RESEARCH

Preventive medicine of von Hippel–Lindau disease-associated pancreatic neuroendocrine tumors

Tobias Krauss[1,*], Alfonso Massimiliano Ferrara[2,*], Thera P Links[3,*], Ulrich Wellner[4], Irina Bancos[5], Andrey Kvachenyuk[6], Karina Villar Gómez de las Heras[7], Marina Y Yukina[8], Roman Petrov[9], Garrett Bullivant[10], Laura von Duecker[11], Swati Jadhav[12], Ursula Ploeckinger[13], Staffan Welin[14], Camilla Schalin-Jäntti[15], Oliver Gimm[16], Marija Pfeifer[17], Joanne Ngeow[18], Kornelia Hasse-Lazar[19], Gabriela Sansó[20], Xiaoping Qi[21], M Umit Ugurlu[22], Rene E Diaz[23], Nelson Wohllk[24], Mariola Peczkowska[25], Jens Aberle[26], Delmar M Lourenço Jr[27], Maria A A Pereira[28], Maria C B V Fragoso[27], Ana O Hoff[27], Madson Q Almeida[27], Alice H D Violante[29], Ana R P Quidute[30], Zhewei Zhang[31], Mònica Recasens[32], Luis Robles Díaz[33], Tada Kunavisarut[34], Taweesak Wannachalee[34], Sirinart Sirinvaravong[34], Eric Jonasch[35], Simona Grozinsky-Glasberg[36], Merav Fraenkel[36], Dmitry Beltsevich[8], Viacheslav I Egorov[9], Dirk Bausch[4], Matthias Schott[37], Nikolaus Tiling[13], Gianmaria Pennelli[38], Stefan Zschiedrich[11], Roland Därr[11,39], Juri Ruf[40], Timm Denecke[41], Karl-Heinrich Link[42], Stefania Zovato[2], Ernst von Dobschuetz[43], Svetlana Yaremchuk[6], Holger Amthauer[44], Özer Makay[45], Attila Patocs[46], Martin K Walz[47], Tobias B Huber[26], Jochen Seufert[48], Per Hellman[49], Raymond H Kim[50], Ekaterina Kuchinskaya[51], Francesca Schiavi[2], Angelica Malinoc[11], Nicole Reisch[52], Barbara Jarzab[19], Marta Barontini[20], Andrzej Januszewicz[25], Nalini Shah[12], William F Young Jr[5], Giuseppe Opocher[53], Charis Eng[54], Hartmut P H Neumann[55] and Birke Bausch[48]

[1]Department of Radiology, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[2]Familial Cancer Clinic and Oncoendocrinology, Veneto Institute of Oncology IOV- IRCCS, Padua, Italy
[3]Department of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[4]Department of Surgery, University of Luebeck, Luebeck, Germany
[5]Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Mayo Clinic, Rochester, USA
[6]Institute of Endocrinology and Metabolism, NAMS of Ukraine, Kiev, Ukraine
[7]Central Services, Servicio de Salud de Castilla-La Mancha (SESCAM), Toledo, Spain
[8]Department of Surgery, Endocrinology Research Center, Moscow, Russia
[9]Department of Surgery, Bakhrushin Brothers Moscow City Hospital, Moscow, Russia
[10]Princess Margaret Cancer Center, University Health Network, Toronto, Ontario, Canada
[11]Department of Medicine IV, Faculty of Medicine, Albert-Ludwigs-University, Freiburg, Germany
[12]Department of Endocrinology, KEM Hospital, Mumbai, India
[13]Interdisciplinary Center of Metabolism: Endocrinology, Diabetes and Metabolism, Charité-University Medicine Berlin, Campus Virchow-Klinikum, Berlin, Germany
[14]Department of Endocrine Oncology, Uppsala University Hospital, Uppsala, Sweden
[15]Endocrinology, Abdominal Center, University of Helsinki and Helsinki University Hospital, Helsinki, Finland
[16]Department of Clinical and Experimental Medicine, Department of Surgery, University of Linköping, Linköping, Sweden
[17]Department of Endocrinology, University Medical Center, Ljubljana, Slovenia
[18]Cancer Genetics Service, Division of Medical Oncology, National Cancer Center Singapore and Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore
[19]Department of Endocrine Oncology and Nuclear Medicine, Center of Oncology, MSC Memorial Institute, Gliwice, Poland
[20]Centro de Investigaciones Endocrinológicas "Dr Cesar Bergada" (CEDIE), Hospital de Niños Ricardo Gutiérrez, CABA, Buenos Aires, Argentina
[21]Department of Oncologic and Urologic Surgery, the 117th PLA Hospital, Wenzhou Medical University, Hangzhou, Peoples Republic of China
[22]Department of General Surgery, Breast and Endocrine Surgery Unit, Marmara University School of Medicine, Istanbul, Turkey
[23]Endocrine Section, Hospital del Salvador, Santiago de Chile, Chile
[24]Department of Medicine, Endocrine Section, Hospital del Salvador, University of Chile, Santiago de Chile, Chile
[25]Department of Hypertension, Institute of Cardiology, Warsaw, Poland
[26]3rd Department of Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[27]Serviço de Endocrinologia, Hospital das Clínicas (HCFMUSP) and Instituto do Cancer do Estado de São Paulo (ICESP), Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
[28]Serviço de Endocrinologia, Hospital das Clinicas (HCFMUSP), Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil
[29]Department of Internal Medicine-Endocrinology, Faculty of medicine-Hospital Universitario Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

[30]Department of Physiology and Pharmacology, Drug Research and Development Center (NPDM), Faculty of Medicine, Federal University of Ceará (UFC), Fortaleza, Brazil
[31]Department of Urology, 2nd Hospital of Zhejiang University, School of Medicine, Hangzhou, China
[32]Hospital Universitari de Girona, Gerencia Territorial Girona, Institut Català de la Salut, Girona, Spain
[33]Unidad de Tumores Digestivos, Servicio de Oncología Médica, Hospital Universitario 12 de Octubre, Madrid, Spain
[34]Division of Endocrinology and metabolism, Siriraj Hospital, Mahidol University, Bangkok, Thailand
[35]Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA
[36]Neuroendocrine Tumor Division, Endocrinology & Metabolism Service, Department of Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel
[37]Department of Endocrinology, Heinrich-Heine-University, Düsseldorf, Germany
[38]Department of Medicine (DIMED), Surgical Pathology Unit, University of Padua, Padua, Italy
[39]Department of Cardiology and Angiology I, Heart Center Freiburg University, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[40]Department of Nuclear Medicine, Faculty of Medicine, Albert-Ludwigs-University, Freiburg, Germany
[41]Department of Radiology, Campus Virchow-Klinikum, Charité – Universitätsmedizin Berlin, Berlin, Germany
[42]Department of Surgery, Asklepios-Paulinen Klinik, Wiesbaden, Germany
[43]Section of Endocrine Surgery, Reinbek Hospital, Academic Teaching Hospital University of Hamburg, Reinbek, Germany
[44]Department of Clinical Nuclear Medicine, Charité – Universitätsmedizin Berlin, Berlin, Germany
[45]Department of General Surgery, Division of Endocrine Surgery, Izmir, Turkey
[46]2nd Department of Medicine and Molecular Medicine Research Group, Hungarian Academy of Sciences, Semmelweis-University, Budapest, Hungary
[47]Department of Surgery, Huyssens Foundation Clinics, Essen, Germany
[48]Department of Medicine II, Faculty of Medicine, Medical Center – University of Freiburg, University of Freiburg, Freiburg, Germany
[49]Department of Surgical Sciences, Uppsala University, University Hospital, Uppsala, Sweden
[50]Department of Medicine, University of Toronto, University Healthy Network & Mount Sinai Hospital, The Fred A Litwin Family Center in Genetic Medicine, Toronto, Ontario, Canada
[51]Department of Clinical Genetics and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[52]Department of Endocrinology, Ludwigs-Maximilians-University of Munich, Munich, Germany
[53]Scientific Direction, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy
[54]Genomic Medicine Institute, Lerner Research Institute and Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA
[55]Section for Preventive Medicine, Faculty of Medicine, Albert-Ludwigs-University, Freiburg, Germany

Correspondence should be addressed to B Bausch: birke.bausch@uniklinik-freiburg.de

*(T Krauss, A M Ferrara and T P Links contributed equally to this work)
C Eng was not involved in the review or editorial process for this paper, on which she is listed as an author

Abstract

Pancreatic neuroendocrine tumors (PanNETs) are rare in von Hippel–Lindau disease (VHL) but cause serious morbidity and mortality. Management guidelines for VHL-PanNETs continue to be based on limited evidence, and survival data to guide surgical management are lacking. We established the European-American-Asian-VHL-PanNET-Registry to assess data for risks for metastases, survival and long-term outcomes to provide best management recommendations. Of 2330 VHL patients, 273 had a total of 484 PanNETs. Median age at diagnosis of PanNET was 35 years (range 10–75). Fifty-five (20%) patients had metastatic PanNETs. Metastatic PanNETs were significantly larger (median size 5 vs 2 cm; $P<0.001$) and tumor volume doubling time (TVDT) was faster (22 vs 126 months; $P=0.001$). All metastatic tumors were ≥2.8 cm. Codons 161 and 167 were hotspots for *VHL* germline mutations with enhanced risk for metastatic PanNETs. Multivariate prediction modeling disclosed maximum tumor diameter and TVDT as significant predictors for metastatic disease (positive and negative predictive values of 51% and 100% for diameter cut-off ≥2.8 cm, 44% and 91% for TVDT cut-off of ≤24 months). In 117 of 273 patients, PanNETs >1.5 cm in diameter were operated. Ten-year survival was significantly longer in operated vs non-operated patients, in particular for PanNETs <2.8 cm vs ≥2.8 cm (94% vs 85% by 10 years; $P=0.020$; 80% vs 50% at 10 years; $P=0.030$). This study demonstrates that patients with PanNET approaching the cut-off diameter of 2.8 cm should be operated. Mutations in exon 3, especially of codons 161/167 are at enhanced risk for metastatic PanNETs. Survival is significantly longer in operated non-metastatic VHL-PanNETs.

Key Words
- PanNET
- von Hippel–Lindau disease
- survival
- management recommendations

Endocrine-Related Cancer (2018) **25**, 783–793

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

Introduction

Preventive medicine uses key evidence to exponentially improve the quality of life and life expectancy. Next to environmental and behavioral parameters, heritable factors can result in major morbidity and mortality but also pose opportunities for early detection and prevention. Hereditary neoplasia syndromes such as von Hippel–Lindau disease (VHL) reflect a major challenge and major opportunity. VHL is characterized by specific tumors in different organs. Optimal surveillance and treatment decisions are based on disease-specific parameters. Hereditary diseases offer the possibility that mutation carriers are detected early, often in an asymptomatic stage. For VHL and its tumor spectrum, tumor-specific surveillance programs and long-term management strategies are of paramount importance.

VHL is an autosomal-dominant neoplasia syndrome caused by germline mutations in the *VHL* tumor suppressor gene (Latif *et al.* 1993). Disease incidence is ~1/36000 live births (Maher *et al.* 1991, Neumann & Wiestler 1991, Lonser *et al.* 2003). Penetrance is high but incomplete (Maher *et al.* 1990). VHL is characterized by hemangioblastomas of retina and the central nervous system (CNS), clear cell renal carcinomas (RCCs), pheochromocytomas, endolymphatic sac tumors of the inner ear, cystadenomas of the epididymis and broad ligament and cysts and tumors of the pancreas (Maher *et al.* 1990, Neumann & Wiestler 1991). Hemangioblastomas and RCCs are associated with a high morbidity and mortality due to potential blindness, life-threatening elevation of intracranial pressure, paraplegia and metastases. Quality of life is dramatically decreased and correlates with the number of operations a patient has undergone (Shuin *et al.* 2006).

Pancreatic neuroendocrine tumors (PanNETs) originate from the islets of the pancreas and may be hormonally active or inactive. PanNETs occur mainly as sporadic tumors, but 9% are also components of three hereditary syndromes, multiple endocrine neoplasia type 1 and 4 (MEN 1 and 4) and VHL; VHL-associated PanNETs represent only a subgroup of about 1% of all PanNETs (Erlic *et al.* 2010, Thakker 2014). In contrast to MEN 1, a disease with neuroendocrine tumors (NETs) also occurring in extra-pancreatic sites, in VHL, NETs have been observed almost exclusively in the pancreas (Neumann & Wiestler 1991). A subset of VHL-associated PanNETs have metastatic potential and should therefore be optimally operated before metastatic spread (Hammel *et al.* 2000, Libutti *et al.* 2000, Blansfield *et al.* 2007, Igarashi *et al.* 2014, Keutgen *et al.* 2016).

Preventive medicine is based on evidence-based guidelines. For VHL, such guidelines have been recently revised (Keutgen *et al.* 2016). These guidelines recommend surgical resection when PanNETs are >30 mm diameter in the pancreatic body and tail and >20 mm in the pancreatic head and uncinate process. However, current guidelines are based on small numbers of patients and only a few studies on VHL-PanNETs. Therefore, we analyzed an independent large series of PanNETs for clinical data, growth kinetics and morbidity and mortality from the population-based European-American-Asian-VHL-PanNET-Registry in order to critically reassess diagnostic and management criteria and to optimize the outcome of patients with VHL-associated PanNETs.

Patients and methods

This study is based on the registry for patients with VHL, the VHL-Registry, which was founded in 1983 in Freiburg, Germany and continuously updated and finally transformed into the European-American-Asian-VHL-PanNET-Registry. The VHL-Registry included mainly German patients with VHL disease. The European-American-Asian-VHL-PanNET-Registry was founded for this study and included registrants with VHL-PanNET. For this purpose, we contacted all centers worldwide with an interest on VHL-PanNET. The requested data provided by each center were collected into a central registry database platform. Inclusion criteria for this study were (i) a confirmed diagnosis of VHL either by the identification of a pathogenic germline mutation of the *VHL* gene (which was the case in about 85%) or clinically by the presence of hemangioblastomas of the retina or CNS; (ii) PanNETs must have been documented by contrast-enhanced MRI or CT and (iii) the diagnosis of PanNET was based on diagnostic imaging or histopathology (Neumann 1987, Lonser *et al.* 2003). PanNET was defined as a solid tumor of the pancreas with early arterial contrast enhancement (Choyke *et al.* 1995, Rha *et al.* 2007). Of the criteria for malignancy, we used in this study metastases and/or gross invasion to adjacent tissue, since not all patients were operated (Bosman *et al.* 2010).

From all registrants, demographic, clinical and molecular genetic data were analyzed, including gender; age at diagnosis; number, size and location of PanNETs; mode of treatment; treatment-associated complications; location of metastases; other manifestations of VHL; clinical outcomes and the specific *VHL* germline mutation. Data were updated till March 31, 2018.

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

The study protocol was approved by the Ethical Committee of the Freiburg University Medical Center and accordingly by the cooperating institutions. All patients provided written informed consent. The following are exceptions: in The Netherlands, data were collected anonymously, and according to Dutch law, no further Institutional Review Board approval is required; in Spain, data were obtained anonymously through Alianza-VHL in collaboration with the patients.

Imaging

The patients had state-of-the-art imaging for detection of PanNETs, which included thin-section and multiphase technique with early arterial-phase images for CT or dynamic contrast-enhanced sequences for MRI (Thoeni *et al.* 2000). Positron-emission tomography/CT (PET/CT) with ^{68}Ga-labeled somatostatin receptor-based tracers (^{68}Ga-DOTANOC/DOTATATE/DOTATOC) and/or ^{111}In-/^{99m}Tc-labeled scintigraphy were also performed if considered necessary for further evaluation. For follow-up, most patients had MRI, others endoscopic ultrasonography. For tumor growth measurements, serial imaging with intervals of at least 12 months was used.

Growth kinetics

For calculation of tumor size, each tumor was analyzed on the initial and all follow-up studies. Diameters measured were the longest transverse and its perpendicular diameter on the largest cross-sectional area of the lesion and the cranio-caudal diameter on the coronal plane. Tumor volume was calculated according to the equation $V=(4/3)\pi r(x^*y^*z)$ (Therasse *et al.* 2000). Growth rate was characterized as tumor volume doubling time (TVDT) and specific growth rate (SGR). TVDT was calculated from SGR according to Schwartz's equation as log(2)/SGR. SGR was calculated as the slope of a linear regression line through the growth curve measurement points defined by natural logarithm of volume on the y-axis and time on the *x*-axis (Schwartz 1961, Mehrara *et al.* 2007).

Statistical analysis

Statistical analyses and plots were performed with R software (www.R-project.org). Scale and categorical variables are expressed as median/range and absolute/relative frequencies. Statistical testing included two-sided Fisher's exact and Kruskal–Wallis tests. Multivariable prediction analysis was performed by least absolute shrinkage and selection operator (LASSO) and covariance test for significance (Lockhart *et al.* 2014). Survival was analyzed by Kaplan–Meier and Cox proportional hazard methods. *P* values of <0.05 were defined as significant.

Results

Clinical characteristics of VHL-associated PanNETs

The European-American-Asian-VHL-PanNET-Registry comprises 2330 patients, of whom 273 patients (12%) had PanNETs. Demographic and clinical characteristics are given in Table 1. PanNETs were detected by screening in 248 (91%) patients with asymptomatic presentations; 25 (9%) patients were symptomatic due to abdominal discomfort, large tumor size, bile duct compression with subsequent pancreatitis or due to metastases. The 273 patients had a total of 484 PanNETs. All VHL-PanPNETs were nonfunctioning. Maximum tumor diameter at diagnosis was <1.5 cm in 76, 1.6–3 cm in 108, 3.1–4.5 cm in 40, 4.6–6 cm in 24 and ≥6.1 mm in 25 patients. Metastatic PanNETs were diagnosed in 55 patients (20%) with a minimum diameter of ≥2.8 cm. Metastases occurred in lymph nodes ($n=29$), liver ($n=35$), lungs ($n=5$) and bones ($n=5$).

Radiological findings

All 273 patients had an MRI (194, 71%) and/or CT (139, 51%) as initial imaging study. In metastatic PanNETs, both the maximum tumor diameter and the tumor volume were significantly larger than those of non-metastatic tumors (median 5 cm vs 2 cm; $P<0.001$; 65.47 cm^3 vs 3.05 cm^3; $P<0.001$) (Table 2). The smallest maximum diameter of metastatic PanNETs was 2.8 cm. Hundred-three patients (40%) received additional somatostatin receptor imaging: PET/CT (^{68}Ga-DOTANOC/DOTATATE/DOTATOC) was positive in 44/45 (98%) patients, scintigraphy (^{111}In-/^{99m}Tc-labeled) in 32/53 (60%).

Growth kinetics of VHL-associated PanNETs

For 111 patients, including 17 with metastatic PanNETs, the change of tumor size was studied by MRI or CT with intervals of ≥12 months. Only the largest tumor size was used for calculation of TVDT and SGR. Median follow-up time was 48 months. TVDTs of metastatic vs non-metastatic PanNETs differed significantly with median 22 months vs 126 months ($P=0.001$) (Table 2).

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

Table 1 Demographics and clinical characteristics of the patients of the European-American-Asian-VHL-PanNET-Registry.

	n
Total of patients with VHL-associated PanNETs	273
Index patients	208/273
Related registrants with PanNETs	65/273
Overall number of PanNETs	484
Nationalities	
German	52 (19%)
Italian	40 (15%)
US American	28 (10%)
Ukrainian	21 (8%)
Spanish	15 (6%)
Dutch	12 (5%)
Russian	12 (5%)
Brazilian	12 (5%)
Canadian	11 (4%)
Swedish	18 (7%)
Indian	8 (3%)
Chinese	6 (2%)
Others	38 (15%)
Gender	
Female	171 (63%)
Male	102 (37%)
Age at diagnosis in years	
Median/range	35/10–75
PanNET location	
Head and uncinate	320 (66%)
Body and tail	164 (34%)
Number of PanNETs per patient	
Single	167 (61%)
2 tumors	40 (15%)
3 tumors	43 (15%)
>3 tumors	24 (9%)
Metastatic PanNET	55 (20%)
Other VHL lesions	
Hemangioblastoma of the CNS	197 (72%)
Retinal hemangiomablastoma	142 (52%)
Pheochromocytoma	144 (53%)
Renal clear cell carcinoma	63 (23%)
Pancreatic cysts	79 (29%)
Endolymphatic sac tumor	9 (3%)

Table 2 Tumor characteristics of patients with non-metastatic and metastatic PanNETs. TVDT, tumor volume doubling time.

	Non-metastatic	Metastatic	*P* value
Sex			
Female	133 (61%)	38 (69%)	0.46
Male	85 (39%)	17 (31%)	
Age at diagnosis in years			
Median	35	33	0.17
Range	10–75	11–68	
Maximal tumor diameter (cm)			
Median	2	5	<0.001
Range	0.4–10	2.8–17	
Maximal tumor volume (cm^3)			
Median	3.05	65.42	<0.001
Range	0.02–376.8	5.86–2571.14	
TVDT overall (months)			
Median	126	22	0.001

Germline mutations in patients with VHL-associated PanNETs

The European-American-Asian-VHL-PanNET-Registry comprises 2,330 total registrants, 2,057 without PanNETs and 273 with PanNETs. Of the 2330, 1770 patients had mutation analysis of the *VHL* gene and *VHL* germline mutations identified; of the 1770, 1539 did not have PanNETs and 231 had PanNETs. Germline mutation testing was not possible in 518 patients without PanNETs but meeting clinical criteria of VHL, and in 42 patients with PanNETs, meeting clinical criteria for VHL. This is due to either the patients not consenting to provide blood samples or the center not having the facility of germline mutation analysis. The 231 *VHL* mutation-positive patients with PanNETs included 194 index patients and 37 relatives with 86 different intra-exonic mutations and 32 large deletions of 1–3 exons (Table 3). We compared the genotype and spectra of *VHL* germline mutations in patients with and without PanNETs. PanNETs were significantly more frequent in patients with intragenic mutations compared to large deletions (191/1249 vs 30/408; $P<0.001$). In addition, intragenic mutations were more common in those patients who had metastatic compared to non-metastatic PanNETs (43/1249 vs 5/408, $P=0.017$). Also, patients with large deletions involving exon 3 developed significantly more often PanNETs compared to those with deletions involving exons 1 and/or 2 (17/133 vs 14/285; $P=0.008$). In contrast, PanNETs and metastatic PanNETs occurred more frequently in patients with intragenic exon 3 mutations compared to those with intragenic mutations in exons 1 and 2 (PanNETs, 107/521 vs 84/728; $P<0.001$ and metastatic PanNETs, 30/521 vs 13/728; $P<0.001$). Further, mutations of codon 161 and 167 were statistically more frequent in patients with PanNETs as well as metastatic PanNETs compared to mutations in the rest of exon 3 (78/273 vs 29/262; $P<0.001$, and metastatic PanNETs 23/273 vs 8/262; $P=0.005$). In contrast, patients with mutations in the third most frequently mutated codon 98 showed rarely PanNETs (6/206 vs 58/267; $P<0.001$).

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

Table 3 Germline mutations of the *VHL* gene in patients with VHL-PanNET. Two-hundred and thirty-one VHL-PanNET patients (194 index patients and 37 relatives) from 27 countries showed 86 different intra-exonic mutations of the *VHL* gene. The 86 different germline mutations were distributed over the 3 exons of the *VHL* gene with a hotspot region in exon 3, codon 161/167, the latter with enhanced risk of metastatic PanNET. Additionally, 32 patients had large deletions from 1 to 3 exons.

VHL-PanNET patients *n*	Non-metastatic/metastatic VHL-PanNET patients *n*	Exon	Nucleotide change	Amino acid change	Center *n*
1	1/0	1	c.167C>T	p.Ala56Val	1
1	1/0	1	c.188T>G	p.Leu63Arg	1
1	1/0	1	c.191G>C	p.Arg64Pro	1
2	2/0	1	c.194C>G	p.Ser65Trp	2
1	1/0	1	c.194C>T	p.Ser65Leu	1
1	1/0	1	c.202T>C	p.Ser68Pro	1
1	0/1	1	c.208G>T	p.Glu70*	1
1	1/0	1	c.219T>G	p.Val74Gly	1
1	0/1	1	c.221T>A	p.Val74Asp	1
3	3/0	1	c.227_229del	p.Phe76del	3
4	4/0	1	c.233A>G	p.Asn78Ser	3
3	3/0	1	c.233A>C	p.Asn78Thr	1
1	1/0	1	c.238A>G	p.Ser80Gly	1
1	1/0	1	c.239G>T	p.Ser80Ile	1
1	0/1	1	c.340-2GGT>TGA	Splice	1
1	1/0	1	c.240T>G	p.Ser80Arg	1
1	1/0	1	c.245G>T	p.Arg82Leu	1
1	1/0	1	c.250G>T	p.Val84Leu	1
5	5/0	1	c.256C>G	p.Pro86Ala	1
1	1/0	1	c.256C>T	p.Pro86Ser	1
1	1/0	1	c.256C>A	p.Pro86Thr	1
1	1/0	1	c.257C>T	p.Pro86Leu	1
1	1/0	1	c.266T>C	p.Leu89Pro	1
1	1/0	1	c.273del	p.Phe91Leufs*68	1
4	4/0	1	c.277G>C	p.Gly93Arg	3
1	1/0	1	c.280G>T	p.Glu94*	1
1	0/1	1	c.286/287	p.Pro+Val86-87Ser+Leu	1
1	1/0	1	c.287_288AG>CC	p.Gln96Pro	1
2	2/0	1	c.292T>C	p.Tyr98His	1
4	1/3	1	c.293A>C	p.Tyr98Ser	1
1	1/0	1	c.319C>G	p.Arg107Gly	1
1	1/0	1	c.333C>G	p.Ser111Arg	1
2	1/1	1	c.340+1G>T	Splice	2
4	3/1	1	c.340G>A	p.Gly114Ser	2
1	0/1	2	c.349dupT	p.Trp117Leufs*15	1
2	2/0	2	c.357C>G	p.Phe119Leu	2
1	1/0	2	c.362A>G	p.Asp121Gly	1
1	1/0	2	c.364_365delinsAT	p.Ala122Ile	1
2	1/1	2	c.374A>C	p.His125Pro	2
1	1/0	2	c.382C>T	p.Leu128Phe	1
1	1/0	2	c.388G>T	p.Val130Phe	1
1	0/1	2	c.392A>C	p.Asn131Thr	1
1	0/1	2	c.393C>A	p.Asn131Lys	1
1	1/0	2	c.394C>T	p.Gln132*	1
1	1/0	2	c.395A>C	p.Gln132Pro	1
1	1/0	2	c.401T>G	p.Leu63Arg	1
3	3/0	2	c.407T>C	p.Phe136Ser	2
1	1/0	2	c.407C>G	p.Ser65Trp	1
1	1/0	2	c.408delT	p.Phe136Leufs*23	1
2	1/1	2	c.412C>A	p.Pro138Thr	1
1	1/0	2	c.434T>G	p.Val74Gly	1
2	2/0	2	c.440delTCT	p.delPhe76	1
1	1/0	2	c.449C>G	p.Leu188Val	1
1	1/0	2	c.449_462del	p.Asn150Serfs*19	1

(Continued)

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

Table 3 Continued.

VHL-PanNET patients *n*	Non-metastatic/metastatic VHL-PanNET patients *n*	Exon	Nucleotide change	Amino acid change	Center *n*
3	3/0	2	c.452T>C	p.Ile151Thr	2
1	1/0	2	c.453C>G	p.Ile151Met	1
1	0/1	2	c.457C>G	p.Phe119Leu	1
1	1/0	2	c.461C>T	Splice	1
1	1/0	2	c.463G>C	Splice	1
1	1/0	2	c.463+3A>T	Splice	1
1	1/0	2	c.463+2T>G	Splice	1
2	1/1	3	c.464-2A>G	Splice	1
1	1/0	3	c.464-1G>A	Splice	1
1	1/0	3	c.464T>G	p.Val155Gly	1
1	1/0	3	c.467A>G	p.Tyr156Cys	1
1	1/0	3	c.472C>G	p.Leu158Val	1
2	1/1	3	c.479_480del	p.Glu160Alafs*13	2
1	1/0	3	c.481C>G	p.Arg161Gly	1
3	3/0	3	c.481C>T	p.Arg161X	3
15	8/7	3	c.482G>A	p.Arg161Gln	8
2	1/1	3	c.488T>A	p.Leu163His	2
1	1/0	3	c.490C>T	p.Gln164Ter	1
1	1/0	3	c.491A>G	p.Gln164Arg	1
3	1/2	3	c.496G>T	p.Val166Phe	1
1	1/0	3	c.497T>C	p.Val166Ala	1
2	2/0	3	c.499C>G	p.Arg167Gly	2
32	24/8	3	c.499C>T	p.Arg167Trp	15
24	16/8	3	c.500G>A	p.Arg167Gln	16
2	2/0	3	c.501G>A	p.Arg167=	2
1	1/0	3	c.509T>G	p.Val170Gly	1
3	2/1	3	c.533T>C	p.Leu178Pro	2
1	1/0	3	c.548C>A	p.Ser183*	1
2	2/0	3	c.583C>T	p.Gln195*	2
2	1/0	3	c.593T>A	p.Leu198Gln	1
1	1/0	3	c.599G>C	p.Arg200Pro	1
1	1/0	3	c.641G>T	p.*214Leuext*14	1

Characteristics of VHL disease in patients with VHL-associated PanNET

Of the 273 VHL-PanNET patients, all had extra-pancreatic VHL-associated tumors. Hemangioblastomas of the retina and CNS occurred in 52% and 72% of the patients, respectively. RCCs and pheochromocytomas were detected in 23% and 53% of the patients, respectively. Patients had an average of 2 (range 0–12) operations for extra-pancreatic VHL tumors.

Severe non-PanNET-related VHL-associated disabilities occurred in 144 (144/215, 67%) living patients. Blindness occurred bilaterally in 2 and unilaterally in 19 patients. Steroid dependency after bilateral adrenalectomy for pheochromocytoma occurred in 26, severe neurological deficits after removal of CNS hemangioblastomas in 24 patients, respectively.

Forty-three patients died of VHL, of whom 26 (60%) were caused by PanNETs (2 due to complications during surgery, 24 due to metastases), 10 of CNS hemangioblastomas, 5 of RCC-related metastases and 2 of adrenal insufficiency. Nine patients died of non-VHL-related reasons, including one each of hepatocellular, rectal and ovarian cancers, three from non-VHL-related cardiac arrest, two from drug abuse and one from sepsis.

Surgical treatment and survival

Indication for surgery were tumor size and metastases diagnosed by MRI, CT and /or nuclear medicine imaging. Removal of PanNETs was performed in 117 (43%) patients; in 80 for non-metastatic and in 37 for metastatic PanNETs. Of the 55 patients with metastatic PanNETs, 16 had tumors in advanced stage, too late for surgery and 2 patients refused operation. Total pancreatectomy with or without removal of adjacent organs was performed in 18 patients, segmental pancreatectomy or enucleation of PanNETs in 99 (69 non-metastatic and 30 metastatic). Perioperative mortality was 2% (2/117). Early postoperative complications like fistula, abscess or cholangitis had

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

23% (27/117) and long-term complications (diabetes and/or exocrine pancreatic insufficiency) occurred in 41% (48/117) of the patients.

Estimation of survival was performed for four groups (Fig. 1): patients with maximum tumor diameters 1.5–2.7 cm and ≥2.8 cm (groups 1 and 2) not operated and operated (A and B) with a median follow-up of 7 years. Operated patients (groups 1B and 2B) experienced significantly longer survival than non-operated patients (94% (1B) vs 85% (1A) by 10 years; $P=0.020$; 80% (2B) vs 50% (2A) at 10 years; $P=0.030$). Comparing patients operated for smaller-to-larger tumors, survival was also significantly longer (groups 1B vs 2B, 94% vs 80%; $P=0.030$). Finally, both groups together showed longer survival when operated (88% (1B+2B) vs 70% [1A+2A]; $P=0.04$). In multivariate modeling, survival was independently reduced by age >35 years (HR 2.5, $P=0.012$), by metastatic PanNET (HR 8.7; $P=0.001$) or if mutations were present in codons 161 or 167.

Multivariate prediction modeling for metastatic PanNET

Multivariate modeling by LASSO regression incorporating maximum tumor diameter and volume, TVDT, age and hotspot mutations disclosed only maximum tumor diameter and TVDT as significant predictor variables. Positive and negative predictive values for metastatic PanNET were 51% and 100% for maximum tumor diameter at cut-off >2.8 cm, and 44% and 91% for TVDT at cut-off <24 months, respectively.

There were no statistical differences found for gene, age at diagnosis and tumor size in predicting long-term survival in those with metastatic PanNETs. Similarly, center of accrual (for those contributing more than ten registrants) did not confound survival data. Further, there are no gender-specific statistically significant differences.

Discussion

Our current study reassesses clinical management recommendations based on a large, independent, population-based registry of 273 patients with VHL-PanNETs from a total of 2330 patients with VHL from 27 different countries and 3 continents. Recently, revised diagnostic and treatment recommendations for VHL-associated PanNETs have been presented (Keutgen *et al.* 2016, Tirosh *et al.* 2018). These guidelines were based on past literature, mainly four smaller referral-based studies from the United States (Blansfield *et al.* 2007, Tirosh *et al.* 2018), France (Corcos *et al.* 2008) and Japan (Igarashi *et al.* 2014) with a total of 175, 108, 53 and 35 patients with VHL-associated PanNETs, from a total of 1239 VHL patients (Table 4). Patients with VHL and PanNET are confronted with the risks of metastatic spread of PanNETs and the consequences of surgeries. Complementary, but in these studies widely neglected, challenges are morbidity and mortality due to the multiplicity of non-pancreatic VHL-associated tumors.

Rates of metastatic PanNETs and key characteristics for early diagnosis differ substantially among these studies with prevalence of metastatic VHL-PanNETs

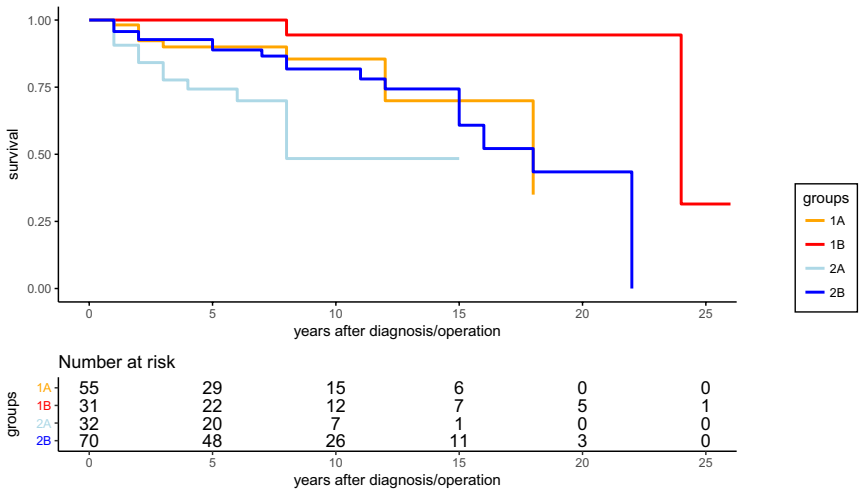

Figure 1
Survival of von Hippel–Lindau disease patients with pancreatic neuroendocrine tumors with a maximum tumor diameter 1.5–2.7 cm not operated/operated vs ≥2.8 cm not operated/operated. Survival was analyzed by Kaplan–Meier Cox proportional hazard methods. Group 1A: patients with maximum tumor diameter 1.5–2.7 cm not operated. Group 1B: patients with maximum tumor diameter 1.5–2.7 cm operated. Group 2A: patients with maximum tumor diameter ≥2.8 cm not operated. Group 2B: patients with maximum tumor diameter ≥2.8 cm operated. Survival was significantly longer in operated patients (group 1B + group 2B) compared to not operated patients (group 1A+ group 2A) (94% (1B) vs 85% (1A) by 10 years, $P=0.020$; 80% (2B) vs 50% (2A) at 10 years, $P=0.030$; 88% (1B+2B) vs 70% (1A+2A) $P=0.040$). Survival was also significantly longer in patients operated for smaller tumors compared to patients operated for larger tumors (groups 1B vs 2B, 94% vs 80% $P=0.030$).

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

Table 4 PanNET characteristics and patient information of current study in comparison with those in the literature. All values indicate number of patients; *values based on 111 growth observations (cases) of this study and 63 growth observations (cases) of Blansfield *et al.* (2007).

	This study	Blansfield *et al.* (2007)	Corcos *et al.* (2008)	Igarashi *et al.* (2014)	Tirosh *et al.* (2018)
Patients total with VHL	2330	633	n.a.	377	229
Patients total with PanNET (%)	273 (11.7)	108 (17.1)	35	53 (14.1)	175
Age at diagnosis in years range (median)	10–75 (35)	16–68 (38)	21–57 (37)	14–55 (34)	n.a.
Non-metastatic PanNETs	218	98	12	49	166
Diameter mm (median)	4–101 (20)	n.a.	10–45 (23)	n.a.	n.a.
Metastatic PanNETs	55	9	17	4	9
Diameter mm (median)	28–170 (50)	n.a.	15–80 (34)	n.a.	n.a.
TVDT (months) non-metastatic/metastatic*	126 (22)	88 (11)	n.a.	n.a.	n.a.
Multiple PanNETs (%)	107 (39)	32 (30)	6 (17)	n.a.	n.a.
Patients' nationalities	27	1	1	1	1
Operated patients	117	39	23	34	29
Total pancreatectomy (%)	18 (17)	2 (5)	14 (61)	3 (9)	n.a.
Early postoperative complications	26 (24)	11 (28)	n.a.	n.a.	n.a.
Long-term complications	46 (43)	n.a.	n.a.	n.a.	n.a.
Perioperative mortality rate (%)	2 (1.8)	1 (3)	n.a.	n.a.	n.a.
Total death (*n*=)	52	n.a.	7	16	n.a.
Death associated with VHL	43				
Death associated with PanNET (%)	26 (50)	n.a.	2 (29)	0	n.a.
Death associated with VHL but not PanNET (%)	17 (32)	n.a.	n.a.	n.a.	n.a.

of 7.5–20% and case numbers of only 2–17 patients (Yamasaki *et al.* 2006, Blansfield *et al.* 2007, Corcos *et al.* 2008, Charlesworth *et al.* 2012, Igarashi *et al.* 2014, Tirosh *et al.* 2018). In contrast, our study contains 55 patients with metastatic PanNETs, 20% of the total series. Multivariate prediction modeling disclosed maximum tumor diameter and TVDT as the only independent predictors of malignancy. And the strongest predictor for metastatic VHL-PanNET shown here is the maximum tumor diameter regardless of location within the pancreas with a cut-off diameter of ≥2.8 cm, a parameter essential for treatment decisions. The key unanswered question has been whether patients with VHL benefit from the operative removal of PanNETs? Our study answers this question based on 117 patients who underwent removal of PanNETs, an operated cohort larger than any previously published study. We found that the 10-year survival was statistically significantly longer in patients operated for PanNETs that measured 1.5–2.7 cm in diameter in contrast to the watch-and-wait approach; the same findings were found for PanNETs ≥2.8 cm. But the improved survival needs to take into account the side effects by the surgical intervention, reported to occur in 28–35% of the patients (Blansfield *et al.* 2007, de Mestier *et al.* 2015). Our study demonstrated permanent postoperative complications in 41% of the patients with exocrine and endocrine pancreatic insufficiency, although 85% of the operations were declared as organ sparing. This seemingly high frequency of complications is confounded by 29% of the patients having organ-sparing PanNET removal having multiple pancreatic cysts as an additional manifestation of VHL. The higher complication rate obtained in our multicenter, multicontinental study probably represents the reality of outcomes.

The second important aspect is the complexity of multi-organ tumor involvement of patients with VHL by their non-pancreatic tumors. In our study, this is characterized by tumors in extra-pancreatic organs or organ systems like the CNS with a need of up to 17 (median 2) surgeries and in addition frequent laser coagulation of retinal hemangioblastomas to prevent blindness. Complications were experienced in nearly two-thirds of the patients with permanent neurological deficits, uni- or bilateral blindness or steroid dependency after bilateral adrenalectomy for pheochromocytomas. Non-PanNET-related mortality in our study (eg, related to other VHL-associated tumors) was high, with 39% of deaths due to CNS hemangioblastomas, metastases of RCC or adrenal insufficiency.

Diagnosis and treatment recommendations for the evaluation and treatment of VHL-associated PanNETs should be revised to detect metastatic PanNET <2.8 cm in diameter in order to impact survival (Kruizinga *et al.* 2014). Screening for PanNETs should be started before age 11 years, the age of our youngest patient with metastatic PanNET. Thus, screening for PanNETs should be a part of the generally recommended screening program for VHL-associated tumors. If a solid pancreatic lesion is detected, functional somatostatin receptor PET-CT using

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100

^{68}Ga-DOTANOC/DOTATATE/DOTATOC (highly sensitive and specific in our and other studies) should be considered to confirm the endocrine nature (Poeppel *et al.* 2011, Prasad *et al.* 2016). A maximum tumor diameter ≥2.8 cm (regardless of location within the pancreas) should guide treatment decisions, which differs from current recommendations (Libutti *et al.* 2000, Blansfield *et al.* 2007, Keutgen *et al.* 2016, Tirosh *et al.* 2018). We recommend yearly monitoring of the pancreas and calculation of TVDT of PanNETs, if a diameter close to 2 cm is documented. Our recommendations differ to those most recently published, since we found metastatic tumors in five patients with diameters 2.8–3.0 cm (Tirosh *et al.* 2018). Our recommendations do not differentiate between missense mutations and mutations in exon 3 vs other *VHL* germline mutations, since four of our patients with metastatic PanNETs not exceeding 3.2 cm in diameter carried missense mutations. MRI or endoscopic ultrasonography can be used for imaging surveillance; the latter is contrast medium-free and may become the method of choice (van Asselt *et al.* 2016). Compared to recently published data our genotype–phenotype correlation based on 1,539 VHL patients without and 231 with PanNETs does not only exceed the existing number of patients, but is important, as VHL-associated PanNETs occurred significantly more often in patients with mutations affecting exon 3 with hotspots in codons 161/167, the latter with enhanced risk for malignancy (Tirosh *et al.* 2017). But an exclusion of surveillance for PanNETs based on specific mutations cannot be recommended, since PanNETs have been observed in carriers of germline mutations of any type and region of the *VHL* gene and genotype as a predictor of malignancy did not emerge in our multivariate analysis.

In summary, this worldwide study of PanNETs associated with VHL provides a unique and broad data platform to guide the management of VHL-PanNETs, demonstrating the complex challenges due to pancreatic and extra-pancreatic VHL-associated-tumors. For effective preventive medicine, clinical and molecular evaluation is essential. Maximum tumor diameter and growth of PanNETs, measured as TVDT, germline mutations and associated tumors need to be defined, since hotspot mutations in some codons in particular predispose to metastases. To improve the outcome and survival, patients with PanNETs ≥2.5 cm in diameter, regardless of location within the pancreas, are strong candidates for surgery. This study exemplifies the comprehensive data, which are needed for best-practice counseling of neoplasia syndromes and optimally established by an international consortium.

Strength and limitations of the study

Our current sample size of patients with VHL and PanNET exceeds by far all previous reports. The international research participants provide realistic (i.e., unbiased) data which help avoidance of fatal outcome by PanNET. The limitations of our study include that this is a retrospective-prospective registry study. Because the registry accrues over time, technical advances of imaging and surgical techniques may currently contribute to a better outcome in the latter years.

Declaration of interest
There is no relevant conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement
H P H N, B B and C E had access to all the data in the study and are responsible for the conduct and content of the study. H P H N, B B, T K and C E conceptualized, designed and directed the study. T K reassessed radiological data. All authors obtained family histories, collected phenotypic documentation, reviewed all clinical information, ensured regulatory compliance and/or analyzed the data. Statistical analysis of the data was performed by U W, H P H N, C E, T K and B B. H P, H N, B B and C E interpreted the data and drafted the manuscript. All authors reviewed and critically revised the manuscript, and approved the final manuscript.

Acknowledgements
The authors are grateful to our patients and their families in participating in our registries and our studies. They thank for support of this study Fabio Azzolin, Padova, Italy. C Eng is the Sondra J and Stephen R Hardis Endowed Chair in Cancer Genomic Medicine at the Cleveland Clinic, and an American Cancer Society Clinical Research Professor. G Opocher, C Eng, H P H Neumann and B Bausch: shared senior authorship.

References

Blansfield JA, Choyke L, Morita SY, Choyke PL, Pingpank JF, Alexander HR, Seidel G, Shutack Y, Yuldasheva N, Eugeni M, *et al.* 2007 Clinical, genetic and radiographic analysis of 108 patients with von Hippel-Lindau disease (VHL) manifested by pancreatic neuroendocrine neoplasms (PNETs). *Surgery* **142** 814–818. (https://doi.org/10.1016/j.surg.2007.09.012)

Bosman FT, Carneiro F, Hruban RH & Theise ND Eds 2010 *WHO Classification of Tumours of the Digestive System*. Lyon, France: IARC Press.

Charlesworth M, Verbeke CS, Falk GA, Walsh M, Smith AM & Morris-Stiff G 2012 Pancreatic lesions in von Hippel-Lindau disease? A systematic review and meta-synthesis of the literature. *Journal of Gastrointestinal Surgery* **16** 1422–1428. (https://doi.org/10.1007/s11605-012-1847-0)

Choyke PL, Glenn GM, Walther MM, Patronas NJ, Linehan WM & Zbar B 1995 von Hippel-Lindau disease: genetic, clinical, and imaging features. *Radiology* **194** 629–642. (https://doi.org/10.1148/radiology.194.3.7862955)

Corcos O, Couvelard A, Giraud S, Vullierme MP, Dermot OT, Rebours V, Stievenart JL, Penfornis A, Niccoli-Sire P, Baudin E, *et al*. 2008 Endocrine pancreatic tumors in von Hippel-Lindau disease: clinical, histological, and genetic features. *Pancreas* **37** 85–93. (https://doi.org/10.1097/MPA.0b013e31815f394a)

de Mestier L, Gaujoux S, Cros J, Hentic O, Vullierme MP, Couvelard A, Cadiot G, Sauvanet A, Ruszniewski P, Richard S, *et al*. 2015 Long-term prognosis of resected pancreatic neuroendocrine tumors in von Hippel-Lindau disease is favorable and not influenced by small tumors left in place. *Annals of Surgery* **262** 384–388. (https://doi.org/10.1097/SLA.0000000000000856)

Erlic Z, Ploeckinger U, Cascon A, Hoffmann MM, von Duecker L, Winter A, Kammel G, Bacher J, Sullivan M, Isermann B, *et al*. 2010 Systematic comparison of sporadic and syndromic pancreatic islet cell tumors. *Endocrine-Related Cancer* **17** 875–883. (https://doi.org/10.1677/ERC-10-0037)

Hammel PR, Vilgrain V, Terris B, Penfornis A, Sauvanet A, Correas JM, Chauveau D, Balian A, Beigelman C, O'Toole D, *et al*. 2000 Pancreatic involvement in von Hippel-Lindau disease. The Groupe Francophone d'Etude de la Maladie de von Hippel-Lindau. *Gastroenterology* **119** 1087–1095. (https://doi.org/10.1053/gast.2000.18143)

Igarashi H, Ito T, Nishimori I, Tamura K, Yamasaki I, Tanaka M & Shuin T 2014 Pancreatic involvement in Japanese patients with von Hippel-Lindau disease: results of a nationwide survey. *Journal of Gastroenterology* **49** 511–516. (https://doi.org/10.1007/s00535-013-0794-1)

Keutgen XM, Hammel P, Choyke PL, Libutti SK, Jonasch E & Kebebew E 2016 Evaluation and management of pancreatic lesions in patients with von Hippel-Lindau disease. *Nature Reviews Clinical Oncology* **13** 537–549. (https://doi.org/10.1038/nrclinonc.2016.37)

Kruizinga RC, Sluiter WJ, de Vries EG, Zonnenberg BA, Lips CJ, van der Horst-Schrivers AN, Walenkamp AM & Links TP 2014 Calculating optimal surveillance for detection of von Hippel-Lindau-related manifestations. *Endocrine-Related Cancer* **21** 63–71. (https://doi.org/10.1530/ERC-13-0308)

Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, Stackhouse T, Kuzmin I, Modi W, Geil L, *et al*. 1993 Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* **260** 1317–1320. (https://doi.org/10.1126/science.8493574)

Libutti SK, Choyke PL, Alexander HR, Glenn G, Bartlett DL, Zbar B, Lubensky I, McKee SA, Maher ER, Linehan WM, *et al*. 2000 Clinical and genetic analysis of patients with pancreatic neuroendocrine tumors associated with von Hippel-Lindau disease. *Surgery* **128** 1022–1027. (https://doi.org/10.1067/msy.2000.110239)

Lockhart R, Taylor J, Tibshirani RJ & Tibshirani R 2014 A significance test for the lasso. *Annals of Statistics* **42** 413–468. (https://doi.org/10.1214/13-AOS1175)

Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, Linehan WM & Oldfield EH 2003 von Hippel-Lindau disease. *Lancet* **361** 2059–2067. (https://doi.org/10.1016/S0140-6736(03)13643-4)

Maher ER, Yates JR, Harries R, Benjamin C, Harris R, Moore AT & Ferguson-Smith MA 1990 Clinical features and natural history of von Hippel-Lindau disease. *QJM* **77** 1151–1163. (https://doi.org/10.1093/qjmed/77.2.1151)

Maher ER, Iselius L, Yates JR, Littler M, Benjamin C, Harris R, Sampson J, Williams A, Ferguson-Smith MA & Morton N 1991 Von Hippel-Lindau disease: a genetic study. *Journal of Medical Genetics* **28** 443–447. (https://doi.org/10.1136/jmg.28.7.443)

Mehrara E, Forssell-Aronsson E, Ahlman H & Bernhardt P 2007 Specific growth rate versus doubling time for quantitative characterization of tumor growth rate. *Cancer Research* **67** 3970–3975. (https://doi.org/10.1158/0008-5472.CAN-06-3822)

Neumann HP 1987 Basic criteria for clinical diagnosis and genetic counselling in von Hippel-Lindau syndrome. *VASA* **16** 220–226.

Neumann HP & Wiestler OD 1991 Clustering of features of von Hippel-Lindau syndrome: evidence for a complex genetic locus. *Lancet* **337** 1052–1054. (https://doi.org/10.1016/0140-6736(91)91705-Y)

Poeppel TD, Binse I, Petersenn S, Lahner H, Schott M, Antoch G, Brandau W, Bockisch A & Boy C 2011 68Ga-DOTATOC versus 68Ga-DOTATATE PET/CT in functional imaging of neuroendocrine tumors. *Journal of Nuclear Medicine* **52** 1864–1870. (https://doi.org/10.2967/jnumed.111.091165)

Prasad V, Tiling N, Denecke T, Brenner W & Plockinger U 2016 Potential role of (68)Ga-DOTATOC PET/CT in screening for pancreatic neuroendocrine tumour in patients with von Hippel-Lindau disease. *European Journal of Nuclear Medicine and Molecular Imaging* **43** 2014–2020. (https://doi.org/10.1007/s00259-016-3421-6)

Rha SE, Jung SE, Lee KH, Ku YM, Byun JY & Lee JM 2007 CT and MR imaging findings of endocrine tumor of the pancreas according to WHO classification. *European Journal of Radiology* **62** 371–377. (https://doi.org/10.1016/j.ejrad.2007.02.036)

Schwartz M 1961 A biomathematical approach to clinical tumor growth. *Cancer* **14** 1272–1294. (https://doi.org/10.1002/1097-0142(196111/12)14:6<1272::AID-CNCR2820140618>3.0.CO;2-H)

Shuin T, Yamasaki I, Tamura K, Okuda H, Furihata M & Ashida S 2006 Von Hippel-Lindau disease: molecular pathological basis, clinical criteria, genetic testing, clinical features of tumors and treatment. *Japanese Journal of Clinical Oncology* **36** 337–343. (https://doi.org/10.1093/jjco/hyl052)

Thakker RV 2014 Multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4). *Molecular and Cellular Endocrinology* **386** 2–15. (https://doi.org/10.1016/j.mce.2013.08.002)

Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, *et al*. 2000 New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *Journal of the National Cancer Institute* **92** 205–216. (https://doi.org/10.1093/jnci/92.3.205)

Thoeni RF, Mueller-Lisse UG, Chan R, Do NK & Shyn PB 2000 Detection of small, functional islet cell tumors in the pancreas: selection of MR imaging sequences for optimal sensitivity. *Radiology* **214** 483–490. (https://doi.org/10.1148/radiology.214.2.r00fe32483)

Tirosh A, Lakis ME, Green P, Nockel P, Patel D, Nilubol N, Gara SK, Keutgen XM, Linehan WM & Kebebew E 2017 In-silico VHL gene mutation analysis and prognosis of pancreatic neuroendocrine tumors in von Hippel-Lindau disease. *Journal of Clinical Endocrinology and Metabolism* [epub]. (https://doi.org/10.1210/jc.2017-02434)

Tirosh A, Sadowski SM, Linehan WM, Libutti SK, Patel D, Nilubol N & Kebebew E 2018 Association of VHL genotype with pancreatic neuroendocrine tumor phenotype in patients with von Hippel-Lindau disease. *JAMA Oncology* **4** 124–126. (https://doi.org/10.1001/jamaoncol.2017.3428)

van Asselt SJ, Brouwers AH, van Dullemen HM, van der Jagt EJ, Bongaerts AH, Koopmans KP, Kema IP, Zonnenberg BA, Timmers HJ, de Herder WW, *et al*. 2016 Potential value of EUS in pancreatic surveillance of VHL patients. *European Journal of Endocrinology* **174** 611–620. (https://doi.org/10.1530/EJE-15-1012)

Yamasaki I, Nishimori I, Ashida S, Kohsaki T, Onishi S & Shuin T 2006 Clinical characteristics of pancreatic neuroendocrine tumors in Japanese patients with von Hippel-Lindau disease. *Pancreas* **33** 382–385. (https://doi.org/10.1097/01.mpa.0000240604.26312.e4)

Received in final form 24 April 2018
Accepted 10 May 2018
Accepted Preprint published online 10 May 2018

http://erc.endocrinology-journals.org
https://doi.org/10.1530/ERC-18-0100