Technische Universität München

Project B8

MENX rats: a unique platform for translational studies of neuroendocrine tumors (NETs)

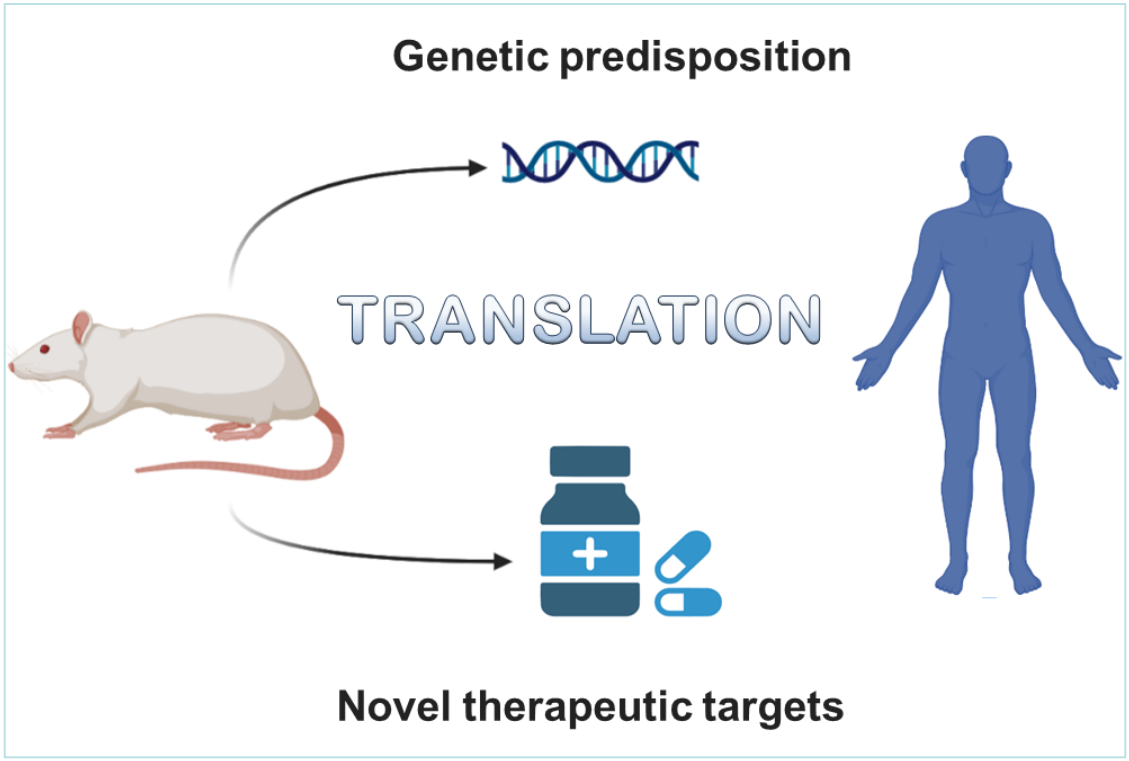
Sebastian Gulde [1], Hermine Mohr [1], Rickmer Braren [2], Johannes Notni [3], Franz Schilling [4], Katja Steiger [3], Natalia S. Pellegata [1]

1. Institute for Diabetes and Cancer, Helmholtz Zentrum München; 2. Institute for diagnostic and interventional Radiology, TUM; 3, Institute of Pathology, School of medicine, TUM; 4. Department of Nuclear Medicine, School of Medicine, TUM.

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

IDC Institute for Diabetes and Cancer

HELMHOLTZ
RESEARCH FOR GRAND CHALLENGES



Gulde S. and Pellegata NS. (2021) *CDKN1B* (p27) defects leading to pituitary tumors, in *Gigantism and Acromegaly 1st Edition: Genetics, Diagnosis, and Treatment*. Ed: C. Stratakis. Publisher: Elsevier..
Mohr H and Pellegata NS. (2017) Animal models of MEN1. Endocr Relat Cancer 24:T161-T177.
Wiedemann T and Pellegata NS. (2015) Animal models of multiple endocrine neoplasia. Mol Cell Endocr, S0303-7207:30013-7.
Lee M and Pellegata NS. (2013) Multiple endocrine neoplasia syndromes associated with mutation of p27. J Endocrinol Invest. 36:781-7.
Lee M and Pellegata NS (2013) MEN4, in *Genetic Endocrine Tumor Syndromes*, Frontiers of Hormone Research, Ed. C. Stratakis, 41:63-78.
Pellegata NS (2012) MENX. Annales d'Endocrinologie, 73:65-70.
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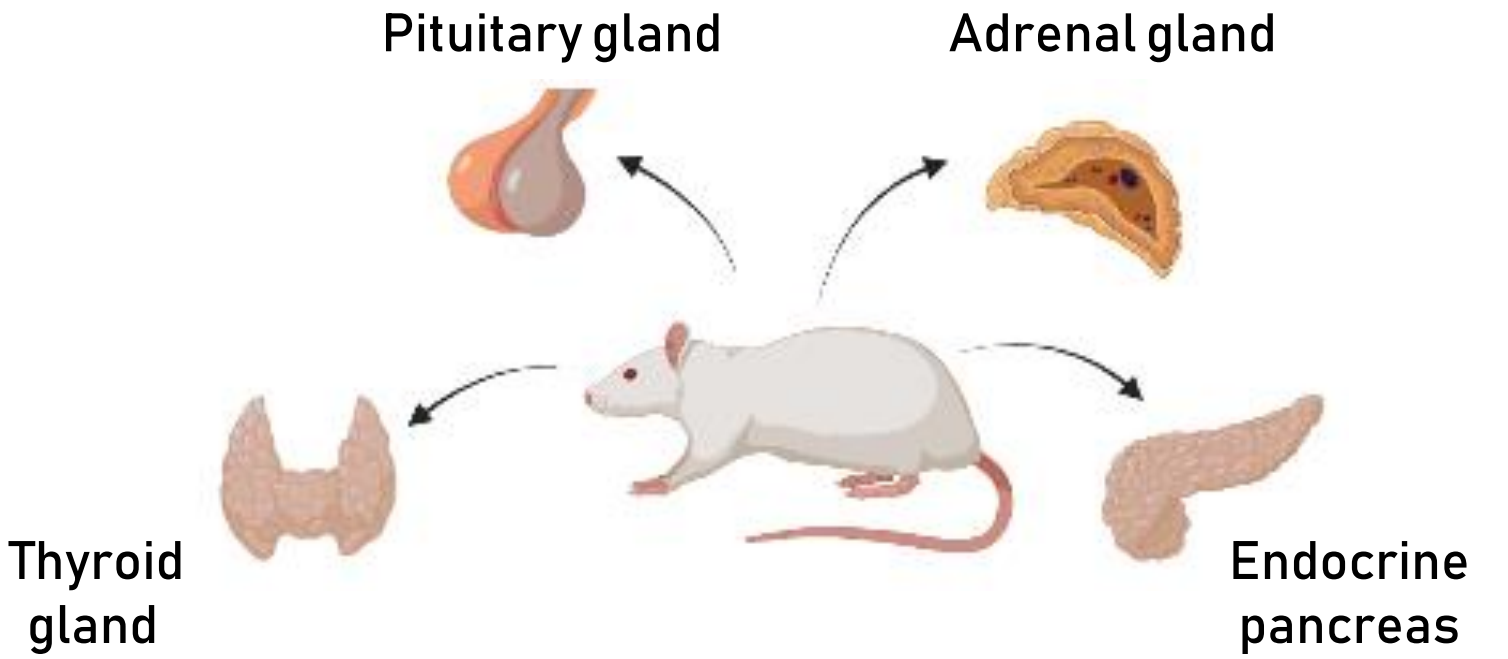
Marinoni I and Pellegata NS. (2010) p27, a novel MEN gene? Neuroendocrinology, 93: 19-28.
Molatore S and Pellegata NS The MENX syndrome and p27: relationships with multiple endocrine neoplasia, in *Progress in Brain Research*: *Neuroendocrinology 2010*.

MENX rats: a model of multiple NETs

phenotype

- Multi-tumor syndrome that spontaneously developed in a rat strain
- MENX affected rats have a loss-of-function mutation in p27
- Pituitary and adrenal tumors develop in 100% of rats by 7-8 months (progression)



Clinical issues to solve for NETs

- Based on the new WHO classification from 2017, all NETs have the potential to become malignant and therefore incurable.

1st question:

how can we predict which tumor will become malignant?

→ New biomarkers

2nd question:

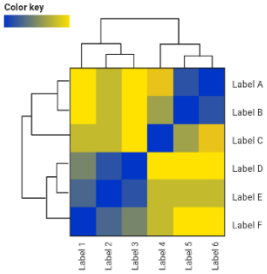
which treatments can work for unresectable/aggressive tumors?

→ New therapeutic targets/therapies

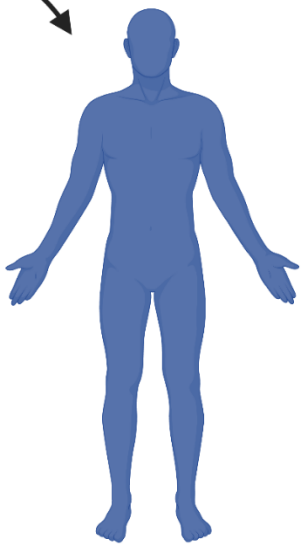
OMICS
Molecular markers

miRNA

mRNA

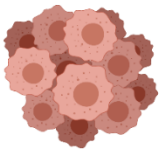


Validation in human NETs

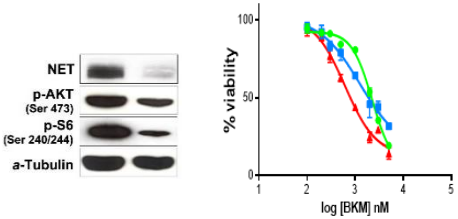


TRANSLATION

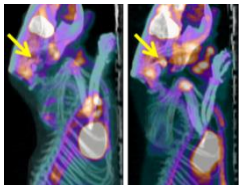
Therapeutic targets



In vitro drug testing

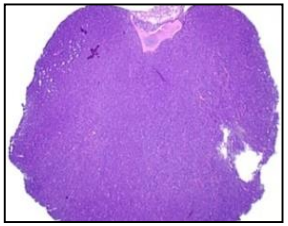


In vivo drug testing



Rat NETs

MENX rats tumor spectrum



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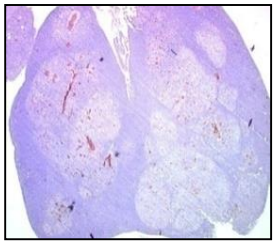
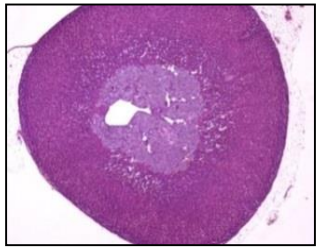


MUT

Pituitary tumors
(multifocal)
100%

WT



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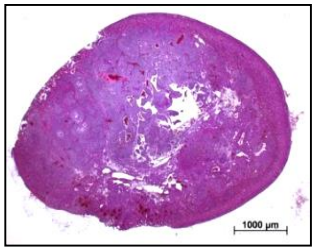
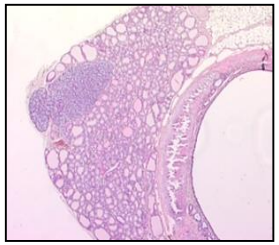


Pheochromocytoma
(bilateral)
100%

WT



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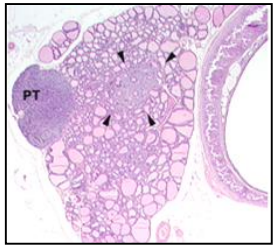
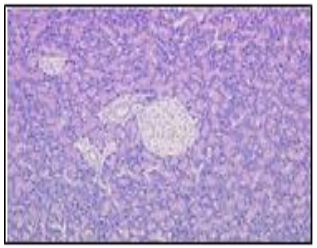


C-cell hyperplasia
(bilateral) 86%



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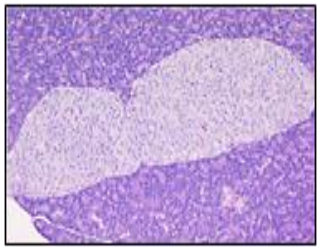


MUT

Islet cell hyperplasia
90%

Hermine Mohr

Sebastian Gulde

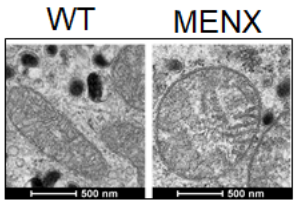
Imaging and treatment of pheochromocytoma (PCCs)

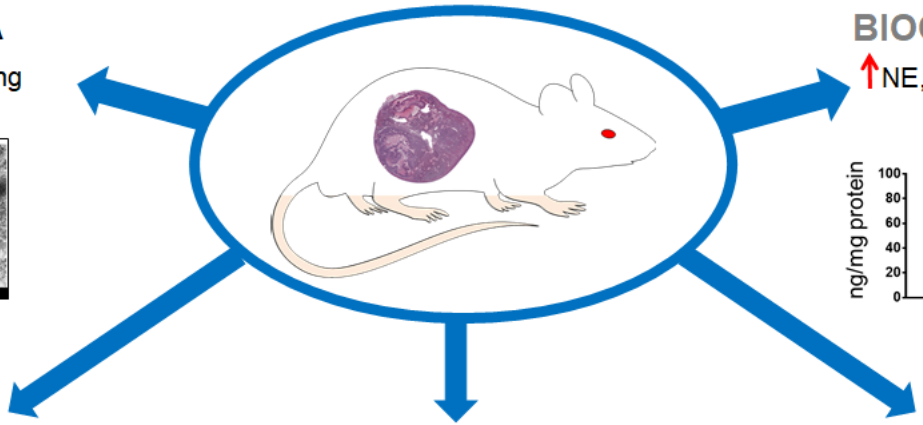
Katja Steiger, Simone Ballke

MENX rats: the only spontaneous, endogenous model of pseudohypoxic PCC (>aggressive subtype)

MITOCHONDRIA

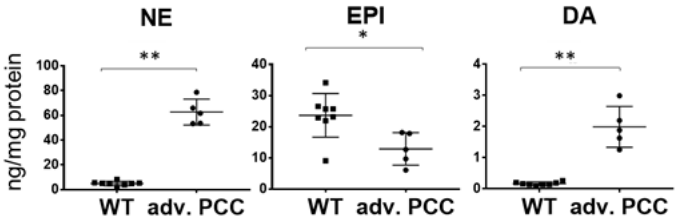
Loss of Cristae, swelling





BIOCHEMICAL

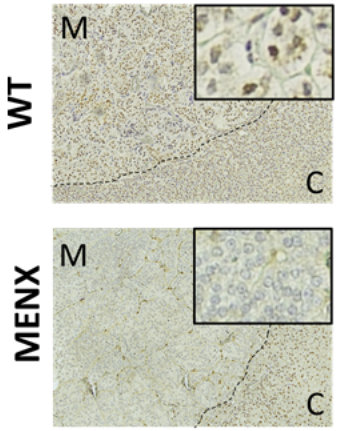
↑NE, ↓EPI, ↓DA



EPIGENETIC

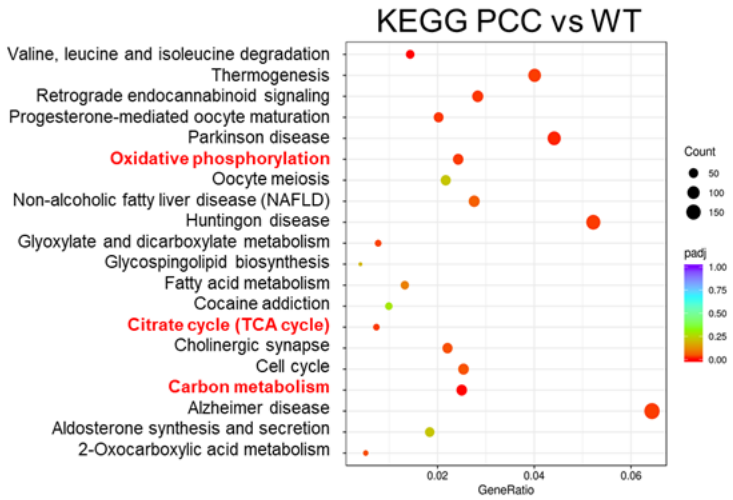
↑ Hypermethylation

5-hmC



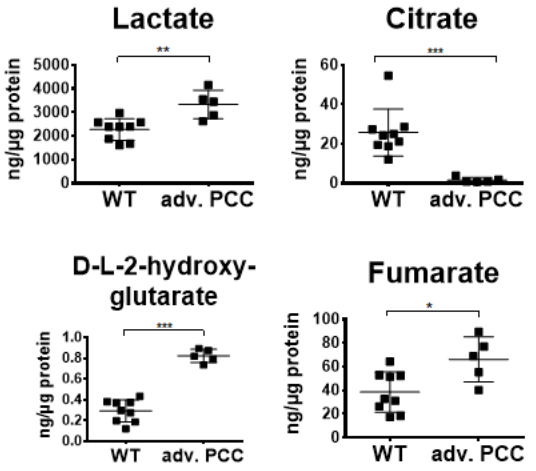
TRANSCRIPTOME

↑OXPHOS, ↑TCA cycle



CELL METABOLISM

↑ Aerobic Gycolysis

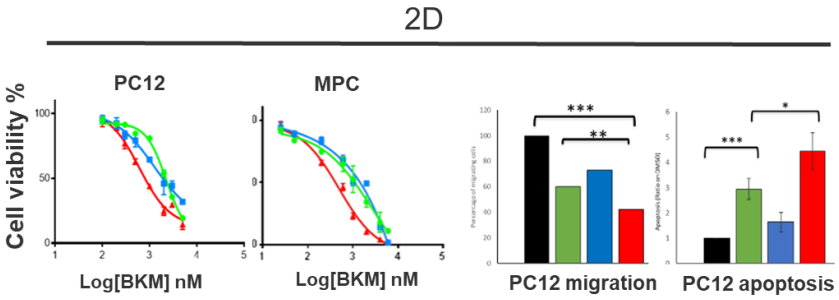


Mohr et al. Cancers 2021; 13:126

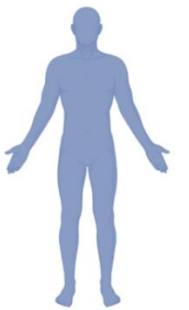
Targeting PI3K and CDK4/6 as effective therapeutic option for PCCs

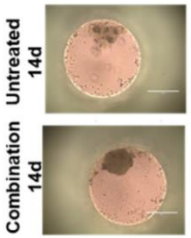
In vitro:

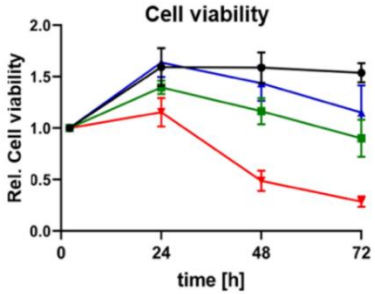
PCC cell lines (2D and 3D)

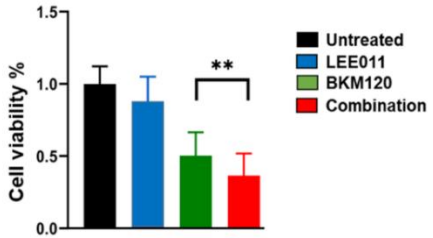


Primary PCC cultures (3D)



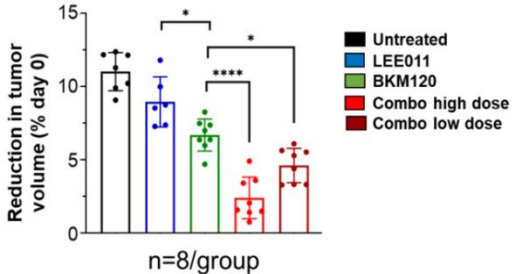






In vivo:

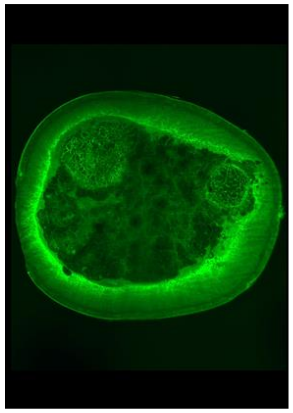
Xenografts of PC12 cells



Manuscript in preparation

Imaging MENX pheochromocytoma (PCC)

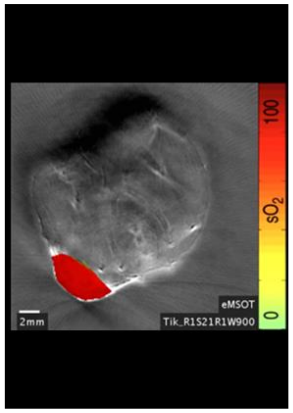
LSM



Advantages:
- Very high resolution
- Immunostaining possible

Disadvantages:
- Only one timepoint

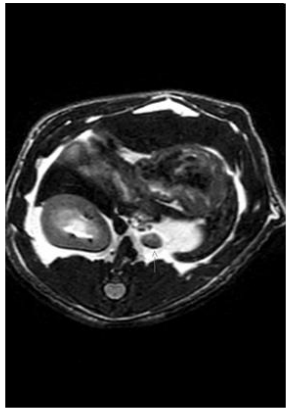
MSOT



Advantages:
- Oxygen saturation
- Compatibility with contrast agents and fluorophores
- Detection of difficult tissue as fat & blood

Disadvantages:
- Limited tissue penetration

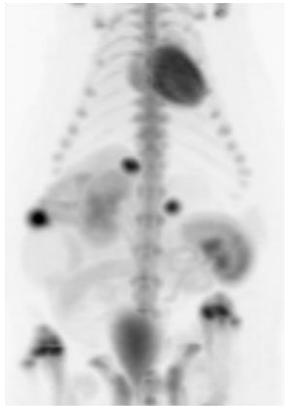
MRI



Advantages:
- High spatial resolution
- No tissue penetrating limit

Disadvantages:
- Relatively low sensitivity
- High cost
- Long imaging time

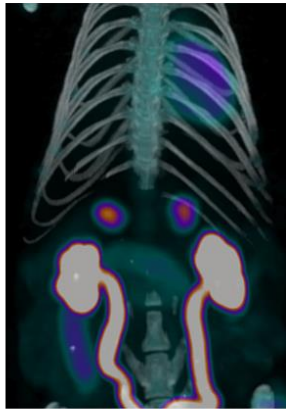
PET



Advantages:
- High sensitivity
- No tissue penetrating limit
- Quantitative
- Whole-body scanning

Disadvantages:
- Radiation risk
- High cost

PET/CT

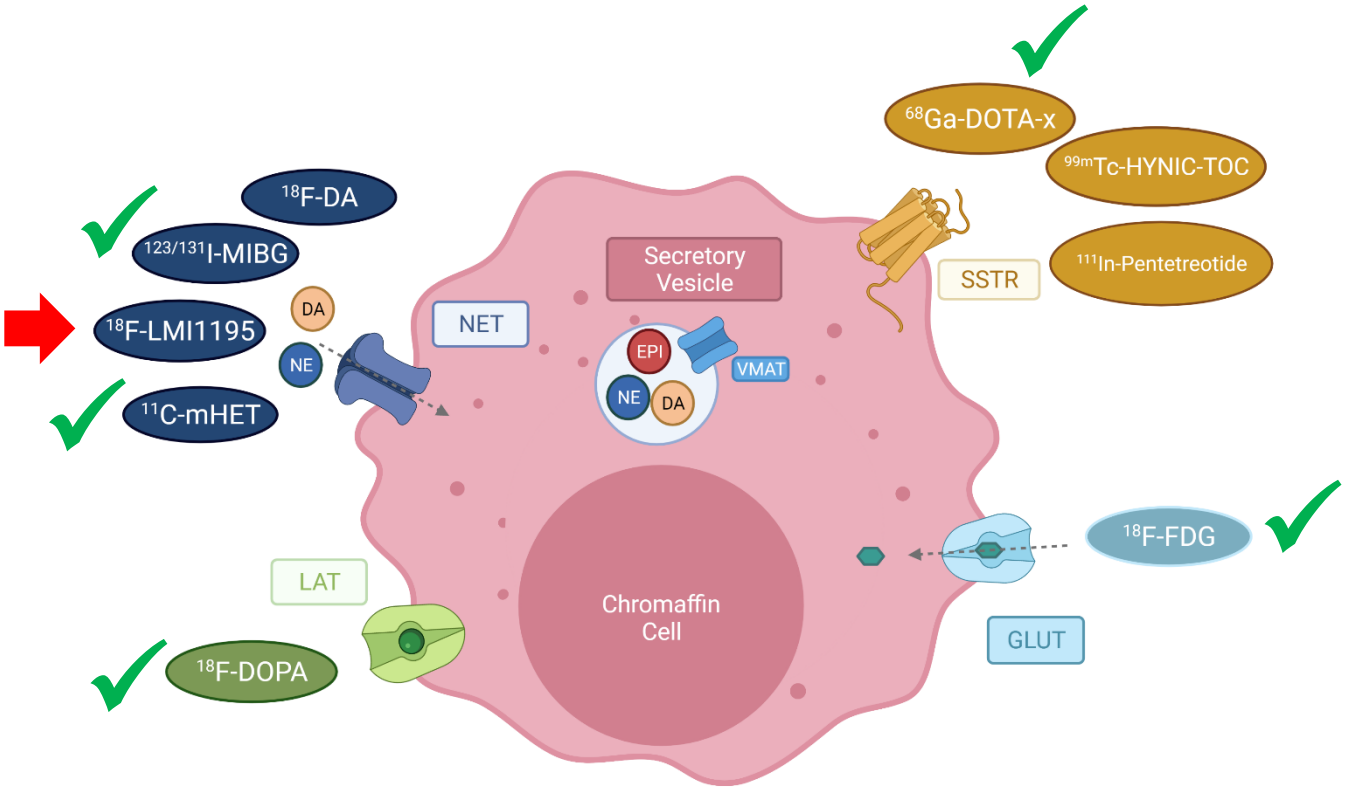


Advantages:
- High sensitivity
- Combination of anatomical and functional images
- Quantitative
- Whole-body scanning

Disadvantages:
- Radiation risk
- High cost

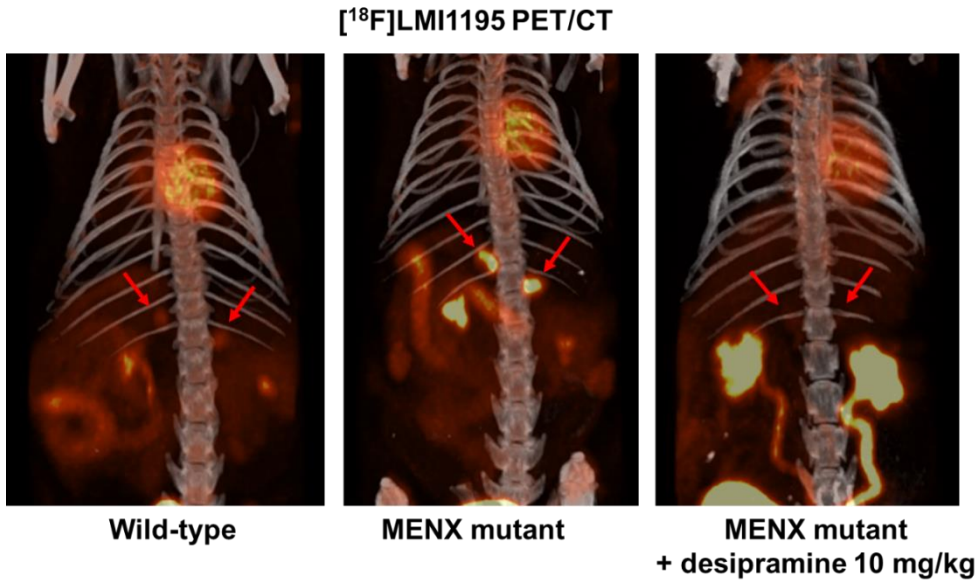
Mohr H, Steiger K, Ballke S, Schilling F, Pellegata NS. Imaging pheochromocytoma in small animals – preclinical models to improve diagnosis and treatment. EJNMMI Res. *Submitted*

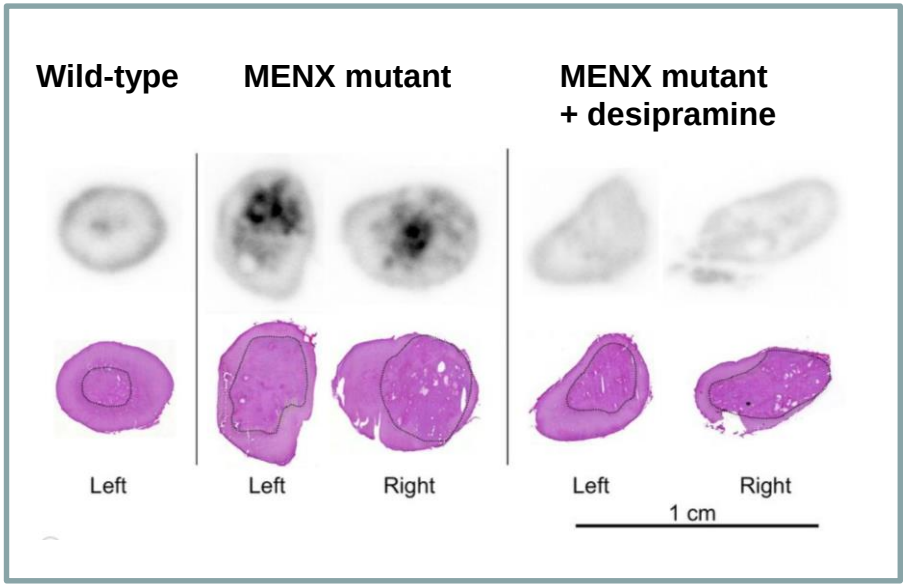
Imaging MENX pheochromocytoma (PCC) -2



Molatore S et al. Proc Natl Acad Sci USA, 2010; 107:18493-8.
Lee M et al. Mol Cancer Ther, 2011; 10:1450-9.
Miederer M et al. Int J Mol Imaging, 2011;175352.
Lee M et al. Mol Endocrinol, 2012;26:1394-405.
Gaertner FC et al. J Nucl Med 2013; 54:2111-7
Leinhäuser I et al. OncoTarget 2015; 6:39111-26.
Wiedemann T et al. Endocrinology 2016; 157:3157-66.
Lee M et al. Endocr Relat Cancer 2017; 24:1-15.
Molatore et al. Endocr Relat Cancer 2018; 25:145-162
Mohr et al. Cancers 2021; 13:126

A novel MIBG analog (LMI1195) allows the detection of PCC in MENX rats

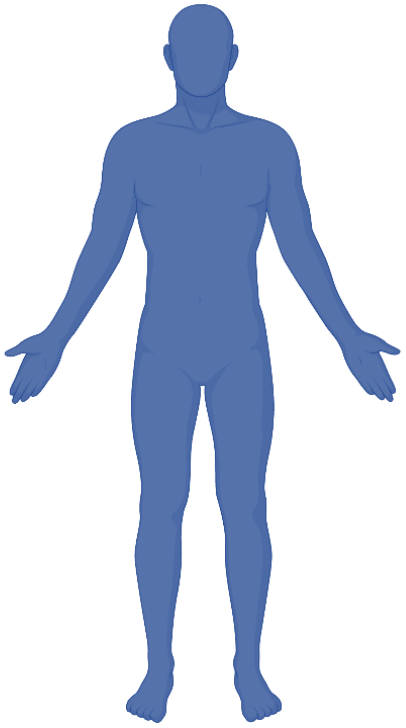




Gaertner FC, et al. (2013) Preclinical evaluation of [18F]LMI1195 for in vivo imaging of pheochromocytoma in the MENX tumor model. J Nucl Med 54:2111-7

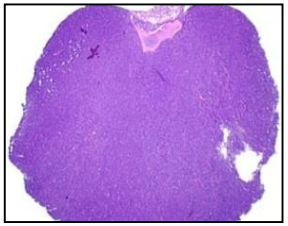
NOVEL PET TRACER FOR PCCs

TRANSLATION



MENX rats phenotype

histology



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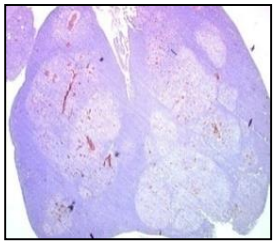
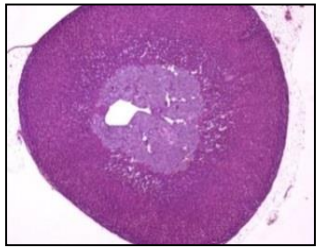


MUT

Pituitary tumors
(multifocal)
100%



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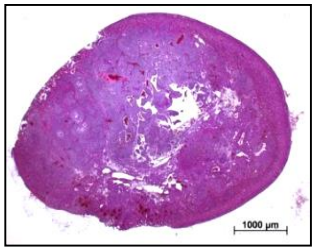
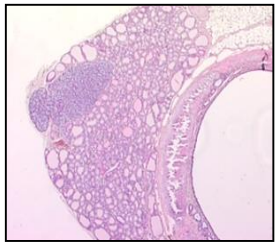


MUT

Pheochromocytoma
(bilateral)
100%



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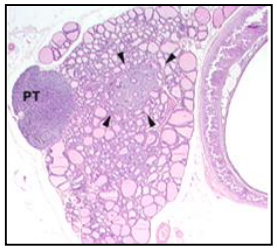
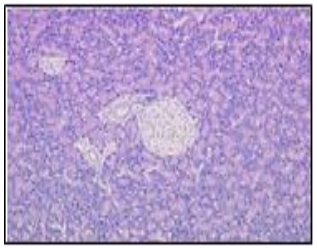


MUT

C-cell hyperplasia
(bilateral) 86%



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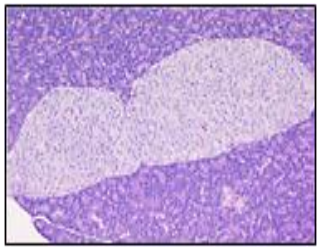


MUT

Islet cell hyperplasia
90%

Clinical issues to solve

Nonfunctioning pituitary tumors

- Often invasive→ they cannot be removed by surgery→ recur
- Don‘t respond to standard therapies with somatostatin analogs

→ New therapeutic targets/therapies

Marinoni I et al. Neuropath App Neurobiol 2013; 39:256-69.
Lee M et al. Acta Neuropathol 2013; 126:137-50
Lee M et al. Endocr Relat Cancer 2014; 22:111-9.
Lee M et al. Clin Cancer Res 2015; 21:3204-3215.
Bogner et al. Int J Cancer 2020; 147:3523-3538.
Gulde S et al. Cancers 2021 epub 22.06.2021

Sebastian Gulde

Tobias Wiedemann

Head-to-head comparison of octreotide LAR (sandostatin) and pasireotide LAR (signifor) for efficacy against pituitary tumors (PTs)

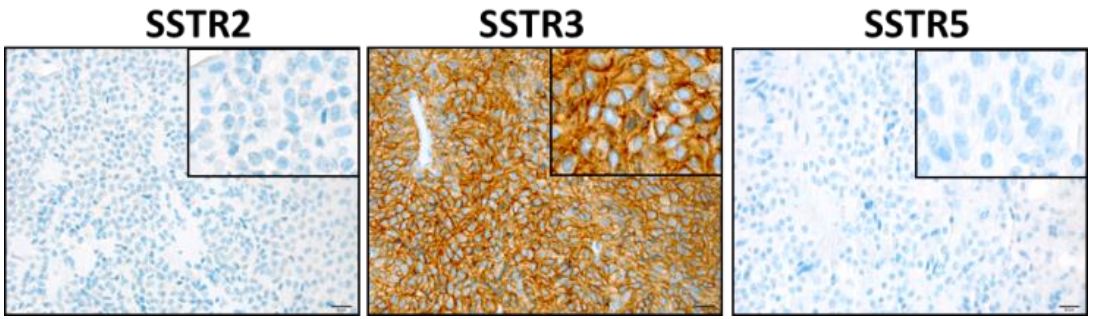
Mathias Schillmaier, Franz Schilling, Johannes Notni, Katja Steiger

Clinical issues to solve

Nonfunctioning pituitary tumors

- Often invasive→ they cannot be removed by surgery→ recur
- Don‘t respond to standard therapies with somatostatin analogs (SSTR2-directed)

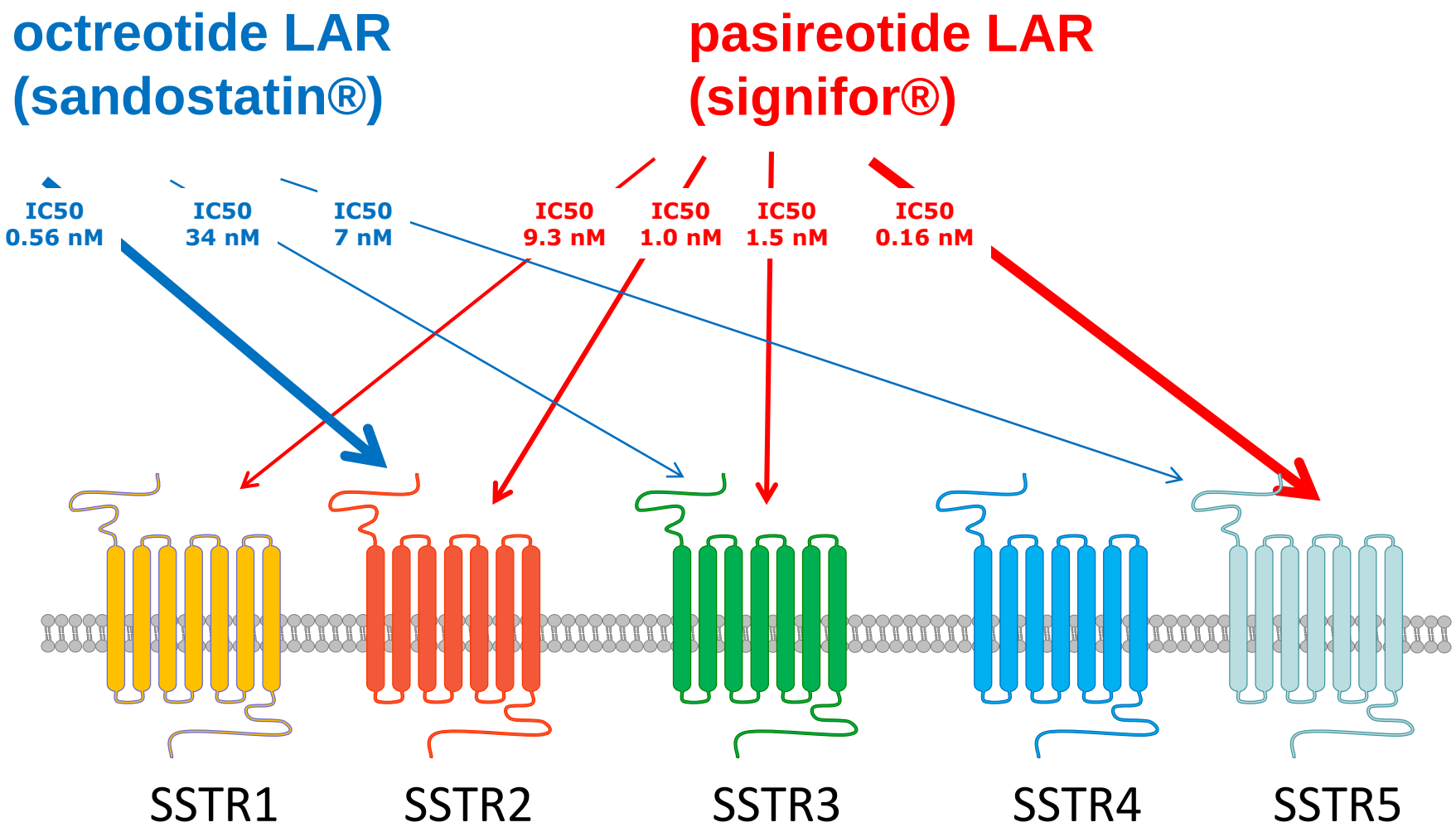
→ New therapeutic targets/therapies



Human NFPTs

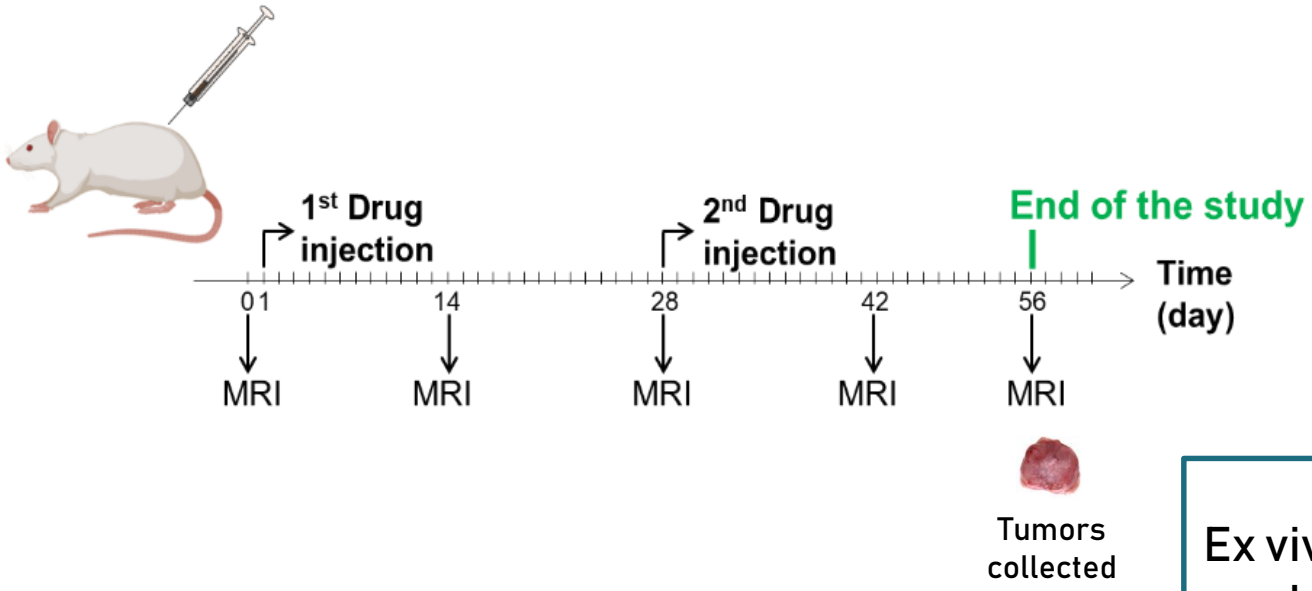
Lee et al. Endocr Related Cancer 2014

Somatostatin analogs and their affinities for somatostatin receptors (SSTRs)



Is pasireotide more effective than octreotide in our model of NFPTs?

Group 1: Mut/mut placebo-treated (control)
Group 2: Mut/mut + sandostatin® (octreotide) [1X/2x 28d]
Group 3: Mut/mut + signifor® (pasireotide) [1X/2x 28d]

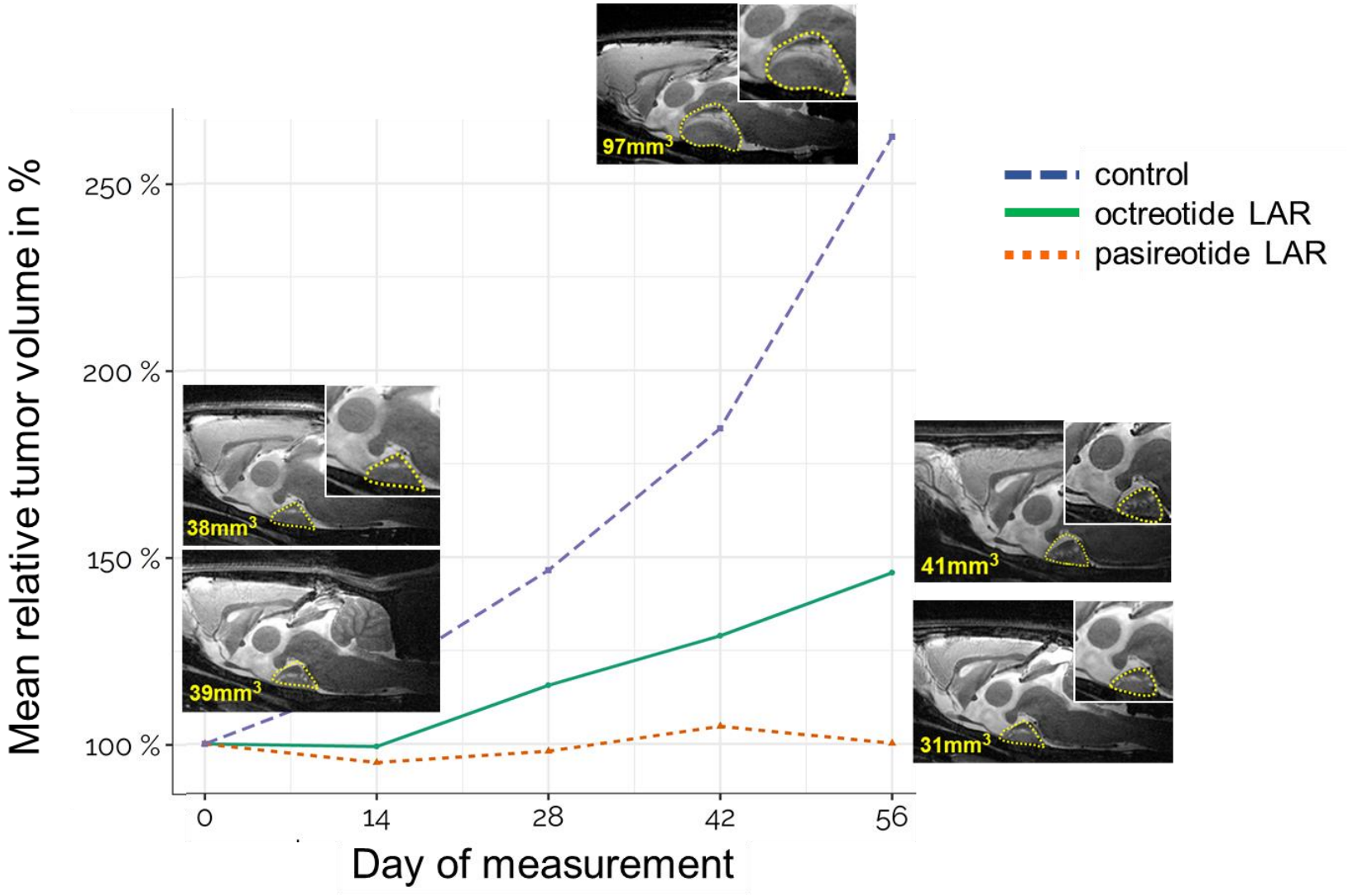


Ex vivo readouts → Ki67, Apoptosis, Sstr expression

Octreotide LAR (sandostatin)
Pasireotide LAR (signifor)
] Novartis Pharma

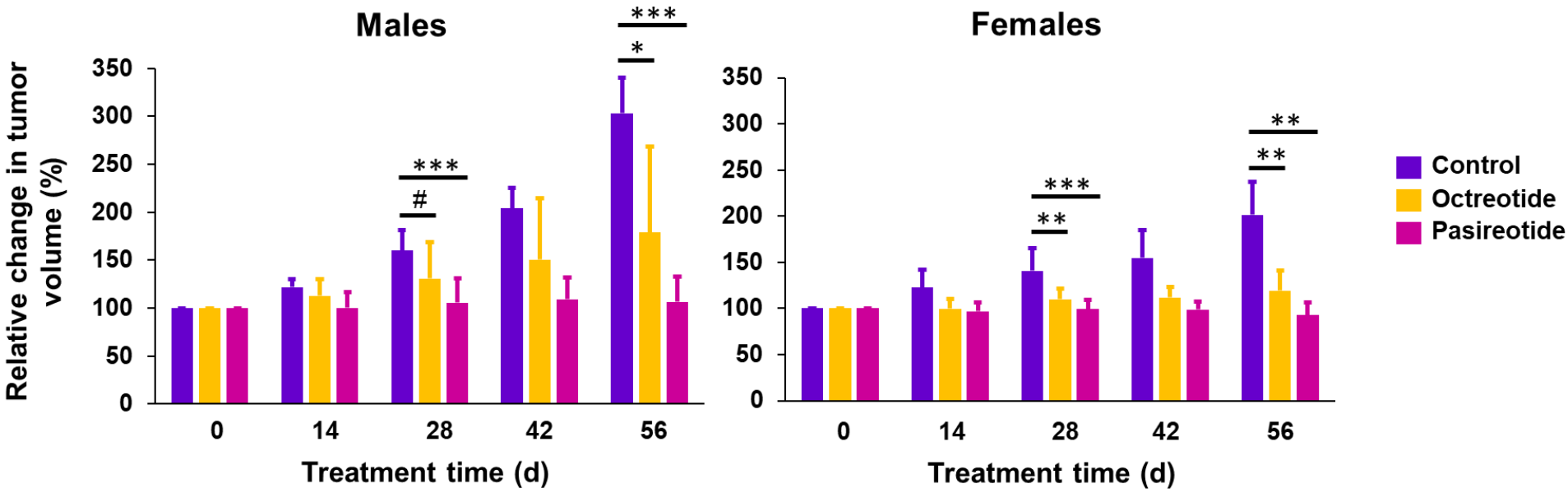
Gulde S et al. Cancers 2021 epub 22.06.2021

Inhibition of tumor growth: pasireotide > octreotide; females > males

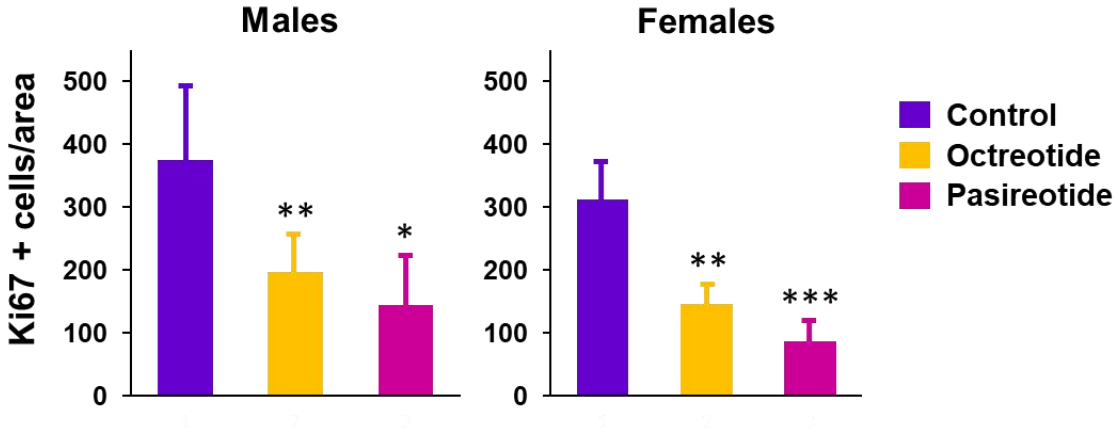


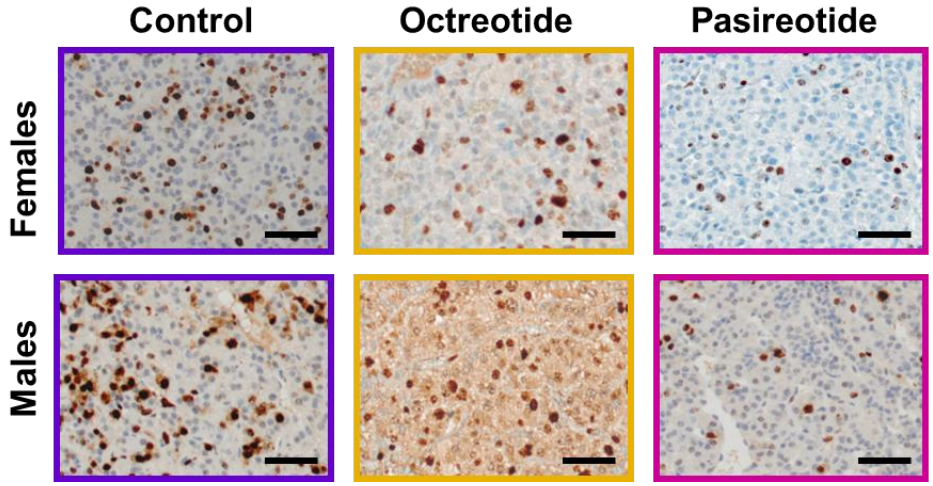
Inhibition of tumor growth: pasireotide > octreotide

Gender effect



Inhibition of tumor growth *in vivo* correlates with Ki67 labelling index *ex vivo* (for pasireotide < than for octreotide)

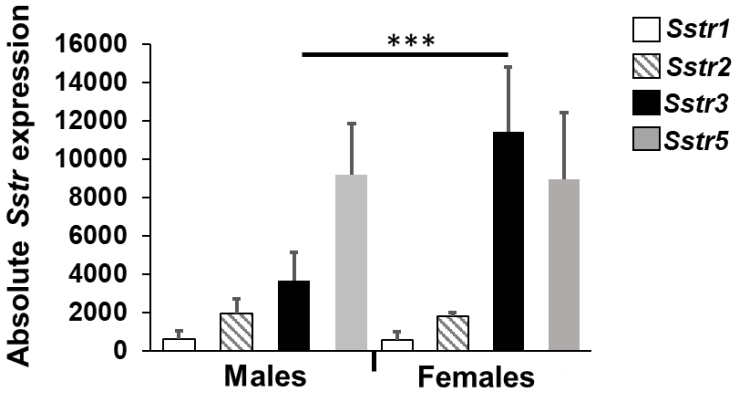




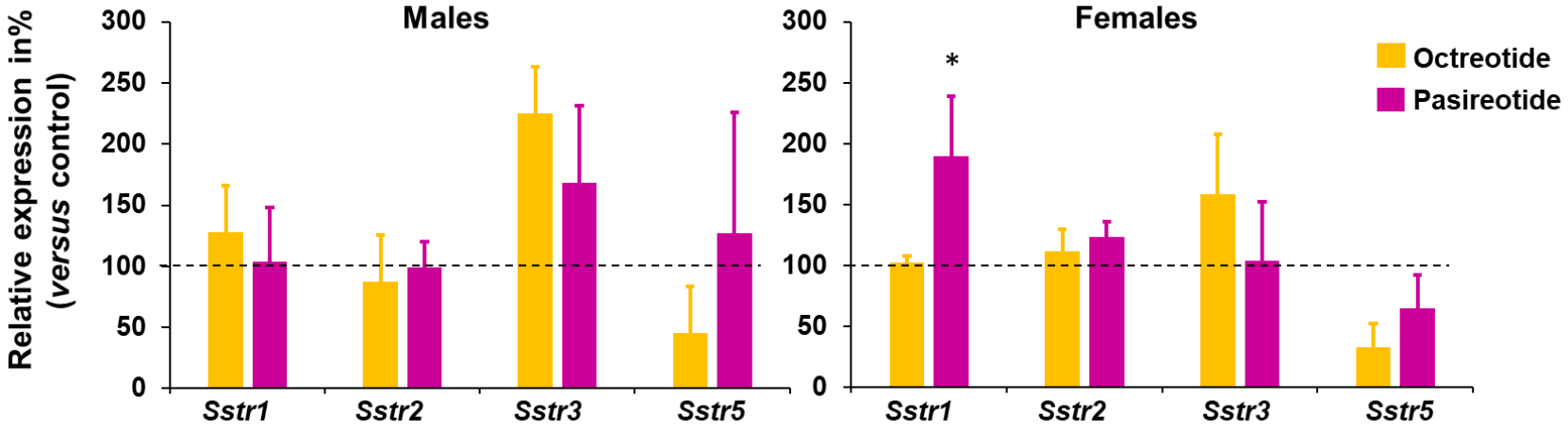
Ki67 immunohistochemistry

Expression of Sstr genes in PTs @ baseline and after treatment

Females have higher *Sstr3* expression at baseline

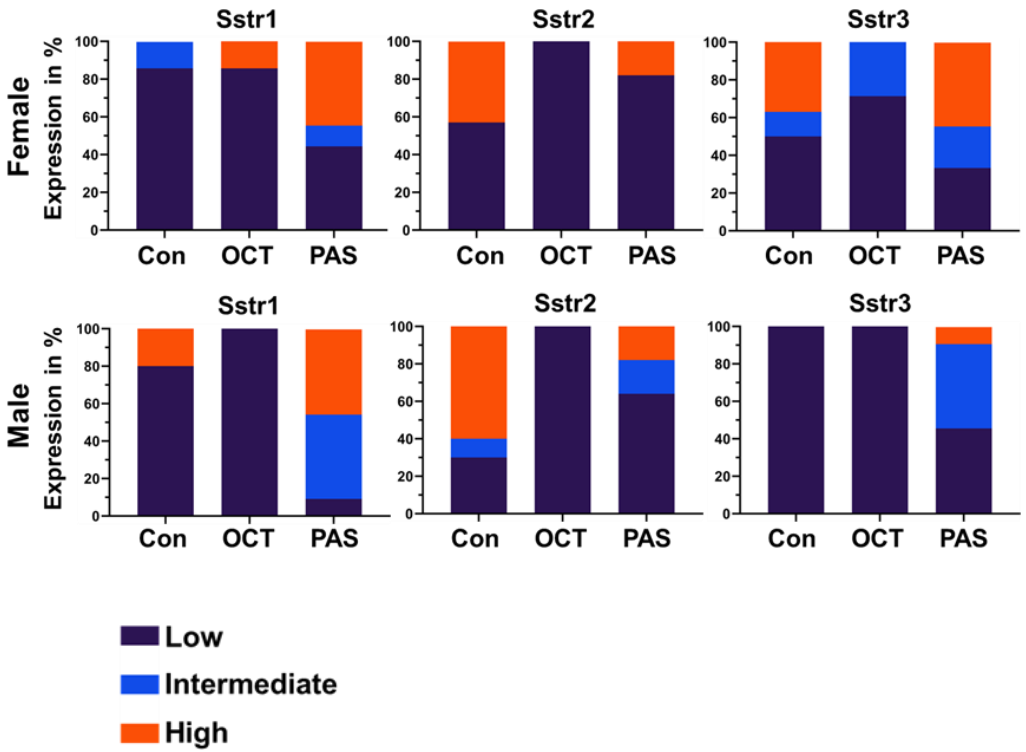


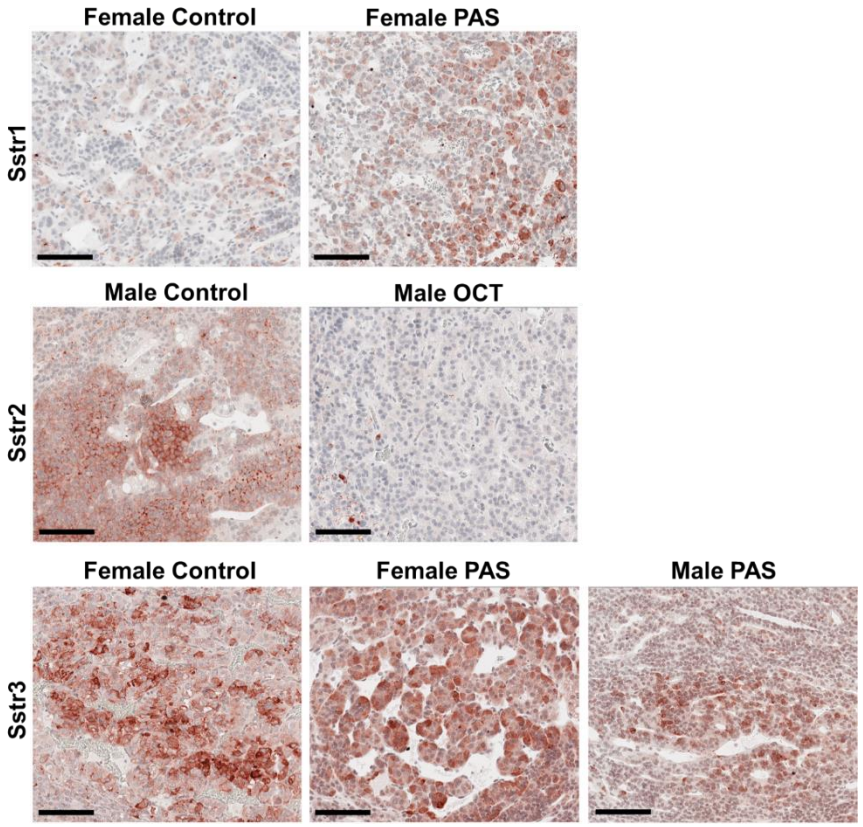
Following treatment



Is the expression of Sstr 1,2,3 receptors modulated by the drugs?

Octreotide reduces Sstr2 protein expression; pasireotide increases Sstr3 expression in males

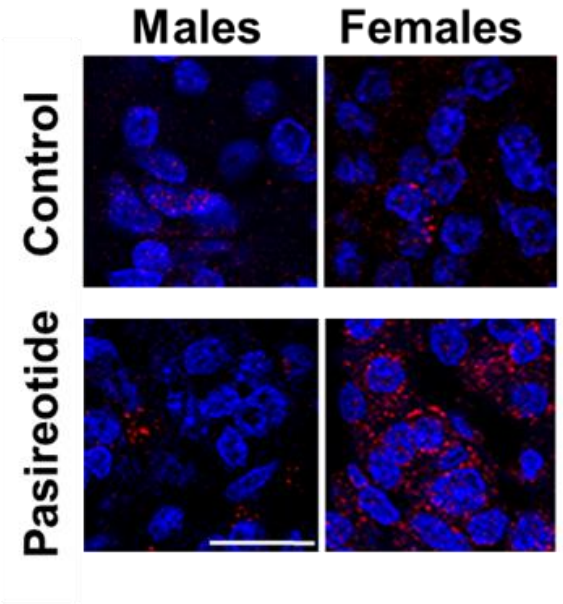


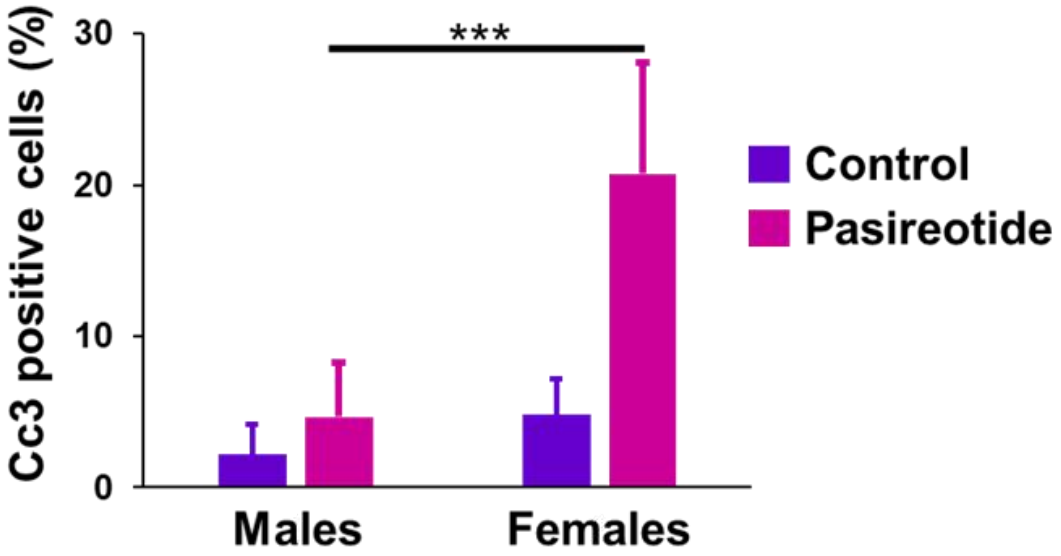


Coll. A. Lupp, S. Schulz (Uni Jena)

Apoptosis as the cause of shrinkage upon pasireotide treatment?

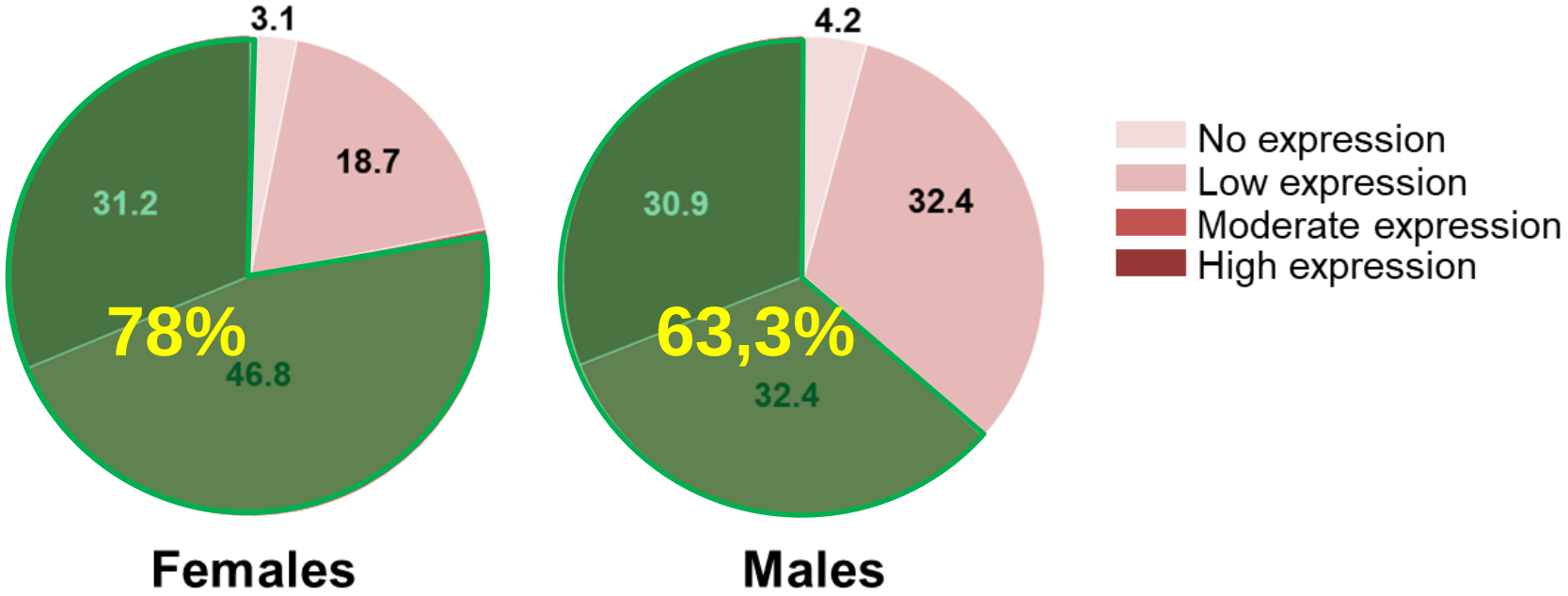
In females there is induction of apoptosis upon pasireotide treatment





Expression of SSTR3 in human NFPT patients (n=108)

In female patients there is a trend for higher SSTR3 expression (patient stratification?)



Gulde S et al. Cancers 2021 epub 22.06.2021

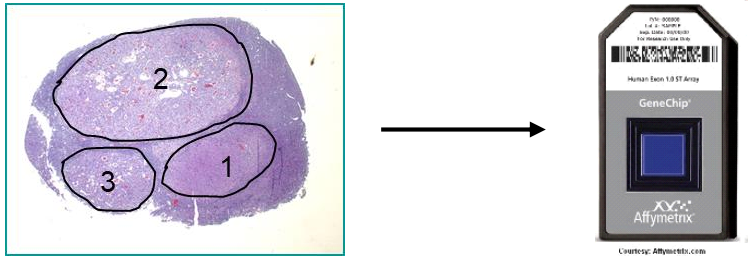
Ninelia Minaskan

Tobias Wiedemann

A novel druggable pathway active in pituitary tumors (PTs)

Mathias Schillmaier, Franz Schilling, George Kaissis, Rickmer Braren

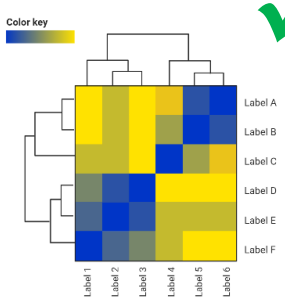
Transcriptome analysis of pituitary tumors (PTs)



OMICS
Molecular markers

miRNA

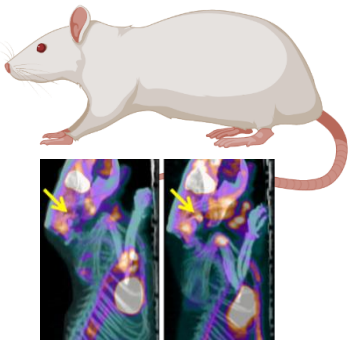
mRNA

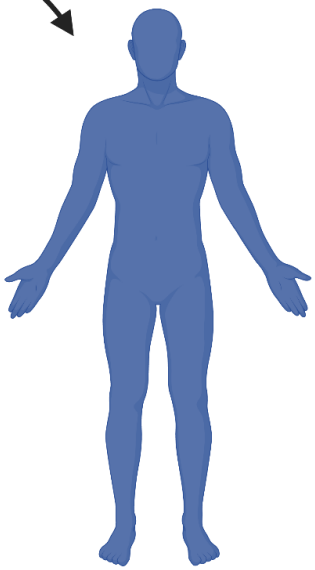


Validation
in human
NETs

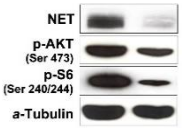
TRANSLATION

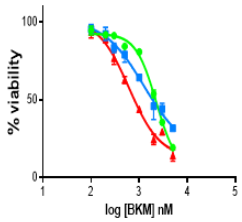
Rat NETs

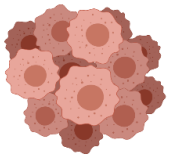




In vivo drug
testing



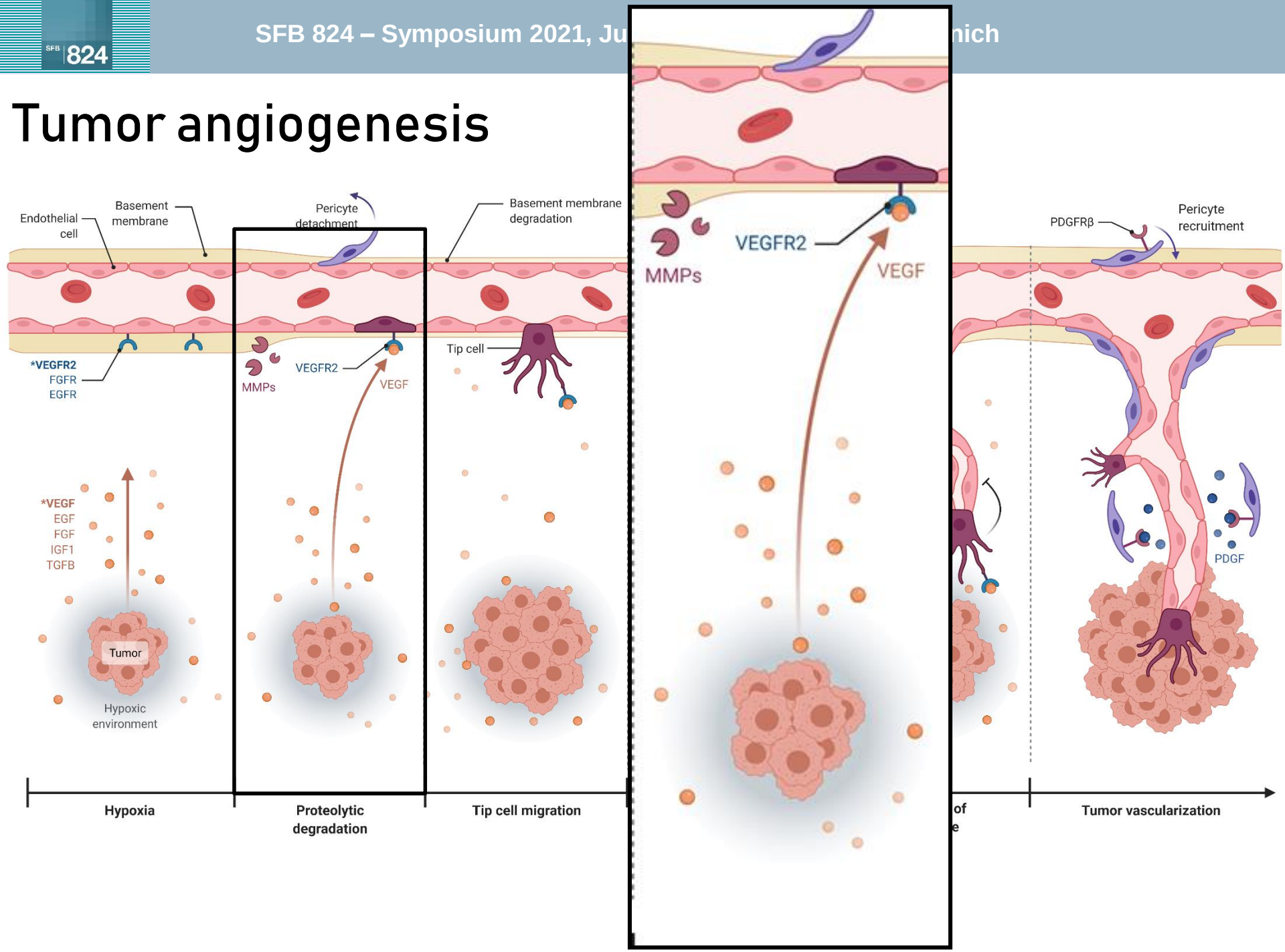




Therapeutic
targets

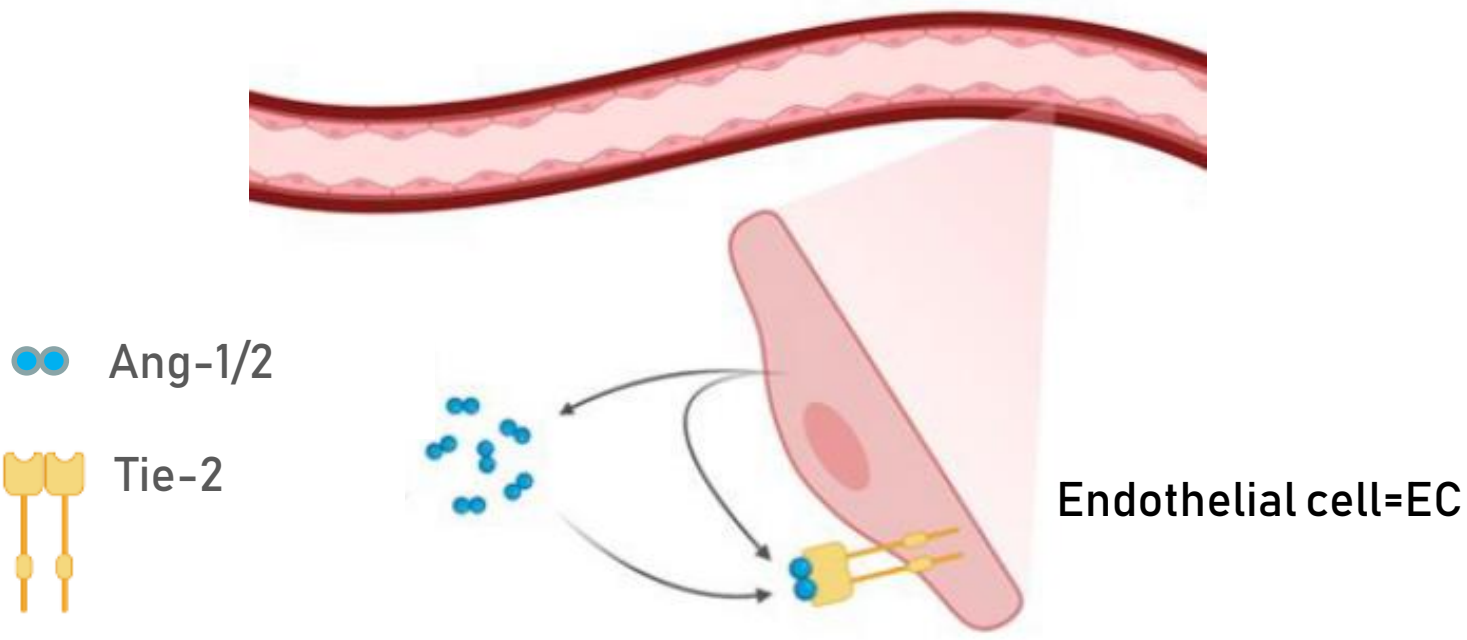
In vitro drug testing

Tumor angiogenesis



Angiopoietins and Tie-2 receptor

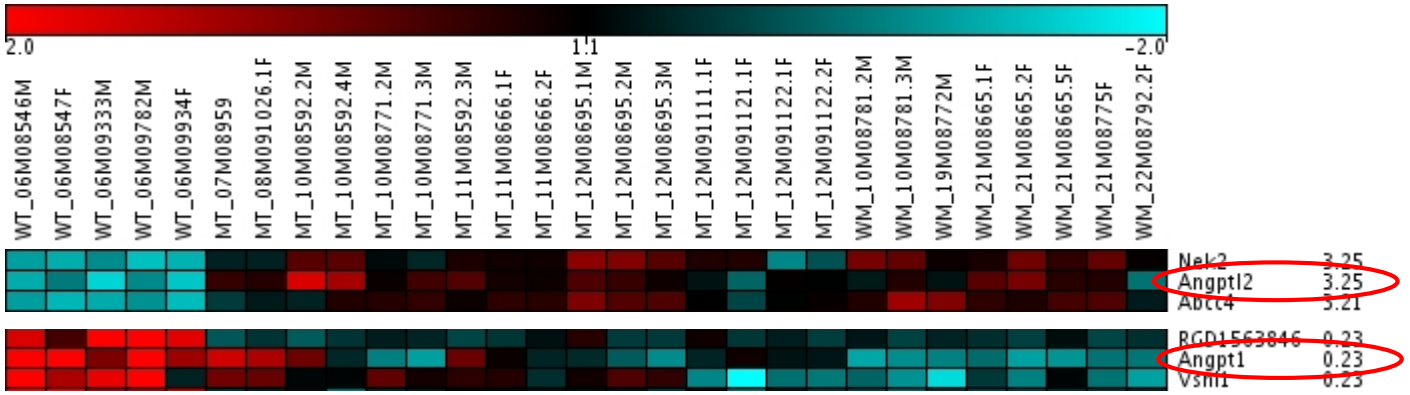
promote survival and proliferation of ECs



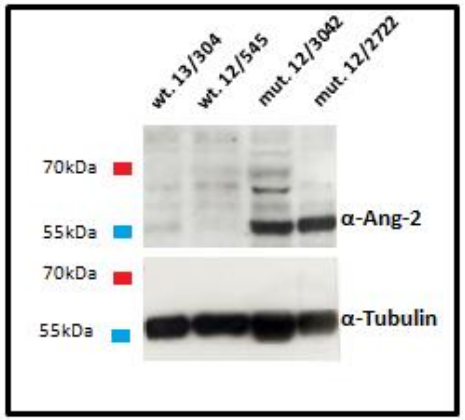
Autocrine signaling

Signature of rat pituitary tumors (PTs)

- Among the genes dysregulated in rat PTs are angiogenic genes: Angiopoietin (Ang)-2 (+2,4 fold) and Ang-1 (-3,4 fold) *versus* normal pituitary



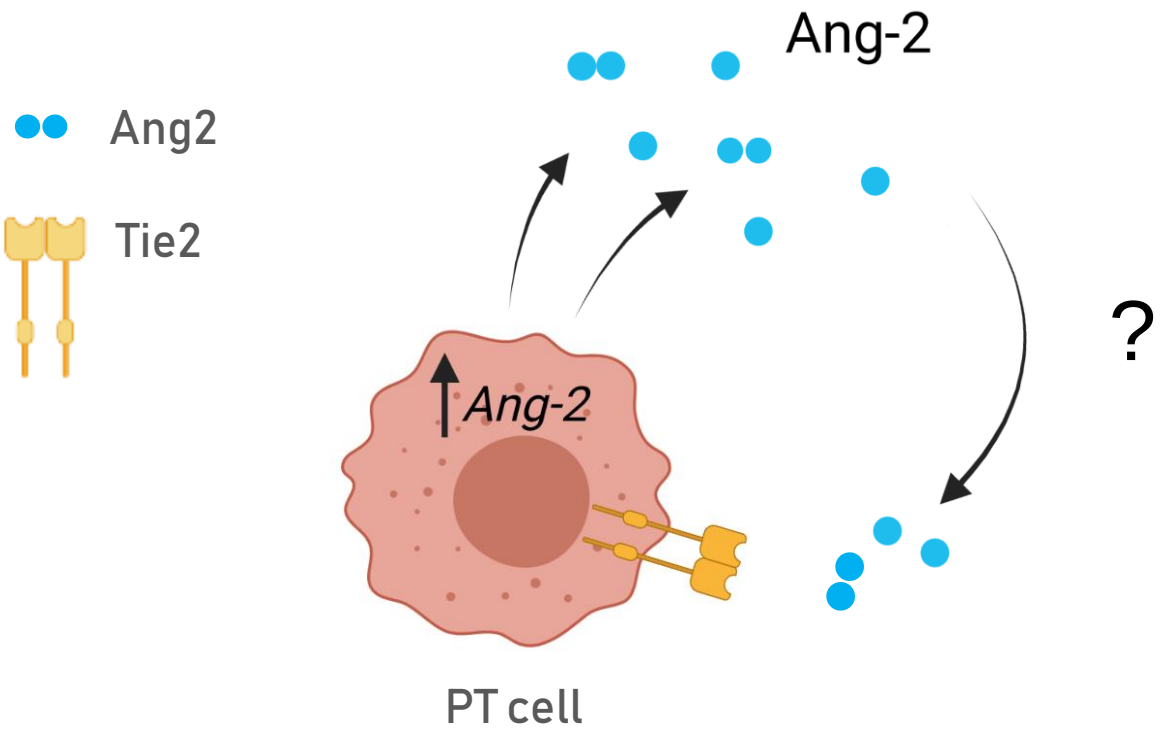
	Age	Ang-2	Angptl2	Vegf
wild-type n=3 (the average is used)	7-8 mo	0,81	0,58	0,75
12/1189 mutant	8.5 mo	5,18	2,40	3,25
12/1791 mutant	8.5 mo	1,89	0,70	1,46
12/1792 mutant	8.5 mo	1,64	0,47	1,63
12/3037 mutant	7 mo	1,00	0,46	0,85
11/447 mutant	9 mo	2,47	1,14	2,10
11/370 mutant	9.5 mo	1,56	2,25	1,78
11/1633 mutant	8 mo	2,14	0,44	1,75



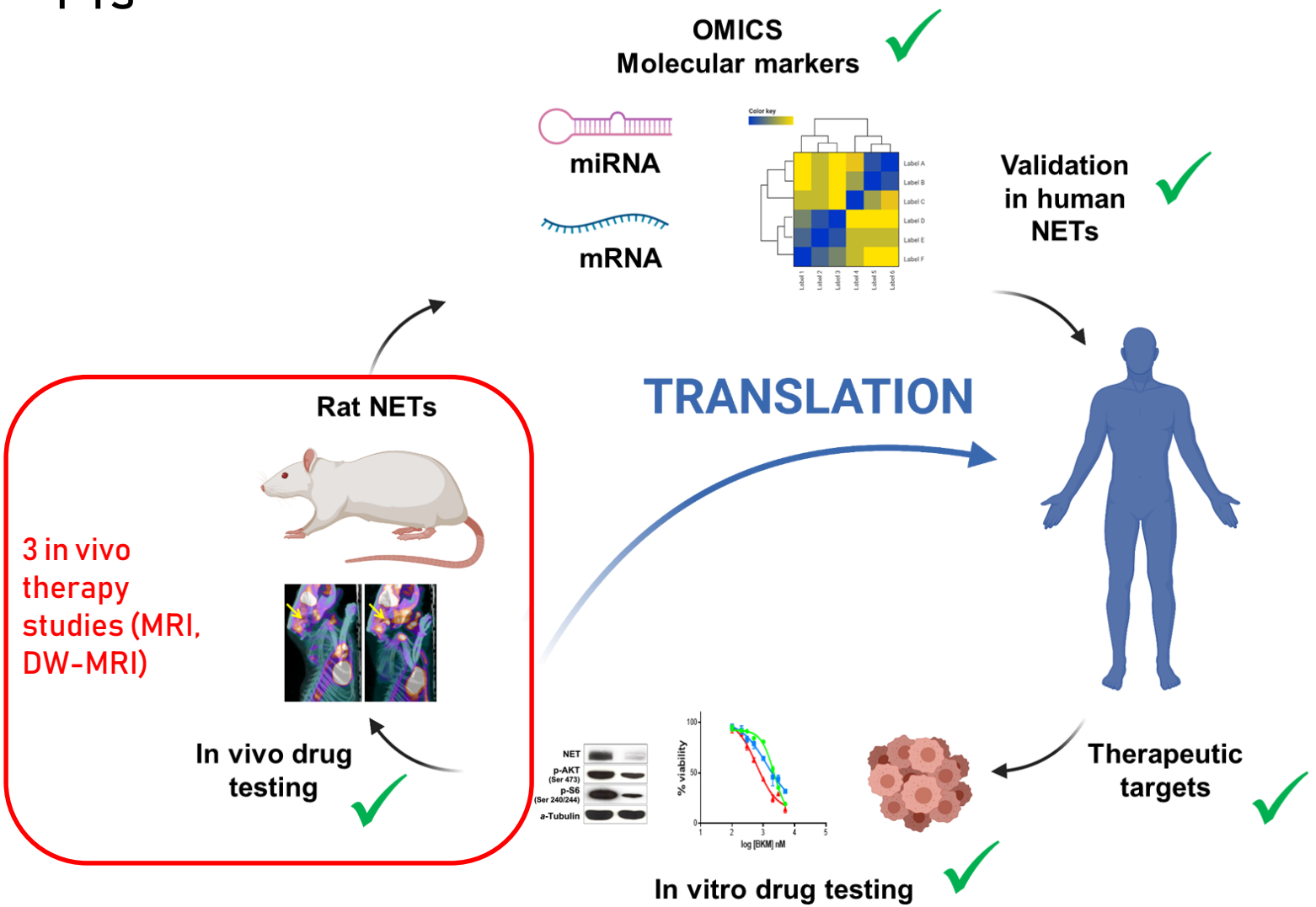
Situation in PT cells:

- High expression of Ang-2 (which is secreted)
- Expression of Tie-2

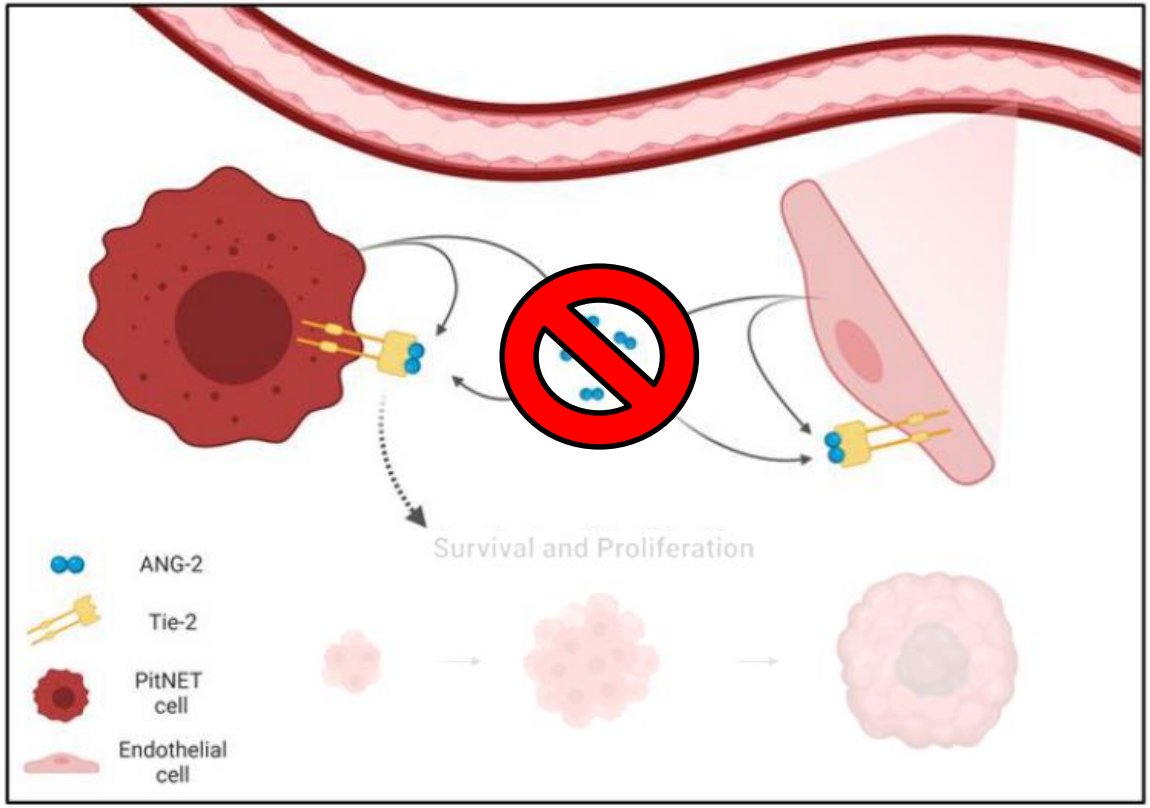
autocrine stimulatory loop as in ECs?



- Ang/Tie-2 signaling in PT cells supports cell viability in vitro and in vivo and represents a therapeutic target for recurrent/aggressive PTs



A new pathway active in PT cells that mediates the cross-talk between tumor and endothelial cells → target for therapy

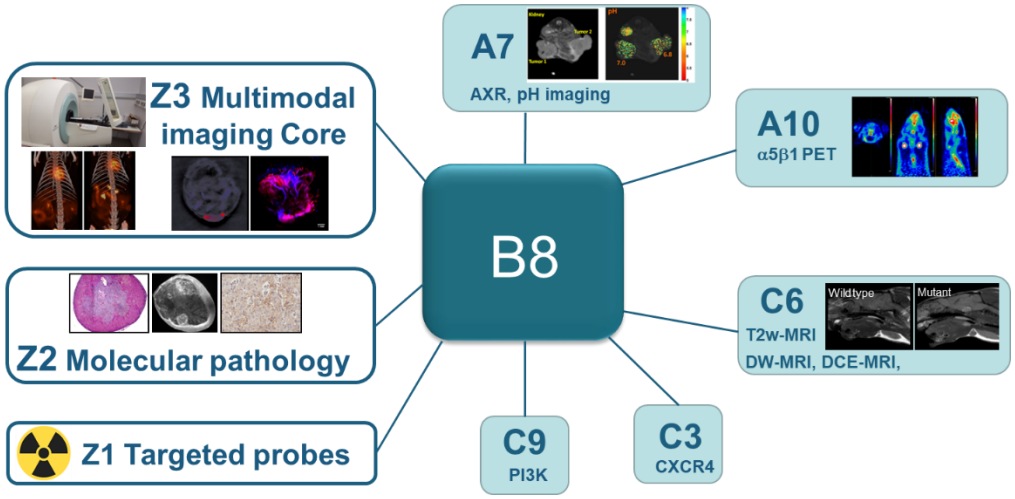


(In revision @ EMBO Mol Med)

Acknowledgements

Prof. M. Schwaiger

SFB 824



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Elke Pulz