Disease biology and principles of biological therapies

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ESMO PRECEPTORSHIP PROGRAM

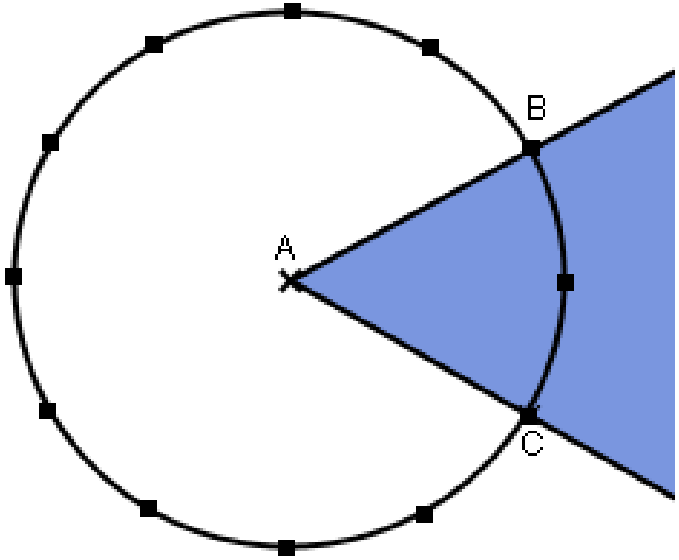
Prague , 2017

Conflict/Link of Interest

- **Advisory board and/or honoraria**
  - Novartis
  - Ipsen
  - Pfizer
  - Roche
  - Sanofi

- **Research support**
  - Pfizer
  - Ipsen
  - HRA
  - Roche
  - Novartis
  - AAA

Clinician angle



Prognostic , predictive roles or, surrogacy demonstrate the relevance of a target
Correlation with well defined NET characteristics as a preliminary step

Heterogeneity : focus on NETs

NETs and NECs are two distinct neoplasms

TABLE 2. Immunohistochemical Features of Pancreatic Neuroendocrine Neoplasms

	Proliferation		Markers of PanNET[20]			Markers of Neuroendocrine Differentiation			Markers of Cell Cycle Regulation			PDAC Marker	Therapeutic Target
Marker	Ki67 (%)	Mitotic Rate	DAXX	ATRX	Pten	CD56	Pdx1	PAX8	p53	Rb	p16	Smad4	Bcl-2
Small cell NEC*													
1	58.1	50	+	+	–	+	+	+	+	–	+	+	+
2	36.9	42	+	+	+	+	–	–	+	–	+	+	+
3	55.9	36	+	+	+	+	–	–	+	–	+	+	+
4	57.1	22	+	+	+	+	–	+	+	+	–	+	+
5	59.7	56	+	+	+	+	–	+	+	–	+	+	+
6	77.6	33	+	+	+	+	NA†	–	+	–	+	+	+
7	55.1	49	+	+	+	+	–	–	+	–	+	+	+
8	57.4	22	+	+	+	+	–	–	+	–	+	+	+
9	85.8	58	+	+	+	+	+	+	+	–	+	+	+
Large cell NEC*													
10	55.2	53	+	+	+	+	–	–	+	–	+	+	+
11	68.4	31	+	+	+	+	+	+	+	–	+	+	–
12	56.1	74	+	+	+	–	–	–	+	–	–	+	+
13	67.1	48	+	+	+	–	+	–	+	–	+	+	–
14	39.5	29	+	+	+	+	+	+	+	+	–	+	+
15	38.7	28	+	+	+	+	–	+	+	–	+	+	+
16	37.2	25	+	+	+	+	–	–	+	–	+	+	+
17	22.0	31	+	+	+	+	–	+	+	+	–	–	–
18	30.1	4	+	+	+	–	–	–	+	+	–	+	–
19	20.0	20	+	+	+	+	+	+	–	+	–	+	–
Well-differentiated NET (G2, intermediate grade)													
20	9.2	3	+	+	+	+	NA	–	–	+	+	+	+
21	8.9	2	+	+	+	–	+	+	–	+	+	+	+
22	3.3	2	+	–	+	+	+	+	–	+	+	+	–
23	5.7	1	+	+	+	–	+	–	–	+	+	+	–
24	2.3	2	+	+	+	+	+	–	–	+	+	+	–
Well-differentiated NET (G1, low grade)													
25	0.4	0	+	+	+	+	NA	+	–	+	+	+	–
26	1.3	0	+	–	+	NA	+	+	–	+	+	+	–
27	1.7	0	+	+	+	–	+	+	–	+	+	+	–
28	0.8	0	+	–†	+	+	+	+	–	+	+	+	–
29	0.8	0	+	+	+	+	+	–	–	+	+	+	–
30	0.2	1	–†	–†	+	+	–	–	–	+	+	+	–

*$P = 0.02$, mean Ki67 labeling index of small cell NEC versus large cell NEC.
†Areas of both positive and negative immunolabeling present in the same section (heterogeneity).
NA indicates not analyzed or technical failure; NET, neuroendocrine tumor.

(*Am J Surg Pathol* 2012;36:173–184)

Yachida t al : surgical series of 19 pa NEC vs 11 paNET

“OMICS” and hypotheses

- Before the “OMICs”, 3 main druggable targets
  - NETs secrete hormones : Somatostatin analogues
  - NETs are hypervascularized : antiangiogenic
  - NETs are part of inherited syndromes : mTOR inh.and antiangiogenic
- After the “OMICs”:
  - “old “ and new targets

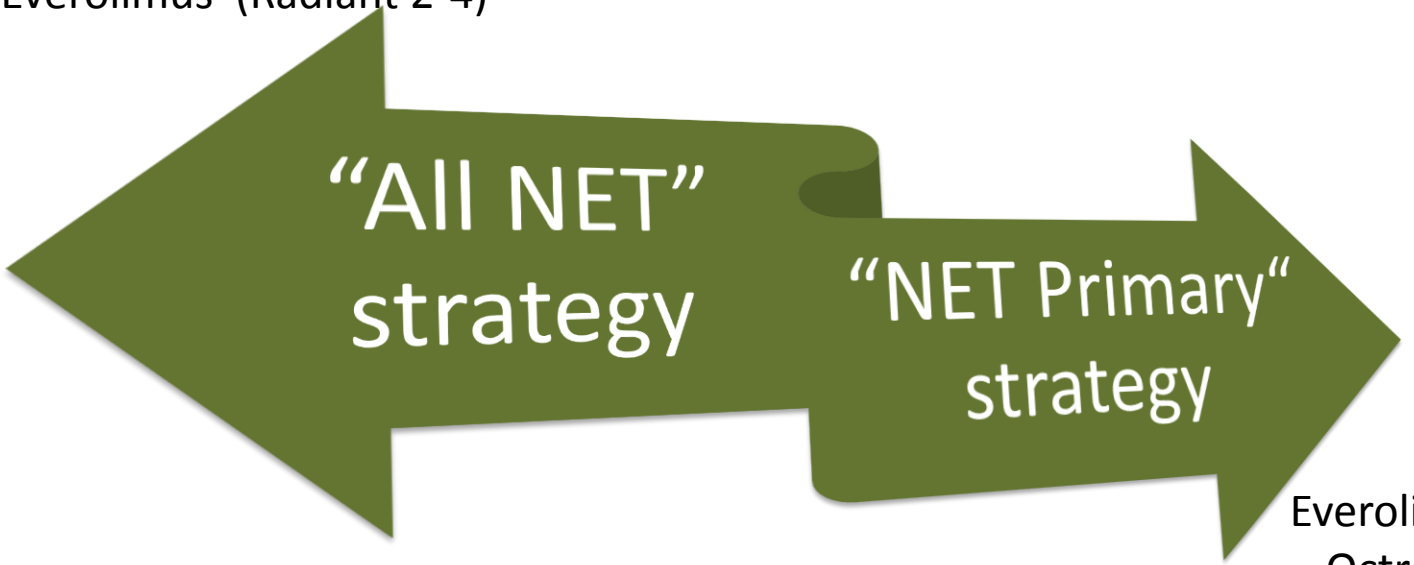
Medical Therapy in Advanced GEP NET: current status

	Bronchial	Ileum	Pancreas
Somatostatin analogs	Expert consensus	**Approved**	**Approved** LAN 120
Chemotherapy	Expert consensus	Expert consensus	**Approved** STZ+Fu/DXR
PRRT	Expert consensus	**Approval pending**	Expert consensus
Everolimus	**Approved "nonfunctioning"**	Approved **"nonfunctioning"**	**Approved**
TKI	-	-	**Approved** sunitinib
Other options	Surgery/ablative Locoregional therapy IFN/ Chemotherapy	**Telotristat ethyl (FDA-approved)** Surgery/ablative Locoregional therapy IFN/Bevacizumab	Surgery/ablative Locoregional therapy Chemotherapy/ Bevacizumab

GEP NET, gastroenteropancreatic neuroendocrine tumor; IFN, interferon; LAN 120, lanreotide 120 mg; PRRT, peptide receptor radionuclide therapy; STZ+Fu/DXR, streptozoticin plus 5-fluorouracil/doxorubicin; TKI, tyrosine-kinase inhibitor

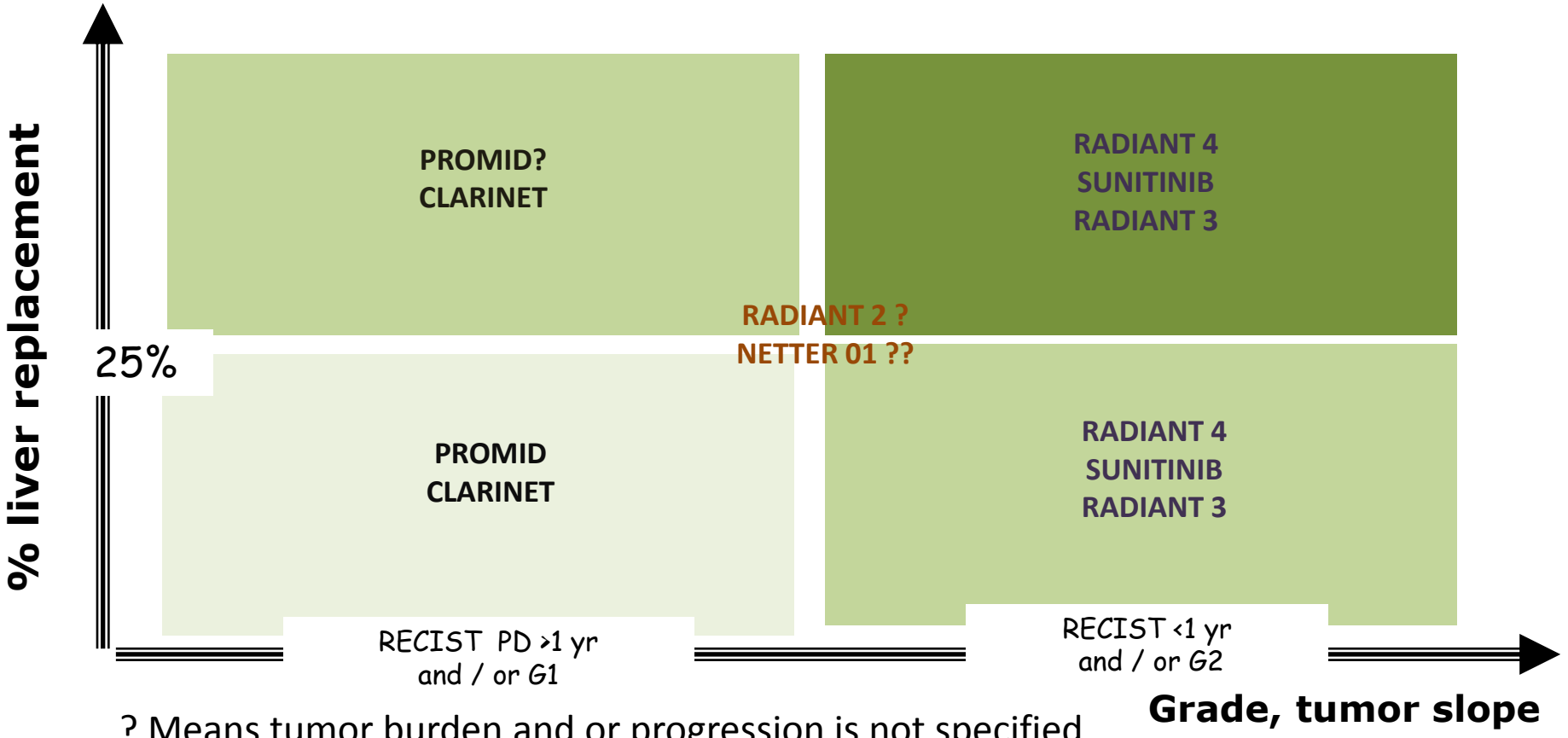
Different strategies of development according to NET primary

Somatostatine analogues (Clarinet)
Everolimus (Radiant 2-4)



Sunitinib Trial
Everolimus (Radiant3)
Octreotide (Promid)
PRRT (Netter 01)

Different strategies of development according to NET tumor burden and Grade/slope



? Means tumor burden and or progression is not specified

Somatostatin is an inhibitor of hormonal secretions

Hypothalamic Polypeptide That Inhibits the Secretion of Immunoreactive Pituitary Growth Hormone

Abstract. *A peptide has been isolated from ovine hypothalamus which, at* 1×10^{-9} *M, inhibits secretion in vitro of immunoreactive rat or human growth hormones and is similarly active in vivo in rats. Its structure is*

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

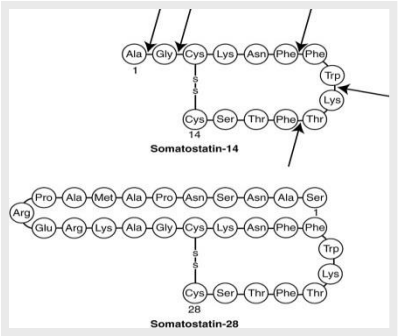
The synthetic replicate is biologically active.

Paul Brazeau, Wylie Vale, Roger Burgus, Nicholas Ling, Madalyn Butcher, Jean Rivier and Roger Guillemin (January 5, 1973)
Science **179** (4068), 77-79. [doi: 10.1126/science.179.4068.77]

Five somatostatin receptors : > 70% NET tumors express at least three sstr by immunohistochemistry which constitute targets for somatostin analogs

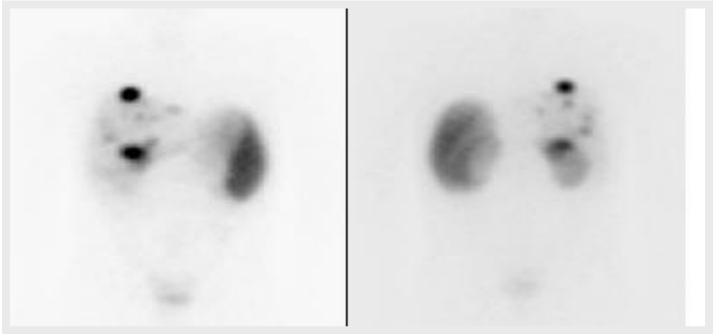
	N pts	sst1	sst2	sst3	sst4	sst5
Asnacios A et al. 2008	60-81	2.5%	63%	28%	38%	63%
Volante M et al. 2007	70	-	79%	44%	-	71%
Nasir A et al. 2006	14	61%	83%	72%	56%	83%
Kulaksiz et al. 2002	104	33%	80%	77%	-	80%
Papotti M et al. 2002	81	-	75%	44%	-	69%

sst 2 >sst1 and 5 > sst 3 and 4



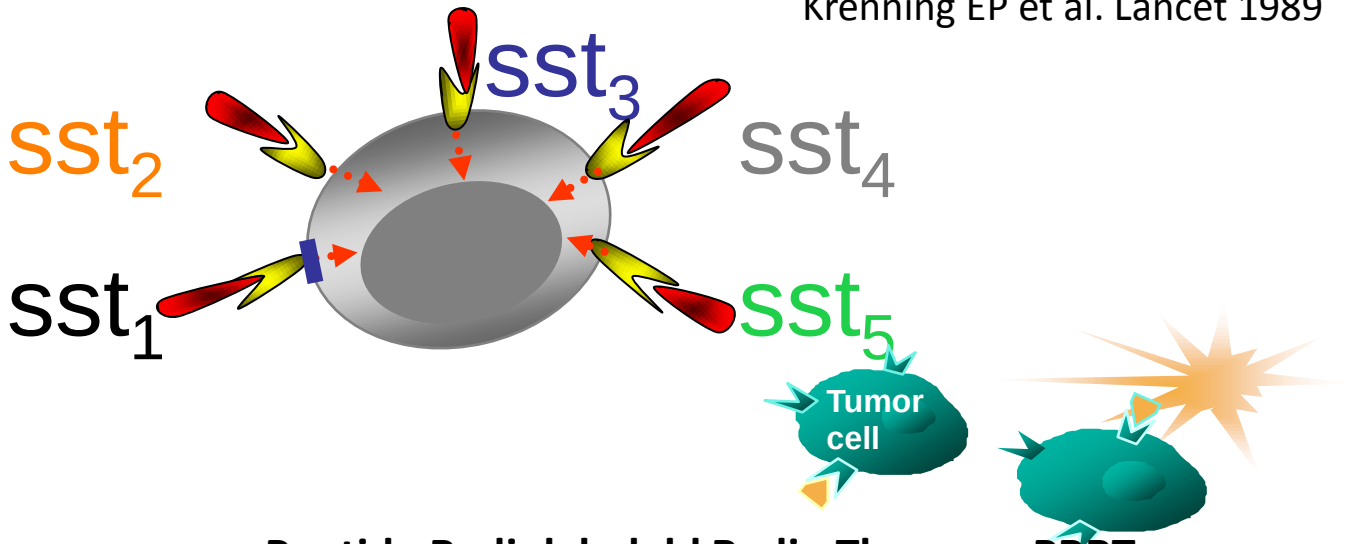
Synthetic SMS analogues

Bauer W et al. Life Science 1982



Imaging : SRS

Krenning EP et al. Lancet 1989



Peptide Radiolabeleld Radio Therapy : PRRT

Otte A et al. EJNM 1997, Kwekkeboom D et al EJNM 2003

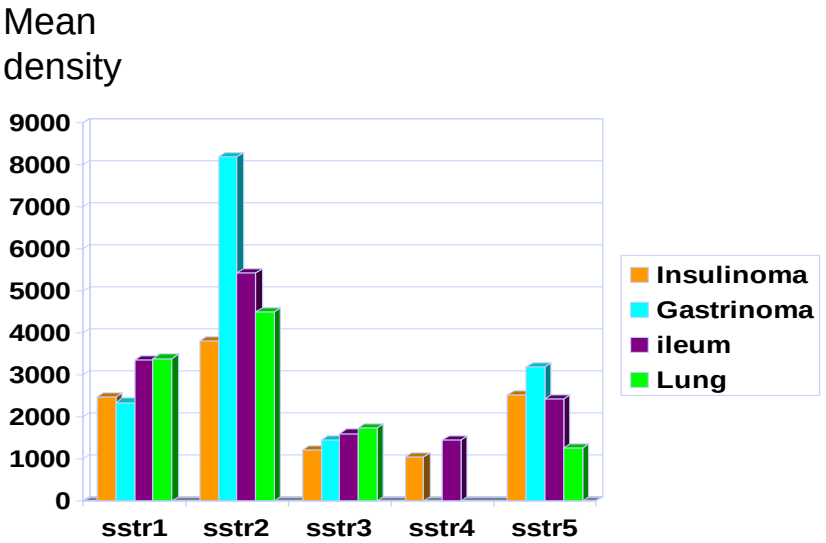
Synthetic derivatives of SMS with high affinity for sst2 > 3-5

Analogs SMS hsst/IC50	1	2	3	4	5
SRIF-14	0.93	0.15	0.56	1.5	0.29
Lanreotide	180	0.54	14	230	17
Octreotide	280	0.38	7.1	$>10^3$	6.3
Pasireotide	9.3	1	1.5	$>10^3$	0.2
In-DTPA-Octreotide	-	22	182	$>10^4$	237
Y-DOTAT-Octreotide	-	11	389	$>10^4$	114
Ga-DOTAT-Ocreotate	-	0.2	$> 10^3$	300	377

Brun C et al. Eur J Endocrinology 2002 / Reubi JC Eur J Nucl Med 2000

Somatostatin receptor expression and density depends on primary and hormonal secretion : autoradiography

Sstr expression	sstr 1	Sstr 2	Sstr 3	Sstr 4	Sstr 5
Insulinoma (26)	52%	69%	35%	4%	20%
Gastrinome (11)	10%	100%	20%	0%	33%
Ileal carcinoid (27)	52%	96%	15%	4%	48%
Lung carcinoid (28)	71%	68%	3%	0%	23%



Reubi JC et al. Eur J Nucl Med 2003 : sstr2>1>5>3-4

Somatostatin receptor scintigraphy posivity rate is affected by Ki 67….and differentiation (Grading)

TABLE 6. Functional Imaging Results Based on Proliferation Index

	SRS		^{123}I-MIBG		^{18}F-FDG	
Ki67 value	Positive	Negative	Positive	Negative	Positive	Negative
<2%	87% (40)	13% (6)	48% (22)	52% (24)	41% (19)	59% (27)
2%–15%	96% (25)	4% (1)	73% (19)	27% (7)	73% (19)	27% (7)
>15%	69% (9)	31% (4)	46% (6)	54% (7)	92% (12)	8% (1)

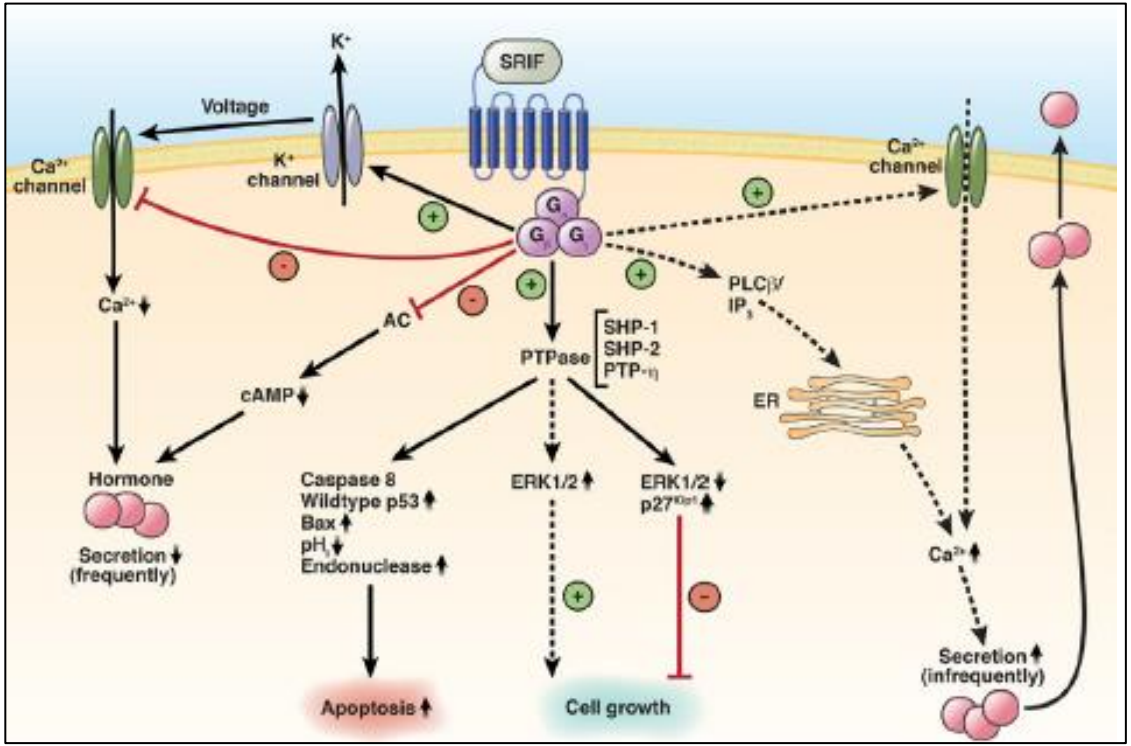
Data in parentheses are numbers of patients.

J Nucl Med 2010; 51:704–712

Binderup T et al

Volante M et al. 2007	***Sstr 2***	***Sstr 3***	***Sstr5***
NET (70 cases)	79%	44%	71%
NEC (18 cases)	44%	17%	28%

SMS analog therapy in NET : preclinical investigation mechanisms of antisecretory and antitumor effect



GASTROENTEROLOGY 2010;139:742–753

SMS analog therapy in NET : mechanisms of antisecretory and antitumor effect

Inhibition of hormone secretion

sst 1, 2, 4, 5

Dimeres sst 2, 3, 5

Heterodimeres sst 5 + D2/sst

Susini et al. Ann Oncol 20006

Inhibition of proliferation

sst 1, 2, 3, 5 (4)

Trigger apoptosis

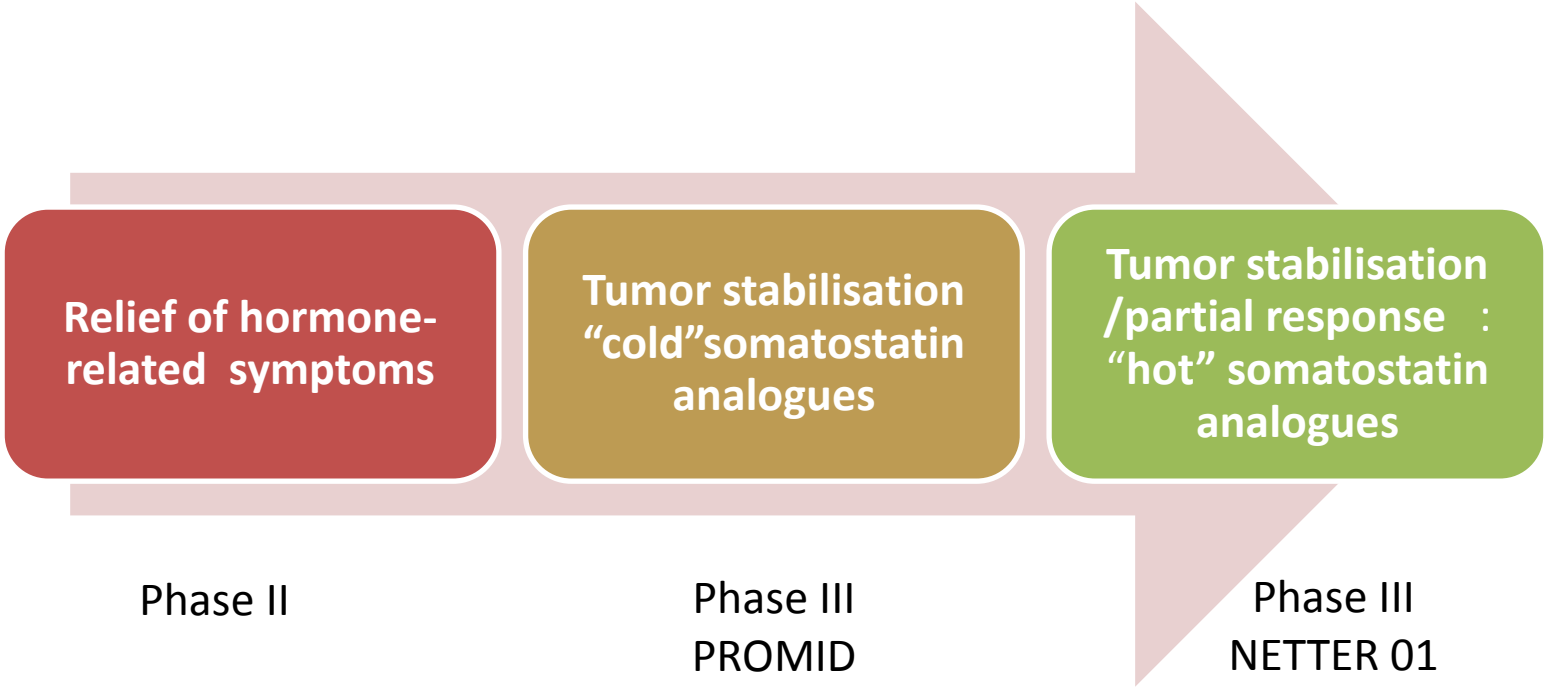
sst 2, 3

Inhibition of growth factor secretion

sst 2, 3, 5

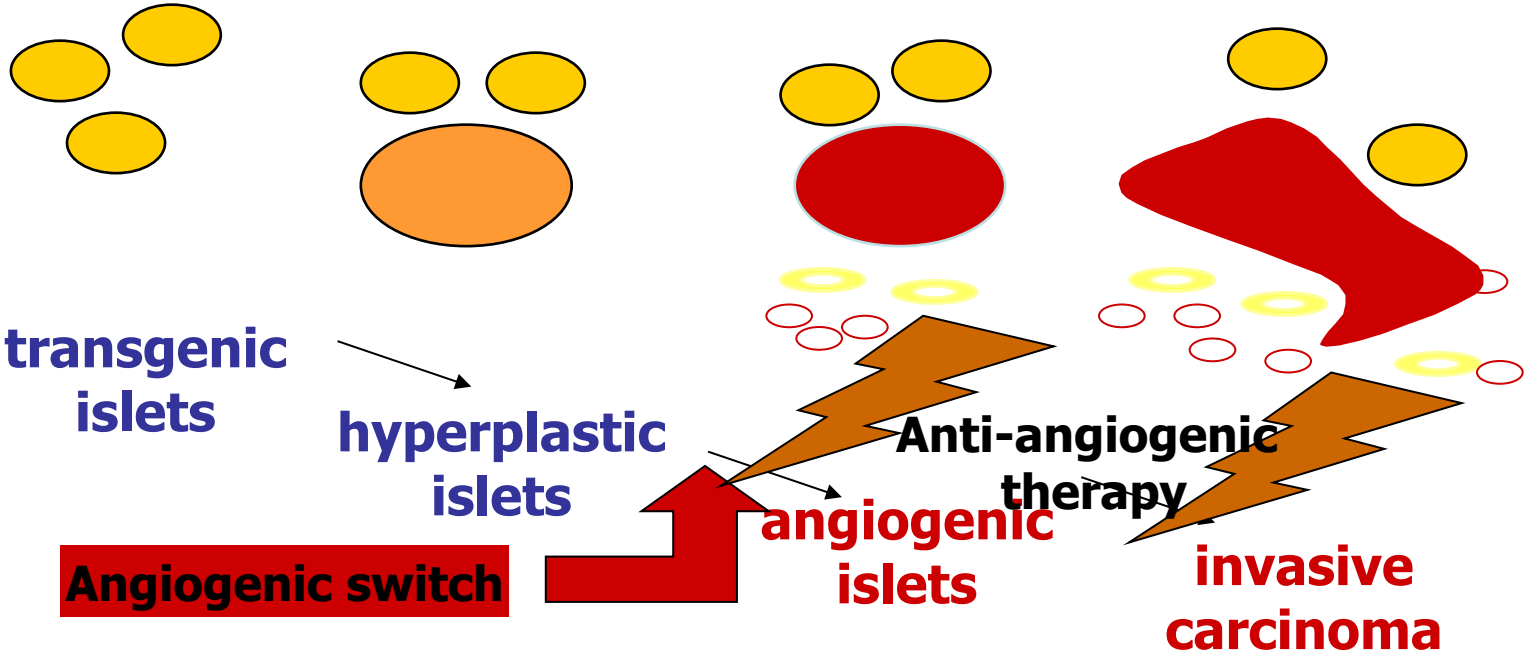
Inhibition of angiogenesis

History of somatostatin analogue therapeutic developement



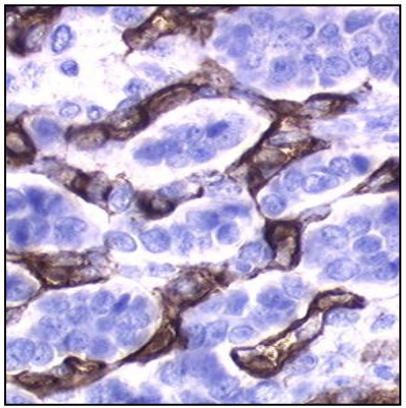
Anti-angiogenesis and endocrine tumors: a strong preclinical rational

Transgenic animal models of beta cell pancreatic tumors (Hanahan)

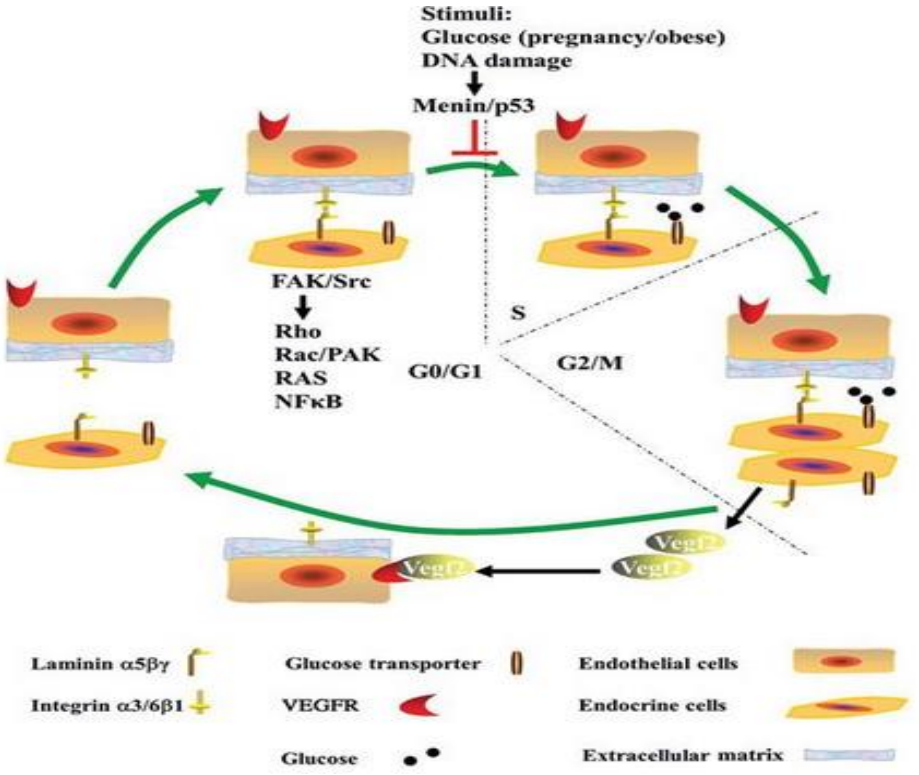


NATURE · VOL 339 · 4 MAY 1989

NET cells are surrounded by an endothelial capillary network which constitutes the extracellular matrix, VEGF is constituvely expressed : pancreatic model



Scoazec courtesy



Zhang J et al JNCI , 2013

Terris B, Scoazec JY et al Histopathology 1998 : expression of VEGF in digestive NETs

Casanovas O et al . Cancer Cell 2005 : paNET also show strong expression of PDGFRs..

“NET paradox “: good prognosis is associated with NETs with high microvascular density (MVD)

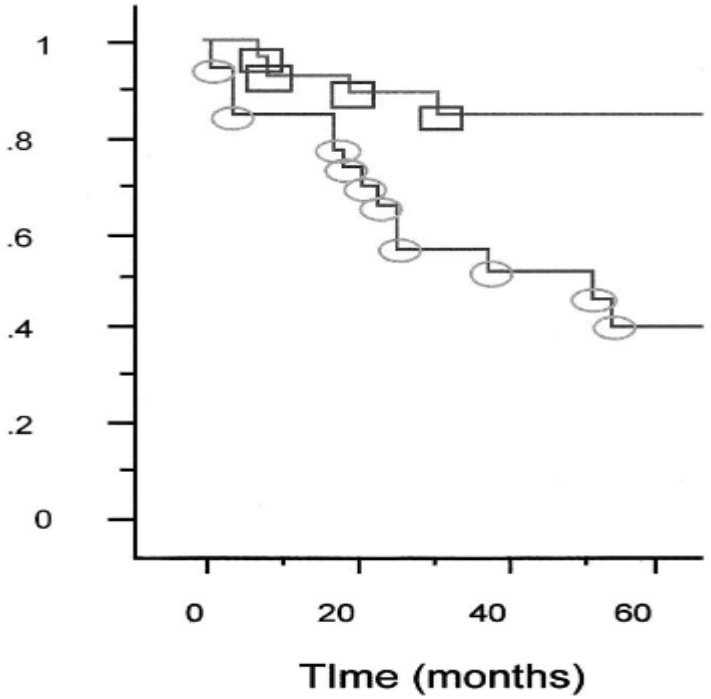


Figure 4. Survival curves (according to Kaplan–Meier) for all the patients of the study group according to intratumoral microvascular density < 30 (open circles) or > 30 (open squares); $P < 0.05$ (log-rank test).

Marion-Audibert et al. Gastro 2003

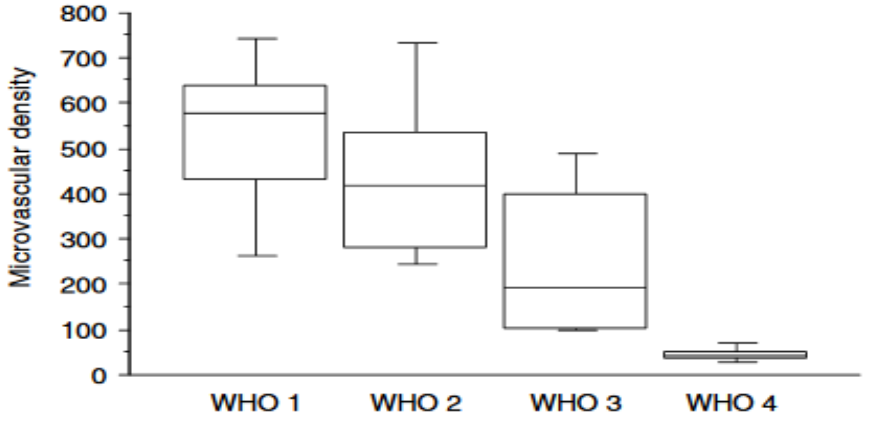


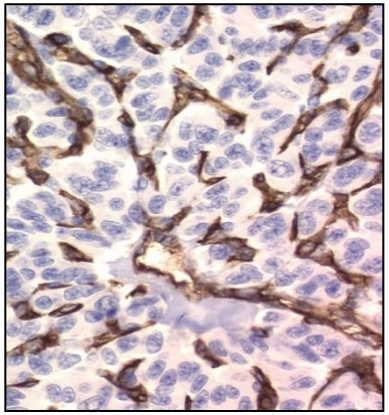
Figure 8 Box plot of MVD recorded in hotspots stratified according to the WHO classification. Differences between groups were statistically significant ($P = 0.0001$).

Couvelard A et al. BJCa2005

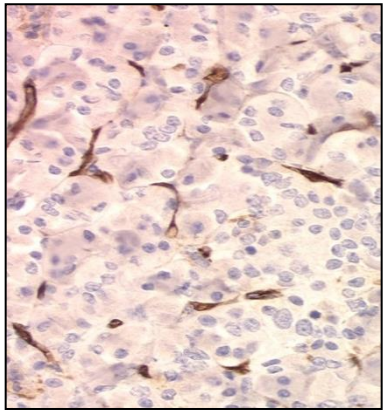
Vascular density in pancreatic endocrine tumors is differentiation dependent

An inverted marker: the most vascular, the least aggressive

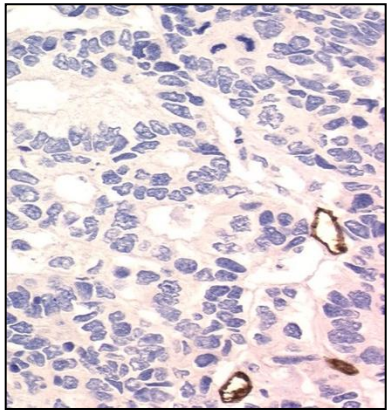
The most vascular, the most differentiated



550 vessels/mm²
Well differentiated tumor, benign



220 vessels/mm²
Well differentiated carcinoma (low grade)



50 vessels/mm²
Poorly differentiated carcinoma (high grade)

Microvascular density

Gastroenterology, 2003;125:1094-1104.

Scoazec JY Neuroendocrinology 2013

Lesson from VHL syndrome-PaNET model : significantly upregalutated genes in VHL vs non VHL (matched)

Gene symbols	Gene definition	patients[a] ($n = 18$)	patients[a] ($n = 16$)	P^b	AUC
HIF-related molecules					
CA9	Carbonic anhydrase IX	480 (1.5–1,907)	14 (0–502)	0.00095	0.843
HIF2A	Hypoxia-inducible factor 2, α subunit	2,393 (319–7,325)	593 (183–14,081)	0.0012	0.835
GLUT	Glucose transporter 1	448 (72–2,423)	138 (20–558)	0.005	0.781
Angiogenesis					
CDH5	VE-cadherin (vascular endothelium)	308 (144–1,151)	83 (18–768)	0.00027	0.878
VEGFR1	FLT1-vascular endothelial growth factor	716 (175–2,267)	235 (64–1,117)	0.0003	0.865
EDNRA	Endothelin receptor type A	213 (33–521)	38 (0–290)	0.0003	0.861
ANGPT2	Angiopoietin 2	148 (41–2,844)	26 (0–450)	0.00048	0.863
CD34	CD34 molecule	105 (29–433)	35 (17–114)	0.0009	0.833
VEGFR2	KDR-kinase insert domain receptor	275 (111–593)	122 (34–342)	0.005	0.781
VEGFA	Vascular endothelial growth factor A	2,688 (734–8,922)	1,118 (181–4,847)	0.008	0.767
ANGPT1	Angiopoietin 1	0.19 (0–2.96)	0 (0–0.61)	0.03	0.725
EMT					
VIM	Vimentin	7,047 (1,840–22,231)	2,110 (19–31,838)	0.00055	0.859
Metastasis-related genes					
LAMA4	Laminin α 4	447 (128–1,459)	176 (47–930)	0.0019	0.813
CXCR4	Chemokine (c-x-c) receptor 4	90 (19–257)	38 (16–1,157)	0.021	0.733
Growth factors and receptors					
PDGFB	Platelet-derived growth factor beta polypeptide	369 (55–1,050)	67 (21–2,485)	0.00048	0.863
IRS1	Insulin receptor substrate 1	209 (65–472)	134 (27–3,092)	0.011	0.765
ERBB1	Epidermal growth factor receptor (EGFR)	259 (10–1,388)	124 (3.8–4,063)	0.016	0.759
Cell cycle					
CCND1	Cyclin D1	2,515 (569–11,322)	559 (336–12,386)	0.0009	0.843
CDKN2A	Cyclin-dependent kinase inhibitor 2A	2.4 (0–6)	0.44 (0–3.38)	0.003	0.799

Speisky D et al Clin Ca Res 2012

VHL inactivation occurs also in 24 % of sporadic paNETs : Schmitt AM et al. ERC 2009

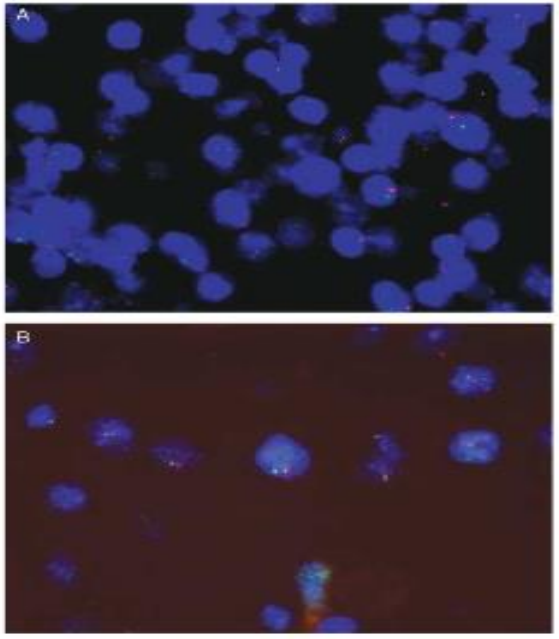


Figure 2 FISH shows a deletion of the 3p25 gene locus (red signal) and of the centromere of chromosome 3 (green signal) in most of the tumor cells in these two PET.

VHL deletion by FISH:
14/78 NETs (18%)

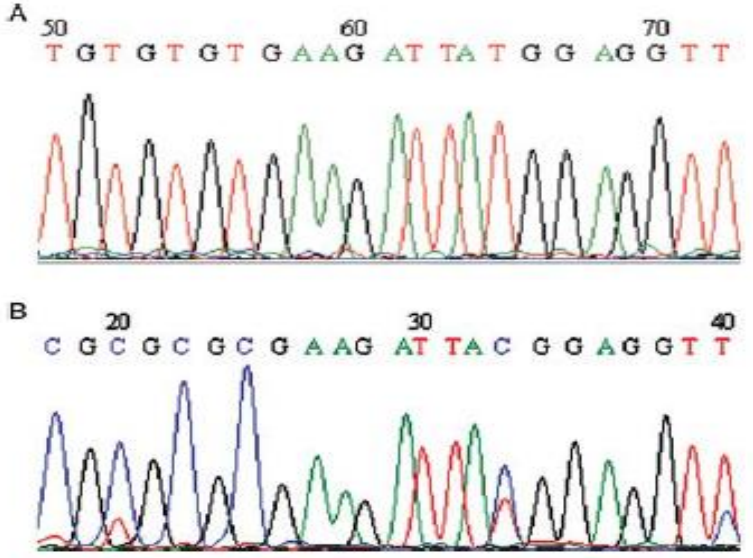


Figure 3 Sequence analysis of the largest CpG island of the VHL promoter: a PET without (A) and one of the two PET with methylation (B). Note that the conversion of cytosine to uracil by bisulfite treatment in the unmethylated tumor in contrast to the methylated tumor, in which all cytosine residues are retained.

Hypermethylation of VHL promoteur :
2/35 pts (6%)

Are genes of the angiogenic pathway mutated ?

Table 3. Commonly mutated genes in pancreatic neuroendocrine tumors in Taiwanese, Chinese, and Caucasian cohorts

Study	Current study (Taiwanese cohort) n = 40	Chinese cohort[7] n = 37	Caucasian cohort[6] n = 68
ATRX	11 (27.5%)	13 (35.1)	12 (17.6%)
MEN1	11 (27.5%)	13 (35.1)	30 (44.1%)
ASCL1	11 (27.5%)	n/s	0
TP53	8 (20%)	5 (13.5%)	2 (2.9%)
mTOR	8 (20%)	n/s	0
ARID1A	8 (20%)	n/s	0
VHL	8 (20%)	15 (40.5%)	0
NF1	7 (17.5)	n/s	0
TSC2	7 (17.5%)	16 (43.2%)	6 (8.8%)
DAXX	6 (15%)	11 (29.7)	17 (25%)
ANGPT2	5 (12.5%)	n/s	0
PIK3CA	3 (7.5%)	n/s	1 (1.5%)
PTEN	3 (7.5%)	7 (18.9%)	5 (7.4%)

n/s: no sequencing

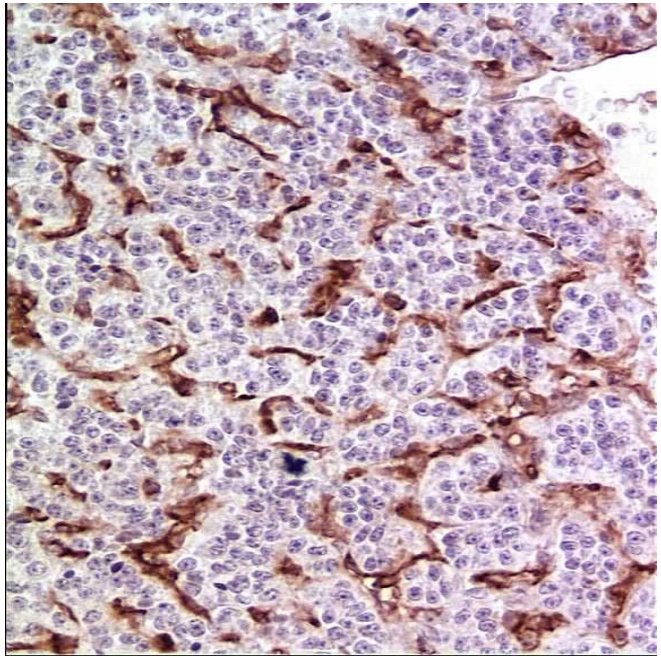
Table 4. Mutation frequencies in cellular pathways in pancreatic neuroendocrine tumors in Taiwanese, Chinese, and Caucasian cohorts

Study	Current study (Taiwanese cohort) n = 40	Chinese cohort[7] n = 37	Caucasian cohort[6] n = 68
MEN1 pathway	48%	35%[a]	44%
DAXX/ATRX	38%	54%	43%
TP53 pathway	20%	14%[b]	3%
VHL pathway	45%	41%[c]	0%
mTOR pathway	48%	54%[d]	15%

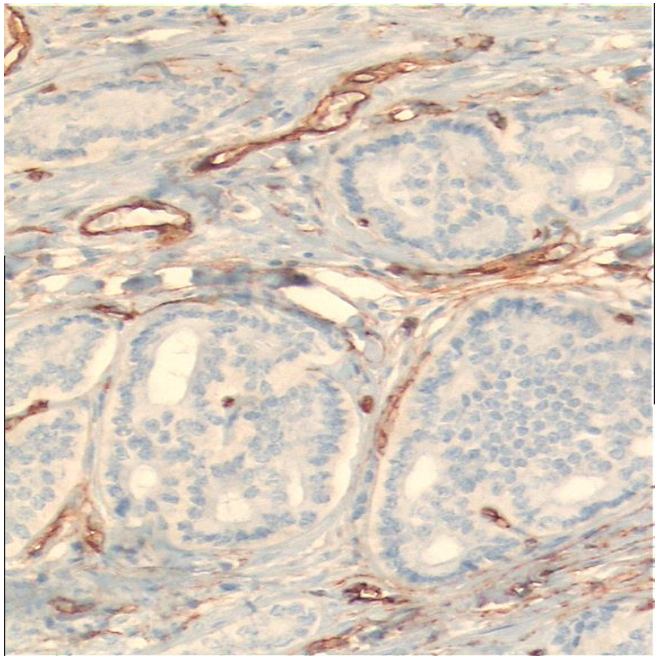
[a]sequencing MEN1 gene only, [b]sequencing TP53 only, [c]sequencing VHL gene only, [d]sequencing PTEN and TSC2 genes only.

Jiao I Science 2011 : 68 pa NETs, 41% stage IV, 45% G1
Yuan F et al. IJBS 2014 : 37 paNETs, 8% stage IV, 59% G1
Chou WC et al. IJBS 2016 : 40 paNETs ; 32% stage III/IV, 77%G1

Vascular architecture in GEP endocrine tumors is site-specific



Pancreas



Ileum : lower MVD,
vessels in the stroma/not admixed,
a few vessels penetrate tumor nests

Scoazec JY et al NE 2013

Biomarkers in RADIANT 3 : not predictive !

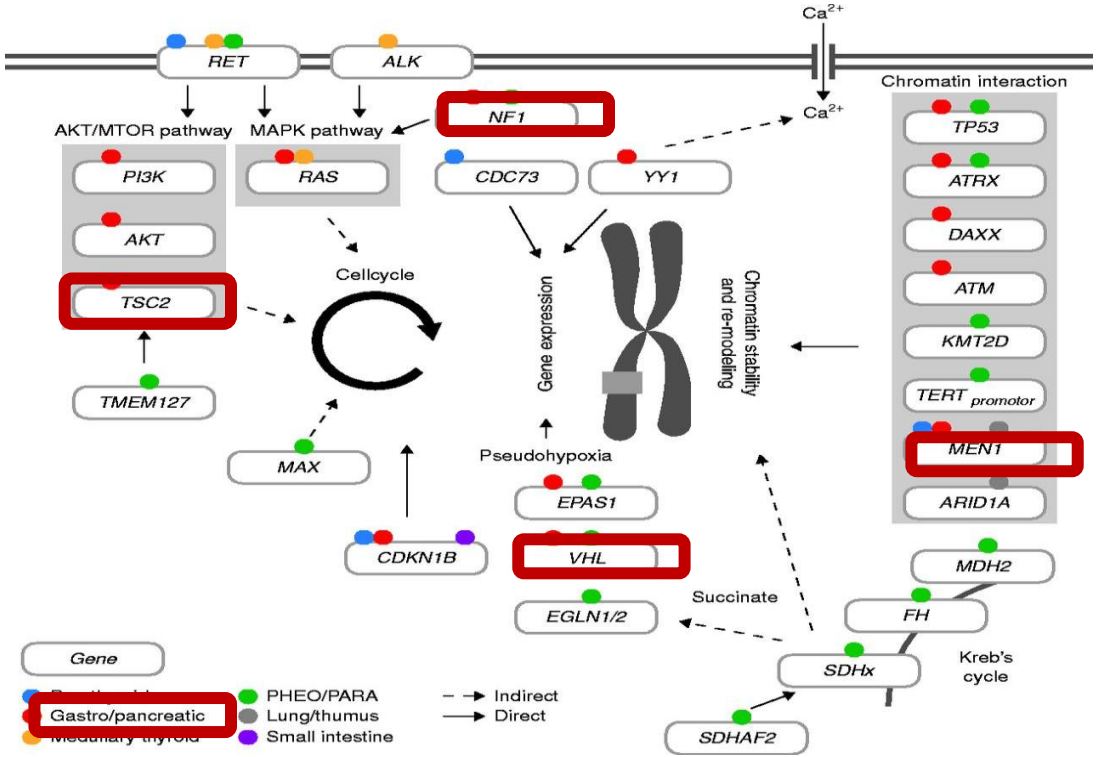
Table A4. Median OS of Subgroups Defined by CgA (2× ULN as cutoff), NSE (1× ULN as cutoff), PlGF, sVEGFR1, sVEGFR2, VEGF, bFGF (median values as cutoff)

Biomarker	Subgroup	No. Patients	No. Events	Median OS (95% CI), months
CgA	High	191	141	27.76 (22.34 to 33.41)
	Low	215	112	57.2 (47.05 to 62.59)
NSE	High	107	86	16.1 (13.57 to 22.08)
	Low	290	158	52.9 (43.1 to 60.91)
PlGF	High	197	140	27.83 (22.24 to 34.53)
	Low	196	105	55.26 (47.61 to 62.59)
sVEGFR-1	High	197	133	30.29 (22.24 to 39.33)
	Low	112	196	50.23 (40.87 to 58.58)
sVEGFR-2	High	195	122	34.76 (28.45 to 47.61)
	Low	195	121	43.83 (39.29 to 51.06)
VEGF-A	High	197	128	30.72 (23.75 to 39.56)
	Low	196	117	49.77 (40.87 to 56.15)
bFGF	High	197	122	37.68 (30.49 to 51.06)
	Low	196	123	42.41 (35.12 to 49.77)

Abbreviations: bFGF, basic fibroblast growth factor; CgA, chromogranin A; NSE, neuron-specific enolase; OS, overall survival; PlGF, placental growth factor; sVEGFR1 and 2, soluble vascular endothelial growth factor receptor 1 and 2; VEGF-A, vascular endothelial growth factor A; ULN, upper limit of normal.

Yao 2016

NETs occur as part four of inherited syndromes (MEN1, VHL, TSC, NF1) : activation of pathways including AKT/MTOR



Joakim Crona, and Britt Skogseid Eur J Endocrinol 2016;174:R275-R290: genes and pathways involved in NET tumorigenesis

PI3K/Akt as a prominent mediator of physiological function regulation through tumor mass regulation (proliferation/apoptosis pathways)

Regulation of pancreatic β-cell growth and survival by the serine/threonine protein kinase Akt1/PKBα

ROBYN L. TUTTLE[1], NAVDEEP S. GILL[2], WILLIAM PUGH[4], JEAN-PYO LEE[4], BRIGITTE KOEBERLEIN[3], EMMA E. FURTH[2], KENNETH S. POLONSKY[4], ALI NAJI[3] & MORRIS J. BIRNBAUM[1]

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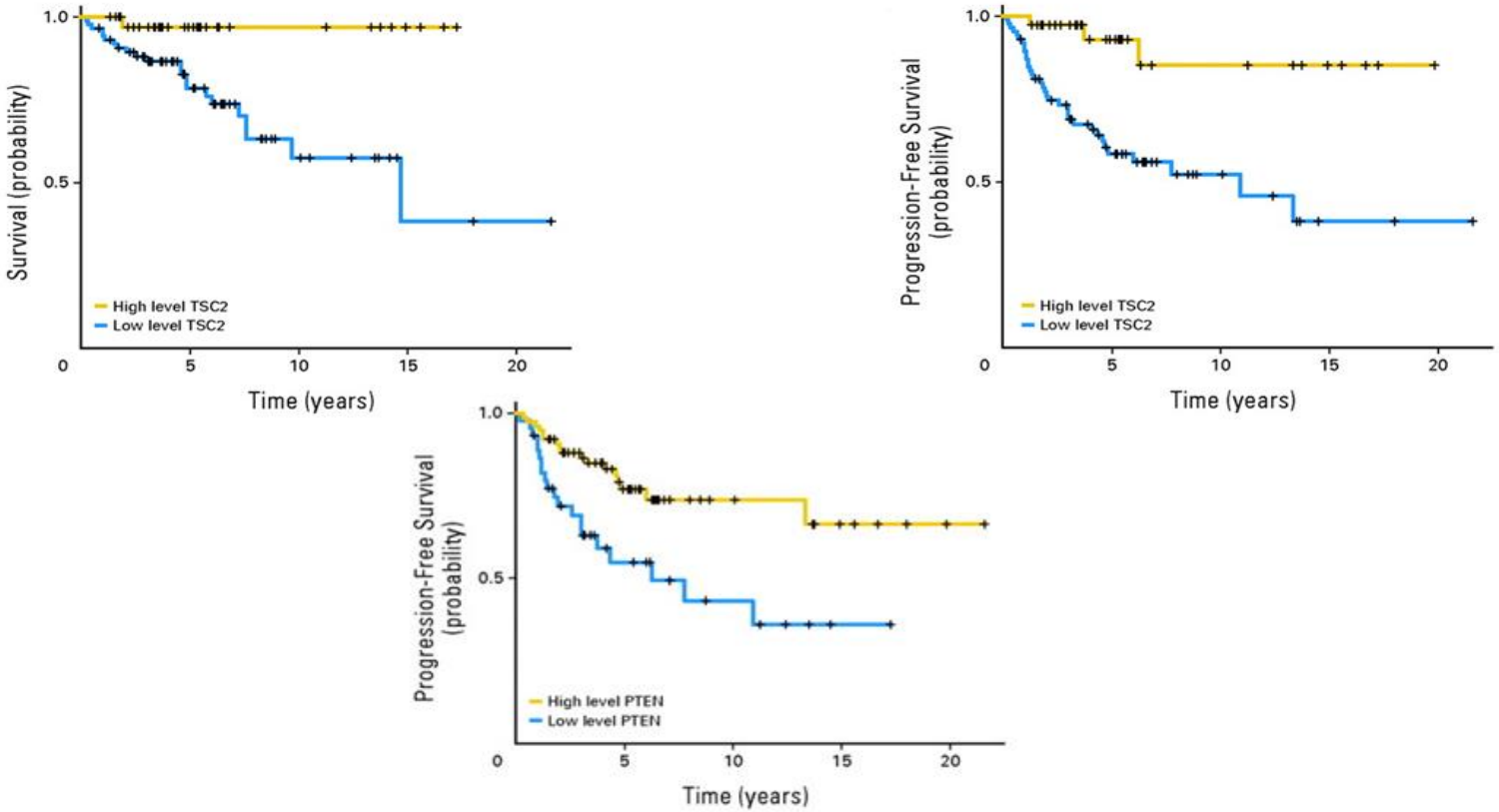
The physiological performance of an organ depends on an interplay between changes in cellular function and organ size, determined by cell growth, proliferation and death. Nowhere is this more evident than in the endocrine pancreas, where disturbances in function or mass result in severe disease. Recently, the insulin signal-transduction pathway has been implicated in both the regulation of hormone secretion from β cells in mammals as well as the determination of cell and organ size in *Drosophila melanogaster*. A prominent mediator of the actions of insulin and insulin-like growth factor 1 (IGF-1) is the 3′-phosphoinositide–dependent protein kinase Akt, also known as protein kinase B (PKB). Here we report that overexpression of active Akt1 in the mouse β cell substantially affects compartment size and function. There was a significant increase in both β-cell size and total islet mass, accompanied by improved glucose tolerance and complete resistance to experimental diabetes.

Tuberous sclerosis 2 (TSC2) protein expression and phosphatase and tensin homolog (PTEN) protein expression : correlation with survival in pancreatic endocrine tumors (PETs).



Missiaglia E et al. JCO 2010;28:245-255 : 141 pts, 71% low / intermediate expression of TSC2, 58% low expression of TSC2 : NS at MV

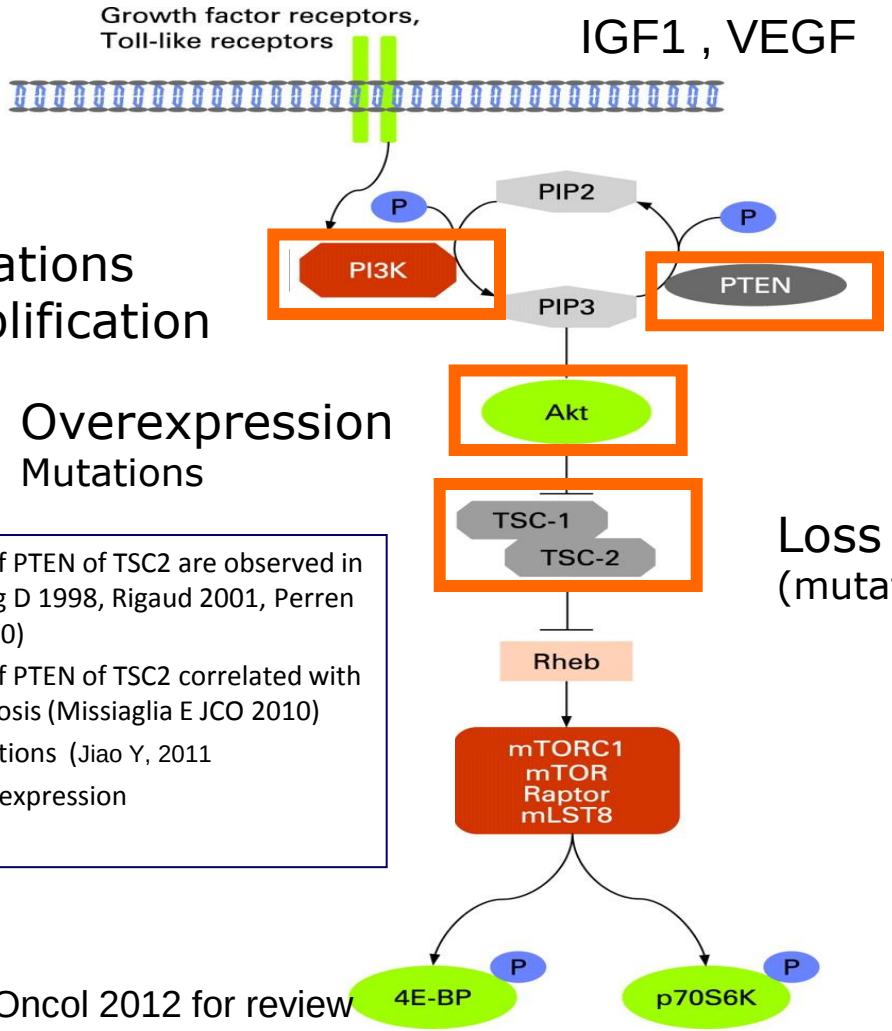
Genetic alterations in pancreatic NETS

Genetic alterations in PanNETs and potential targeted therapies

Gene	Mutation frequency (%)*	Protein function	Targeted therapy
MEN1	44	Histone remodeling	NA
DAXX	25	Chromatin assembly	NA
ATRX	18	Chromatin assembly	NA
TSC2	9	GTPase-activating protein	Everolimus, sirolimus, temsirolimus
PTEN	7	Dual-specificity protein phosphatase	Everolimus, sirolimus, temsirolimus
PIK3CA	1	Phosphoinositide 3-kinase	Everolimus, sirolimus, temsirolimus
TP53	5	Cell-cycle arrest	NA

Voie mTOR

Jiao Y, Shi C, Edil BH, et al: DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors.
Science 331:1199-203, 2011



Mutations
Amplification

Overexpression
Mutations

Loss of fonction
(mutations, deletions,
LOH, hypermethylation)

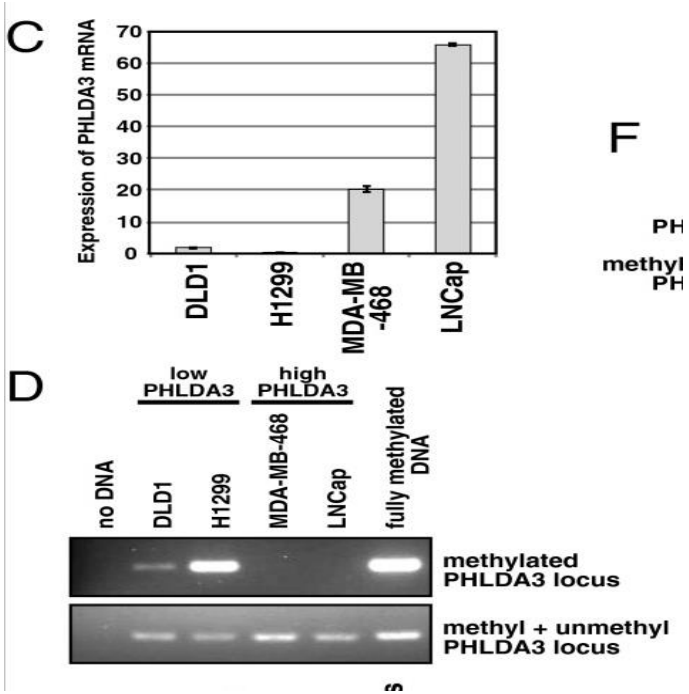
Loss of fonction
(mutations, methylation)

- Loss/dowregulation of PTEN of TSC2 are observed in 10-60% of NET (Chung D 1998, Rigaud 2001, Perren 200O, Missiaglia E 2010)
- Loss/dowregulation of PTEN of TSC2 correlated with pancreatic NET prognosis (Missiaglia E JCO 2010)
- TSC2, PTEN,PI3K mutations (Jiao Y, 2011
- VEGF, IGF1 et Rc IGF1 expression

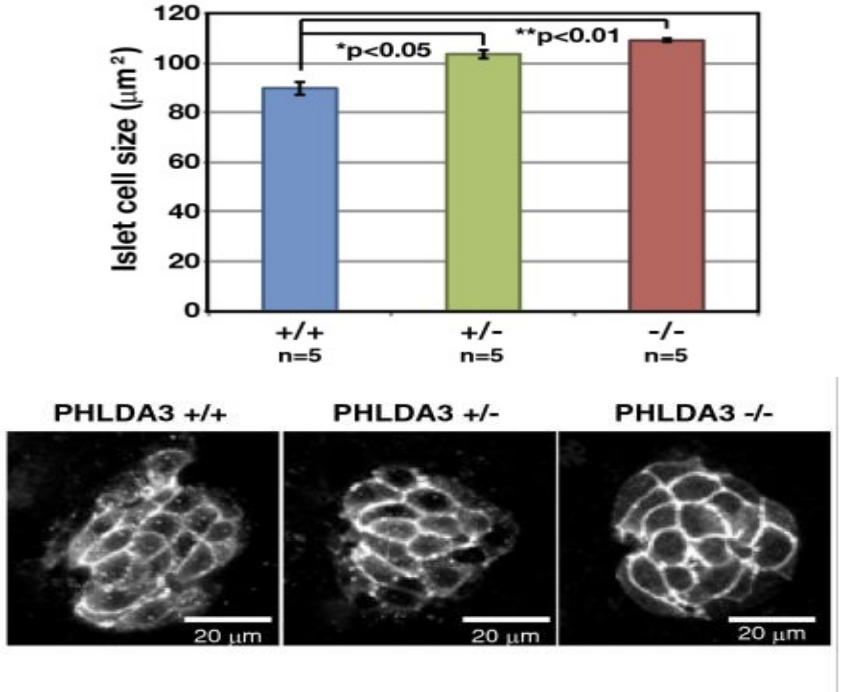
mTOR pathway is activated in NET

Cingarlini S et al Target Oncol 2012 for review

Alternative model : PHLDA3 (Akt supressor) is inactivated in 72% of paNETs (54 pts) and cell lines



LOH and hypermethylation causes mRNA down regulation of PHLDA3 in cell lines



PHLDA3 deficiency is associated with increase cell size

Ohki R et al. 2014

Are genes of the mTOR pathway mutated ?

Table 3. Commonly mutated genes in pancreatic neuroendocrine tumors in Taiwanese, Chinese, and Caucasian cohorts

Study	Current study (Taiwanese cohort) n = 40	Chinese cohort[7] n = 37	Caucasian cohort[6] n = 68
ATRX	11 (27.5%)	13 (35.1)	12 (17.6%)
MEN1	11 (27.5%)	13 (35.1)	30 (44.1%)
ASCL1	11 (27.5%)	n/s	0
TP53	8 (20%)	5 (13.5%)	2 (2.9%)
mTOR	8 (20%)	n/s	0
ARID1A	8 (20%)	n/s	0
VHL	8 (20%)	15 (40.5%)	0
NF1	7 (17.5)	n/s	0
TSC2	7 (17.5%)	16 (43.2%)	6 (8.8%)
DAXX	6 (15%)	11 (29.7)	17 (25%)
ANGPT2	5 (12.5%)	n/s	0
PIK3CA	3 (7.5%)	n/s	1 (1.5%)
PTEN	3 (7.5%)	7 (18.9%)	5 (7.4%)

n/s: no sequencing

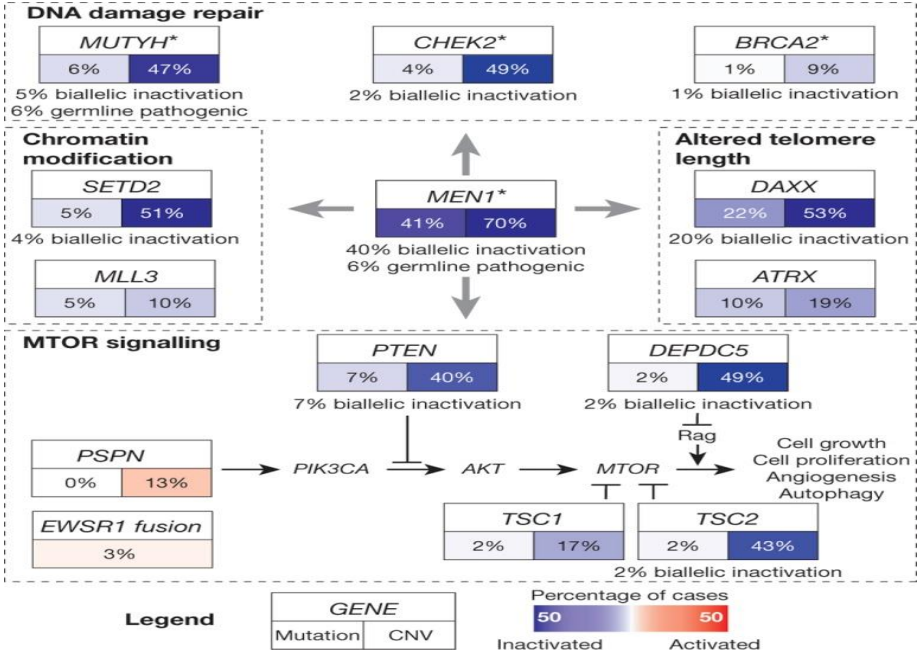
Table 4. Mutation frequencies in cellular pathways in pancreatic neuroendocrine tumors in Taiwanese, Chinese, and Caucasian cohorts

Study	Current study (Taiwanese cohort) n = 40	Chinese cohort[7] n = 37	Caucasian cohort[6] n = 68
MEN1 pathway	48%	35%[a]	44%
DAXX/ATRX	38%	54%	43%
TP53 pathway	20%	14%[b]	3%
VHL pathway	45%	41%[c]	0%
mTOR pathway	48%	54%[d]	15%

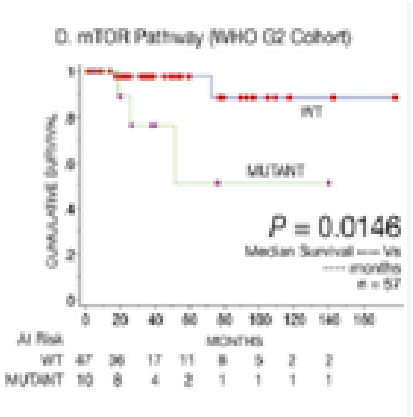
[a]sequencing MEN1 gene only, [b]sequencing TP53 only, [c]sequencing VHL gene only, [d]sequencing PTEN and TSC2 genes only.

Jiao I Science 2011 : 68 pa NETs, 41% stage IV, 45% G1
Yuan F et al. IJBS 2014 : 37 paNETs, 8% stage IV, 59% G1
Chou WC et al. IJBS 2016 : 40 paNETs ; 32% stage III/IV, 77%G1

Whole genome landscape of pancreatic NETs



mTOR patway alteration
1.12% mutations : PTEN, TSC2,TSC1, et DEPDC5
2.16% of EWSR1 fusion or PSPN
CN alterations



A Scarpa *et al. Nature* 1–7 (2016) :The frequency of somatic mutations and copy number change are shown for key genes

Genomic landscape of small intestin is different

CDKN1B : 10% of cases

Somatic mutation of *CDKN1B* in small intestine neuroendocrine tumors

Joshua M Francis[1,2,18], Adam Kiezun[1,18], Alex H Ramos[1,2,17,18], Stefano Serra[3], Chandra Sekhar Pedamallu[1,2], Zhi Rong Qian[2], Michaela S Banck[4,5], Rahul Kanwar[4], Amit A Kulkarni[4], Anna Karpathakis[6,7], Veronica Manzo[2], Tanupriya Contractor[8], Juliet Philips[2], Elizabeth Nickerson[1], Nam Pho[1], Susanne M Hooshmand[2], Lauren K Brais[2], Michael S Lawrence[1], Trevor Pugh[1], Aaron McKenna[1], Andrey Sivachenko[1], Kristian Cibulskis[1], Scott L Carter[1], Akinyemi I Ojesina[1,2], Samuel Freeman[2], Robert T Jones[9], Douglas Voet[1], Gordon Saksena[1], Daniel Auclair[1], Robert Onofrio[1], Erica Shefler[1], Carrie Sougnez[1], Jonna Grimsby[1], Lisa Green[1], Niall Lennon[1], Tim Meyer[6,7], Martyn Caplin[7], Daniel C Chung[10,11], Andreas S Beutler[4,5], Shuji Ogino[2,12,13], Christina Thirlwell[6,7], Ramesh Shivdasani[2], Sylvia L Asa[3,14], Chris R Harris[8,15,16], Gad Getz[1], Matthew Kulke[2] & Matthew Meyerson[1,2,9]

NATURE GENETICS VOLUME 45 | NUMBER 12 | DECEMBER 2013

The genomic landscape of small intestine neuroendocrine tumors

Michaela S. Banck,[1,2] Rahul Kanwar,[1] Amit A. Kulkarni,[1] Ganesh K. Boora,[1] Franziska Metge,[1] Benjamin R. Kipp,[3] Lizhi Zhang,[3] Erik C. Thorland,[3] Kay T. Minn,[1] Ramesh Tentu,[1] Bruce W. Eckloff,[4] Eric D. Wieben,[4] Yanhong Wu,[4] Julie M. Cunningham,[4] David M. Nagorney,[5] Judith A. Gilbert,[6] Matthew M. Ames,[6] and Andreas S. Beutler[1,2]

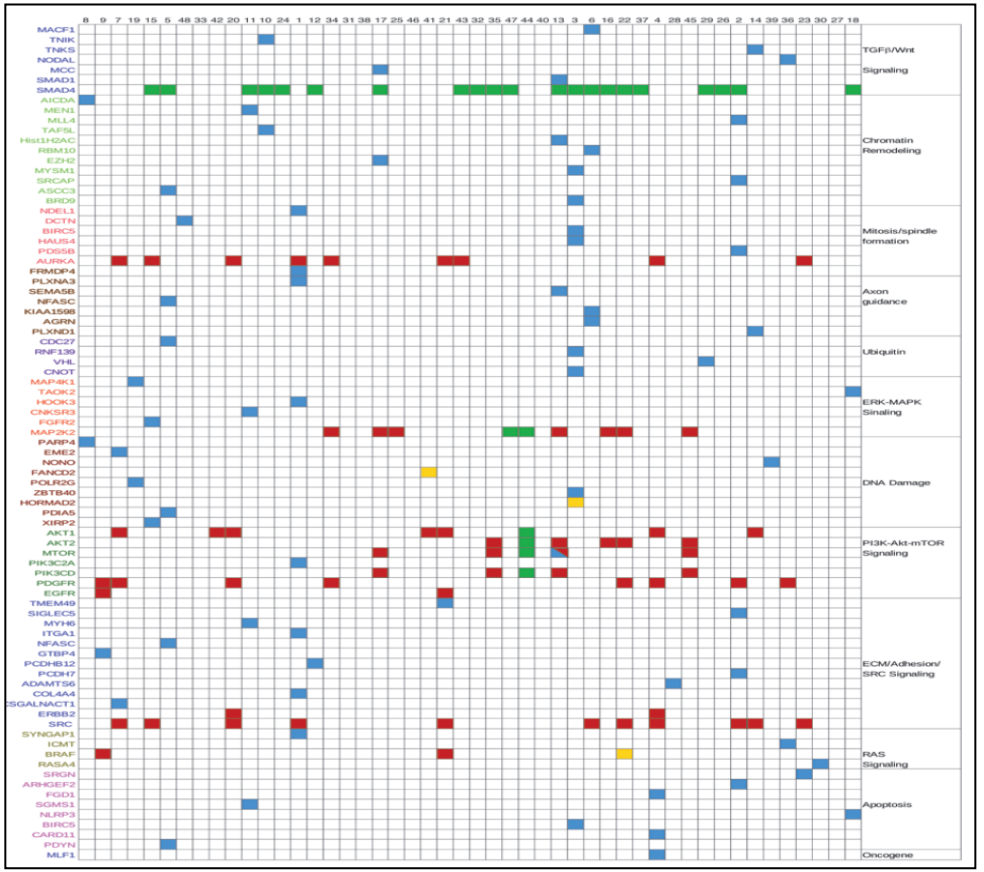
AKT/mTOR pathway: 16/48 (33%)

Other pathways: 35/48

PDGFR, EGFR, HSP90

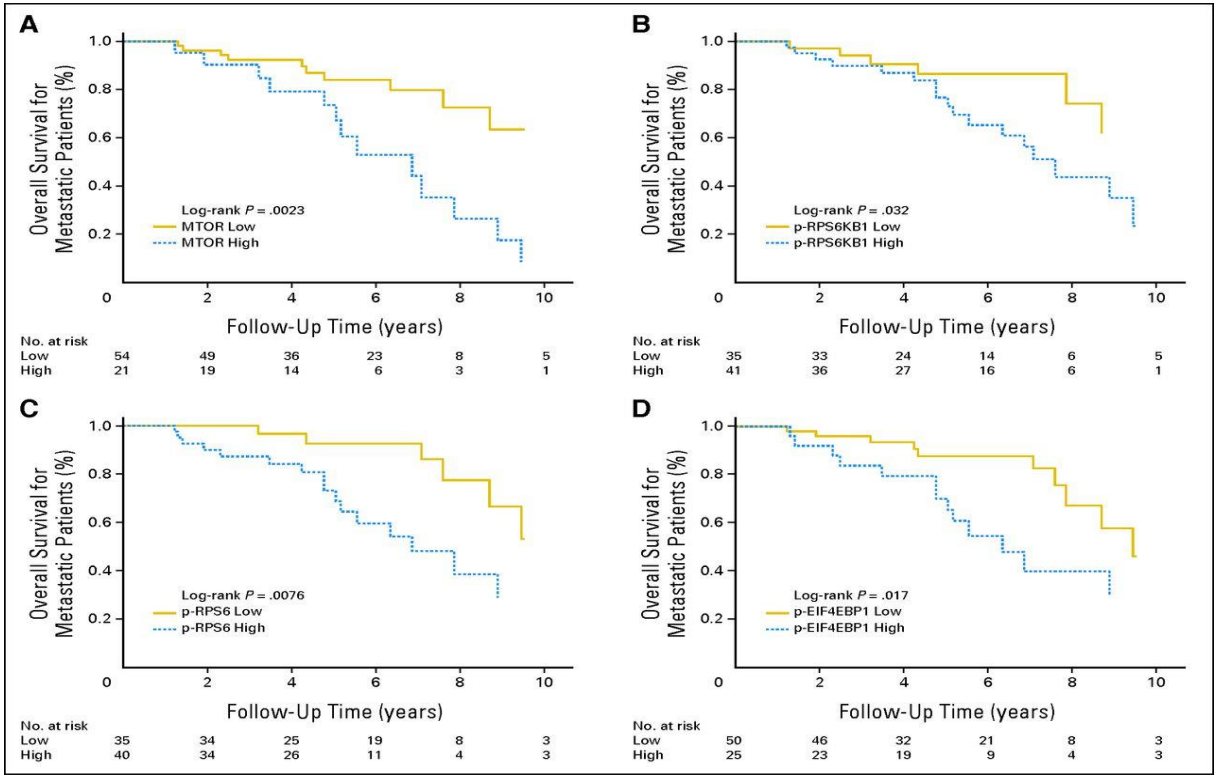
SRC, SMAD, AURKA

J Clin Invest. 2013;123(6):2502–2508.



mTOR pathway activation in small intestin is different:

mTOR and its activated downstream mTOR targets (but not PTEN, TSC2) play a prognostice role (OS) in metatastatic small intestinal NET



Zhi Rong Qian et al. JCO 2013;31:3418-3425

Kasajima A 2011 : strong p6K expression correlates with short DSS in 39 stage IV ileum NETs

Conclusions

- Three "druggable" hypotheses led to positive phase III trials based on gain in PFS/TTP with placebo as a control arm
  - Mainly stabilisation
  - No prognostic / predictive role of each target has been confirmed or demonstrated
- Role and mechanisms of activated pathways/targets in NETS are primary specific
- Biology of NET tumors is increasingly understood but still insufficiently "integrated" : frequency of activation, first vs secondary event, organ specificity, NET characteristics, microenvironment…
- New hypotheses and models together with active translational research expected

Thank you

1 informative patient = 1 informative sampling