VHL Family Alliance

Annual Meeting

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Prepared by

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VHL FAMILY ALLIANCE
ANNUAL MEETING HIGHLIGHTS

I recently attended the Annual Conference of the von Hippel-Lindau Family Alliance which was held at the prestigious Dana Farber Cancer Institute in Boston on November 17th, 2012. Below are some selected highlights and my own personal comments on certain talks at this meeting.

In addition to the daylong meeting, the VHLFA also held a Fundraising and Awards dinner where awards were presented to Joyce Graff, Founder and Former Executive Director of the VHLFA, for her tireless service since 1993, and to Dr. William G. Kaelin Jr., a prominent cancer researcher whose important work in this field has tipped him as a future Nobel Prize winner.

Note: *Hyperlinks to definitions of certain terms are included in this document.*

SUMMARY

In all, 10 talks were given, with the subjects ranging from new research, potential new treatments, insights from related research, current clinical trials and palliative/complementary therapies. Highlights of some of these talks are summarized below and copies of many of the slide presentations from the meeting are now available on the VHLFA website (click here).

Suzanne Hanser EdD, MT-BC

Chair of Music Therapy Department, Berklee College of Music—Music Therapist

"Setting the Tone: Music Therapy: Harmonius Stress Management"

Suzanne gave an introductory talk to outline the use of music therapy in pain management. Her work has previously focused on pain management in several different settings. Her previous work used evidence-based strategies employing music therapy for stress management. In her introduction, she linked the impact of music on the stress response—more commonly known as the '*fight-or-flight*' response of the Autonomic Sympathetic Nervous System (ASNS). The stress response is an evolutionary adaptation to acute threat. It is initiated by a rush of the stress hormone adrenalin (epinephrine) resulting in increased heart and respiration rate, increased blood flow to muscles and a simultaneously decreased blood flow to the digestive tract.

In contrast, the '*relaxation response*' employs the Parasympathetic Nervous System (PSNS) which promotes rest and recuperation through signals that decrease heart rate and respiration, decrease blood flow to muscles and increase blood flow to the digestive tract. The relaxation response is associated with signaling through the neurotransmitter acetylcholine and has also been linked with another neurotransmitter—nitric oxide (NO) which is released by the brain on listening to music. These neurotransmitters counteract stress hormones—in particular cortisol, which is associated with chronic stress—ultimately resulting in a delicate balance between the '*fight-or-flight*' and '*relaxation response*'.

With this in mind, music therapy can be beneficial in managing stress responses and can be helpful in calming individuals who are susceptible to chronic stress—such as people experiencing post-traumatic stress disorder (PTSD), chronic depression and stress experienced by women in labor.

Previous studies suggested that music is positively associated with imagery, memories, associations, positive mood changes, and breathing rhythm. Suzanne's own published work has shown that music therapy can assist women in labor by inducing a relaxed state of mind and to subsequently decrease pain (Hanser et al, 1983 Birth Psych Bulletin). In this study, music therapy helped by providing an auditory 'focal point' to focus attention pace breathing and to induce visualization (imagery) when the participants were in a closed eye state.

In her work on depressed individuals she reported "significant differences" between individuals treated with intensive music therapy versus a control group. Using a questionnaire based study, those treated individuals reported significant improvements in depression, distress, self-esteem and mood—'gains' which were maintained up to 9 months later. (Hanser & Thompson: Effects of music Tx on depressed older adults—*J. Geront.* 1994).

More recently, Suzanne has begun to use music therapy as an antidote to pain in cancer patients. She reported a case study of an individual with cancer who was successfully treated with music therapy to reduce anxiety of acute pain during a blood draw.

In summary, music therapy is an underused complementary treatment which has many unexplored benefits, especially in the treatment of acute pain experienced by cancer patients. Suzanne's ongoing work in this area is being carried out at the Dana Farber Cancer Institute in Boston.

Nikolaos Stathatos MD

Instructor in Medicine (Endocrinology), Massachusetts General Hospital

A Balancing Act*: VHL in the Adrenal Glands*

Dr. Stathatos has a special interest in the treatment of patients with genetic disorders of the endocrine glands and treats patients with von Hippel-Lindau syndrome who are susceptible to tumors within the adrenal glands. His work with VHL patients has focused on clinical care and management of endocrine dysfunction (hormonal changes) caused by tumors called pheochromocytomas.

The adrenal glands are comprised of two main tissues—the (outer) adrenal cortex produces hormones called glucocorticoids (such as the hormone cortisol, involved in chronic stress response), mineralocorticoids (e.g., aldosterone—hormones involved in salt and water balance) and sex steroids.

The inner adrenal medulla produces signaling molecules called catecholamines (kat-eh-kol-ay-meenz). These include are epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine; all of which are produced from precursor amino acids. Release of epinephrine and norepinephrine from the adrenal medulla is part of the '*fight-or-flight*' response.

Pheochromocytomas are seen in 10-20% of patients with VHL, resulting in disturbances in stress hormone levels. This produces a number of characteristic symptoms which include; palpitations headaches, increased sweating, pallor and feelings of nausea. Dr. Stathos stated that following these clinical presentations, the diagnosis of pheochromocytoma in VHL patients can be confirmed by biochemical tests which detect increased levels of metabolites of adrenaline in the blood stream. Treatment of these tumors involves blocking excess catecholamines with drugs (such as phenoxybenzamine) before surgery but surgical treatment (adrenalectomy) is recommended. Following surgery, a further problem is 'Adrenal Insufficiency'—the lack of production of these necessary hormones. The acute symptoms of which can include cardiovascular system failure, while chronic symptoms include weight loss, darkening of the skin and salt craving. These symptoms can be treated by hormone replacement therapy using synthetic glucocorticoids (e.g. hydrocortisone dexamethasone or prednisone). Overtreatment can produce symptoms resembling those of Cushing's syndrome—including rapid weight gain, 'moon face' and high blood sugar levels (hyperglycemia). Because of this, the dosage of these drugs should be carefully adjusted in close consultation with patients, especially since cortisol is needed in increased levels during periods of stress.

COMMENT: Although HLRCC patients are not susceptible to pheochromocytomas, the adrenal glands are susceptible to 'macronodular lesions' (Matyakhina et al., *J. Clin. Endocrinol. & Metab.* 90 (6): 3773 2005—Hereditary Leiomyomatosis Associated with Bilateral, Massive, Macronodular Adrenocortical Disease and Atypical Cushing Syndrome: A Clinical and Molecular Genetic Investigation). A separate, more recent study conducted by Marston W. Linehan's group has concluded "To date, no patient has been found to have adrenal malignancy"—Shuch et al., *J. Urol.* 2012

John Libertino MD

Chairman, Institute of Urology, Lahey Clinical Medical Center; Professor of Urology, Tufts University School of Medicine

Treating the VHL Kidney Tumor*:*
Novel Techniques in Partial Nephrectomy

In his talk, Dr. Libertino suggested that it is now clear that in patients with RCC, removal of the diseased kidney (full nephrectomy) should be avoided if it all possible. Techniques for removal of only part of the kidney (partial nephrectomy) has until recently been carried out by clamping the renal artery during surgery. However, this is a somewhat risky procedure due to 'ischemic reperfusion injury'—a problem caused by lack of blood to the cells of the kidney which can cause unwanted damage to healthy tissue. Following ischemic reperfusion injury the kidney may be susceptible to 'renal insufficiency' (lack of ability to carry out normal function) and this is a significant problem in cases where there are bilateral tumors (i.e. in both kidneys either simultaneously or concurrently).

Dr. Libertino's team pioneered new techniques to avoid clamping of major blood vessels referred to as 'nephron sparing surgery'. This technique employs 3D imaging (CT)—a technique that came into use around 2000. The technique helps a surgeon to avoid damaging healthy tissue during certain procedures but its use was initially slow to be adopted by the Urological community. Dr. Libertino's team were undaunted by this and he has developed several pioneering methods for nephron sparing surgery combining delicate surgical procedures with non-clamping techniques while using intra-operative ultrasound to help guide the surgeon to the correct spot. His work has been published in a series of high-profile journal articles and is now accepted as something of a 'Gold-Standard' and is now recognized by other leaders in the field.

Where next? Dr. Libertino described the use of minimally invasive robotic surgery augmented by the use of novel polymers which can be used to block vessels during surgery. Lumagel, is a polymer that is heat reactive and forms a gel (i.e., solidifies) in the artery. This polymer solidifies when warmed, but when cooled (with an ice solution), the gel depolymerizes and kidney reperfusion is normal. Ultimately, this reduces the time that blood vessels are sealed thereby decreasing the time that blood flow is prevented from reaching the healthy tissue.

William G. Kaelin, Jr.

Professor of Medicine, Harvard Medical School, Dana-Farber Cancer Institute, and Brigham & Women's Hospital

VHLFA Research Grants: *Steps Towards a Cure*

History of VHL Research

The first scientific paper on von Hippel-Lindau was actually published in English in 1894 by E. Treacher Collins in "Transactions of the Opthalmological Society of the United Kingdom". The paper detailed two cases of a brother and sister with "*Intra-Ocular Growths*" with "*peculiar vascular new growth, probably primarily retinal, affecting both eyes*". At that time, German was the predominant scientific language and this paper went largely ignored by the scientific and medical community.

Ten years later in 1904, a paper by Eugen von Hippel was published in German which described "*a rare disorder of the retina*", and in 1911, he discovered the anatomical basis of this disease, which he named "*angiomatosis retinae*".

However, it wasn't until 1926 that Swedish pathologist Arvid Lindau recognized "*…an association between angiomatosis of the retina with hemangioblastomas of the cerebellum and other parts of the central nervous system*". This condition is known today as the von Hippel-Lindau Disease (VHL).

The gene for VHL was first identified in 1993, in a landmark paper by a large international research team. At the time, molecular biological techniques were laborious, time and resource consuming. Through collaboration, the team were able to amplify their ability to discover the gene responsible, using samples gathered from multiple families affected by VHL disease.

In 2001 the Human Genome Project was 'completed'. This has provided a reference sequence for the entire complement of genes that make up a human being. This information has since revolutionized the discovery of new genes associated with disease, reducing the time to identify disease causing mutations from years to a matter of months or weeks.

The VHL gene provides instructions to a cell to produce VHL protein (pVHL) which is important in the process called "transcription", where an initial DNA message is first turned into multiple copies of a simpler, RNA message, which then provides the cell with a set full blueprint for the construction of a specific protein (see below).

DNA	**GENE = MASTER BLUEPRINT**
RNA	**TRANSCRIPTION = XEROX COPIES**
PROTEIN	**TRANSLATION = CONSTRUCTION**

A number of different genetic mutations in the same gene are now known to cause VHL. Although the mutations are all in the VHL gene, they are located at different positions within the DNA code. These various different mutations found in the VHL gene provide faulty instructions to cells which then make faulty versions of the VHL protein that do not function properly.

The von Hippel–Lindau protein is a tumor suppressor also known as pVHL that is involved in the degradation of a hypoxia-inducible factor (HIF), which is a transcription factor that plays a central role in the regulation of gene expression by oxygen.

The VHL protein acts as a brake on HIF—when it does not work, this leads to the formation of new blood vessels via increased expression of different signaling molecules. The VHL protein is a guardian which destroys HIF through a process called ubiquitination. This process has been called the "kiss of death" for a protein. In ubiquitination, a protein is inactivated by attaching a small molecule called ubiquitin to it. Ubiquitin acts as a tag that signals the cellular protein-transport machinery to ferry the protein to another site in the cell (the proteasome) for degradation.

When HIF is not degraded it builds up which acts as a cellular trigger to respond to low oxygen. The cell responds by increasing production of growth factors that boost blood vessel growth, blood cell production and other 'wound healing' responses (i.e. in response to hypoxic tissue damage).

Loss of one copy of the VHL gene is not sufficient in itself to cause renal cell cancer. Loss of both copies of the VHL gene causes RCC. Loss of one copy occurs via inheritance from a parent, the second 'good' copy of the gene remains active, but in certain cells, this 'good' copy of the gene is inactivated or lost, which leads to HIF accumulation and other downstream responses related to blood vessel growth and renal cell cancer.

Currently, there are four approved drugs to treat renal cell cancer, Bevacizumab, Sunitinib, Sorafinib, Pazopanib with a further compound Axitinib currently in trials and 30 more are in the pipeline.

James Gnarra PhD

Chair, Research Council and Board Member, VHLFA, Associate Professor of Urology and Pathology, University of Pittsburgh Cancer Institute.

VHL Research: *Where is it going?*

Dr. Gnarra provided a perspective on previous work, current trends and future directions in VHL research. He started with a concise history of scientific publications on VHL, showing that the publication rate from 1970–1993 produced an average of 30–50 publications per year. During this time, the field remained somewhat obscure. However, following a ground-breaking study which identified the VHL gene in 1993, there has been a rapid expansion in the number of published articles on VHL. At present, the field averages 250–300 scientific research papers per year, many published in leading scientific journals, bringing some of the best scientific talent in the world to work on this rare disease.

Current Research on VHL

The VHL gene was first identified in 1993 as a tumor suppressor gene that is responsible for limiting cell growth and acting as a 'brake' to stop the formation of tumors. In patients with VHL, tumors develop when the VHL gene is inactivated.

VHL disease has an incidence of one in 36,000 births. There is over 90% penetrance (proportion of individuals with the mutation who exhibit clinical symptoms) by the age of 65.

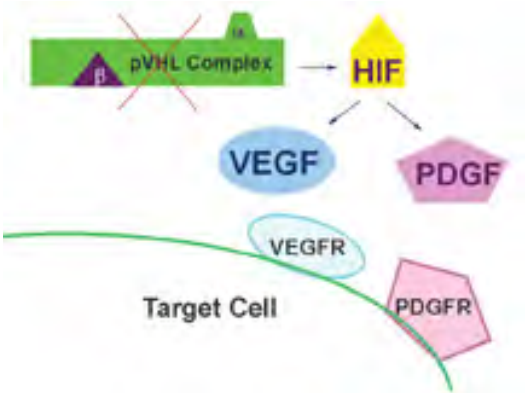
pVHL Controls Levels of Hypoxia Inducible Factor-1 Protein

The VHL protein (pVHL) is involved in the regulation of another protein known as hypoxia inducible factor (also referred to as HIF, HIF1 or HIF1α). HIF plays an essential role in cellular responses to changes in available oxygen in the cellular environment, specifically, to decreases in oxygen or hypoxia. When oxygen levels are normal, HIF is highly regulated by pVHL which binds to HIF and controls its turnover via degradation. In low oxygen conditions—or in cases of VHL disease where the VHL gene is mutated—pVHL does not bind to HIF. This causes the HIF protein to become activated thus leading to the transcription of a number of genes, including genes involved in vascularization, cell growth proliferation and other genes involved in glucose uptake and metabolism.

HIF Induces Growth Factors Involved in Response to Low Oxygen

When the levels of active HIF rise, the cell responds as if to a low oxygen situation, ramping up the production of proteins critical for a response to this 'emergency'—these include two important growth proteins called Vascular Endothelial Growth Factor (VEGF), and Platelet-derived Growth Factor (PDGF) plus others involved in ramping up blood cell production. These growth factors act as chemical messages to the surrounding tissues instructing them to help with regeneration and repair. Together with other growth factors, they promote the formation of new blood vessels and direct the surrounding tissue to replace dead or injured cells and repair structures within.

Growth Factor Receptors—VEGF and PDGF growth factors work via their corresponding receptors (called VEGF-R and PDGF-R) which are located on the surface of a target cell population (see diagram below).



The VHL-HIF1 Signaling mechanism (W. G. Kaelin, Clin Cancer Res. 2004 Sep 15;10 (18 Pt 2):6290S-5S).

When these receptors bind their respective growth factors, a signal is sent to the target cell that instructs the cell to perform a certain function (e.g. ,'grow new blood vessels'). In renal cell cancer, this signaling process is co-opted thus allowing the cancer cells to grow and reproduce unchecked. Cancer cells also respond to other co-opted signals which promote their movement out of a growing tumor and into other tissues.

Therapeutic Targets for VHL: In order to stop these critical cancer-enabling signals from getting through, scientists are continuing to develop newly emerging classes of drugs which aim to halt the signals, trap the signals in transit, or alternatively block the receptors from binding their growth factors. The central role of HIF in VHL-related clear-cell RCC has spurred interest in the development of drugs targeting HIF for treating this disease. Unfortunately, due to its localization in the nucleus few drug-like small molecules exist that are capable of inhibiting transcription factors like HIF.

Recent Research Findings:

Much of the recent work done in the field has focus on the precise role of the VHL protein in kidney cells and towards understanding the mechanisms that lead cells which lack VHL protein to develop into kidney cancer. VHL research has recently begun to expand into new areas that could not be predicted previously, providing greater understanding and novel targets for therapeutic intervention.

Gene Regulation Proteins Associated with VHL: The regulation of gene expression involves a number of different mechanisms that are used by cells to increase or decrease the production of specific "gene products" (proteins). A variety of enzymes are important for the gene regulation process—their function is to help the cell "unravel" its tightly coiled DNA, thereby exposing the genes within to the cells transcription processes. Recent work has shown that certain enzymes involved in the gene regulation process were mutated in clear cell RCC tumors and these proteins are thought to play a role in the development of cancer.

VHL is linked to Epidermal Growth Factor: Epidermal Growth Factor (EGF) plays an important physiological role in the maintenance of tissue integrity. When EGF binds to its receptor EGFR, the receptor is "activated" and the activated receptor sends signals to promote cellular proliferation and resistance to cell-death. Failure to turn off the activated EGFR can drive tumor generation and this mechanism is co-opted in many common cancers. It is known that HIF can enhance EGFR activity to promote tumor growth. A recent study led by Dr. Haifeng Yang's team at the Thomas Jefferson University (Philadelphia, PA), has shown that the VHL protein acts in several different ways to keep in check the level EGFR. Several classes of drugs have been approved which successfully target the EGFR in certain other cancers. However von Hippel-Lindau is somehow resistant to EGFR treatment. Dr. Yang's laboratory is currently focusing on understanding the underlying mechanisms of this drug resistance with a view to developing better targeted treatment strategies.

Future Directions:

VHL Patient Registry

The VHL Family Alliance is currently in the process of assembling a patient registry. This will be a global database containing information about VHL (and HLRCC) patients through patient-entered information. Each record will have anonymous information on a patient's medical history—such as the number of lesions, sizes, types of scans, demographics, follow-up imaging, treatment and detailed information on each patient's molecular mutation.

The registry will provide a number of benefits to the research community, allowing investigators to address questions of genetic-environmental interactions, improve understanding on the development of lesions, and enable clinicians to better predict responses to treatment

The VHLFA Patient Registry Task Force, was recently created with representatives from the VHLFA Research Council and Clinical Care Centers plus members of the HLRCC FA Medical Advisory Board. The patient registry will include patient submitted data and will follow patients over the course of their disease. The registry will address many important clinical research questions and issues relevant to the development of both diseases. The patient registry will also provide important background information in preparation for clinical trials.

Othon Iliopoulos MD

Associate Professor of Medicine, Harvard Medical School

What is happening in the world of clinical trials?

The clinical spectrum of VHL patients shows that they are at high risk for the development of several different types of tumor. These tumors include; clear cell renal cell cancer (RCC), hemangioblastomas, pheochromocytomas and pancreatic neuroendocrine tumors. In absence of a functioning pVHL protein, activated HIF turns on hypoxia-responsive genes, in these tumors. The expression of these genes leads to the production of several growth factor proteins, which in turn result in the growth of highly vascularized and malignant tumors.

Research has identified several growth factors linked with von Hippel-Lindau associated tumors, expressed at very high levels. These include:

- Vascular Endothelial Growth Factor (VEGF)
- Platelet Derived Growth Factor(PDGF)
- Fibroblast Growth Factor
- Possibly other Growth Factors

Currently there are 4 approved drugs to treat RCC, with another compound (Axitinib) in trials 30 In addition, 30 more compounds are in the drug pipeline.

Current treatments employ two approaches to targeting growth factors and their receptors:

- small molecule drugs that block growth factor receptor signaling
  - these drugs are commonly referred to as "receptor tyrosine kinase inhibitors" (RTKi's)
- engineered antibodies that block the receptor or scavenge the signaling molecule (ligand)
  - these drugs are commonly referred to as "monoclonal antibodies" (MAb's)

Targeting tyrosine kinases and their receptors has been shown to reduce tumor growth, metastasis, and angiogenesis. The drugs which target tyrosine kinases and their receptors often have more than one target—the rationale is that the more targets they block, the better chance they have of successfully treating different mechanisms behind tumor growth. The table below shows a side-by-side comparison of several different drugs and the degree to which they are effective on different targets.

Targets of Current Tyrosine Kinase Inhibitor Drugs

	Sunitinib	Axitinib	Pazopanib	Cabozantinib
VEGFR1	++	++	++	+
VEGFR2	+	++	+	++
PDGF	++	++	+	++
c-Met				++
FGF			++	
RET	++			

RCC Treatment—Pilot Clinical Trial with Sunitinib: Recently a pilot Phase I tolerance and toxicity study was carried out in 15 VHL patients treated with the Pfizer drug sunitinib, (Sutent®). The study primarily looked at how well the drug was tolerated and whether patients showed any adverse (toxic) effects from the drug. As a secondary goal, two clinical endpoints were examined—the effect of treatment on clear cell RCC and hemangioblastomas.

Adverse effects of the treatment included fatigue in five patients, and dose reductions were needed in 10 patients. When measuring clear cell RCC and hemangioblastomas, six of the patients with RCC responded partially, however none of those treated showed any effect on hemangioblastomas. (Jonasch et al., *Annals Oncol.* 2011)

Based on this study, a new trial has opened using a similar compound, the GSK drug pazopanib (Votrient®). This study is openly recruiting and currently is being carried out at the MD Anderson Cancer Center (Houston, TX), and recently began recruiting patients from a satellite site at the University of North Carolina (Chapel Hill, NC).

Hemangioblastoma treatment

Hemangioblastomas are tumors of the central nervous system that originate from the vascular system. These tumors can also occur in other sites such as the spinal cord and the retina.

Retinal hemangioblastomas are the most common manifestation of von Hippel-Lindau disease. These tumors are believed to result from the significantly increased expression of VEGF which stimulates new blood vessel growth within the retina of the eye.

Although retinal hemangioblastomas can be treated using thermal lasers, any lesions occurring close to the optic nerve or macula (where central vision occurs) are much more difficult to treat and commonly result in blindness. Previously a novel anti-VEGF therapy has proven successful in treating two unrelated conditions, where unchecked growth of new blood vessels in the eye can cause blindness: Age-related, and Diabetic Macular Degeneration (AMD, DMD). In both conditions, clinical researchers have successfully employed a novel antibody-based therapy called Bevacizumab (Avastin®), which is a potent VEGF-receptor inhibitor. Treatment is administered by injecting the drug into the vitreous part of the eye. Consequently, similar intra-ocular injections of the drug were explored as a treatment for retinal hemangioblastomas in VHL.

Several clinical case studies have now been conducted using this approach but with slightly differing experimental design:

- Michels et al., examined one patient over a period of 4 months
- Wong et al., examined 5 patients over a period up to 61 weeks
- Hrisomalos et al., examined one patient (a monocular subject with progressive visual loss due to VHL) over 60 months

Unfortunately, despite its initial promise, this treatment strategy has produced mixed results—with variable changes in both lesion size and preventing vessel leakage. (Michels S et al., *Klin Monbl Augenheilkd*., 225(4):292-4 2008; Wong WT et al., *Ophthalmology* 2008; Hrisomalos et al., *Open Ophthalmol J*. 4: 66–69 2010).

Since the intra-ocular injection may not be ideal, clinicians are considering treatment using an alternative delivery method. This follows a previous case study with a single patient who showed rapid and durable recovery of visual function after systemic therapy with an experimental VEGF inhibitor (SU5416). (Aiello LP et al., *Opthalmol*. 2002 Sep;109).

Next Steps

The next challenges facing the clinical researchers and medical oncologists are how to determine which is the best therapy for each VHL lesion (HB, RCC, Retina, Phaeo), when best to start and stop treatments, how to best combine different approaches to treatment (i.e., combining medical oncology with surgery and/or radiation therapy). To this end, the VHLFA has set up a 'Clinical Adviory Council' which is currently being chaired by Dr. Eric Jonasch at the MD Anderson Cancer Center (Houston, TX) and staffed by Dr. Ilene Sussman, Executive Director of the VHLFA. The task for will enable clinicians to discover and test new preventative treatments for those persons who are known to be at risk.

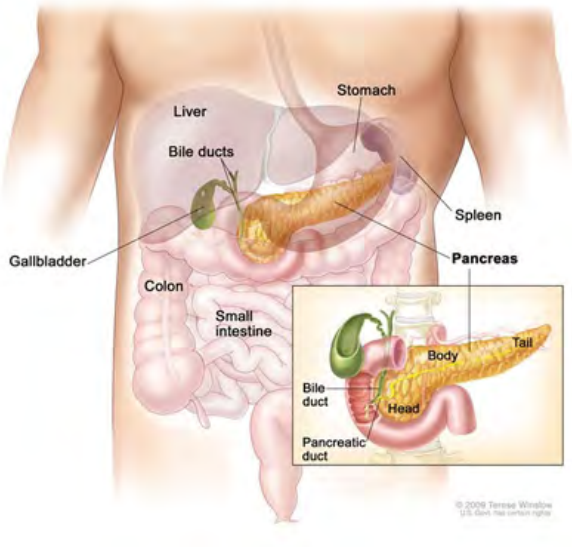
Steven K. Libutti MD FACS

Director and Vice Chair, Deptartment of Surgery; Professor, Department of Surgery & Genetics

Update on Diagnosing and Treating Pancreatic Neuroendocrine Tumors (pNETS)

VHL patients are at risk of developing highly vascularized tumors within multiple organs, including hemangioblastomas of the retina and central nervous system (CNS), clear cell renal carcinomas and pheochromocytomas associated with the adrenal glands. Pancreatic lesions also feature prominently in the disease and these include cysts and neuroendocrine tumors of the pancreas.

The pancreas is an elongated gland which lies behind the stomach and in front of the spine and adjacent to the small intestine (see diagram below) where it releases enzymes for digestion .



The pancreas contains two cell types:

- Exocrine cells which make enzymes that are released into the small intestine to help the body digest food.
- Endocrine cells which make several kinds of hormones including insulin to control blood sugar.

The endocrine cells are organized together in small clusters called 'islets' (or 'islets of Langerhans') situated throughout the pancreas.

Clinical features of pNETs Tumors

Most pNETs tumors are well characterized and under close examination resemble normal cell-types (specifically α, β, and δ cells) found within the islets of Langerhans in the pancreas. These tumors typically have a slow clinical progression and the median survival for patients with metastatic disease may exceed 5 years. The cells ability to secrete neuropeptides can result in characteristic clinical syndromes.

Pancreatic Lesions & VHL

Pancreatic cysts are typically observed in 70% of VHL patients, these can be individual, or multiple. Generally these cysts have no malignant potential; however, in some patients pancreatic cysts can lead to pancreatic insufficiency—the progressive loss of exocrine cells that make digestive enzymes

Pancreatic neuroendocrine tumors (pNETs) arise in the hormone-producing endocrine cells of the pancreatic islets. Most pNETs are *functional* benign tumors; however, they can secrete excess hormones which may cause symptoms. Some pNETs are non-functional, malignant tumors which also produce pancreatic secretions (these do not cause symptoms). In these non-functional tumors, any observable symptoms are observed as a result of tumor growth and metastasis.

Non-functional, malignant pNETs are detected in 12–17% of vHL patients, and of those, metastasis is seen in 8% of patients. The initial size of the primary tumors at diagnosis is thought to be predictive of their metastatic potential. In a 1998 study, large primary tumors were associated with increased risk of spread to the liver (Libutti et al., *Surgery*, 1998).

Diagnosis, Detection and Prognostic Indicators

Pancreatic imaging to identify neuroendocrine tumors early can therefore prevent the development of liver metastases *if surgery is performed before these tumors reach 2 to 3 cm in size.*

The incidence and prevalence of pNETs is increasing; early estimates put incidence at 1–2 per 100,000 population and prevalence at >100,000 cases in the United States. This increase is partly due to the improved awareness, classification, and diagnosis of pNETs tumors primarily as a result of improved scanning techniques and their widespread adoption.

The techniques used to image pNETs include Positron Emission Tomography (PET) and Computed Tomography (CT) with contrast (particularly effective given the highly vascular presentation of these tumors).

A general staging system used for neuroendocrine tumors is applicable to VHL neuroendocrine tumors. It has now been established that the size of these tumors is particularly important with respect to clinical course and disease management.

- Tumors <2cm—patients do well when no metastasis is present—median survival = 5+ years
- Tumors >3–4cm—patients do not fare as well—when metastatic disease is present, patients median survival is <2 years

Predicting Risk of pNETs

No strong features predictive of malignancy or metastatic potential can be observed on close examination of pNETs tumors using various tissue staining techniques (histopathology). In addition, circulating hormone levels (e.g., insulin) also are not prognostic indicators of whether these tumors are malignant and metastatic. However, researchers have begun to develop better ways to predict the course and outcome of disease using several different criteria. Using these criteria, the outlook for pNETs patients is improving incrementally.

Predicting Risk of pNETs: Tumor Size & VHL Mutation Type

A study which evaluated tumor size before surgery on pancreatic lesions, also sought to determine if different types of VHL gene mutations predict disease severity. This type of analysis is extremely helpful in predicting the clinical course and outcomes of a disease.

Overall, 389 patients with VHL gene mutations and a clinical diagnosis of pNETs were screened. In the study, 44 patients with pNETs were identified; of these, 25 underwent surgery, 5 had metastatic disease, and 14 monitored further.

- No patient who underwent surgery based on tumor size developed metastases.
- Of the patients screened those with pNETs, were more likely to have a specific type of gene mutation called a missense mutation (58% in total).
- In patients whose tumors had spread, 4 out of 5 (80%) had mutations in a specific region of the VHL gene (Exon-3), compared with 18 of 39 (46%) patients who did not have metastases.

Analysis of germline mutations may therefore help identify patients at risk for pNET and which patients may benefit from early surgical intervention (Libutti et al., *Surgery*, 2000).

Comparisons of tumor diameter in patients with versus those without metastasis have shown that patients without metastatic disease generally have smaller tumors than patients with metastases. In addition the rate of growth of primary pNETs also tracks with metastases—i.e., the faster the growth rate, the more likely it is that the tumor is metastatic (*Surgery* 142 (6): 814-8 2007).

In this way, measuring levels of circulating proteins can be used as biomarkers of disease progression. Biomarkers—such as the Ki-67 protein—are an excellent means to determine the growth fraction of a given cell population. The fraction of Ki-67-positive cells is correlated with the clinical course and progression of cancers; therefore increased levels of Ki67 are indicative of rapid proliferation and overall reduced survival.

In summary:

Three significant criteria have been linked with pNETs prognosis and outcome—the more of these factors present, the higher the probability of metastasis and the worse the overall prognosis and survival.

1) Mutations in the Exon-3 region of pVHL
2) Diameter of the lesion ≥ 3cm
3) Rapid growth rate (doubling time <500 days)

While these criteria are still being refined, these indicators help oncologists to select patients as candidates for surgery—patients with 2 or more of these criteria present have the poorest prognosis and therefore the most likely to be selected for surgery.

Recent Research on pNETs

Signaling pathways are good and are very helpful in allowing researchers find underlying patterns that bring us closer to finding ways to intervene and prevent disease progression. Developing mouse models of disease can help researchers better understand the pathways which lead to tumor growth and progression.

First-generation mouse models used to understand signaling in VHL associated tumors used genetically modified mice which either over-expressed or knocked-out specific genes implicated in the growth and progression of tumors. Unfortunately, certain signaling pathways perturbed by these techniques are critical to embryonic and postnatal development. For this reason, knocking out the genes involved in these pathways can prove to be embryonically lethal.

These early techniques are now being superseded by mouse models that are based on new genetic techniques that allow researchers to finely regulate specific genes in certain tissues. In these mice, mutations can be induced in a tissue-specific and time-controlled fashion, which more faithfully mimics sporadic tumor formation. These second-generation models have provided new opportunities to gain insight into the contribution of known and unknown genes in the initiation, progression and treatment of cancer, and better mimic human cancer than ever before.

Inactivation of tumor suppressor genes in the pancreas

Tissue specific mouse models have been developed recently which "knockout" genes in specific pancreatic cell types. Specifically, the VHL gene was inactivated in different pancreatic cell populations distinguished by their roles during embryonic development and their endocrine type. These gene knockout technologies have been used to target the VHL gene in only α-cells or β-cells in the pancreatic islets, showing that deletion of VHL is dispensable for normal functions and is insufficient to induce tumor development.

However, when VHL was inactivated in pancreatic *progenitor* cells, highly vascularized microcystic adenomas and hyperplastic islets developed that exhibited clinical features very similar to VHL patients. This work showed that common precursor cells may be involved in the development and progression of pancreatic tumors.

Although VHL predisposes to several different forms of tumors, it has been observed that there is no overall increase (above the general population) in other more common types of cancer. In other words, although the same risk factors are present in people with the VHL gene mutation (i.e., smoking, diet, environmental toxins, etc.) it is very intriguing that these individuals are not at any higher risk of developing cancers of the lung, colon or other type of cancer associated with life-style factors. This suggests that a certain degree of tissue specificity exists where some tissues are more vulnerable due to the genetic mutation and others are relatively spared.

"The Highest Attainable Standard of Health is one of The Fundamental Rights of Every Human Being"

Inscription: Harvard School of Public Health.