL. In addition, some patients with erythrocytosis have been found to be heterozygous rather than homozygous for the expected alteration.
**Methods:** We performed a comprehensive study of families with VHL-related disease presenting a such unexpected genotype: cloning and sequencing of all the mRNA VHL isoforms, segregation studies, phylogenetic analysis, RNA sequencing, measurement of mRNA and proteins expression, functional studies of the potential new VHL protein isoform and splicing reporter assays.

**Results:** We identified a new VHL cryptic-exon (termed E1') deep in intron 1 that is naturally expressed in many tissues. More importantly, we identified mutations in E1' in seven families with erythrocytosis and in one large family with typical VHL disease but without any alteration in the other VHL exons. In this study, we have shown that the mutations induced a dysregulation of the VHL splicing with excessive retention of E1' and are associated with a downregulation of VHL protein expression. In all the studied cases, the mutations differentially impact splicing, correlating with phenotype severity (erythrocytosis *versus* cancer).

**Conclusion:** This study demonstrates that cryptic-exon-retention is a new VHL alteration and reveals a novel complex splicing regulation of the VHL gene. These findings open new avenues for diagnosis and research into the VHL-related-hypoxia-signaling pathway.

**Funding:** This study was supported by grants from the Région Pays de la Loire, Project EryCan; the Agence Nationale Recherche (Programme de Recherche Translationnelle en Santé 2015 GenRED) and the Laboratory of Excellence GR-Ex (reference #ANR-11-LABX-0051).
VHL Information Sharing International Consortium (VISION)


1 Baylor College of Medicine, Houston TX, USA; 2 University Health Network, Princess Margaret Cancer Center, Toronto, Ontario, CA; 3 Invitae, San Francisco, CA, USA; 4 University Medical Center, Utrecht, NL; 5 McDonnell Genome Institute, Washington University, St. Louis, MO, USA; 6 Ambry Genetics, Aliso Viejo, CA; 7 GeneDx, Gaithersburg, MD, USA; 8 St. Jude's Children's Research Hospital, Memphis, TN, USA; 9 Children's Hospital of Philadelphia, Philadelphia, PA USA; 10 Prevention Genetics, Marshfield WI, USA; 11 University of Cambridge, Cambridge, UK; 12 National Institutes of Health, Bethesda, MD, USA

Due to the rarity and complexity of von Hippel-Lindau disease, fragmented information about patients and mutations has hindered improvement in the understanding and treatment of VHL. To address these issues, we assembled a consortium of VHL clinicians and researchers (VISION) to promote VHL data collection and exchange on a global scale. VISION focused on three main efforts to deliver a more widespread availability of VHL information through freely available means. This includes: 1) Establishment of the largest searchable VHL disease collection in the Clinical Interpretations of Variants in Cancer (CIViC) database; 2) Creation of an NIH ClinGen VHL Expert panel to create VHL gene-specific rules; 3) Development of a ready-to-use, head-to-toe VHL patient RedCap database. These efforts will advance clinical data collection strategies, disseminate the knowledge of VHL mutations resulting in improved diagnosis, surveillance, and treatment of VHL patients.
Bioinformatics tools to gain insight into proteomic and genomic data

Christine B. Peterson
Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

In this talk, I will discuss tools for making the most of protein and gene expression data sets relevant to ccRCC research. While standard analysis of gene expression typically begins with assessing differential abundance of individual genes, multivariate methods that look across pathways or sets of genes can often improve power and provide more interpretable results. In particular, gene set enrichment analysis (GSEA) and single-sample gene set enrichment analysis (ssGSEA) can be used to detect coordinated changes in expression for a given pathway, while deconvolution methods such as CIBERSORT can be used to identify the abundance of different cell types within a sample. I will discuss these methods and provide examples of their application to publicly available data sets, in particular, to The Cancer Genome Atlas (TCGA) and The Cancer Proteome Atlas (TCPA) KIRC data.
SESSION TITLE

New Developments in Imaging Technology
Novel Imaging Approaches in VHL

Ivan Pedrosa
Departments of Radiology, Advanced Imaging Research Center, and Urology, Simons Comprehensive Cancer Center, The University of Texas Southwestern, Dallas, TX, USA

Patients with von Hippel-Lindau (VHL) disease, a rare hereditary autosomal dominant disease, suffer from the development of benign and malignant tumors in multiple organs during their lifetime. There are currently no optimal biomarkers to screen or monitor the progression of these tumors. Accordingly, imaging plays an essential role in the screening, initial diagnosis/staging and monitoring of the progression of these disease-associated neoplasms. Apart from retinal hemangioblastomas, which are diagnosed by direct ophtalmoscopic examination, the most common tumors assessed by imaging involve the central nervous system (CNS), kidneys, pancreas, adrenals/retroperitoneum, and epididymis. The wide variety of organs involved require extensive anatomic coverage while maintaining high-resolution strategies that permit the evaluation of complex anatomy. Furthermore, because many of these tumors can develop early in life, patients require multiple diagnostic imaging examinations during their lifetime. Moreover, these patients are susceptible to renal insults because they commonly suffer renal function impairment secondary to renal tumors or their treatment. Accordingly, exposure to repeated doses of ionizing radiation and/or nephrotoxic contrast during computed tomography (CT) exams is particularly problematic in this patient population. During this presentation, we will review novel CT technologies that offer an opportunity to reduce the radiation dose and intravenous contrast requirements. Magnetic resonance imaging (MRI) provide exquisite detail of the CNS and abdomen, the most common locations for VHL-related tumors. However, it is frequently associated to long examination times. Similarly, recent concerns have been raised about the administration of repeated doses of gadolinium-based contrast agents for MRI. Here, we will review the novel approaches for short MRI protocols and non-contrast MRI techniques. The role of contrast-enhanced ultrasound will be discussed, particularly in the context of abdominal neoplasms. Finally, specific scenarios for the use of molecular imaging will be presented.
Overview of Imaging Modalities in the Management of VHL Lifecycle

W. Kimryn Rathmell
Division of Hematology and Oncology, Departments of Medicine and Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, USA

The clinical management of VHL has recently undergone a series of changes in recommendations and guidelines for early detection and interventions. A recent publication by Rednam, et al., has suggested more aggressive surveillance and earlier attention to the prospective identification of lesions. Now, therapeutics targeting key pathways common to all VHL deficient cells are underway. This presentation will overview the current clinical guidelines, emerging clinical trials, and factors to consider when developing a patient and family centered care plan.
F18-FAZA PET Imaging Reveals Precise Pharmacodynamics in vivo of the Novel Chemotherapeutic IACS-010759

Seth Gammon

Department of Cancer Systems Imaging, Division of Diagnostic Imaging, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Genetic deletions and mutations resulting in defects in glycolysis, force these tumors to depend on oxidative phosphorylation (OxPhos) for growth. IACS-010759 is a nanomolar inhibitor of Complex I, and in phase-I clinical trials at MD Anderson for both AML and solid tumors. While ex vivo monitoring of inhibition of oxygen consumption in leukocytes was sufficient for AML, non-invasive methods of monitoring target engagement in solid tumors was desired. Tumors, particularly those that rely on oxidative phosphorylation, yield hypoxic and reducing environments. These conditions are ideal for trapping 2-nitroimidazole based imaging agents, such as F18-labeled fluoroazomycin arabinoside ([18F]FAZA). IACS-010759 inhibited oxygen consumption in vitro with IC50 values ranging from 1.3 nM to 6 nM across a variety of cell lines with a diversity of glycolysis defects. In H460 NSLC, SKMEL5 melanoma, A375R melanoma, and D423-Fluc orthotopic GBM in vivo, at the MTD 10 mg/kg, a robust, up to 6 fold, (2 way ANOVA, p<0.0001) decrease in FAZA T/B ratio was observed. A slight, 1.5-fold, but detectable increase in [18F]FDG was also observed at 10mg/kg in A375 and A375R cells (p=0.002), but smaller than the decrease [18F]FAZA retention. Thus neither cell death nor loss of perfusion could explain the reduction in [18F]FAZA retention. To further test the robustness of the mechanism of inhibition, the converse experiment was conducted. Both 2,4-dinitrophenol and pyruvate were utilized to stimulate oxygen consumption in vivo, and as predicted [18F]FAZA retention increases (p<0.05 in both cases). [18F]FAZA retention by PET yielded the same IC50 for IACS-010759 as by IHC in a dose-dependent manner, with an apparent IC50 of 1.4 mg/kg (95%CI 0.48 to 4.1 mg/kg, n=12 mice) for A375R melanoma tumors. The PET measurement was more precise than an independently scored IHC metric based upon staining for pimonidazole from the same mice. In renal cell carcinoma 786-0 cells the FAZA retention is size unlike the above tumor types, thus a test/retest imaging protocol is even more important for appropriate interpretation of the imaging PD data. [18F]FAZA can be a powerful PD marker for the complex-I inhibitor IACS-010759 in preclinical models, and is translatable to upcoming clinical trials in patients.
For individuals with von Hippel-Lindau (VHL) disease, the burden of imaging is not insignificant. Beginning at age 16, the current guidelines recommend abdominal imaging with contrast-enhanced magnetic resonance imaging (MRI) annually, although this can be replaced with a quality ultrasound every other year. Central nervous system contrast-enhanced MRI is recommended every 2 to 3 years. For an individual who lives to their 70s, this can mean upwards of 45 lifetime MRI scans which also means exposure to high lifetime doses of gadolinium-based contrast agents. Historically, these contrast agents have been known to be quite safe with few significant adverse effects, but more recent findings have suggested that gadolinium can deposit in certain areas of the brain, although the clinical import of this is not yet known. In addition, MRI is also costly and can be difficult to tolerate for some patients. Patients with metal implants cannot get MRI. Computed tomography (CT) is an appropriate alternative, but repeating CT scans lead to high doses of radiation. Identification of a safe imaging technique that has equivalent sensitivity to contrast-enhanced MRI for detection of the abdominal pathologies seen with VHL would allow minimization of total lifetime MRI exposure. Contrast-enhanced ultrasound (CEUS) is an emerging ultrasound technique that has been in use for decades for cardiac imaging but has been more recently investigated for abdominal applications. This presentation will address the possibility for the application of this technology in individuals with VHL with the goal of reducing lifetime exposure to MRI.
in silico Exploration of von Hippel-Lindau (pVHL) Tumor Suppressor Molecular Functions: Correlations between Disease Mutations, Interactors and Oathways

Giovanni Minervini¹, Federica Quagilia¹, Francesco Tabaro¹, Silvio C.E. Tosatto¹²*

¹ Department of Biomedical Sciences, University of Padova, Viale G. Colombo 3, 35121, Padova, Italy;
² CNR Institute of Neuroscience, Padova, Viale G. Colombo 3, 35121, Padova, Italy;
*Corresponding Author: silvio.tosatto@unipd.it

Objectives: Here, we present our efforts in deciphering the role of the von Hippel-Lindau tumor suppressor protein (pVHL) in cancer insurgence. In particular, we aim at unveiling correlations between the effects of pVHL mutations and the plethora of different phenotypes characterizing the von Hippel-Lindau syndrome (VHL).

Background: VHL, a familiar predisposition to develop different cancers, represents a privileged point of view for studying complex cellular events inducing tumor transformation. This condition is associated to functional inactivation of pVHL and is characterized by cancer development in different target organs. Since its discovery, efforts in correlating pVHL mutations and types of developed cancers have been made. Evidences of patients developing different phenotypes despite harboring the same mutations suggest, however, that molecular details sustaining VHL insurgence are far from being clear.

Methods: We collected high quality information on ca. 1,600 pVHL mutations and ca. 160 interactors from VHLdb, a freely accessible database dedicated to pVHL (1). These were investigated in terms of association between patient phenotypes, mutated surfaces and impaired interactions. These data were used to drive molecular docking of pVHL with its interactors and guide Petri net simulations of the most promising alterations.

Results: Our data suggests that different phenotypes correlate with localized perturbations of the pVHL structure, with specific cell functions associated to different protein surfaces. We propose five pVHL interfaces to be selectively involved in modulating proteins regulating gene expression, protein homeostasis as well as to address ECM and ciliogenesis associated functions. We predict that the disruption of pVHL association with certain interactors can trigger tumor transformation, inducing metabolism imbalance and ECM remodeling.

Conclusions: Collectively taken, our findings provide a novel insight into VHL-associated tumorigenesis. This highly integrated in silico approach may help to elucidate novel treatment paradigms for VHL disease.

References:
SESSION TITLE

CNS Hemangioblastomas
KEYNOTE ADDRESS

HIF Reprograms Cancer Metabolism in VHL-Deficient Tumors: Opportunities for Targeted Therapy of VHL Disease

Othon Iliopoulos
Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Glutamine dependence is a critical feature and a vulnerability of VHL-deficient clear cell renal cell carcinoma (ccRCC), suggesting that glutaminase 1 (GLS1) inhibitors are putative novel therapeutic agents for ccRCC. The glutaminase inhibitor CB-839 is now in clinical trials for the treatment of ccRCC and other Hypoxia Inducible Factor (HIF)-driven cancers. We recently showed that ccRCC cells treated with GLS1 inhibitors become impaired in nucleotide synthesis and exhibit DNA damage and replication stress. This observation led us to discover that Olaparib, an inhibitor of DNA repair, acts synergistically with GLS1 inhibitors to suppress growth of ccRCC both in vitro and in vivo.

In order to identify adaptive responses of HIF-expressing cells to glutamine deprivation by GLS1 inhibition, we generated and analyzed gene expression profiles in VHL deficient (VHL-/-) and isogenic VHL-replete (VHL+) ccRCC cells treated with CB-839. Gene set enrichment analysis (GSEA) identified known signaling pathways that are involved in cellular adaptive response to glutamine deprivation. In addition, we discovered novel signaling and metabolic pathways that can putatively modulate the cellular response to GLS1 inhibitors, which will be presented. We validated these metabolic targets either pharmacologically or via shRNA. Therapeutic targeting of these signaling pathways would enhance the therapeutic value of GLS1 inhibitors as a single agent or in combination with PARP inhibitors.
CLINICAL DEBATE

Optimal Treatment of Hemangioblastoma

Ian McCutcheon
Professor, Department of Neurosurgery, The University of Texas MD Anderson Cancer Center

Ashok Asthagiri
Neurological Surgery, University of Virginia School of Medicine

The three essential elements of tumor treatment in oncology are surgery, radiotherapy, and medical therapy. Although targeted therapy has been studied in patients with VHL, its results are much better for those tumors located outside the central nervous system than for the hemangioblastomas that VHL causes in the brain and spinal cord. For that reason, for hemangioblastomas, the two main methods of tumor control (and thus control of the secondary effects of the tumor, such as edema, local pressure, and diffuse pressure from hydrocephalus) are currently surgery and radiotherapy. Surgery has the advantages of separating the patient from the disease, but it is invasive and poses risk of injury to adjacent structures. Radiotherapy is less invasive but tumors show differential sensitivity to irradiation, and unless the treatment is delivered in a highly targeted fashion, risk to adjacent structures can also occur. Both methods are also operator-dependent; more experienced surgeons will achieve better results, and the same is true for radiation oncologists. The issue of durability of treatment will also be addressed. This session describes the techniques that form the state of the art in both surgery and radiotherapy, explores the relative advantages and disadvantages of these two methods for controlling hemangioblastomas, and discusses which method makes the most sense for different subsets of patients with this tumor type.
PROFFERED PAPER

New Radio-Surgical Technique for Endolymphatic Sac Tumor Treatment: Results for the First Two Patients

Nevoux, J.,1,2 Le Pajolec, C.,1 Herbrecht, A.,3 Parker, F.,2,3 Benoudiba, F.,4 Ducreux, D.,2,4 Richard, S.,2,5,6 Spelle, L.,2,7 Papon, J.F.1,2

1 AP-HP, Hôpital Bicêtre, service d'Oto-Rhino-Laryngologie, Le Kremlin-Bicêtre, France; 2 Université Paris-Saclay, Faculté de Médecine, Le Kremlin-Bicêtre, France; 3 AP-HP, Hôpital Bicêtre, service de neurochirurgie, Le Kremlin-Bicêtre, France; 4 AP-HP, Hôpital Bicêtre, service de neuroradiologie, Le Kremlin-Bicêtre, France; 5 AP-HP, Hôpital Bicêtre, Réseau Expert National pour Cancers Rares de l’Adulte PREDIR labellisé par l’INCa, Le Kremlin-Bicêtre, France; 6 Génétique Oncologique, Ecole Pratique des Hautes Etudes, PSL Research University, Paris, France; INSERM U1186, Institut de Cancérologie Gustave Roussy, Villejuif, France; 7 AP-HP, Hôpital Bicêtre, service de neuroradiologie interventionnelle, Le Kremlin-Bicêtre, France

Objectives: To improve the management of patients with endolymphatic sac tumor (ELST) combining surgery and embolization.

Introduction: ELST are challenging to remove because these tumors are extremely well vascularized and are encased in the temporal bone. The standard treatment is an extensive endovascular embolization followed as complete a resection as possible. Frequently, endovascular embolization after the preoperative angiography cannot be completed due to risk to embolize the feeding vessels with potential neurological complications or because catheterization is not possible. In such cases, the surgeon struggles with massive bleeding and surgery cannot be complete without risk of complications. To avoid these issues, we developed, a new approach consisting of a four-step procedure to treat this tumor.

Methods: In the first step of the procedure, the surgeon drills into the bone surrounding the tumor and opens an access for the radiologist. In the second step, classical endovascular angiography was realized. The third step was the direct puncture technique with needle under permanent fluoroscopic guidance using the 3D XperGuide planning software. The last step was the ablation of the tumor by the surgeon.

Results: Two cases of ELST were treated with our new protocol.

A 25-year-old man with an ELST of 6.5 cm had a previous partial surgery because of an intra-operative massive bleeding after an only partial classical embolization. One year after surgery, the tumor imaging showed that the tumor was growing. We performed our new technique without any complications confirmed by the conventional angiography, which revealed complete devascularization of the tumor.

A 7-year-old girl with an ELST of 3 cm with an initial endovascular angiography revealed that it would be impossible to catheterized the main feeder. The second part of the procedure was the classical endovascular angiography. In the third step, the interventional radiologist punctured the tumor for injection. No side effects were observed in the postoperative period. In the fourth step, the surgeon resected the tumor by a retro-labyrinthine approach. Intra-operative bleeding was less than usual for an ELST.
Conclusions: To improve the management of ELST, we modified the direct puncture approach used previously only for treatment of accessible tumors. A window was created in the temporal bone by the surgeon that allowed a combined endovascular and 3D-guided direct puncture embolization, which resulted in excellent devascularization and permitted a safe surgery. This new technique opens a new field for treatment of temporal bone tumors.
SESSION TITLE

Renal Cell Carcinoma
HIF-2 In Renal Cancer

James Brugarolas

Department of Internal Medicine’s Division of Hematology/Oncology and the Department of Developmental Biology, The University of Texas Southwestern Medical Center, Dallas, TX, USA

*VHL* is arguably the most important gene in the pathogenesis of clear cell renal cell carcinoma (ccRCC). *VHL* is inactivated in the majority of tumors, represents the only consistently mutated truncal event, and its inactivation may precede tumor development by several years. Furthermore, *VHL* reconstitution into *VHL*-deficient ccRCC cell lines inhibits tumor formation. The VHL protein, pVHL, functions as the substrate recognition subunit of an E3 ubiquitin ligase complex. While multiple pVHL substrates have been identified, VHL reconstitution has limited consequences *in vitro* and its inhibitory effects *in vivo* can be largely overcome by expression of a HIF-2α protein that evades pVHL-mediated degradation. Complementarily, HIF-2α downregulation (like VHL reconstitution), inhibits tumor growth. Thus, HIF-2α represents an attractive target for drug development. Nevertheless, as a transcription factor of the non-nuclear hormone receptor family, HIF-2α was regarded as undruggable. However, structural analyses of the HIF-2α protein at UT Southwestern identified a potential vulnerability. In the PAS-B domain, a 300Å³ cavity was discovered, which could provide a foothold for a drug. The HIF-2α PAS-B domain is implicated in dimerization with HIF-1β, and this is essential for DNA binding and transactivation. Thus, investigators reasoned that a chemical that bound the cavity and altered the domain conformation may preclude dimerization and inhibit HIF-2. A screen of the UT Southwestern chemical library identified several lead compounds, which were licensed to Peloton Therapeutics, Inc. and underwent extensive medical chemistry. One such compound, PT2399, was evaluated extensively in preclinical models were it demonstrated activity against ccRCC. A related compound, PT2385, was evaluated in a phase I clinical trial, where it showed remarkable safety (which is consistent with preclinical data demonstrating its high specificity), as well as activity against heavily pretreated ccRCC. Targeting HIF-2 thus represents a promising strategy against ccRCC.
CLINICAL DEBATE

Partial Nephrectomy or Percutaneous Ablation for VHL-Related RCC

MODERATOR
Surena Matin
Professor with Tenure in the Department of Urology, Division of Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Sharjeel Sabir
Assistant Professor, Interventional Radiology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Brian Shuch
Associate Professor and Alvin & Carrie Meinhardt Endowed Chair in Kidney Cancer Research; Director of the UCLA Kidney Cancer Program, UCLA Medical Center, Los Angeles, CA, USA

The Clinical Debate will include a debate on partial nephrectomy versus percutaneous ablation, highlight the surgical priorities and challenges in managing patients with bilateral, multifocal and recurring tumors. Selecting a therapeutic strategy for multifocal RCC in patients with VHL requires balancing the risk of RCC metastasis with the risk of worsening renal function. Percutaneous ablation offers a less invasive and renal sparing modality for patients at risk for multiple interventions over time, and can be easily repeated. The majority of VHL patients have cystic renal lesions, including benign cysts and cystic RCC, regardless of germline mutation type. In mixed cystic-solid tumors, the size of the solid portion guides the need for intervention. Ultrasound as well as MRI can be a useful tool to estimate the solid component of complex cysts. Surgical intervention for cystic RCC utilizes real-time intraoperative ultrasound and enucleation to ensure complete removal while minimizing normal parenchymal loss.
DNA Damage Signaling and Therapeutic Opportunities in VHL Disease

Eric Jonasch1, Lijun Zhou1, Christine B. Peterson3, Yang Peng4, Guang Peng4, Lawrence Bronk5, Xian-De Liu1, Xuesong Zhang1, Christopher G. Wood6, Jose A. Karam6, Kanishka Sircar7, Carl M. Gay2, Timothy A. Yap8, Xizeng Mao9, Jianhua Zhang9, P. Andrew Futreal9, Patrick Pilie2

1 Department of Genitourinary Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 2 Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 3 Department of Biostatistics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 4 Department of Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 5 Department of Radiation Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 6 Department of Urology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 7 Department of Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 8 Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 9 Department of Genomic Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Clear cell renal cell carcinoma (ccRCC) displays a moderate level of genomic instability, suggesting deficiencies in DNA damage response (DDR). However, ccRCC does not harbor frequent mutations in canonical DDR genes, but does have near ubiquitous mutation in the von Hippel-Lindau (VHL) gene and loss of chromosome 3p. In this study, we show in clinical samples and preclinical models that biallelic VHL loss is sufficient to cause homologous repair deficiency (HRD) early in ccRCC carcinogenesis. Early stage ccRCC tumors display dysregulated DDR signaling, and in silico data show a functional HRD signature in ccRCC tumors that has prognostic and predictive potential and shows association with VHL mutation and early stage disease. Preclinical modeling shows VHL loss/mutation leads to defective binding/activation of Tip60 (KAT5), a key activator of DDR signaling pathways. Taken together, these findings establish VHL as a key regulator of DDR signaling in early RCC carcinogenesis.
Clinical and Surgical Management of VHL-Related Cysts and Cystic RCC

Mark Ball
Urologic Oncology Branch, National Cancer Institute (NIH), Bethesda, MD, USA

Renal cysts and cystic renal cell carcinoma are common manifestations of von Hippel-Lindau (VHL). Management of these lesions can include active surveillance and surgical removal. This talk will include strategies to appropriately risk-stratify patients with cystic lesions, differentiate benign cysts from cystic renal cell carcinoma and operative techniques to remove cystic tumors.
Evolution of Clinical Studies in VHL Disease

Ramaprasad Srinivasan
Urologic Oncology Branch, National Cancer Institute (NIH), Bethesda, MD, USA

Patients with von Hippel-Lindau (VHL) are at risk for developing neoplasms in multiple organs, including the kidney (clear cell renal cell carcinoma), the pancreas (pancreatic neuroendocrine tumor), pheochromocytomas, CNS hemangioblastomas and retinal angiomas. VHL associated neoplasms are managed surgically in most organ systems affected, with tumors resected periodically to minimize the risk of metastatic disease (clear cell RCC, pancreatic tumors etc.) or local effects (CNS hemangioblastomas). Since repeated surgical resection is associated with significant long term morbidity, there is significant interest in the development of nonsurgical approaches for VHL patients. VHL is caused by germline mutations in the VHL tumor suppressor gene, with subsequent somatic loss of the second VHL allele in tumors. One of the better characterized consequences of loss of VHL function is the dysregulated expression of hypoxia inducible factors, particularly HIF2, and consequent overexpression of proangiogenic and growth factors such as Vascular Endothelial Growth Factor (VEGF) and Epidermal Growth Factor Receptor (EGFR). A variety of approaches targeting the downstream consequences of VHL inactivation have been evaluated in the clinic in the context of proof-of-principle phase 2 studies. While approaches targeting the VEGF-axis are associated with antitumor activity, the attendant toxicity profile is not acceptable to many VHL patients and the long term clinical utility of these approaches remains unclear. More recently, approaches targeting HIF-2α have attracted significant interest and are currently undergoing clinical evaluation in VHL patients.
SESSION TITLE

VHL Endocrine Manifestations
Germline Genotype Analysis as a Clinical Tool in the Management of Patients with VHL Associated Pancreatic Neuroendocrine Tumors

Amit Tirosh
Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel; Endocrine Oncology Bioinformatics Lab and Neuroendocrine Tumors Service, Sheba Medical Center, Tel-Hashomer, Israel

Background: The type of mutation in the VHL gene is associated with clinical phenotype in von Hippel-Lindau (VHL). We aimed to determine whether VHL genotype may be associated with pancreatic neuroendocrine tumor (PNET) phenotype in patients with VHL.

Methods: A prospective study of patients with VHL and pancreatic lesions with imaging follow-up (n=301). The risk for a metastatic disease and for requiring a surgical intervention during follow-up was compared by largest lesion diameter, and by germline VHL genotype parameters. In silico prediction of the variant using five computational prediction models were performed for patients with a known variant of missense germline VHL mutation (n=69). Patients with >80% prediction for disease-causing mutations in all models (high predicted risk, HPR) were compared with others (low predicted risk, LPR).

Results: One hundred seventy-five patients with a solid pancreatic lesion (156 with a known VHL genotype) were followed for a median of 53.0 months (range 12–84 months). Twenty-nine patients (16.6%) required surgical intervention, and seven (4.5%) developed metastatic disease. Patients with a missense mutation (n=76) had a shorter disease-free interval (P=0.03), and a higher rate of metastases (P=0.006) and of requiring surgical intervention during follow-up (P=0.01) as compared to patients with a non-missense VHL mutation (n=80). Patients with solid pancreatic lesions <1.2 cm in diameter had a low risk for metastasis or requiring surgical intervention, whereas those with solid lesions >3 cm had a higher risk for metastases in a multivariable analysis. Patients with a tumor diameter between 1.2 and 3 cm with missense VHL mutation, and/or mutations in exon 3 had a higher rate of requiring surgical intervention in multivariable analysis (hazard ratio 8.8, P=0.02).

Patients with a missense VHL mutation, with a high risk consensus in the five prediction models (HPR group, n=13) had a higher rate of disease progression than the LPR group (n=43) in multivariable analysis (HR 3.6, P=0.037), and a higher risk of developing metastases (P=0.015). Among patients with codon 167 hotspot mutations (n = 26), those in the HPR group had a higher risk for disease progression (P=0.03) compared to other patients.

Conclusion: VHL genotyping may be used to assess the risk of patients with VHL for having a solid pancreatic lesion, and is a complementary tool to tumor diameter for determining the risk of metastases and requiring surgical intervention. Computational models for predicting the impact of missense VHL gene mutations may further define the prognosis of patients with pancreatic manifestations of VHL.
Precise Surgical Management of VHL Endocrine Manifestations

Electron Kebebew
Division of General Surgery, Endocrine Oncology, Stanford Cancer Center, Stanford University, Stanford CA, USA

Von Hippel-Lindau (VHL) disease is a heritable cancer-predisposition syndrome with multiorgan involvement. Pheochromocytoma (PHEO) and pancreatic neuroendocrine tumors (PNETs) are common manifestations of VHL that require a multidisciplinary team. There have been significant advances in our understanding of the natural history of VHL-associated PHEO and PNETs, genotype-phenotype associations that impact patient outcome, new generation tumor specific imaging studies, and treatment alternatives for these tumors. We will discuss these advances and their application to precise surgical management for VHL-associated PHEO and PNETs.
Malignant Pheochromocytomas in VHL

Camilo Jimenez
Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Patients with von Hippel-Lindau disease are prone to develop pheochromocytomas and sympathetic paragangliomas. These tumors frequently secrete excessive amounts of noradrenaline predisposing patients to hormonal disease. 90% of these tumors are non-metastatic and could be cured with surgery. 10% of these tumors are metastatic to organs such as the skeleton, lymph nodes, liver and lungs. Patients with malignant tumors are at risk for cardiovascular (heart attacks, arrhythmias) and gastrointestinal complications (severe constipation) because of the excessive secretion of noradrenaline. Furthermore, the metastatic spread predisposes to severe tumor burden related complications such as skeletal related events (fractures, cord compression, hypercalcemia), lung and liver failure, and urinary tract obstruction. Therapies for patients with metastatic disease have been limited. Nevertheless, over the last decade the value of chemotherapy has been recognized and several potential systemic therapies have been identified. During this lecture we will discuss the benefits derived from surgery and chemotherapy and the preliminary/final results of phase 2 clinical trial with the high specific activity MIBG, tyrosine kinase and hypoxia inducible factor inhibitors.
VHL IN THE GENOMIC LANDSCAPE OF PHEOCHROMOCYTOMA AND PARAGANGLIOMA

Anne-Paule Gimenez-Roqueplo1,2,3

1 INSERM, UMR970, Paris-Centre de Recherche Cardiovasculaire, F-75015, Paris, France ; 2 Université Paris Descartes, PRES Sorbonne Paris Cité, Faculté de Médecine, F-75006 Paris, France ; 3 Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Génétique, F-75015 Paris, France

Paragangliomas and pheochromocytomas (PPGL) are neuroendocrine tumors with the strongest genetic component1. A germline mutation in one of the fifteen susceptibility genes identified so far explains about 40% of all cases and about 7% to 10% of these mutations are located on the VHL gene. While whole-exome sequencing studies showed that PPGL is characterized by a low mutation rate of 0.3 mutations per megabase similar to other neural crest-derived tumors, the first integrative genomic analysis of a large collection of PPGL, carried out by the French COMETE network, demonstrated that mutation status in PPGL susceptibility genes is strongly correlated with multi-omics data and revealed the crucial role of predisposing mutations as being the main drivers of PPGL tumorigenesis 2. PPGL subtypes can be defined by a set of unique genomic alterations that represent different molecular entities. Transcriptomic studies identified two main molecular pathways, activating either the hypoxic pathway (cluster C1) or the MAP kinase/mTOR signalling (cluster C2). Interestingly, the VHL-mutated tumors, at germline but also somatic level, were all classified in the cluster C1B in the transcriptome and were distinguishable from the other pseudo-hypoxic tumors, the SDHx-mutated tumors (cluster C1A), by a distinct Warburg effect3 and by a different DNA methylation profile in the methylome4. Currently, next-generation sequencing (NGS) is the ideal technology to screen in routine the high number of PPGL susceptibility genes and to give an access to a precise molecular classification at tumoral level. New immunohistochemical tools have been developed for helping the interpretation of genetic variants identified by NGS in the genes encoding for the Krebs cycle proteins and in the VHL gene. Finally, ‘omics’ data have helped the deciphering of tumors, and notably the tumors caused by the inactivation of the VHL protein, within the genomic landscape of PPGL and then, have opened the way to a personalized medical management for the affected patients and their relatives.

References:

1. Favier, Nat Rev Endocrinol, 2015
4. Letouzé, Cancer Cell 2013
PROFFERED PAPER

Synonymous but Not Silent: A Synonymous VHL Mutation Confers Susceptibility to Pheochromocytomas in a Four-Generation Family

Shahida K. Flores¹, Ziming Cheng¹, Angela Jasper¹, Richard W. Tothill², Patricia L.M. Dahia¹

¹ Department of Medicine, Division of Hematology/Oncology, UT Health San Antonio, San Antonio, TX; ² Peter McCallum Cancer Centre, Melbourne, Victoria, Australia

Pathogenic mutations in the von Hippel-Lindau (VHL) gene predispose individuals to VHL disease comprising of renal cell carcinomas, pheochromocytomas (PCCs), hemangioblastomas of the central nervous system and other manifestations with clinical presentation varying remarkably. In VHL disease type 2, PCC risk is higher, with type 2C manifesting PCC only. We report on a four-generation family with a history of PCCs in a pattern consistent with autosomal dominant inheritance. The proband developed a unilateral PCC at age 32. Several of her family members, including her brother, father, paternal aunt and cousin, have also developed unilateral PCCs. Whole exome sequencing of her germline DNA revealed a heterozygous, synonymous mutation (c.414A>G, p.P138P) in VHL exon 2. No other candidate genes were identified. Sanger sequencing showed that the mutation segregated with the PCC phenotype in the family. The variant was not observed in population databases (ExAC) whereas ClinVar had 3 entries with conflicting interpretations (2 uncertain significances, 1 likely pathogenic). Nanostring-based Pheo-Type profiling of the proband's archival PCC was consistent with a VHL subtype. In PCC from another relative, loss of the WT allele was observed at the variant locus. PCC cDNA analysis showed absence of the full length VHL transcript and presence of the shorter transcript lacking exon 2 in contrast to other PCCs which expressed both transcripts. Leukocyte cDNA analysis of carrier and WT relatives supported this finding. VHL encodes a tumor suppressor with ubiquitin ligase activity for degradation of hypoxia inducible factors (HIFs). VHL exon 2 is critical for the HIF binding domain; predominant expression of the shorter isoform leads to elevated HIF targets associated with oncogenesis. Most genetic screening workflows exclude synonymous variants. Our findings show that synonymous variants in coding regions of VHL should be taken into consideration as they may have splicing disruptions and affect protein function. Based on our findings, the c.414A>G variant is pathogenic and carriers should undergo routine follow-up for early detection. Although this family's clinical profile suggests VHL type 2C disease, broader surveillance is recommended as the consequences of this variant are not yet fully defined.
SESSION TITLE
Retinal Hemangioblastomas
Retinal hemangioblastomas are often the earliest manifestation of VHL. Therapies are directed toward focal management with vision and globe preservation. Treatment is highly personalized as individual lesions are best managed with modalities that balance tumor ablation with potential impact on vision. The smallest lesions may be managed with observation but require close serial assessment. Laser photocoagulation & cryotherapy remain standard therapies however; both can lead to secondary retinal exudation and detachment. Alternatives techniques such as photodynamic therapy have shown select benefit. Early reports suggested encouraging results with intravitreal ranibuzimab but further studies demonstrated limited efficacy. Surgical resection is not common but may have outstanding results. Radiation therapy has been described in advanced lesion however secondary toxicity may include neovascular glaucoma and subsequent enucleation.
Murine Model of VHL Retinal Hemangioblastomas

Herui Wang1, Matthew J. Shepard2, Chao Zhang2, Lijin Dong3, Dyvon Walker2, Liliana Guedez3, Stanley Park3, Yujuan Wang3, Shida Chen3, Ying Pang4, Qi Zhang1, Chun Gao3, Wai T. Wong3, Henry Wiley3, Karel Pacak4, Emily Y. Chew3, Zhengping Zhuang1, Chi-Chao Chan3

1 Neuro-Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, 2 Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA; 3 National Eye Institute, National Institutes of Health, Bethesda, MD, USA; 4 Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

Von Hippel-Lindau (VHL) disease is an autosomal dominant tumor predisposition syndrome characterized by the development of highly vascularized tumors and cysts. Loss of heterozygosity (LOH) of the VHL gene results in aberrant upregulation of hypoxia-inducible factors (HIF) and has been associated with tumor formation. Hemangioblastomas of the central nervous system and retina represent the most prevalent VHL-associated tumors, but no VHL animal model has reproduced retinal capillary hemangioblastomas (RCH), the hallmark lesion of ocular VHL. We developed a murine model of VHL-associated RCH by conditionally inactivating Vhl in a hemangioblast population using a Scl-Cre-ERT2 transgenic mouse line. In tamoxifen-induced Vhl conditional knockout mice, 64% exhibited various retinal vascular anomalies. Affected Vhl mutant mice demonstrated retinal vascular lesions associated with prominent vasculature, anomalous capillary networks, hemorrhage, exudates, and localized fibrosis. Histological analysis showed RCH-like lesions characterized by tortuous, dilated vasculature surrounded by "tumorlet" cell cluster and isolated foamy stromal cells, which are typically associated with RCH. Fluorescein angiography suggested increased vascular permeability of the irregular retinal vasculature and hemangioblastoma-like lesions. Vhl deletion was detected in "tumorlet" cells via microdissection. Our findings provide a phenotypic recapitulation of VHL-associated RCH in a murine model that may be useful to study RCH pathogenesis and therapeutics aimed at treating ocular VHL.
Vascular Abnormalities with VHL Mutations

Alexandra Arreola¹, Laura Beth Payne², Morgan H. Julian²,³, Aguirre A. de Cubas⁴, Anthony B. Daniels⁵,⁶,⁷,⁸, Sarah Taylor², Huaning Zhao²,⁹, Jordan Darden²,¹⁰, Victoria L. Bautch¹,¹¹,¹², W. Kimryn Rathmell⁴,⁶,⁸, John C. Chappell²,³,⁹,¹⁰

¹ Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill (UNC-CH), Chapel Hill, North Carolina, USA; ² Center for Heart and Regenerative Medicine and ³ Department of Basic Science Education, Virginia Tech Carilion School of Medicine and Research Institute, Roanoke, Virginia, USA; ⁴ Department of Medicine, Division of Hematology and Oncology, ⁵ Department of Ophthalmology and Visual Sciences, ⁶ Department of Biochemistry, ⁷ Department of Radiation Oncology, and ⁸ Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, Tennessee, USA; ⁹ Department of Biomedical Engineering and Mechanics, ¹⁰ Graduate Program in Translational Biology, Medicine, and Health, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA; ¹¹ Department of Biology and ¹² McAllister Heart Institute, UNC-CH, Chapel Hill, North Carolina, USA

Mutations in the von Hippel-Lindau (VHL) gene often give rise to hemangioblastomas in neural tissue in addition to highly vascular clear cell-renal cell carcinoma (ccRCC) lesions. Hypoxia Inducible Factors (HIFs) and Vascular Endothelial Growth Factor-A (VEGF-A) are among many downstream genes and pathways that contribute to pathological vascular remodeling. We recently found that Vhl mutations also compromise Notch signaling, leading to vascular perturbations specific to each mutation type, e.g. Type 1 (null) vs. Type 2B (murine G518A corresponding to human R167Q)¹. Induction of a conditional Vhl-null mutation in the developing mouse retina had relatively little effect on early stages of vessel branching, though arterial and venous branching was severely reduced at later time points. The Vhl-null mutation also accelerated maturation of vessels towards an arterial phenotype, reflected in retina vessel dysmorphogenesis (e.g. increased artery diameter and arterial-venous shunts) and aberrant expression of alpha-smooth muscle actin, particularly by vascular pericytes. RNA sequencing analysis of whole retina tissue from conditional Type 1 and 2B Vhl mutants revealed gene transcription changes within several vascular-related pathways, including VEGF-A, Notch, and smooth muscle cell contractility. Disrupting Notch signaling in the Vhl-null mutant background did not reverse later-stage branching dysmorphogenesis but did rescue the accelerated arterial phenotype. Inducing the Type 2B Vhl mutation caused stage-specific changes in vascular branching as well as an accelerated progression towards arterialization. Notch inhibition in this context (i.e. Type 2B) increased vessel branching within arterial and venous regions, and rescued arterial maturation back toward non-mutant outcomes. The differential effects of the Type 1-null and Type 2B Vhl mutations on retina vessel branching and maturation offer insight into the variability of VHL-related vascular anomalies, specifically the heterogeneity and aggressiveness of ccRCC vascular remodeling. These observations also suggest the Notch pathway as a viable option for treating vascular-related complications in VHL syndrome.

References:
1. Arreola et al. JCI Insight 201.
Propranolol a Repurposed Medicine Designed Orphan Drug by the EMA for the Treatment of von Hippel-Lindau Disease

Albiñana, V.¹,², Escribano, R.M.J.³, González, B.³, Padial, L.R.³, Recio-Poveda, L.¹,², Aguirre, D.⁴, Cuesta, A.¹,², Villar Gómez de Las Heras, K.⁵, Botella, L.M.¹,²

¹ Department Molecular Biomedicine. Centro de Investigaciones Biológicas, CSIC, Madrid, Spain; ² Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain; ³ Ophtalmology Department. Hospital Virgen de la Salud (SESCAM), Toledo, Spain; ⁴ Neurosurgery Department. Fundación Jiménez-Díaz. Madrid. Spain; ⁵ Servicio de Salud de Castilla-La Mancha (SSCC-SESCAM), Toledo, Spain

Background: Von Hippel-Lindau (VHL) disease is a rare oncological disease with an incidence of 1:36,000, and is characterized by the growth of different types of tumors. Hemangioblastomas in the central nervous system (CNS) and retina; clear cell renal carcinoma and pheochromocytomas are the most common tumors. The absence of specific medicines for VHL, leads to repeated surgeries as the only option to treat these patients. Targeting VHL-derived tumors with safe and efficient drugs with is urgent to delay/avoid repeated surgeries. Recently propranolol, a β-blocker commonly used for hypertension and other cardiac and neurological diseases, has shown to be the best option for infantile hemangioma (IH) replacing surgeries in 90% of the cases. In this context propranolol may be efficient to control haemangioblastoma growth in VHL disease given its antiangiogenic and proapoptotic in primary cultures of VHL hemangioblastomas, as was recently published by us.

The main objective of the present study was the assessment of efficiency and safety of propranolol on retinal hemangioblastoma in patients with von Hippel-Lindau disease (VHL).

Methods: 7 VHL patients, suffering from juxtapapillary or peripheral haemangioblastomas were administered oral propranolol daily in a clinical trial (CT) during 12 months, with a follow up at 1, 3, 6, 9, and 12 months, at the Virgen de la Salud Hospital (Toledo).

Results: Propranolol was initiated with a progressive increase up to a final dose of 120 mg daily. All tumours remained stable, and no new tumors appeared. The reabsorption of retinal exudation was noted in the two patients suffering from them. No adverse effects were recorded. VEGF and miRNA 210 levels were monitored in the patient's plasma as potential biomarkers of VHL progression in the CT follow up. Their levels decreased in all cases from the first month of treatment.

Conclusions: Although more studies are necessary, the results of this work suggest that propranolol is a drug to be considered in the treatment of VHL patients and that VEGF and miRNA 210 may be used as biomarkers of VHL disease activity. As a consequence of these results, the European Medicines Agency (EMA) designed propranolol as the first orphan drug for the treatment of VHL disease in January 2017.

Trial Registration: The study has a clinical trial design and was registered at EU Clinical Trials Register and Spanish Clinical Studies Registry, EudraCT Number: 2014-003671-30.
Exophytic retinal hemangioma formation in a new in vivo model of von Hippel-Lindau (VHL) syndrome

Anna Matynia, Sachin Parikh, Steven Nusinowitz, Michael B. Gorin
Jules Stein Eye Institute, David Geffen School of Medicine, UCLA, Los Angeles, CA, USA

Background: Retinal hemangioblastomas are often the first manifestations of VHL syndrome and have significant consequences for vision. At presentation, retinal hemangiomas are often large, highly vascular, characterized by giant foamy cells of unidentified origin, located in the superficial layers of the retina and project into the vitreous. Retinal hemorrhage and exudation can lead to retinal damage and detachment resulting in vision loss. We have generated a mouse model of retinal hemangiomas in which the effects of loss of expression of the Vhl gene is limited to the eyes so as to not compromise their overall health and survival.

Methods: Intravitreal injections of AAV2 virus expressing Cre recombinase and eGFP under a general promoter were performed in mice homozygous for the floxed VHL allele. Confocal microscopy of retinal whole mounts was used to assess expression of eGFP. Retinas were monitored by indirect ophthalmic endoscopy, fluorescence angiography (FA), and spectral domain optical coherence tomography (sd-OCT) over the course of 8 weeks. A subset of mice was perfused and fixed eyes stained for histology.

Results: GFP expression was visualized three days post-injection. Retinal growths that were either vascular or avascular, and flat or elevated, were observed by indirect ophthalmoscopy in 44% of eyes (15/34). Vascular leakage that appeared intermittently and/or lesions were observed by FA in 30% of eyes (5/17). Structural alterations included inclusions and infiltrations in the outer retina, retinal and/or choroidal thickening or thinning, retinal fluid-filled cavities, and/or disorganization of retinal layers were observed by sd-OCT in all eyes (17/17). Vascularization near the photoreceptor/retinal pigment epithelium layers (4/11), infiltration by presumptive macrophages/microglia (4/11), growths associated with disruption of photoreceptors (3/11) or other abnormalities were observed histologically in 64% of eyes.

Conclusions: Vascular alterations, consistent with exophytic retinal hemangiomas described in VHL patients prior to formation of overt hemangiomas, are observed as an early phenotype in this novel ocular mouse model of VHL syndrome. We hypothesize that the earliest lesions form at the RPE/photoreceptor layer, grow inwards to break through the retina where unrestricted growth leads to a classical hemangiomas with exudation and bleeding that threatens vision. This unbiased targeting of retinal cells with virally-expressed Cre recombinase causing genetic loss of VHL enables us to identify key cell types in the initiation and progression of disease. A major use of this model system will be determining and testing new strategies for hemangioma prevention and treatment.
SESSION TITLE
Clinical Guidelines
Universal Reflex Referral to VHL Comprehensive Clinical Care Center of Patients Presenting to Ophthalmologists Leads to Dramatic Improvement in Guideline-Concordant Screening: Results of a Pilot Study

Anthony B. Daniels1,2, Alexis Flowers1, Debra L. Friedman2,3, W. Kimryn Rathmell2,4

1 Department of Ophthalmology and Visual Sciences, Vanderbilt Eye Institute, Vanderbilt University Medical Center, Nashville, TN, USA; 2 Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA; 3 Department of Pediatrics, Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, TN, USA; 4 Department of Medicine, Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, TN, USA

Objective: To determine if instituting a policy of universal reflex referral to a von Hippel-Lindau (VHL) Comprehensive Clinical Care Center (CCCC) improves guideline-concordant screening.

Introduction: VHL affects many organ systems and requires treatment by multiple specialists. Thus, care is often fragmented. It is difficult for a physician seeing a VHL patient for the first time to ascertain which other specialists are ordering screening studies, and whether the patient’s screening is up-to-date and guideline-concordant. In 2017, Vanderbilt became one of a dozen CCCCCs in the United States. We instituted a quality improvement initiative to improve guideline-concordant screening by referring all VHL patients presenting to our ophthalmology clinic to the Vanderbilt CCCC for surveillance imaging, regardless of whether they were being followed by other specialists.

Methods: Retrospective case series of patients presenting to Vanderbilt Eye Institute, both before institution of the CCCC in 2017 as well as afterwards. Beginning in 2017, all patients were referred to the CCCC medical or pediatric oncologist for evaluation and surveillance. Patients referred to ophthalmology from the CCCC oncologists were excluded. Rates of CCCC referral from ophthalmology to oncology were measured. Guideline-concordant screening status was determined for patients prior to seeing ophthalmology, as well as afterwards. These rates were determined in both the pre-2017 and post-2017 cohorts. Tumors identified on initial screening were recorded.

Results: 100% of VHL patients presenting to ophthalmology were referred to CCCC oncologists. Almost all patients were already followed by other specialists. Prior to creating the CCCC in 2017, 0% of patients were guideline-concordant at the time they presented to ophthalmology, and 29% were concordant afterwards. After creating the CCCC and the reflex referral initiative, 20% of patients were guideline-concordant at presentation, and 100% were concordant after seeing the CCCC oncologist. 50% of patients referred from ophthalmology to CCCC oncology had tumors requiring intervention at the time of initial screening imaging. These included renal cell carcinomas (>3cm), pheochromocytomas, metastatic rhabdoid tumors, and central nervous system hemangioblastomas.

Conclusions: Rates of guideline-concordant screening have historically been poor, even for patients being followed for VHL-related tumors by subspecialists. Universal reflex referral of VHL patients to a CCCC dramatically improved guideline-concordant screening rates to 100%. Half of all patients have a (non-ocular) tumor requiring treatment at the time they present to ophthalmology, underscoring the importance of expeditious referral.
Recommendations for von Hippel–Lindau Tumor Surveillance in Childhood and Adolescence

Surya P. Rednam1, Ayelet Erez2, Harriet Druker3, Katherine A. Janeway4, Junne Kamihara4, Wendy K. Kohlmann5, Katherine L. Nathanson6, Lisa J. States7, Gail E. Tomlinson8, Anita Villani3, Stephan D. Voss9, Joshua D. Schiffman5,10, Jonathan D. Wasserman11

1 Department of Pediatrics, Baylor College of Medicine, Texas Children's Cancer Center, Texas Children's Hospital, Houston, TX, USA; 2 Department of Biological Regulation, Weizmann Institute of Science, Rehovot, Israel; 3 Division of Haematology/Oncology, The Hospital for Sick Children, Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada; 4 Department of Pediatric Oncology, Dana-Farber Cancer Institute and Children's Hospital, Boston, MA, USA; 5 Huntsman Cancer Institute, University of Utah, Salt Lake City, UT, USA; 6 Department of Medicine, Division of Translational Medicine and Human Genetics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA; 7 Department of Radiology, Children's Hospital of Philadelphia, Philadelphia, PA, USA; 8 Department of Pediatrics, Division of Hematology and Oncology and Greehey Children’s Cancer Research Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; 9 Department of Radiology, Children’s Hospital, Boston, MA, USA; 10 Department of Pediatrics, University of Utah, Salt Lake City, UT, USA; 11 Division of Endocrinology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

Von Hippel–Lindau disease (VHL) is a hereditary tumor predisposition syndrome that places affected individuals at risk for multiple tumors, which are predominantly benign and generally occur in the central nervous system or abdomen. Although the majority of tumors occur in adults, children and adolescents with the condition develop a significant proportion of VHL manifestations and are vulnerable to delayed tumor detection and their sequelae. Although multiple tumor screening paradigms are currently being utilized for patients with VHL, surveillance should be reassessed as the available relevant clinical information continues to expand. We propose a new VHL screening paradigm similar to existing approaches, with important modifications for some tumor types, placing an emphasis on risks in childhood. This includes advancement in the timing of surveillance initiation and increased frequency of screening evaluations.
SESSION TITLE

The Patient's Perspective
MyVHL: Patients Contributing to VHL Research

Ilene Sussman, Joshua Mann, VHL Patients and Researchers
VHL Alliance, Boston, MA, USA

Over the past number of years, the rare disease community has embraced the importance of natural history studies focused on data entered by the patient. Since information known only by the given patient is entered, this approach enhances existing and developing knowledge of registries/databanks collected by the clinical and research communities.

MyVHL: Patient Natural History Study (formally known as Cancer in Our Genes International Patient (CGIP) Databank) was recreated in response to a mandate from the VHL Alliance's Research Council. Launched in 2014, this IRB approved longitudinal study, includes a comprehensive series of surveys designed to collect data on each organ impacted by VHL. In addition, information about lifestyle factors (nutrition, exercise, mood) is collected with the hope of understanding how these factors influence VHL progression.

With over 600 consented participants, we now have a good understanding of the populations entering data and have identified some interesting findings. It is important to expand the reporting patient population. As such, we need to fully engage the VHL patient community to participate in MyVHL on at least an annual basis. It is also incumbent on the VHL medical and research communities to encourage them to do so.
New VHLA Programmatic Initiatives

Joshua Mann, VHL Patients and Families
VHL Alliance, Boston, MA, USA

People with a hereditary disease commonly experience a heightened sense of stress and anxiety\(^1\).\(^2\). The unpredictable nature of VHL can cause an even greater risk in patients, as well as their families and loved ones. It can help to connect with others who are on the same journey. The VHL Alliance has developed a portfolio of initiatives meant to help mitigate the psychosocial impact of VHL. This presentation will provide an overview of the programmatic initiatives offered by the VHL Alliance and how they achieve the organization’s mission: VHLA is dedicated to research, education, and support to improve awareness, diagnosis, treatment, and quality of life for those affected by VHL.

References:
Medical Coaching: What Is It All About?

Leona deVinne

VHL Alliance, Boson, MA, USA; Accendo Consulting, Calgary, AB, Canada

Medical Coaching involves addressing the entire person, not simply addressing their medical situation. A Medical Coach works with patients that are currently undergoing medical care for a recently diagnosed condition or chronic illness. Medical Coaches have expertise in the condition they're supporting the patient with, as well as coaching skills to have a collaborative, instead of a directive approach, to achieve desired outcomes set out by the patient and medical care team.

Research shows that the impact is long lasting and results in decreased health care costs, increased overall well-being and more informed and greater adherence to advised medical protocols.

This presentation will provide an overview of Medical Coaching and its positive impact on patients as well as look at the recently developed VHL Medical Coaching Program created specifically for VHL Clinical Care Centers (CCC) teams. The VHL medical coaching program was designed specifically to give extra tools for medical personnel in order to provide the best care for VHL patients and their families.
The VHL Alliance's Clinical Care Center (CCC) program recognizes healthcare organizations that are providing multi-specialty, coordinated care for patients with VHL. In an effort to continue to improve the experience for both patients and CCC care teams, the VHL Alliance created the Clinical Care Center Process Improvement Committee in 2018. This presentation will provide an overview of the current CCC program, outline some opportunities for improvement, and introduce attendees to the CCC Process Improvement Committee's current initiatives, progress to-date, and proposed future work. Some specific CCC topics targeted for the discussion include accountability, promotion of MyVHL, integration of the patient liaisons, and tackling psychosocial issues with patients.
Poster Abstracts
Proteomic and Transcriptomic Signatures of pVHL Isoform Expression to Reveal pVHL Functions in Cancer Progression

CNRS UMR 6290-IGDR – Molecular Bases of Tumorigenesis: VHL disease – Université de Rennes 1, Rennes, France

The vhl gene is a tumor suppressor responsible for von Hippel-Lindau Syndrome, an inherited autosomal dominant pathology that causes high susceptibility to developing cancers including clear cell renal cell carcinoma (ccRCC). The vhl gene encodes three proteins pVHL213, pVHL160 and the pVHL172 isoform which is translated from a mRNA variant #2, resulting from an alternative splicing (exon 2 skipping). This is the less characterized pVHL isoform and we have for the first time demonstrated the expression of the protein pVHL172 in human tissues through the use of a specific monoclonal antibody of pVHL that we have developed (Chesnel et al., 2015).

Currently, the most described function of pVHL is its role in oxygen sensing since it is part of an E3 ubiquitin ligase complex in which pVHL is the substrate recognition subunit. The main target of this complex is the α subunit of the Hypoxia Inducible Factor (HIF-α) which is polyubiquitylated and targeted to the proteasome for its degradation in normoxia. Non canonical functions were credited to pVHL during the last decade. pVHL is implicated in microtubules stabilization and primary cilium regulation, cell cycle regulation, assembly of a proper extracellular fibronectin matrix and regulation of apoptosis. Most of these functions were studied with pVHL213/160 and few was known about pVHL172.

The von Hippel-Lindau isoform pVHL172 is not a tumor suppressor protein. Its expression in 786-O cells triggers the formation of higher sarcomatoid xenograft tumors compared with parental 786-O cells that do not express pVHL. Moreover, the expression of pVHL172 did not suppress cell aggregation in 3D culture as observed with the expression of pVHL213. PVHL172 does not down regulate HIF2α expression even if the protein still participates to the E3 ubiquitin ligase complex. We showed that expression of pVHL172 in renal tumoral cells (786-0) stimulated TGFβ signaling and upregulation of the metalloproteases MMP13 and MMP1, while pVHL213 expression downregulated these genes (Hascoet et al. 2017). The presence of pVHL172 in cells may provide a growth advantage to affect the tumor progression and the physiological impact of the balance of expression of pVHL213 and /or pVHL172 remain to be explored (Hascoet et al 2017).

These results prompted us to go further in understanding pVHLs functions through transcriptomic and proteomic analyses to search for differentially expressed genes and new pVHLs partners, respectively.
Characterization of Proteostasis Network of Human pVHL Isoforms

Le Goff, Xavier, Chesnel, Franck, Couturier, Anne, Le Goff, Cathy, Arlot-Bonnemains Yannick
CNRS UMR 6290-IGDR - Molecular Basis of Tumorigenesis: VHL disease – Université de Rennes 1, Rennes, France

Mutations in the von Hippel-Lindau (VHL) tumor suppressor gene are responsible for VHL syndrome and are found in many sporadic tumor types as well. Missense mutants of the pVHL protein may exhibit protein folding defects and instability. Quality control mechanisms promote aggregation and degradation of misfolded proteins.

We investigate the aggregation of three pVHL isoforms (pVHL213, pVHL160 and pVHL172) in fission yeast. The full-length pVHL213 isoform aggregates in highly dynamic small puncta and in large spherical inclusions. The pVHL160 isoform forms dense foci and large, irregularly shaped aggregates. In silico prediction of pVHL aggregation propensity identified a key aggregation-promoting region within exon 2. Consistently, the pVHL172 isoform, which lacks exon 2, forms rare reduced inclusions.

We studied the aggregation propensity of pVHL variants harboring missense mutations found in kidney carcinomas. We identified aggregation-prone as well as destabilizing mutations for pVHL. Using yeast genetics, we initiated the characterization of the proteostasis network of pVHL and showed the involvement of the Hsp90 chaperone and the prefoldin complex in pVHL stability and aggregation. Reduction of soluble functional pVHL may be critical in VHL-related diseases.
CAIX Immunohistochemistry as a Tool to Predict or Validate Germline and Somatic VHL Mutations in Pheochromocytoma and Paraganglioma – A Retrospective and Prospective study

Nelly Burnichon1,2,3, Tchao Meatchi1,2,5, Estelle Robidel1,2, Cécile Badoual1,2,5, Mathilde Sibony2,6, An Thach Nguyen1,2, Anne-Paule Gimenez-Roqueplo1,2,3,4,7, Judith Favier1,2

1 INSERM, UMR U970, Paris Cardiovascular Research Center-PARCC, Paris, France; 2 Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Paris, France; 3 Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Service de Génétique, Paris, France; 4 PREDIR (Labellised network of centres of expertise for Von Hippel Lindau disease and hereditary predispositions for adult kidney carcinomas), Institut National du Cancer, France; 5 Assistance Publique-Hôpitaux de Paris (AP-HP), Hôpital Européen Georges Pompidou, Département d'anatomopathologie, Paris, France; 6 Assistance Publique-Hôpitaux de Paris, Hôpital Cochin, Service d'anatomie-pathologie, Paris, France; 7 Rare Adrenal Cancer Network-Cortico Médullosurrénale Tumeur Endocrine, Institut National du Cancer, Paris, France

**Background:** The development of pheochromocytomas and paragangliomas (PPGL) is strongly linked to the presence of germline mutations in more than 15 predisposing genes. Germline and somatic VHL mutations account for approximately 10% of all cases. In contrast with SDHA and SDHB immunohistochemistry that are routinely used to validate SDHx mutations, there was no such tool available to characterize VHL mutations.

The aim of this study was to assess whether immunohistochemistry with carbonic anhydrase IX (CAIX) antibody could be used as a tool to predict or validate VHL gene mutations in PPGL. Methods Immunohistochemistry with anti-CAIX antibody was performed on 203 paraffin-embedded PPGL. A retrospective series of 100 PPGLs with known germline mutation status for PPGL susceptibility genes was first investigated. Then, a prospective series of 103 PPGLs was investigated for CAIX immunostaining followed by germline and/or somatic genetic testing of all PPGL susceptibility genes by NGS.

**Results:** Cytosolic CAIX protein expression was heterogeneous in the different samples. However, we observed that a membrane CAIX staining was almost only observed in VHL-mutated cases. Forty-two (42) of 47 (89.4%) VHL cases showed at least one cell exhibiting a membrane CAIX immunostaining. In contrast, 139 out of 154 (90%) of non-VHL mutated tumors presented no membrane CAIX localization.

**Conclusion:** Our results demonstrate that in PPGL, VHL gene mutations can be predicted or validated reliably by an easy-to-perform and low-cost immunohistochemical procedure and suggest that CAIX immunohistochemistry should improve the diagnosis of VHL-related tumors.
Phenotype-genotype correlations in Hungarian VHL patients

Henriett Butz\textsuperscript{1,2}, István Likó\textsuperscript{2}, Vince Grolmusz\textsuperscript{2}, Balázs Sarkadi\textsuperscript{3}, Sára Zakariás\textsuperscript{3}, Miklós Tóth\textsuperscript{3}, Péter Igaz\textsuperscript{3}, Attila Patócs\textsuperscript{1,2}

\textsuperscript{1} Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary; \textsuperscript{2} Lendulet” Hereditary Endocrine Tumours Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary; \textsuperscript{3} 2nd Department of Medicine, Semmelweis University, Budapest, Hungary

Background: VHL disease a hereditary tumor syndrome caused by germline mutations or deletions of the VHL tumor suppressor gene. Association of various neoplasms including hemangioblastomas, clear cell renal cell carcinoma (ccRCC), neuroendocrine tumors and pheochromocytoma (PCC) can be detected. Some strong genotype-phenotype associations are well documented but atypical presentations can also be observed.

Objective: Our aim was to summarise the genotype-phenotype correlations observed in Hungarian VHL patients.

Methods: 51 members from 30 unrelated Hungarian families with VHL disease were retrospectively investigated. Demographic and disease-specific signs and symptoms together with genetic data were collected and analysed. Genetic analysis included Sanger sequencing, multiplex ligation-dependent probe amplification, quantitative real time PCR and next-generation sequencing were applied.

Results: 19 different (2 small and 7 large deletions, 4 splice site and 6 missense) mutations were identified in our patients. No founder mutation has been identified. Missense mutations associated almost exclusively with Type 2 VHL, while frameshift/nonsense mutations and splice site mutations were detected in 5% and 15%, of cases, all in pheochromocytoma negative patients.

Large deletions of VHL gene associated only in Type 1 VHL. However, patients with VHL large deletion showed heterogenic manifestations even with the same genetic background. We observed that in 3 unrelated cases the sole manifestation was ccRCC. Two of these cases harboured a \textit{de novo} exon 2 deletion: they presented with bilateral ccRCC at the age of 20-40 years of age. Family screening revealed no other relatives harbouring this alteration.

Conclusions: In Hungarian VHL patient's distribution of the VHL gene mutations is comparable with literature data. However, our data showed that patients with large VHL deletion present heterogenic clinical manifestations. We found three cases with RCC only phenotype which extend our previous knowledge about the cryptic VHL disease. Pheochromocytoma only and haemangioblastoma only phenotypes have been already described in literature and now we recommend that for cases with bilateral, apparently sporadic renal cell carcinoma VHL gene testing should be performed.

Funding: National Research, Development and Innovation Office K12531 to Attila Patocs
Propranolol as a Therapy for VHL: in vitro and Clinical Data for Hemangioblastoma and Renal Carcinoma.

Angel Cuesta1,5, Virginia Aliñana1, Beatriz González2, Daniel Aguirre3, Karina Villar4, Luisa Botella1,5

1 Centro de Investigaciones Biológicas, (CIB-CSIC), Madrid, Spain; 2 Servicio de Ofalmología, Complejo Hospitalario de Toledo, Spain; 3 Servicio de Neurocirugía, Fundación Jiménez Díaz, Madrid, Spain; 4 SSCC del Servicio de Salud de Castilla-La Mancha (SESCAM), Toledo, Spain; 5 Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain

Objectives: To find a non-invasive, safe, and long-term therapy for VHL disease, able to impair or even stop the growth of central nervous system (CNS) and retinal hemangioblastomas and renal cell carcinoma.

Introduction/Background: The most frequent tumors in VHL disease are CNS and retinal hemangioblastomas and renal carcinoma. Since the systemic medical approaches have not provided long-term cessation of tumor’s growth up to now, the standard treatments involve repeated surgeries and other invasive procedures.

Propranolol, a non-selective β-blocker with more than 50 years in clinics, has proven effective for infantile hemangioma due to its antiangiogenic and proapoptotic properties, and therefore could be a useful treatment for hemangioblastomas and renal carcinomas in VHL disease. In line with these findings, propranolol has been also recently used in combinatorial therapies for cancer.

In order to avoid the multiple surgical interventions and provide a non-invasive, safe, and long-term therapy for VHL disease, propranolol has been tested for its therapeutic application in VHL clinics.

Methods: CNS hemangioblastomas from VHL patients were isolated and cultivated. Hemangioblastoma cultures and the renal carcinoma cell line 786-O were treated with propranolol in vitro and viability, apoptosis, and other biomarkers were quantified.

An open clinical trial to evaluate propranolol effectiveness and safety on retinal hemangioblastomas was developed (EudraCT Number: 2014-003671-30).

Results: In vitro assays revealed a downregulation of HIF protein expression and its corresponding HIF target genes such as VEGF, EPO, and SOX2. Furthermore, propranolol also inhibited cell proliferation in parallel with induction of apoptosis.

The results from the clinical trial showed a stabilization of all tumors and the absence of new tumors. Reabsorption of retinal exudation was noted in the two patients having exudates and no adverse effects were recorded but hypotension in one patient. Analysis of VEGF, EPO, and SOX2 had shown a reduction after propranolol treatment, becoming as possible biomarkers of VHL.

Finally, propranolol showed a synergistic effect together with temozolomide in a metastatic paraganglioma case by potentiating the apoptosis and decreasing the viability in the VHL derived treated cells.

Conclusions: Propranolol shows its properties as therapeutic drug for retinal hemangioblastomas and also shows its capabilities for CNS hemangioblastomas and renal carcinomas in VHL disease.

To highlight, the potential uses of propranolol in combinatorial therapies that open a new strategy for malignant metastatic carcinomas.
Endocrine Manifestations in von Hippel-Lindau Disease at VHL Clinical Care Center (CCC) in Argentina

Valeria de Miguel¹, Constanza Ramacciotti², Andrea Paissan⁴, Julia García Arabehety¹, Javier de Arteaga², Miriam Wyor², Eduardo Cohen², Gabriela Sansó³, Patricio García Marchiñena³, Juan Pekolj¹, Marcelo Serra¹, Patricia Fainstein Day¹

¹ Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; ² Hospital Privado Universitario de Córdoba, Córdoba Argentina; ³ Centro de Investigaciones Endocrinológicas (CEDIE), Hospital de Niños Ricardo Gutierrez, Buenos Aires, Argentina

Background: Von Hippel-Lindau disease (VHL) is an autosomal dominant, heritable cancer-predisposition syndrome caused by mutation in the VHL suppressor gene that is characterized by the occurrence of multiple endocrine and non-endocrine lesions. Pancreatic neuroendocrine tumors (PNETs) and pheochromocytoma/paraganglioma (Pheo/PGL) are important endocrine manifestations in VHL disease. PNETs are typically nonfunctional and occur in an average of 15% of patients with pancreatic manifestations of VHL disease, while pheochromocytomas (Pheo) are more frequently reported in VHL, paragangliomas (PGL) can also be founded and they are usually parasympathetic.

Multidisciplinary approach in specialized units with trained professionals is mandatory. The Hospital Italiano of Buenos Aires and Hospital Privado Universitario of Córdoba were designated by the VHL Alliance as VHL Clinical Care Center (CCC) in 2014.

Objective: The aim of this study is to describe the endocrine manifestations in patients with VHL disease seen at the VHL CCC of Argentina.

Methods: We retrospectively reviewed the clinical records of ten patients seen at the VHL CCC of Córdoba and Buenos Aires since 2013 until 2018. Genetic testing of the VHL gene was performed by Sanger and MLPA (Multiplex Ligation-dependent Probe Amplification).

Results: Ten (N=10) patients, median age of 36 years old. Nine women, 5/10 had central nervous system hemangioblastomas and retinal angiomas. Pheo manifested in 8/10 patients, median of age of 19 years old as first manifestation of VHL disease. Five were bilateral (synchronous: 3, metachronous: 2), noradrenergic biochemical phenotype: 6/8, biochemical silent: 2/8, sympathetic biochemical silent PGL: 2/8, mean tumor size was 4 cm (ranging from: 2 to 7 cm), cortical sparing adrenalectomy: 3/8, recurrence: 1 (6 years after surgery), adrenal insufficiency: 3/8, metastatic disease: 0/8. PNETs manifested in 5/10 patients, median age of manifestation 53 years old, multiple tumors: 4/5, mean size 2.4 cm (ranging from: 1 to 5.5 cm), located in the pancreatic head: 5/5, non-functional: 4/5, partial pancreatectomy: 4/5, exocrine pancreatic insufficiency: 4/4, pancreatic insufficiency 0/4, metastatic disease: 0/5. Genetic testing was offered to all patients and missense and nonsense mutations were found.

Conclusion: Pheo/PGL was the first clinical manifestation in most patients and was characterized by a noradrenergic biochemical phenotype and multiple tumors. PNETs were located in the pancreatic head and were surgically removed in the majority of patients because of the tumor size. We highlight the importance of the multidisciplinary approach should be undertaken for treatment and proper patient follow up.
VHL Clinical Care Center (CCC) in Buenos Aires. Epidemiological Report

Valeria de Miguel¹, Patricio García Marchiñena¹, Fátima Barragán¹, Guillermo Gueglio¹, Alberto Jurado¹, Matteo Baccanelli¹, Carlos Videla¹, Gabriela Pérez Raffo¹, Carolina Gentile¹, Federico Cayol¹, Pablo Kalfayan¹, Gabriela Sansó², Marcelo Serra¹

¹Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; ²Centro de Investigaciones Endocrinológicas (CEDIE), Hospital de Niños Ricardo Gutierrez, Buenos Aires, Argentina

Background: Von Hippel-Lindau syndrome (VHL) is a rare autosomal dominant disorder characterized by the development of cysts, benign and malignant tumors in different organs that may lead to multi-organic affection and health impairment. Is caused by germline mutations of the tumor suppressor gene located on short arm of chromosome 3. While the majority of affected individuals have a positive family history, up to 20% of the cases arise from the novo mutations. Patients presents with spectrum of manifestations in central nervous system (CNS), kidneys, adrenal glands, pancreas and reproductive organs.

Multidisciplinary approach in specialized units with trained staff is mandatory. The Hospital Italiano of Buenos Aires was designated by the VHL Alliance as VHL Clinical Care Center (CCC) in 2014

Objectives: To describe the clinical manifestations and genetics of patients with VHL disease

Methods: We retrospectively reviewed the electronic medical records of 27 VHL patients seen at VHL CCC of Buenos Aires since 2014 until 2018. Genetic testing of the VHL gene was performed by Sanger and MLPA (Multiplex Ligation-dependent Probe Amplification).

Results: Twenty-seven (N= 27) patients were reviewed, mean age of 35 years (SD:15, ranging from 14 to 68-year-old). Nineteen female (74,1%), 70,4% had a positive family history. The most frequent tumors found were: CNS hemangioblastomas (74,1%), pancreatic cysts and neuroendocrine tumors (57,7%), renal cell carcinoma and cysts (55.6%), retinal angiomas (53,3%), pheochromocytoma (29.9%), endolymphatic sac tumors (3.8%). Epididymal cystadenomas (3), broad ligament cystadenomas (unknown). Mean age of first manifestations was 26 years old (SD:13, ranging from 10 to 68 years old).

Ten (37%) patients had complications: unilateral amaurosis (5), bilateral hearing loss with cochlear implant (1), hemiplegia (1), exocrine pancreatic insufficiency (2), adrenal insufficiency (2) and end stage renal disease (3). One of the patients died because of advanced renal cell carcinoma disease. Genetic testing was offered to all patients and twenty-five individuals were studied. We found missense, nonsense mutations by sanger and large rearrangements by MLPA.

Conclusion: The frequency of clinical manifestations and age of presentation coincide with the epidemiological data. Unilateral amaurosis was the most frequent complications. Multidisciplinary approach in clinical referral centers is very important for the appropriate patients and families follow up.
RSUME is Involved in Missense VHL Mutation-Loss of Function

Lucas Tedesco¹, Belen Elguero¹, David Gonilski Pacin¹, Mariana Fuertes¹, Eduardo Arzt¹,²
¹ Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) - CONICET - Partner Institute of the Max Planck Society, Buenos Aires, C1425FQD, Argentina; ² Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, C1428EGA, Argentina

Introduction: In VHL disease HIFs-a deregulation caused by mutations in VHL results in the development of high vascularized tumors such as renal clear-cell carcinomas (RCC). Some missense mutations on VHL, described in the majority of Type 2 VHL tumors, retain a partial function on HIF downregulation.

RSUME or RWDD3 (RWD-domain-containing sumoylation enhancer) is highly expressed in VHL-related tumors. A recent analysis carried out by The Human Protein Atlas shows that 20.07% of RCC tumors present high levels of RSUME mRNA, which correlates with a decrease in survival rate.

Objectives: Our aim is to investigate the action of RSUME on VHL Type 2 loss of function and the mechanism by which RSUME acts on type 2 VHL-related angiogenesis.

Methods: RSUME and HIF2α expression or stability in RCC-786-O and in COS-7 cells were evaluated by Western Blot (WB). HIF2α activity was evaluated by HRE-LUC. RSUME/VHL variants interaction was studied by co-immunoprecipitation. VHL sumoylation was analyzed by affinity purification of sumoylated proteins.

To assess RSUME action on VHL disease, we generated RCC-786-O clones expressing either Leu188Val Type2 VHL mutant or a variant defective for sumoylation (VHLL188V/K171R), in which RSUME was silenced by shRNA. VEGF mRNA was measured in theses clones, they were used for tubulogenesis “in vitro” assay and injected in nod-scid mice (n=4 each group, two independent experiments) for “in vivo” angiogenesis assay.

Results: VHL Type 2 representative mutants (Tyr112His, Arg167Gln and Leu188Val) failed to downregulate RSUME protein levels compared to VHL WT, generating a permissive context for high RSUME found in VHL-related tumors. RSUME potentiated missense VHL mutants loss of function, increasing HIF-2α stabilization and activity.

RSUME mechanism of action involves a decrease. Type 2 VHL-HIF binding. RSUME disrupts ECV complex assembly. type 2 VHL mutants are targets of sumoylation, but HIF-2α stabilization was mediated by a VHL sumoylation independent mechanism confirmed by using type 2 VHL variants deficient for sumoylation.

RCC clones in which RSUME was knocked-down resulted in a gain of function of wild type and mutant VHL. These clones showed impaired VEGF-A expression and vascularization both in vitro and in vivo.

Conclusion: These results show a mechanism for VHL type 2 loss of function and highlight RSUME as a potential biomarker of the VHL disease outcome.
The e3 Ubiquitin-Protein Ligase mdm2 is a Novel Interactor of the von Hippel-Lindau Tumor Suppressor

Antonella Falconieri1#, Giovanni Minervini1#, Raissa Bortolotto1, Damiano Piovesan1, Raffaele Lopreiato1, Geppo Sartori1, Silvio C.E. Tosatto1,2*

1 Department of Biomedical Sciences, University of Padova, Viale G. Colombo 3, 35121, Padova, Italy; 2 CNR Institute of Neuroscience, Padova, Viale G. Colombo 3, 35121, Padova, Italy; # contributed equally; * corresponding author: silvio.tosatto@unipd.it

Objectives: The identification and characterization of new isoform-specific protein-protein interactions aimed at developing an enlarged pVHL pathway characterization at the molecular level. These findings can clarify pVHL interplay with other cellular pathways pointing out the molecular mechanisms disrupted in VHL syndrome.

Background: Von Hippel-Lindau (VHL) syndrome is a dominantly inherited familiar condition predisposing to the development of different cancers due to mutations in pVHL protein. pVHL exists in two main isoforms, termed pVHL19 and pVHL30, both of them overlapping in regulating degradation of hypoxia-inducible factor 1-alpha (HIF-1α) and ultimately, modulating the hypoxia response. Previous studies, however, proposed pVHL30 to form an isoform-specific interaction with p14ARF, suggesting a connection between oxygen sensing and apoptosis. This evidence suggested a functional asymmetry among the two canonical pVHL isoforms.

Methods: We used a highly integrated bioinformatic and experimental approach. In particular, in silico analyses have been paired with yeast two-hybrid and mammalian cell assays in order to validate the predicted interactions and characterize them at the molecular level.

Results: Here, we present a novel direct interaction between pVHL30 and MDM2. Data show that the interaction is isoform-specific and sustained by both the pVHL30 N-terminal tail and β domain. These regions were found to bind two interacting motifs localizing within MDM2 intrinsically disordered regions.

Conclusions: We identified a novel pVHL isoform-specific interaction. This data is a further evidence of functional specializations between pVHL30 and pVHL19. We found that the N-terminal acidic tail of pVHL30 is required for its association with MDM2, suggesting this region to be an additional pVHL binding surface. Indeed, pVHL19 lacking of this portion was demonstrated not to bind MDM2. Collectively, our data point to a novel pVHL30 HIF-1α-independent function, suggesting pVHL30 to play a role in MDM2 stabilization and mutual regulation between oxygen sensing and apoptosis.
Genetic Tests for Patients with VHL Syndrome with Elusive Abnormalities in VHL

Caitlin Mauer, Brian Reys, Remington Fenter, Theodora Ross
Cancer Genetics Program, The University of Texas Southwestern Medical Center, Dallas, Texas, USA

Introduction: Genetic testing for von Hippel-Lindau (VHL) syndrome has one of the highest expected sensitivities and specificities of any known hereditary cancer predisposition syndrome\(^1\). Thus clinical testing is routinely used to confirm a diagnosis of VHL. Here we present five generations of a large Hispanic family who met clinical diagnostic criteria for VHL syndrome\(^2\). However, despite testing many family members with several different clinical genetic tests for VHL, no mutation in \(VHL\) has been identified.

Methods: Affected members of this family (n=23) presented with one or more classic VHL lesions including: hemangioblastomas (n=15), clear cell renal cell carcinomas (ccRCC) (n=7), renal cysts (n=6), pancreatic cysts (n=5), pancreatic neuroendocrine tumors (n=2), retinal angioma (n=1), pheochromocytoma (n=1) and endolymphatic sac tumor (n=1). \(VHL\) gene testing for multiple family members through the Mayo Clinic, University of Alabama, Children's Hospital of Philadelphia, Ambry Genetics and Invitae laboratories did not identify abnormalities in the \(VHL\) gene. Thirteen family members' germlines were further evaluated on a research basis in addition to the clinical tests for \(VHL\) via whole genome sequencing (Complete Genomics, Inc.) or whole exome sequencing (WES, Personalis, Inc.). Two tumors, a ccRCC and a hemangioblastoma, were also evaluated with WES and RNA-seq.

Results: A cluster of three rare missense variants in \(ATG7\), \(TSEN2\) and \(DPH3\), all linked and located within six megabases of \(VHL\) on Chromosome 3, were identified in affected family members but not in the unaffected 84-year-old female member in the first generation of this family. The \(ATG7\) and \(TSEN2\) variants have been observed several times in the ExAC database, while the \(DPH3\) variant is a rare Hispanic variant found in four individuals in the ExAC database. WES and RNA-seq on the two tumors demonstrated a loss of heterozygosity of the \(VHL\) region. This led to loss of expression of the common variants of \(ATG7\), \(TSEN2\) and \(DPH3\) in the tumors and sole expression of the rare variants.

Conclusion: Despite the high clinical sensitivity and specificity of \(VHL\) gene testing, there remain classic VHL families with no identifiable germline \(VHL\) mutation. These data suggest evaluation for \(ATG7\), \(TSEN2\) and \(DPH3\) rare variants may be of use in uncommon cases where \(VHL\) mutations are not identified.

Furthermore, this family exemplifies why \(VHL\) gene evaluation alone cannot be used, especially in the context of a clinical evidence, to rule out a diagnosis of VHL.

References:
**Ophthalmology Outcomes of a Clinical Trial on VHL Patients with Ocular Affection Treated with Oral Propranolol.**

Beatriz González-Rodríguez¹, Karina Villar Gómez de las Heras², Ángel Cuesta³⁴, Virginia Albiñana³⁴, Luisa María Botella³⁴, Rosa María Jiménez Escribano¹

¹ Ophthalmology Department, Retina Service, Complejo Hospitalario de Toledo (SESCAM), Toledo, Spain; ² Pharmacy Department, Servicio de Salud de Castilla-La Mancha (SESCAM), Central Services, Toledo, Spain; ³ Centro de Investigaciones Biológicas, (CIB-CSIC), Madrid, Spain; ⁴ Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain

**Introduction and Objectives:** Von Hippel-Lindau disease is an inherited autosomal dominant, multisystem cancer syndrome that leads to the development of both malignant and benign tumors. Retinal hemangioblastomas are the most frequently and the earliest tumors in appear. Its complications, such as retinal exudates, macular edema, or retinal detachment, may lead to visual impairment and eventually to complete blindness. So, these patients require ophthalmic treatments and continuous follow-up. Classical treatments include observation, laser photocoagulation, cryotherapy, photodynamic therapy, or intravitreal anti-VEGF agents. There is no pharmacological treatment with a long term success so far. Juxtapapillary and optic nerve hemangioblastomas are difficult and risky to treat: in most cases these patients will lose their vision without any alternative. So, new treatments need to be found.

Propranolol is a-non selective β-blocker. Recent studies proved that its use in different tumors - may increase the efficacy of chemotherapy used in combined therapies. Albiñana et al. in *in vitro* assays showed that propranolol decreases HIF expression levels and increases apoptosis in hemangioblastomas suggesting a potential role of propranolol as vasoconstrictor, antiangiogenic agent, and proapoptotic drug.

**Methods:** To evaluate therapeutic effect of propranolol in VHL disease, a clinical trial including 7 VHL patients with juxtapapillary or peripheral hemangioblastomas was developed at Virgen de la Salud Hospital in Toledo, Spain. Patients took 120 mg/day propranolol and were monitored at baseline and at 1, 3, 6, 9 and 12 months after. On every visit alongside the treatment, funduscopy as well as different biomarkers from blood samples were analyzed.

**Result:** As the main clinical outcomes, the number and size of tumors present on the retina remained stable, and no new tumors appeared. To highlight, the reabsorption of the exudation in the only two patients who had it initially, being progressive and clear. These outcomes correlated with a decrease of VEGF plasma levels from the first month of treatment in a significant manner (p<0.001), reaching normal levels in all cases after 3 months of treatment.

**Conclusions:** Higher propranolol doses should be tried, up to 3mg/kg body weight/day. It is also necessary to adjust the dose of the drug to the particular needs of each patient, depending if they have exudation or not, or the severity of the ocular affection. It may be interesting as well to explore the effect of intravitreal injection of propranolol in clinical trials. These results are encouraging to continue our research for the treatment of VHL with propranolol in Ophthalmology.
A Model for the Initiation of ccRCC: Cellular and Genomic Analyses of Vhlh Conditional Knockout Mice Displaying Metabolic Abnormalities, Inflammation, Hyperplastic Lesions and Genome Instability In the Kidney

Tien Hsu1*, Chan-Yen Kuo1, Hannah Bader2, Yuki Hagiwara1, Li-Ching Wu1
1 Department of Biomedical Sciences and Engineering, National Central University, Taoyuan City, Taiwan, 2 Department of Medicine, Boston University School of Medicine, Boston, MA, USA

Objectives: We are interested in understanding the mechanism of clear-cell renal cell carcinoma (ccRCC) initiation, which can provide new avenues for ccRCC prevention and early detection for VHL patients.

Introduction: It has been suggested that ccRCC is a metabolic disease. This can explain how the tumor sustains itself and progresses. However, it is not yet clear how such metabolic abnormality can drive ccRCC initiation.

Methods: We generated a mouse strain with conditional Vhlh knockout in a subset of kidney tubule cells using the Hoxb7-Cre-GFP driver, followed by cellular and genomic analyses.

Results: Vhlh inactivation resulted in hyperplasia and abnormal clear cells exhibiting genome instability, accompanied by severe inflammation and fibrosis, as shown below. In VHL loss-of-function kidney tubule cells, imbalance in proteostasis induced by metabolic abnormalities led to ER stress. Two critical inflammation-inducing master switches, NFκB and JNK, were activated in cells under ER stress via the IRE1α pathway. Such condition then leads to activation of inflammatory responses. We demonstrated that these potential tumorigenic phenotypes could be relieved by administering an IRE1α kinase inhibitor APY29. Furthermore, transcriptome analysis validated the inflammation signature in the Vhlh KO kidney. In addition, genes involved in dedifferentiation were also up-regulated in the knockout kidney, consistent with the notion that inflammation may be the initiator of ccRCC. Interestingly, we noted a group of genes that are up-regulated specifically in Vhlh knockout mice and in human stage 1 ccRCC (representing early stage disease), but not in stage 3 ccRCC (representing late stage disease). We reasoned that these genes, including the well-known oncogene Myc, might be involved in inflammation-induced ccRCC initiation. On the other hand, genes specifically up-regulated in Vhlh knockout kidney and in stage 3 ccRCC, but not in stage 1 ccRCC, including chemokine CXCL13, may be involved in immune cell infiltration and tumor metastasis.

Conclusion: Our Vhlh knockout model provides strong support for the notion that inflammation is an inducer of ccRCC. Comparative transcriptome analyses identified several interesting markers that are potential ccRCC-initiator genes or metastasis genes. These may serve as new therapeutic targets.
Risk Factors for Survival in Patients with von Hippel-Lindau Disease

Gong Kan, Wang Jiangyi, Liu Shengjie, Zhou Jingcheng, Hong Baoan

Department of Urology, Peking University First Hospital, Beijing, P.R. China; Institute of Urology, Peking University, Beijing, P.R. China; National Urological Cancer Center, Beijing, P.R. China; Department of Urology, Beijing Hospital, Beijing, P.R. China

Objectives: In this study, we aim to assess the overall and VHL-related survival of Chinese patients with VHL disease based on a large VHL cohort and determine how survival is affected by sex, family history, genotype, birth year, birth order, onset age and first presenting symptom.

Introduction: Von Hippel-Lindau (VHL) disease is a rare hereditary kidney cancer syndrome characterized by a poor survival. Although genotype-phenotype correlation has been described in many studies, the risk factors for VHL survival remains unclear. This study aims to evaluate the median survival of Chinese VHL patients, and explore whether VHL survival is influenced by genetic and clinical factors.

Methods: In this retrospective study, we recruited 340 patients from 127 VHL families. Kaplan Meier plot and Cox regression model were used to evaluate the median survival and assess how survival was influenced by birth year, birth order, sex, family history, mutation type, onset age and first presenting symptom.

Results: The estimated median life expectancy for Chinese VHL patients was 62 years. Patients with early onset age, positive family history and truncating mutation types had poorer overall and VHL-related survival. Patients with hemangioblastoma as their first presenting symptom were related to higher risk of death from central nervous system hemangioblastoma than those with abdominal lesions (HR=8.84, 95% CI 2.04 to 38.37, p=0.004).

Conclusions: This largest to date VHL survival analysis indicates that onset age, family history, mutation type and first presenting symptom have an effect on the survival of VHL patients, which is helpful to genetic consulting and clinical decision making.
Genotype and Phenotype Correlation in von Hippel–Lindau Disease Based on Alteration of the HIF-α Binding Site in VHL Protein

Gong Kan, Liu Shengjie, Wang Jiangyi, Zhou Jingcheng, Hong Baoan
Department of Urology, Peking University First Hospital, Beijing, P.R. China; Institute of Urology, Peking University, Beijing, P.R. China; National Urological Cancer Center, Beijing, P.R. China; Department of Urology, Beijing Hospital, Beijing, P.R. China

Objective: We aimed to construct a more valuable genotype–phenotype correlation based on alterations in VHL protein (pVHL).

Methods: VHL patients (n = 339) were recruited and grouped based on mutation types: HIF-α binding site missense (HM) mutations, non-HIF-α binding site missense (nHM) mutations, and truncating (TR) mutations. Age-related risks of VHL-associated tumors and patient survival were compared.

Results: Missense mutations conferred an increased risk of pheochromocytoma (HR = 1.854, p = 0.047) compared with truncating mutations. The risk of pheochromocytoma was lower in the HM group than in the nHM group (HR = 0.298, p = 0.003) but was similar between HM and TR groups (HR = 0.901, p = 0.810). Patients in the nHM group had a higher risk of pheochromocytoma (HR = 3.447, p < 0.001) and lower risks of central nervous system hemangioblastoma (CHB) (HR = 0.700, p = 0.045), renal cell carcinoma (HR = 0.610, p = 0.024), and pancreatic tumor (HR = 0.382, p < 0.001) than those in the combined HM and TR (HMTR) group. Moreover, nHM mutations were independently associated with better overall survival (HR = 0.345, p = 0.005) and CHB-specific survival (HR = 0.129, p = 0.005) than HMTR mutations.

Conclusion: The modified genotype–phenotype correlation links VHL gene mutation, substrate binding site, and phenotypic diversity (penetrance and survival), and provides more accurate information for genetic counseling and pathogenesis studies.
An Open-Label Phase 2 Study to Evaluate PT2977 for the Treatment of von Hippel-Lindau Disease-Associated Renal Cell Carcinoma

E. Jonasch¹, E. Park², S. Thamake², M. Hirmand², R. Srinivasan³
¹ Department of GU Medical Oncology Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, ² Clinical Development, Peloton Therapeutics, Dallas, TX, USA, ³ Center for Cancer Research, NCI, Bethesda, MD, USA

Objectives: To evaluate the efficacy of PT2977 for the treatment of von Hippel-Lindau (VHL) disease-associated renal cell carcinoma (RCC) as measured by overall response rate (ORR) per Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST 1.1).

Background: In VHL disease, RCC is known to be of clear cell histology (ccRCC). HIF-2α has been established as an oncogenic driver in ccRCC, where VHL deficiency is the underlying genomic alteration. With VHL gene inactivation, HIF-2α accumulates under normoxic conditions, driving expression of genes associated with progression of ccRCC, including vascular endothelial growth factor A (VEGFA), cyclin D1 and other factors that contribute to tumor growth and proliferation. Clinical management of VHL ccRCC involves active surveillance until surgery is required for tumors larger than 3 cm to prevent metastasis. Repeated surgical procedures can carry significant morbidity. Systemic therapy options that can delay or obviate the need for surgery by reducing tumor size are needed.

Methods: This open-label Phase 2 study will evaluate the efficacy and safety of PT2977, a highly selective small molecule inhibitor of HIF-2α, in patients with VHL disease who have at least 1 measurable ccRCC (as defined by RECIST 1.1). PT2977 will be administered orally at a dosage of 120 mg once daily. Key inclusion criteria include a germline VHL alteration and at least 1 measurable solid ccRCC but no tumor >3.0 cm requiring immediate surgical intervention. Patients may have VHL disease-associated tumors in other organ systems. Key exclusion criteria include prior systemic therapy for VHL disease, an immediate need for surgical intervention, metastatic disease, and history of non-VHL invasive malignancy in the past 2 years. Primary endpoint is ORR of ccRCC tumors per RECIST 1.1. Secondary endpoints include duration of response (DOR), time to response (TTR), progression-free survival (PFS), and time to surgery (TTS) for ccRCC tumors and efficacy evaluations for non-ccRCC VHL disease-associated tumors. Safety/tolerability and pharmacokinetics of PT2977 will also be evaluated. Patient recruitment is ongoing.
Dysregulated Acyl-CoA Metabolism Drives the Clear Cell Phenotype and Tumor Growth in Renal Cell Carcinoma

Edward L. LaGory, Timothy D. Klasson, Marjan Rafat, Amato J. Giaccia
Division of Radiation and Cancer Biology; Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA, USA

Objective: To determine the molecular drivers of the clear cell phenotype in renal cell carcinoma, thus enabling the development of novel therapeutic and imaging approaches to treat and identify renal cell carcinomas.

Introduction: Von Hippel-Lindau disease is associated with a high lifetime risk of the development of benign renal cysts and clear cell renal cell carcinomas (ccRCC) which can negatively impact kidney function and present the threat of metastatic dissemination. ccRCC tumors display a characteristic clear cell histology driven by the pronounced accumulation of lipid droplets in the cytoplasm of tumor cells. However, the molecular mechanisms that drive lipid accumulation, and any contribution of this aberrant metabolic phenotype to ccRCC initiation or progression remains elusive.

Methods: We use a variety of in vitro and in vivo preclinical models to characterize the molecular pathways that contribute to lipid droplet formation and assess the efficacy of targeting lipid metabolism as a therapeutic strategy for the treatment of ccRCC.

Results: Our studies provide evidence that uptake of fatty acids predominantly drives lipid droplet formation in ccRCC. Using genetic and pharmacologic approaches, we further describe an essential role for long chain acyl-CoA synthetase (ACSL) in the subsequent metabolism of fatty acids into triglycerides, the major component of lipid droplets. We employ genetic approaches to determine that one ACSL isoform is the predominant driver of the clear cell phenotype in ccRCC cell lines. Genetic suppression of this isoform or pharmacologic targeting of ACSL activity attenuates cell growth in in vitro models, suggesting that ACSL-mediated lipid droplet formation is essential for ccRCC growth. In addition to ablating lipid droplet formation, ACSL inhibition results in a pronounced accumulation of lipid peroxidation products, indicating that oxidative stress may underlie cell death upon ACSL inhibition.

Conclusions: Our findings provide evidence that uptake of exogenous fatty acids drives lipid droplet formation through an ACSL-dependent mechanism. These results highlight the potential of lipid-based imaging modalities for ccRCC. We also demonstrate that ablation of lipid droplet formation by ACSL inhibition results in growth arrest and cell death, suggesting that targeting this metabolic pathway may be a useful therapeutic approach worthy of further investigation.
Exon Skipping Represents New VHL Alteration Associated with von Hippel-Lindau Disease or Congenital Erythrocytosis.

Marion Lenglet1,2,3, Florence Robriquet2,3, Klaus Schwarz4, Anne Couturier5, Sophie Gad1,6, Sophie Couvé1,6, Franck Chesnel5, Mathilde Pacault2,7, Pierre Lindenbaum3, Sylvie Job8, Solene Dumont2, Thomas Besnard,3,7 Marine Cornec,3 Sophie Deveaux,9 Nelly Burnichon,9,10,11 Jean-Michæel Mazzella9,10,11, Laurence Heidet12, Sabine Irland13, Elpis Mantadakis14, Karim Bouchireb12, Brigitte Bressac-de Paillerets6, Bin Tean Teh15, François Girodon16,17, Maria-Luigia Randi18, Antonis Kattamis20, Nicolas Janin21, Yannick Arlot-Bonnemains5, Stéphane Richard1,6,98, Anne-Paule Gimenez-Roqueplo9,10,118, Holger Cario23*, Betty Gardie1,2,3,17*

1 Ecole Pratique des Hautes, EPHE, PSL research University, France; 2 CRCINA, INSERM, Université de Nantes, Université d'Angers, Nantes, France; 3 L'institut du thorax, INSERM, CNRS, UNIV Nantes, Nantes, France; 4 Institute for Transfusion Medicine, University of Ulm and Institute for Clinical Transfusion Medicine and Immunogenetics Ulm, German Red Cross Blood Service Baden-Wurttemberg-Hessen, Ulm, Germany; 5 Univ Rennes, CNRS, IGDR (Institut de génétique et développement de Rennes) - UMR 6290, F- 35000 Rennes, France; 6 INSERM UMR 1186, Institut Gustave Roussy, Université Paris-Saclay, Villejuif, France; 7 Service de Génétique Médicale, CHU de Nantes, Nantes, France; 8 Programme Cartes d'Identité des Tumeurs, Ligue Nationale Contre le Cancer, F-75013 Paris, France; 9 Réseau Expert National pour Cancers Rares de l'Adulte INCa ”PREDIR” and Réseau d'Oncogénétique National INCa "Maladie de VHL et prédispositions au cancer du rein." Service d'Urologie, Assistance publique, Hôpitaux de Paris, Hôpital Bicêtre, Le Kremlin-Bicêtre, France; 10 INSERM UMR970, Paris-Cardiovascular Research Center at HEGP, Paris, France; 11 Assistance Publique Hôpitaux de Paris, Hôpital européen Georges Pompidou, Service de Génétique, Paris, France; 12 Assistance Publique Hôpitaux de Paris Centre de Référence des Maladies Rénales Héréditaires de l'Enfant et de l'Adulte (MARHEA), Service de Néphrologie Pédiatrique, Hôpital Universitaire Necker-Enfants malades, Paris, France; 13 Assistance Publique Hôpitaux de Paris, Département de Chirurgie Pédiatrique, Hôpital Universitaire Necker-Enfants malades, Université Paris Descartes-Sorbonne Paris Cité, Paris,France; 14 Democritus University of Thrace Faculty of Medicine Alexandroupolis, Thrace, Greece; 15 SingHealth/Duke-NUS Institute of Precision Medicine, National Heart Centre Singapore,Singapore; 16 Service d'hématologie Biologique, Pôle Biologie, CHU Dijon, Dijon, France; 17 Laboratory of Excellence GR-Ex; 18 First Medical Clinic, Department of Medicine- DIMED, University of Padua, Padua, Italy; 19 Clinic of Pediatric Hemato-Oncology, Department of Woman's and Child's Health, University of Padua, Padua, Italy; 20 First Department of Pediatrics, National and Kapodistrian University of Athens, Greece; 21 Centre de Génétique Humaine, Cliniques universitaires Saint-Luc, B-1200 Bruxelles, Belgium; § ß * These authors contributed equally to this work

**Background:** Germline mutations in the von Hippel-Lindau (VHL) tumor suppressor gene predispose patients to different phenotypes: VHL disease characterized by the development of specific tumors (heterozygous mutations) or congenital erythrocytosis without tumors (homozygous or compound-heterozygous mutations). The full molecular mechanisms at the origin of these different phenotypes remains to be elucidated. All previous studies have focused on the impact of missense mutations on the protein function. However, in several unexplained cases, patients with a VHL-related phenotype carry synonymous genetic variants with no impact on the amino-acid sequence.

**Methods:** We performed a comprehensive study of five families carrying the synonymous variants D143D or P138P, in addition to mutations P138L and G144R located nearby. We performed segregation studies, quantification of the different mRNA and VHL protein isoforms, Hypoxia-Responsive-Element-luciferase reporter assay, splicing reporter assay and RNA sequencing of patient's blood samples and tumors.
**Results:** We demonstrated that synonymous variants in Exon 2 affect splicing with a consequent exon skipping. This exon skipping is correlated with a downregulation of VHL protein expression, which constitutes a different mechanism of VHL inactivation (compared to alteration of protein function). RNAsequencing confirmed the dysregulation of the VHL/HIF pathway in tumor bearing the VHL synonymous mutation. We observed that, depending on the mutation in Exon 2, the impact on splicing can be moderate (D143D, G144R, P138L) or severe (P138P), which correlates with the severity of the disease developed by individuals carrying these VHL mutations (erythrocytosis versus cancers).

**Conclusion:** This study demonstrates that Exon 2 skipping is a new VHL alteration and that synonymous variants should be considered as pathogenic mutations. We recommend to evaluate the impact of all the nucleotide changes described in VHL-E2. This discovery may help to better understand some complex genotype/phenotype correlation observed in VHL-related diseases.

**Funding:** This study was supported by grants from the Région Pays de la Loire, Project EryCan; the Agence Nationale Recherche (Programme de Recherche Translationnelle en Santé 2015 GenRED) and the Laboratory of Excellence GR-Ex (reference #ANR-11-LABX-0051).
New Experimental Models for VHL Disease by Using Patient-Derived Induced Pluripotent Stem Cells

Eijiro Nakamura
DSK Project, Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan

Introduction/Background: One of the major problems of developing new therapy for VHL disease is the lack of disease model. To resolve these, we are now establishing new models by using VHL patient-derived induced pluripotent stem cells (VHL iPS) and focusing on recapitulating disease phenotypes both in vitro and in vivo.

Methods: We have successfully established iPS cells from Japanese patients with VHL disease. Those patients have developed RCC, hemangioblastoma, pheochromocytoma, and pancreatic neuroendocrine tumors in their clinical courses. First, we injected VHL iPS cells into testes if they could form mature teratomas to confirm that those cells retained the pluripotency. We also examined if they could differentiate into the possible tumor origin of VHL-related tumors such as chromaffin cells for pheochromocytoma.

Results and Conclusions: Our results indicated that VHL iPS possess the ability to differentiate all three germ layers, ectoderm, mesoderm, and endoderm. Based on these, we mainly focus on establishing hemangioblastoma models through the differentiation of VHL iPS to its tumor origin since there exist no cell lines from disease cells. Our preliminary results from both in vitro and in vivo experiments indicated that differentiated VHL iPS successfully express several marker molecules of hemangioblastoma tumors. Collectively, VHL iPS might be useful tools for the recapitulation and the drug discovery of VHL related tumors.
Von Hippel-Lindau Disease and Steroid Cell Tumor of the Ovary – A New Association?

Ha Nguyen, Anita K. Ying, Elizabeth D. Euscher, Larissa Meyer, Samuel Hyde, Gilbert Cote, Eric Jonasch, Steven G. Waguespack*

The University of Texas MD Anderson Cancer Center, Houston, TX, USA
* primary author

Objectives: Ovarian involvement in von Hippel–Lindau (VHL) disease is rare. Previously, only 3 cases of steroid cell tumor of the ovary (SCTO) have been reported. We hereby describe 2 additional VHL patients with SCTO.

Case 1: A 22-year-old female-to-male transgender patient who had prior manifestations of bilateral pheochromocytoma (PHEO), nonfunctioning pancreatic neuroendocrine tumors (nPNET), and retinal and central nervous system (CNS) hemangioblastomas reported amenorrhea for 2 years. He had had oligomenorrhea since menarche at age 13. Workup showed high total testosterone 56 ng/dl (8-48) and normal DHEAS 18.4 mcg/dl (44-332). Pelvis US revealed enlargement of both ovaries (right 4.6 x 4.7 x 3.3 cm and left 5.9 x 4.1 x 4.3 cm). He underwent a left salpingo-oophorectomy (SO). Pathology was consistent with SCTO. Due to the uncertain malignant potential of SCTO and his desire to undergo gender-affirming surgery, the patient had a right SO 10 months later. Postoperative testosterone was unevaluable due to the exogenous use of testosterone. There was no evidence of recurrent disease on imaging studies after 3 years follow up.

Case 2: An 18-year-old female with history of bilateral PHEO, nPNET and CNS hemangioblastoma presented with irregular periods and hirsutism. Workup showed high total testosterone 269 ng/dl (12-60) and normal DHEAS 280 mcg/dl (44-332). A pelvis MRI showed a 4.9 x 4.1 x 5.6 cm lipid containing solid mass in the right adnexa with no abnormality in the left. The patient had a right SO and pathology reported SCTO. One month postoperatively, her testosterone level was < 7ng/dl (12-60). However, 1 year later, symptoms of hirsutism recurred and testosterone was elevated at 190 ng/dl (5-38). Pelvis US revealed a new enlarged left ovary measuring 4.3 x 2.7 cm and the patient subsequently underwent a SO of the remaining ovary. Pathology of the left ovary also confirmed SCOT. At 6 month postoperatively, testosterone level decreased to 42 ng/dl (8-48). Patient had no evidence of disease after 2 years follow up.

Conclusions: SCTO is a rare ovarian neoplasm that could be associated with VHL disease and should be in the differential diagnosis for women with VHL and hyperandrogenism.
Inactivation of Vhlh in Mouse Kidney Epithelial Cells Induces Inflammatory Response in Endothelial Cells

Hieu-Huy Nguyen-Tran, Li-Jen Su, Tien Hsu

Department of Biomedical Sciences and Engineering, National Central University, Taoyuan City, Taiwan

Introduction: Inactivation of tumor suppressor gene von Hippel–Lindau (VHL) contributes to development of clear-cell renal cell carcinoma (ccRCC). However, the exact mechanism of VHL mutation-induced tumor formation has remained unclear. In our Vhlh conditional knockout mouse model (Hoxb7-Cre-GFP; VhlhloxP/loxP), we found a high penetrance of interstitial inflammation and fibrosis in the kidney in addition to hyperplasia and the appearance of transformed clear cells. These results support the notion that inflammation is a precursor of ccRCC. Interestingly, we also found that the expression of inflammatory marker p-JNK was enriched in endothelial cells (ECs) in the inflamed Vhlh knockout kidney, even though the Vhlh knockout occurs in epithelial tubule cells, not in endothelial cells. This raised the possibility that endothelial cells may play a significant role in pre-cancerous inflammation and may present a novel therapeutic target for ccRCC.

Objective: We aimed to investigate gene expression profile of ECs in inflammation induced by Vhlh knockout in kidney epithelial cells.

Methods: ECs were isolated from kidneys of 3-month old mutant and wild-type mice using PCAM1-coupled Dynabead, and total RNA extracted immediately from the isolated ECs without further manipulation. Illumina NextSeq was employed for RNA sequencing. HUVECs were stimulated by conditioned media (CM) from shVHL knockdown HK-2 cells to investigate the response of ECs to mutant epithelial cells in vitro.

Results: We found 139 up-regulated and 389 down-regulated genes in ECs from Vhlh knockout kidneys with a minimum of two-fold (log2) change. The affected biological processes include inflammatory response, cell adhesion, response to hypoxia, and positive regulation of angiogenesis. The molecular pathways analysis showed a number of up-regulated pathways involved in EC inflammation, including cell adhesion, TNF signaling, leukocyte transendothelial migration, and VEGF signaling. IHC staining of Vhlh knockout mouse showed high expression of EC activation markers, some of which were also up-regulated in in vitro stimulated HUVECs.

Conclusions: We found that endothelial cells, a quiescent component in non-inflamed tissues, are activated in mutant mouse kidney with Vhlh deletion in tubule cells. The results support the hypothesis that endothelium plays a central role in mediating inflammatory response. Many of these EC inflammatory markers could be used as potential targets for treatment of kidney inflammation, and for early diagnosis or treatment of ccRCC.
VHL Knockdown Kidney Cells Induces Macrophage Extravasation and Polarization toward Tumor-Associated Macrophage (TAM) in Progression of Clear-Cell-Renal Cell Carcinoma

Thi-Ngoc Nguyen, Keng-Wei Liu, Tien Hsu
Institute of Systems Biology and Bioinformatics, National Central University, Taoyuan City, Taiwan

Introduction: There is a very close causal correlation between clear-cell renal cell carcinoma (ccRCC) and inactivation of the tumor suppressor gene von Hippel-Lindau (VHL). To study this correlation, we previously generated a Vhlh conditional knockout mouse mode (Hoxb7-Cre-GFP; VhlhloxP/loxP). We demonstrated a critical link between tissue inflammation and ccRCC formation. Our results showed an abundance of infiltrating macrophages and lymphocytes in inflammatory kidney tissue of Vhlh knockout mouse, accompanied by abnormal clear cells and hyperplastic cysts. Monocyte-derived tissue effector cells, macrophage, is a crucial player in connection of inflammation and cancer formation. Macrophage infiltration in the inflammatory microenvironment has been observed in previous studies of ccRCC models containing VHL mutations. However, the mechanism by which VHL loss-of-function cells attract macrophage during ccRCC formation has remained unclear.

Objective: We aimed to study the interaction between VHL loss-of-function kidney cells and macrophage in progression of clear-cell-renal cell carcinoma.

Methods: Transwell co-culture system was used to mimic the extravasation process in vitro, containing three types of cell lines including human kidney cells (HK-2 with or without VHL knockdown), human umbilical vein endothelial cells (HUVECs) and monocytic macrophage precursor THP-1 cells. The conditioned media (CM) from HK-2 cells were prepared for proteomics analysis of the secreted proteins. Invasive macrophages were quantified using ICC/IF staining, and their polarization analyzed by RT-qPCR for M1 or M2/TAM markers. Immunohistochemistry of Vhlh knockout mouse kidney tissue were used to validate the in vitro results.

Results: The results show that VHL knockdown cells secreted different cytokines and chemokines that recruit macrophage endothelial extravasation (such as IFN-γ, IL-6, GM-CSF, MIF, Serpin E1). Interestingly, we found that recruited macrophage tend to polarize toward M2/TAM phenotype with increased expression of IL10, CCL22 and TGF-α. IHC staining results also confirmed this finding with highly expressed Arginase-1—a M2/TAM macrophage marker—in Vhlh knockout mouse mode.

Conclusions: We found that VHL knockdown cells recruit macrophages through secreting relevant cytokines. We hypothesize that in the inflammatory microenvironment induced by Vhlh inactivation, macrophages aggregate and polarize toward TAM phenotype and thus promote tumor formation. These secreted cytokines/chemokines from VHL loss-of-function cells or tumor-promoting cytokines released by invasive macrophage may present novel targets for anti-ccRCC therapy.
**Differences in Genetic and Epigenetic Alterations between von Hippel-Lindau Disease-Related and Sporadic Hemangioblastomas of the Central Nervous System**

**Shunsaku Takayanagi**, Akitake Mukasa, Shota Tanaka, Masashi Nomura, Mayu Omata, Shunsuke Yanagisawa, Shogo Yamamoto, Koichi Ichimura, Hirofumi Nakatomi, Keisuke Ueki, Hiroyuki Aburatani, Nobuhito Saito

Department of Neurosurgery, The University of Tokyo, Tokyo, Japan (S.T., A.M., S.T., M.O., S.Y., H.N., N.S.); Genome Science Division, Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan (S.Y., H.A.); Division of Brain Tumor Translational Research, National Cancer Center Research Institute, Tokyo 104-0045, Japan (K.I.); Department of Neurosurgery, Dokkyo Medical University, Tochigi, Japan (K.U.)

**Background:** Although inactivation of the *von Hippel-Lindau (VHL)* gene, which is located on chromosome 3p25, is considered to be a major cause of hemangioblastomas, the incidence of biallelic inactivation of *VHL* is reportedly low. The aim of this study was to determine the prevalence of *VHL* alterations in hemangioblastomas, as well as to identify additional molecular aberrations.

**Methods:** Genetic and epigenetic alterations were comprehensively and comparatively analyzed in 11 *VHL*-related and 21 sporadic hemangioblastomas.

**Results:** *VHL* alterations detected by sequencing and multiplex ligation-dependent probe amplification analysis were more frequent in *VHL*-related hemangioblastomas than in sporadic hemangioblastomas (100% vs. 62%; *P* = 0.029). *VHL* alterations were found only in four sporadic hemangioblastomas by direct sequencing; however, targeted deep sequencing detected nine additional alterations. Loss of heterozygosity (LOH) on chromosome 3 was found in 64% and 57% of *VHL*-related and sporadic hemangioblastomas, respectively by single nucleotide polymorphism array analysis. Among 19 tumors with chromosome 3 LOH, five were classified as copy-neutral LOH. *VHL* promoter hypermethylation was detected only in sporadic hemangioblastomas (33%), indicating that epigenetic suppression of *VHL* is a common mechanism in sporadic hemangioblastomas. The rate of biallelic *VHL* inactivation among *VHL*-related and sporadic hemangioblastomas was 64% and 52%, respectively. LOH either on chromosome 6 or 10 was detected only in sporadic hemangioblastomas (43%).

**Conclusion:** Although biallelic inactivation of *VHL* is a dominant mechanistic cause of the pathogenesis of hemangioblastoma, other unknown mechanisms may also be involved, and such mechanisms may be different between *VHL*-related and sporadic hemangioblastoma.
STF-62247 Blocks Late Stages of Autophagy by Disrupting Lysosomal Physiology in VHL-Deficient Renal Cell Carcinoma

Nadia Bouhamdani¹,², Dominique Comeau¹,², Kevin Cormier¹,², Sandra Turcotte¹,²
¹ Department of Chemistry and Biochemistry, Université de Moncton, Moncton, Canada; ² Atlantic Cancer Research Institute, Moncton, Canada

Introduction: VSTF-62247 was previously identified as a promising compound able to selectively target the loss of the tumor suppressor gene von Hippel-Lindau (VHL) in renal cell carcinomas.

Objectives: The present work focuses on investigating the effect of STF-62247 on the autophagic flux to better characterize this underused tool and its potential of action.

Methods: Several autophagic assays exploring both later stages of autophagy as well as crucial signaling pathways leading to autophagy initiation clearly show that STF-62247 blocks later stages of autophagy through lysosomal disruption.

Results: Our investigations show that STF-62247 localizes at lysosomes and causes unregulated swelling of these acidic compartments in VHL-mutated cells, linking a potential role for VHL in lysosomal integrity. CRISPR/Cas9 knock-outs of BECN1 and ATG5 were able to rescue the viability of VHL-mutated cells in response to STF-62247 but did not rescue the lysosomal swelling. In fact, neutralizing the lysosomal pH by inhibiting the vacuolar H⁺-ATPase completely rescued this phenotype. Moreover, we show that STF-62247 disrupts endocytic routes and causes cathepsin D trafficking defects.

Conclusions: This mechanistic study is the first to characterize STF-62447 as a novel lysosomotropic compound and re-classifies STF-62247 as a blocker of later stages of autophagy. These results highlight the STF-62247 potential usage as a new tool for endocytic and autophagy-related research that could be further study in the context of VHL disease.
**microRNA Profiling Revealed Overexpression of miR-2355 in VHL-Inactivated Renal Cell Carcinoma and the Sushi Domain-Containing Protein 4 as Predicted Target**

Sonia A Dastous¹², Nicolas Crapoulet², Dominique Comeau¹², Rodney J Ouellette¹², Sandra Turcotte¹²

¹ Department of chemistry and biochemistry, Université de Moncton, Moncton, New Brunswick, Canada; ² Atlantic Cancer Research Institute, Moncton, New Brunswick, Canada

**Introduction**: Clear cell Renal Cell Carcinoma (ccRCC) is the most common malignant form of neoplasm among adult kidney cancers. Mutations that inactivate the von Hippel-Lindau (VHL) tumor suppressor gene is one of the major driver events in ccRCC carcinogenesis. MicroRNAs (miRNAs) are frequently dysregulated in various type of cancer including ccRCC.

**Objectives**: This study aims to identify differently expressed miRNAs in VHL-inactivated ccRCC and potential targets.

**Methods and Results**: Using deep sequencing in VHL-deficient cells compared to cells with the functional gene, we found 183 differently expressed miRNAs. These results were integrated with clinical data obtained from a cohort of VHL-mutated ccRCC patients found in The Cancer Genome Atlas (TCGA) compared to patient-matching adjacent normal tissue data. This comparison identified a profile of 32 clinically relevant miRNAs. We selected miR-2355, overexpressed in 97% of patients, for further validation and showed its regulation through the hypoxia inducible factor-2α, which promote ccRCC tumorigenesis. To determine potential targets for this miRNA, we stably repressed miR-2355 expression using the CRISPR/Cas9 system. By analyzing mRNA sequencing data of VHL-/- CRISPR miR-2355 cells combined to miRWalk algorithm predictions and mRNA patient data, our findings revealed the Sushi Domain-Containing Protein 4 (SUSD4) as potential miR-2355 target gene. Known as complement inhibitor, SUSD4 decreased in VHL-inactivated tumors and significantly increased with miR-2355 downregulation.

**Conclusion**: This study used clinical data and *in vitro* screening to identify miRNAs aberrantly expressed in VHL-mutated ccRCCs that could provide new insights and potential tools for ccRCC pathogenesis.
Expression of Hemangioblast Proteins in von Hippel-Lindau Related Tumors

Vergauwen, E1, Forsyth, R2, Lefesvre, P2, Michotte, A2, Van Velthoven, V1, Gläsker, S1*

1 Department of Neurosurgery, University Hospital Brussels, Belgium; 2 Department of Anatomopathology, University Hospital Brussels, Belgium
*Corresponding author

Introduction: The incomplete understanding of von Hippel-Lindau (VHL) disease tumorigenesis has impeded the development of a targeted pharmacological therapy. We and others have previously shown that VHL associated hemangioblastomas share developmental and morphological features with the hemangioblast, an embryonic blood and vascular precursor cell. We have therefore suggested that VHL hemangioblastomas are derived from a developmentally arrested hemangioblast with the potential for differentiation. We hypothesize that also other VHL tumors are derived from the same precursor cell, and that intratumoral development of the hemangioblast is demonstrated by the sequential expression of its proteins throughout progressive hemangioblastoma stages.

Objectives: To better understand VHL tumorigenesis, we investigated the expression of hemangioblast proteins in VHL tumors other than hemangioblastomas. Furthermore, we investigated the differences in hemangioblast protein expression between early reticular and late cellular hemangioblastoma stages.

Methods: The expression of embryonic hemangioblast proteins Brachyury, TAL1 (T-cell acute lymphocytic leukemia protein 1) and VEGFR2 (vascular endothelial growth factor receptor 2) was assessed by immunohistochemistry staining on 73 VHL related tumors of 57 patients: 45 hemangioblastomas, 13 clear cell renal cell carcinomas, 8 pheochromocytomas, 5 pancreatic neuroendocrine tumors and 2 extra-adrenal paragangliomas.

Results and Conclusions: Brachyury and TAL1 expression was respectively seen in 54% and 54% of hemangioblastomas, 33% and 0% of clear cell renal cell carcinomas, 75% and 0% of pheochromocytomas, 50% and 50% of pancreatic neuroendocrine tumors, and 50% and 50% of paragangliomas. The expression of hemangioblast proteins in other VHL tumors provides evidence for an embryological origin of VHL associated tumors. Brachyury and TAL1 expression was only observed in cellular hemangioblastomas. This exclusive expression may illustrate the progression from a developmentally arrested hemangioblast in the early reticular stage, to a mature hemangioblast in the cellular stage. Furthermore, we are the first to describe the presence of three different vascular subpopulations in all investigated VHL tumors: large CD31+VEGFR2- structures, small CD31+VEGFR2+ structures and variably sized CD31-VEGFR2- structures; possibly illustrating different stages of vascular development.
Diagnosis and Treatment Strategy of Hemangioblastomas of the Optic Nerve and Chiasm

Evelynn Vergauwen¹, Marie T. Krüger², Jan-Helge Klingler², Vera Van Velthoven¹, Sven Gläsker¹*
¹ Department of Neurosurgery, University Hospital Brussels, Belgium; ² Department of Neurosurgery, Freiburg University Medical Center, Freiburg, Germany
* Corresponding author

Introduction: Optic nerve and chiasm hemangioblastomas are rare tumors, occurring sporadically or in the context of von Hippel-Lindau (VHL) disease. They have only been portrayed in isolated case reports and small cohorts. Their natural history and therapeutic strategies are therefore not well described. To better characterize these rare tumors, we retrospectively analyzed our hemangioblastoma patient series.

Objectives: By combining our own experience to a review of all known cases in literature, we intended to create the first treatment standard for optic nerve and chiasm hemangioblastomas.

Methods: We reviewed 2 electronic databases in the hospitals of our senior authors, searching for VHL patients with optic nerve or chiasm hemangioblastomas. Clinical data were summarized. Tumor size and growth rate were measured on contrast enhanced MRI and correlated to visual symptoms. If available, comparable data were collected from cases already described in literature.

Results: Ten of 269 VHL patients had optic nerve or chiasm hemangioblastomas. In 9 of 10 patients, tumors were diagnosed incidentally upon annual MRI screening. Subsequent radiological follow-up periods ranged from 3 months to 11 years. Seven of these 9 asymptomatic patients had absent or very slow annual progression, without developing visual deficits. Two of these initially asymptomatic patients suffered from rapid tumor growth and progressive visual deficits. Both underwent late stage surgery, resulting in incomplete resection and progressive visual deficits. One VHL patient already had partial visual loss at time of diagnosis. A watchful-waiting approach was preferred because the hemangioblastoma was ineligible for vision-sparing surgery.

Conclusions: When optic nerve and chiasm hemangioblastomas are diagnosed, annual MRI follow-up is sufficient, as long as patients do not develop visual deficits. If tumors grow fast and/or patients develop visual deficits, surgical resection must be considered because neurological deficits are irreversible and resection of large tumors carries a higher risk of further visual decline.
## Participant List

<table>
<thead>
<tr>
<th>Name</th>
<th>E-mail</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daniel Aguire Mollehuanca</td>
<td><a href="mailto:dtaguirre@fjd.es">dtaguirre@fjd.es</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Farzan Ali</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Yannick Arlot</td>
<td><a href="mailto:yannick.arlot@univ-rennes1.fr">yannick.arlot@univ-rennes1.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Ashok Asthagiri</td>
<td><a href="mailto:ara5x@hscmail.mcc.virginia.edu">ara5x@hscmail.mcc.virginia.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Masaya Baba</td>
<td><a href="mailto:babam@kumamoto-u.ac.jp">babam@kumamoto-u.ac.jp</a></td>
<td>Japan</td>
</tr>
<tr>
<td>Mark Ball</td>
<td><a href="mailto:mark.ball@nih.gov">mark.ball@nih.gov</a></td>
<td>United States</td>
</tr>
<tr>
<td>Jane Beasley</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Luisa Botella</td>
<td><a href="mailto:cibluisa@cib.csic.es">cibluisa@cib.csic.es</a></td>
<td>United States</td>
</tr>
<tr>
<td>Sarah Bottomley</td>
<td><a href="mailto:sjbottom@mdanderson.org">sjbottom@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>James Brugarolas</td>
<td><a href="mailto:James.Brugarolas@UTSouthwestern.edu">James.Brugarolas@UTSouthwestern.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Erinn Bruno Rankin</td>
<td><a href="mailto:erankin@stanford.edu">erankin@stanford.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Nelly Burnichon</td>
<td><a href="mailto:nelly.burnichon@aphp.fr">nelly.burnichon@aphp.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Henriett Butz</td>
<td><a href="mailto:butz.henriett@med.semmelweis-univ.hu">butz.henriett@med.semmelweis-univ.hu</a></td>
<td>Hungary</td>
</tr>
<tr>
<td>Marie Canning</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Emily Chang</td>
<td><a href="mailto:emily_chang@med.unc.edu">emily_chang@med.unc.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>John Chappell</td>
<td><a href="mailto:jchappell@vtc.vt.edu">jchappell@vtc.vt.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Daniel Chung</td>
<td><a href="mailto:daniel.chung@sparktx.com">daniel.chung@sparktx.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Kevin Courtney</td>
<td><a href="mailto:kevin.courtney@utsouthwestern.edu">kevin.courtney@utsouthwestern.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Angel Cuesta Martinez</td>
<td><a href="mailto:acme@cib.csic.es">acme@cib.csic.es</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Patricia Dahia</td>
<td><a href="mailto:dahia@uthscsa.edu">dahia@uthscsa.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Anthony Daniels</td>
<td><a href="mailto:anthony.b.daniels@vumc.org">anthony.b.daniels@vumc.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Valeria de Miguel</td>
<td><a href="mailto:valeria.demiguel@hospitalitaliano.org.ar">valeria.demiguel@hospitalitaliano.org.ar</a></td>
<td>Argentina</td>
</tr>
<tr>
<td>Ruhee Dere</td>
<td><a href="mailto:ruhee.dere@bcm.edu">ruhee.dere@bcm.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Leona deVinne</td>
<td><a href="mailto:leona@accendoconsulting.ca">leona@accendoconsulting.ca</a></td>
<td>Canada</td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
<td>Country</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>M. Belén Elguero</td>
<td><a href="mailto:belguero@ibioba-mpsp-conicet.gov.ar">belguero@ibioba-mpsp-conicet.gov.ar</a></td>
<td>Argentina</td>
</tr>
<tr>
<td>Antonella Falconieri</td>
<td><a href="mailto:antonella.falconieri@hotmail.it">antonella.falconieri@hotmail.it</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Alberto Feletti</td>
<td><a href="mailto:alberto.feletti@gmail.com">alberto.feletti@gmail.com</a></td>
<td>Italy</td>
</tr>
<tr>
<td>Remington Fenter</td>
<td><a href="mailto:remington.fenter@utsouthwestern.edu">remington.fenter@utsouthwestern.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Shahida Flores</td>
<td><a href="mailto:floressk@liveemail.uthscsa.edu">floressk@liveemail.uthscsa.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Debra Friedman</td>
<td><a href="mailto:debra.l.friedman@vumc.org">debra.l.friedman@vumc.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Seth Gammon</td>
<td><a href="mailto:stgammon@mdanderson.org">stgammon@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Betty Gardie</td>
<td><a href="mailto:betty.gardie@univ-nantes.fr">betty.gardie@univ-nantes.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Giannicola Genovese</td>
<td><a href="mailto:hhenovese@mdanderson.org">hhenovese@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Anne Paule Gimenez-Roqueplo</td>
<td><a href="mailto:anne-paule.gimenez-roqueplo@aphp.fr">anne-paule.gimenez-roqueplo@aphp.fr</a></td>
<td>United States</td>
</tr>
<tr>
<td>Sven Gläsker</td>
<td><a href="mailto:sven.glaesker@uzbrussel.be">sven.glaesker@uzbrussel.be</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Dan Gombos</td>
<td><a href="mailto:dgombos@mdanderson.org">dgombos@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Beatriz Gonzalez Rodriguez</td>
<td><a href="mailto:glezrbeatriz@gmail.com">glezrbeatriz@gmail.com</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Manuel Greco</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Samantha Greenberg</td>
<td><a href="mailto:samantha.greenberg@hci.utah.edu">samantha.greenberg@hci.utah.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Mohammad Hirmand</td>
<td><a href="mailto:mohammad.hirmand@pelotontx.com">mohammad.hirmand@pelotontx.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Markus Holling</td>
<td><a href="mailto:hollingm@ukmuenster.de">hollingm@ukmuenster.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Tien Hsu</td>
<td><a href="mailto:tienhsu@ncu.edu.tw">tienhsu@ncu.edu.tw</a></td>
<td>Taiwan</td>
</tr>
<tr>
<td>Othon Iliopoulos</td>
<td><a href="mailto:oiliopoulos@partners.org">oiliopoulos@partners.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Camilo Jimenz</td>
<td><a href="mailto:cjimenez@mdanderson.org">cjimenez@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Eric Jonasch</td>
<td><a href="mailto:ejonasch@mdanderson.org">ejonasch@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>John Josey</td>
<td><a href="mailto:john.josey@pelotontx.com">john.josey@pelotontx.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Electron Kebebew</td>
<td><a href="mailto:kebebew@stanford.edu">kebebew@stanford.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Raymond Kim</td>
<td><a href="mailto:raymond.kim@uhn.ca">raymond.kim@uhn.ca</a></td>
<td>United States</td>
</tr>
<tr>
<td>Timothy Klasson</td>
<td><a href="mailto:tklasson@stanford.edu">tklasson@stanford.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Mei Koh</td>
<td><a href="mailto:mei.koh@utah.edu">mei.koh@utah.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Werner Kovacs</td>
<td><a href="mailto:werner.kovacs@biol.ethz.ch">werner.kovacs@biol.ethz.ch</a></td>
<td>Switzerland</td>
</tr>
<tr>
<td>Edward LaGory</td>
<td><a href="mailto:elagory@stanford.edu">elagory@stanford.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Phillip Lander</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Shannon Largent</td>
<td><a href="mailto:shannon.largent@pfizer.com">shannon.largent@pfizer.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Marion Lenglet</td>
<td><a href="mailto:marion.genglet@univ-nantes.fr">marion.genglet@univ-nantes.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Heidi Leone</td>
<td><a href="mailto:heidi.leone@vhl.org">heidi.leone@vhl.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Sandy Liu</td>
<td><a href="mailto:stliu@mednet.ucla.edu">stliu@mednet.ucla.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Stacy LLoyd</td>
<td><a href="mailto:stacy.lloyd22@gmail.com">stacy.lloyd22@gmail.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
<td>Country</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Cheryl Madsen</td>
<td><a href="mailto:gumnuttbaby@yahoo.com">gumnuttbaby@yahoo.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Heba Mahmoud</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Erika Maka</td>
<td>upon request</td>
<td>Hungary</td>
</tr>
<tr>
<td>Holly Mandell</td>
<td><a href="mailto:holly.mandell@novartis.com">holly.mandell@novartis.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Joshua Mann</td>
<td><a href="mailto:josh.mann@vhl.org">josh.mann@vhl.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Jesusa Martinez Gomez</td>
<td>upon request</td>
<td>Spain</td>
</tr>
<tr>
<td>Surena Matin</td>
<td><a href="mailto:surmatin@mdanderson.org">surmatin@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Anna Matynia</td>
<td><a href="mailto:matynia@jsei.ucla.edu">matynia@jsei.ucla.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Benjamin Maughan</td>
<td><a href="mailto:benjamin.maughan@hci.utah.edu">benjamin.maughan@hci.utah.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Ian McCutheon</td>
<td><a href="mailto:imccutch@mdanderson.org">imccutch@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Holly McDonald</td>
<td><a href="mailto:holly.mcdonald@pelotontx.com">holly.mcdonald@pelotontx.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Gautam Mehta</td>
<td><a href="mailto:gmehta@houseclinic.com">gmehta@houseclinic.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Eijiro Nakamura</td>
<td><a href="mailto:hap@kuhp.kyoto-u.ac.jp">hap@kuhp.kyoto-u.ac.jp</a></td>
<td>Japan</td>
</tr>
<tr>
<td>Katherine Nathanson</td>
<td><a href="mailto:knathans@upenn.edu">knathans@upenn.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Jérôme Nevoux</td>
<td><a href="mailto:jerome.nevoux@aphp.fr">jerome.nevoux@aphp.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Ha Nguyen</td>
<td><a href="mailto:hanguyenmd@gmail.com">hanguyenmd@gmail.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Huy Tran Hieu Nguyen</td>
<td><a href="mailto:nguyentranhieuhuy@g.ncu.edu.tw">nguyentranhieuhuy@g.ncu.edu.tw</a></td>
<td>Taiwan</td>
</tr>
<tr>
<td>Ngoc Thi Nguyen</td>
<td><a href="mailto:ngocnguyen@g.ncu.edu.tw">ngocnguyen@g.ncu.edu.tw</a></td>
<td>Taiwan</td>
</tr>
<tr>
<td>Sarah Nielsen</td>
<td><a href="mailto:snielsen@medicine.bsd.uchicago.edu">snielsen@medicine.bsd.uchicago.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Eric Park</td>
<td><a href="mailto:eric.park@pelotontx.com">eric.park@pelotontx.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Ivan Pedrosa</td>
<td><a href="mailto:ivan.pedrosa@UTSouthwestern.edu">ivan.pedrosa@UTSouthwestern.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Christine Peterson</td>
<td><a href="mailto:cbpeterson@mdanderson.org">cbpeterson@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>W. Kimryn Rathmell</td>
<td><a href="mailto:kimryn.rathmell@vanderbilt.edu">kimryn.rathmell@vanderbilt.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Surya Redman</td>
<td><a href="mailto:sprednam@texaschildrens.org">sprednam@texaschildrens.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Sharjeel Sabir</td>
<td><a href="mailto:shsabir@mdanderson.org">shsabir@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Susanne Schlisio</td>
<td><a href="mailto:susanne.schlisio@ki.se">susanne.schlisio@ki.se</a></td>
<td>Sweden</td>
</tr>
<tr>
<td>Brian Shuch</td>
<td><a href="mailto:bshuch240@yahoo.com">bshuch240@yahoo.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Ramaprasad Srinivasan</td>
<td><a href="mailto:ramasrin@mail.nih.gov">ramasrin@mail.nih.gov</a></td>
<td>United States</td>
</tr>
<tr>
<td>Andrea Stacy</td>
<td><a href="mailto:astacy@wustl.edu">astacy@wustl.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Ilene Sussman</td>
<td><a href="mailto:ilene.sussman@vhl.org">ilene.sussman@vhl.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Shunsaku Takayanagi</td>
<td><a href="mailto:takayanagi-nsu@umin.ac.jp">takayanagi-nsu@umin.ac.jp</a></td>
<td>Japan</td>
</tr>
<tr>
<td>Kaushik Thakkar</td>
<td><a href="mailto:kthakkar@stanford.edu">kthakkar@stanford.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Sanjay Thamake</td>
<td><a href="mailto:sanjay.thamake@pelotontx.com">sanjay.thamake@pelotontx.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Karynne Thim</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
<td>Location</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Amit Tirosh</td>
<td><a href="mailto:amit.tirosh@nih.gov">amit.tirosh@nih.gov</a></td>
<td>United States</td>
</tr>
<tr>
<td>Sandra Turcotte</td>
<td><a href="mailto:sandra.turcotte@umoncton.ca">sandra.turcotte@umoncton.ca</a></td>
<td>Canada</td>
</tr>
<tr>
<td>Kim van Bloois</td>
<td>upon request</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Rachel van Leeuwaarde</td>
<td><a href="mailto:r.vanLeeuwaarde@umcutrecht.nl">r.vanLeeuwaarde@umcutrecht.nl</a></td>
<td>Netherlands</td>
</tr>
<tr>
<td>Evelynn Vergauwen</td>
<td><a href="mailto:evelynn-v@hotmail.com">evelynn-v@hotmail.com</a></td>
<td>Belgium</td>
</tr>
<tr>
<td>Karina Villar Gómez de las Heras</td>
<td><a href="mailto:kvillar@jccm.es">kvillar@jccm.es</a></td>
<td>Spain</td>
</tr>
<tr>
<td>Steven Waguespack</td>
<td><a href="mailto:swagues@mdanderson.org">swagues@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Cheryl Walker</td>
<td><a href="mailto:cheryl.walker@bcm.edu">cheryl.walker@bcm.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>John Wallace Jr.</td>
<td><a href="mailto:wallyjohnmd@gmail.com">wallyjohnmd@gmail.com</a></td>
<td>United States</td>
</tr>
<tr>
<td>Anna Waller</td>
<td>upon request</td>
<td>United States</td>
</tr>
<tr>
<td>Herui Wang</td>
<td><a href="mailto:herui.wang@nih.gov">herui.wang@nih.gov</a></td>
<td>United States</td>
</tr>
<tr>
<td>Xia Wang</td>
<td><a href="mailto:xia.wang@moffitt.org">xia.wang@moffitt.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Ronald Westerlaken</td>
<td>upon request</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Juliane Willecke</td>
<td><a href="mailto:juliane.willecke@uniklinik-freiburg.de">juliane.willecke@uniklinik-freiburg.de</a></td>
<td>Germany</td>
</tr>
<tr>
<td>Ashley Woodson</td>
<td><a href="mailto:lhill@mdanderson.org">lhill@mdanderson.org</a></td>
<td>United States</td>
</tr>
<tr>
<td>Haifeng Yang</td>
<td><a href="mailto:haifeng.yang@jefferson.edu">haifeng.yang@jefferson.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Qing Zhang</td>
<td><a href="mailto:qing_zhang@med.unc.edu">qing_zhang@med.unc.edu</a></td>
<td>United States</td>
</tr>
<tr>
<td>Jingcheng Zhou</td>
<td><a href="mailto:zhjc1021@126.com">zhjc1021@126.com</a></td>
<td>China</td>
</tr>
<tr>
<td>Naseem Zojwalla</td>
<td><a href="mailto:naseem.zojwalla@pelotontx.com">naseem.zojwalla@pelotontx.com</a></td>
<td>United States</td>
</tr>
</tbody>
</table>
Join Us for the 14th International VHL Medical/Research Symposium

The Netherlands

2020

More details to follow
vhl.org/symposium