# Overview of Circulating Biomarkers

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4th Milan NET Conferences 2018


*Eva Tiensuu Janson*

*Professor of Medicine*

*Endocrine Oncology*

*Uppsala University*


OECI
Organisation of European Cancer Institutes
European Economic Interest Grouping

European ENETS Neuroendocrine Tumor Society

European Reference Network
for rare or low prevalence complex diseases
Network Adult Cancers (ERN EURACAN)
Member Uppsala University Hospital — Sweden

# ENETS Guidelines Biochemical Markers

**Table 1.** General and specific biomarkers currently used for the management of patients with neuroendocrine tumors

| General tumor markers | Related indications |
|---|---|
| Chromogranin A | Almost all NETs (follow-up, limited in diagnosis) |
| Neuron-specific enolase | Atypical carcinoids, lung NEC, microcytoma |
| Pancreatic polypetide | Pancreatic NET |
| α-Subunit, β-hCG | Pancreatic, lung NET |
| **Specific tumor markers** | **Related indications** |
| Serotonin, 5-HIAA | Well differentiated NET |
| Gastrin | Zollinger-Ellison syndrome |
| Insulinoma | Insulin-secreting pancreatic NET |
| Glucagon, VIP, somatostatin | Well differentiated pancreatic NET |
| Catecholamines | Pheocromocytoma/paraganglioma |
| Calcitonin | Medullary thyroid cancer and pancreatic NET |
| PTHrp, ACTH, CRH, GHRH | Syndromes from (ectopic) mainly lung or pancreatic NET |
| NTpro-BPN (marker of ventricular dysfunction) | Carcinoid syndrome (carcinoid heart disease) |

NET, neuroendocrine tumor; NEC, neuroendocrine carcinoma; 5-HIAA, 5-hydroxyindolacetic acid; VIP, vasoactive intestinal peptide; PTHrp, parathormone-related peptide; ACTH, adrenocorticotropin hormone; CRH, corticotropin-releasing hormone; GHRH, growth hormone-releasing hormone; NTpro-BPN, N-terminal pro-brain natriuretic peptide.

Öberg et al Neuroendocrinology 2017

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# General vs. Specific Marker

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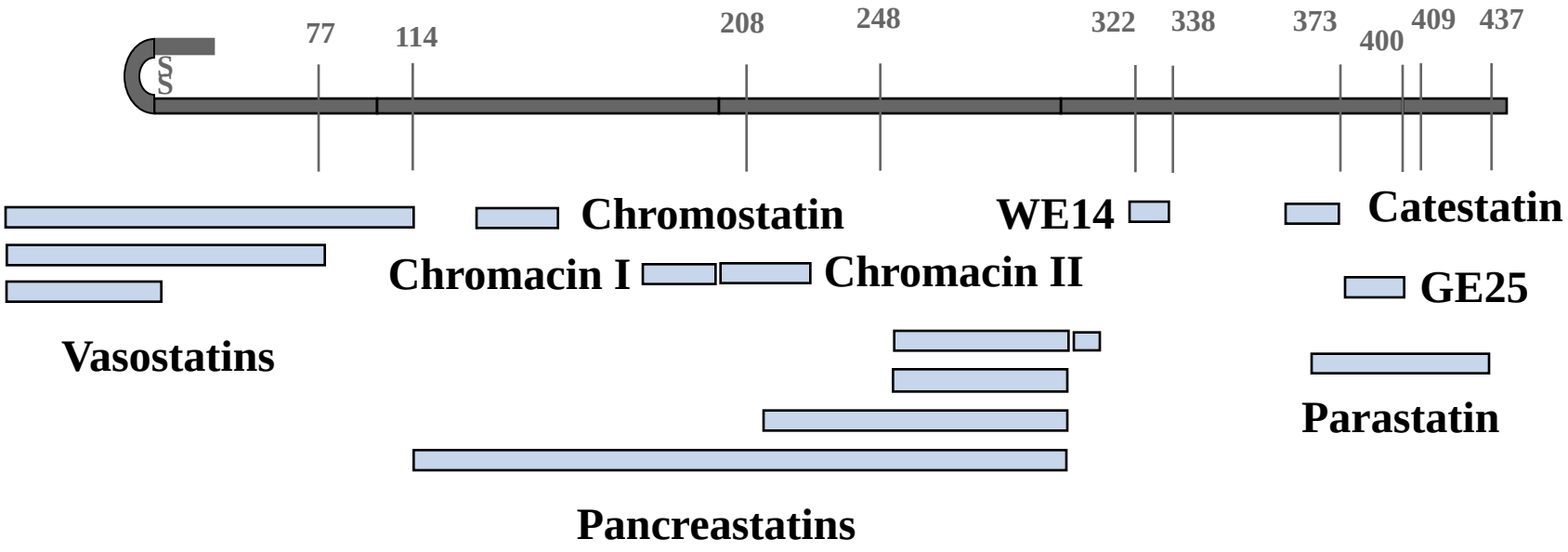
- A general marker can be found in many different (neuroendocrine) cells– indicates that the cell is of neuroendocrine type

- A specific marker can be found in one certain type of cell – indicates which kind of cell it is. (EC-cell, beta-cell, alpha-cell…)

# Chromogranin A – a general biomarker

Belongs to a family of acidic proteins which are produced in neuroendocrine cells and stored and secreted together with the specific hormones



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# Chromogranin A

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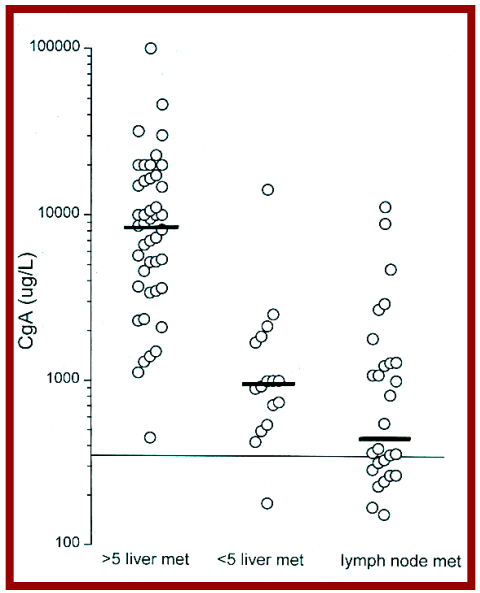
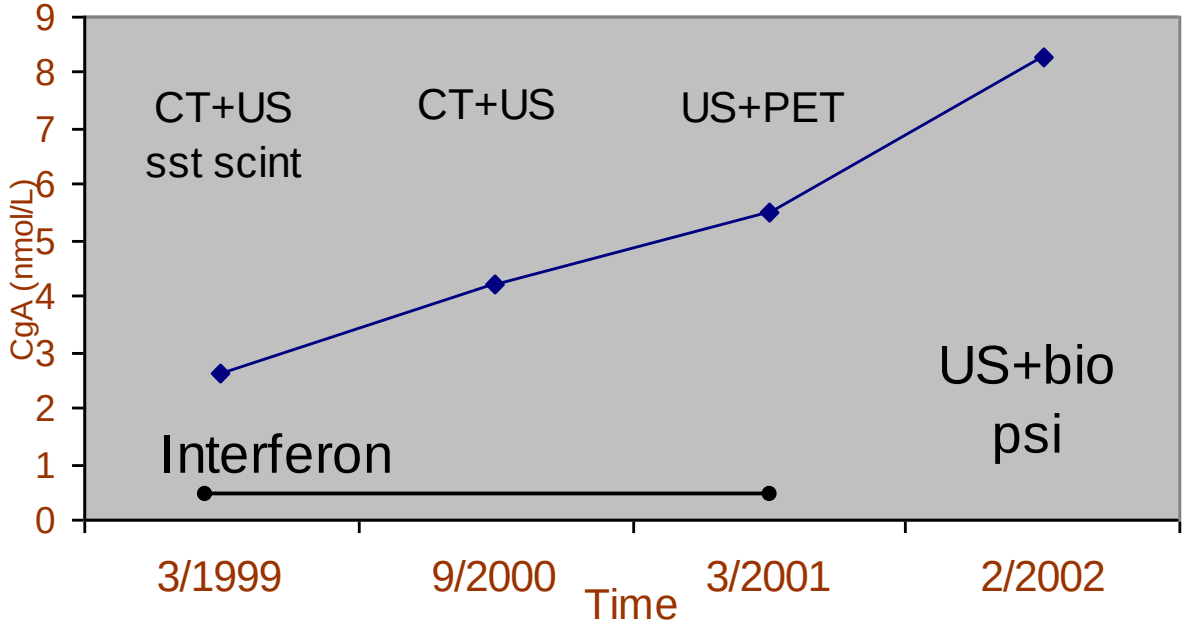



Chromogranin A levels are related to metastatic spread in untreated patients

Janson et al Ann Oncol 1997

The chromogranin A level in plasma is a sensitive marker for recurrent disease in radically operated patients (8 mo *vs*. 32 mo)

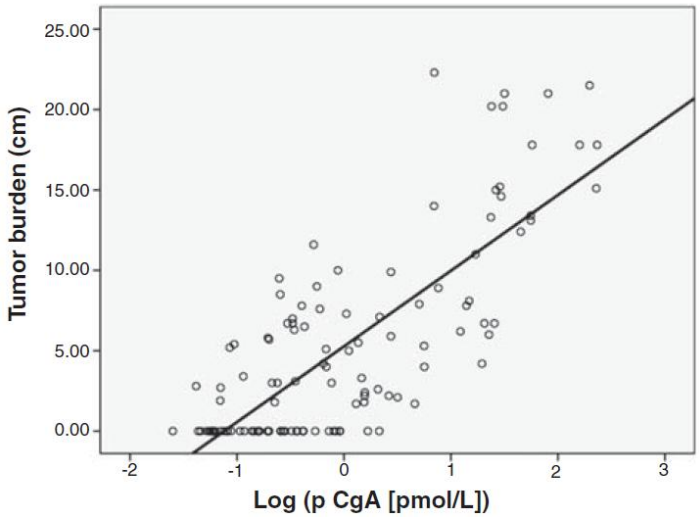
Welin et al, Neuroendocrinology 2009

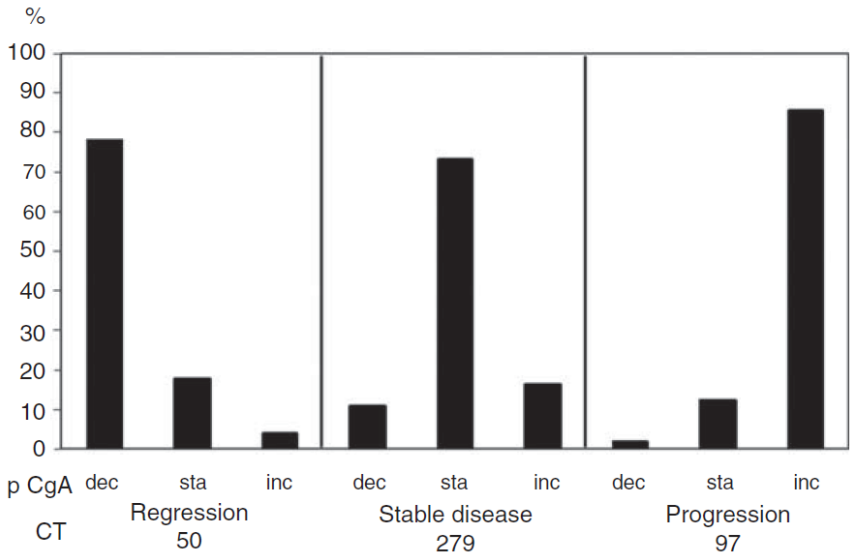
# Chromogranin A – prediktiv marker

**Chromogranin A is a sensitive marker of progression or regression in ileo-cecal neuroendocrine tumors**

KENNETH HØJSGAARD JENSEN[1,4], LINDA HILSTED[2], CLAUS JENSEN[3], TOMMIE MYNSTER[1,5], JENS F. REHFELD[2] & ULRICH KNIGGE[1]

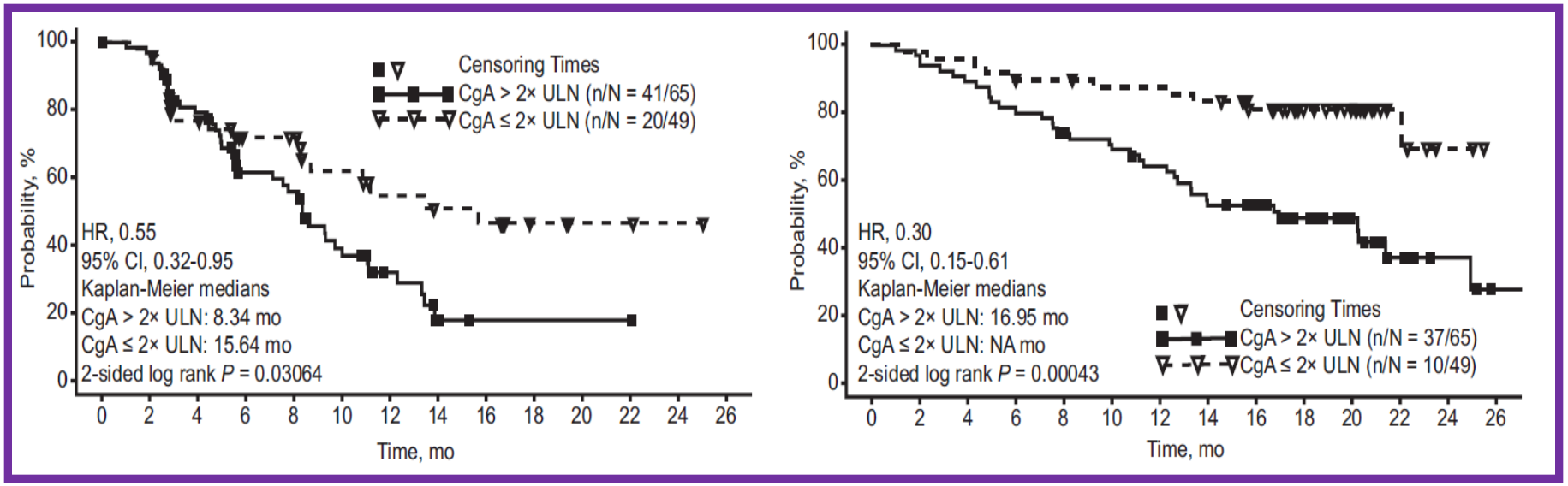
106 SI NET patients




*Scandinavian Journal of Gastroenterology.* 2013; 48: 70–77

# Plasma Chormogranin A as prognostic marker in pNET - Radiant-1 study




Progression-free survival

Overall survival

Yao *et al.* J Clin Endocrinol Metab, December 2011, 96(12):3741

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# Chromogranin A and NSE

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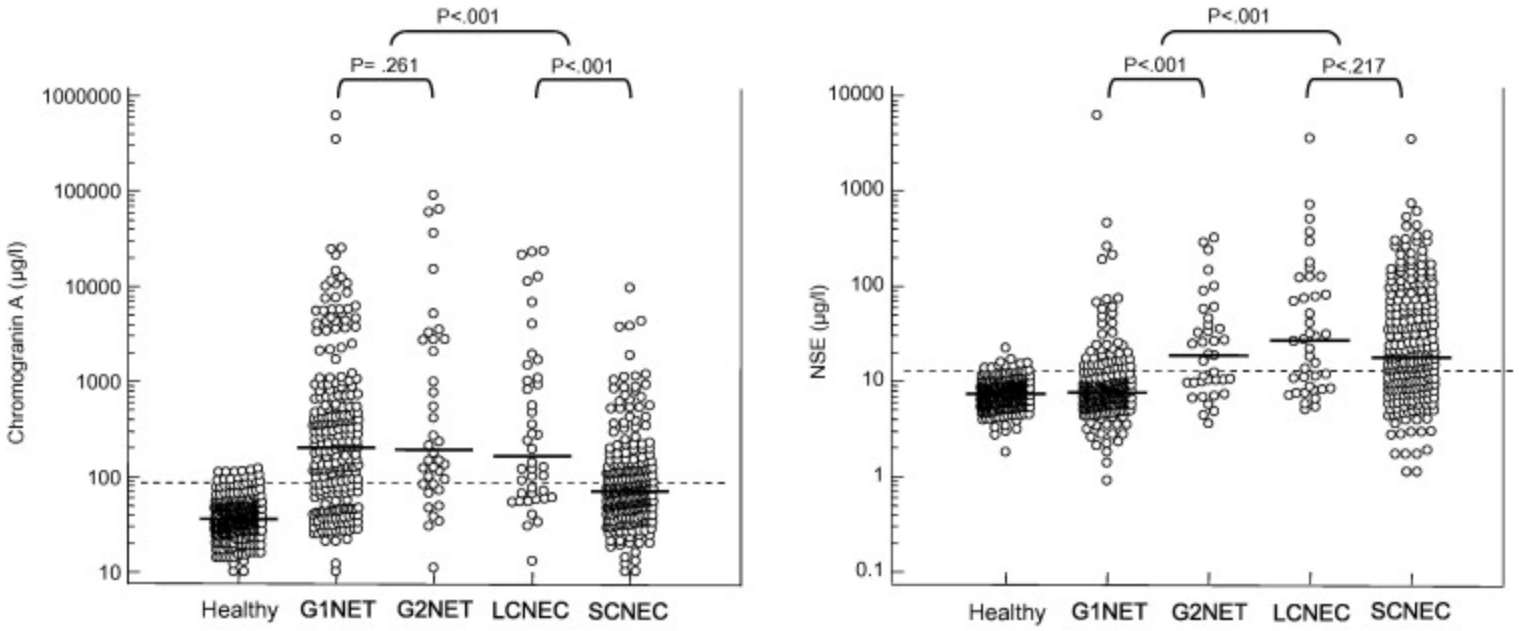
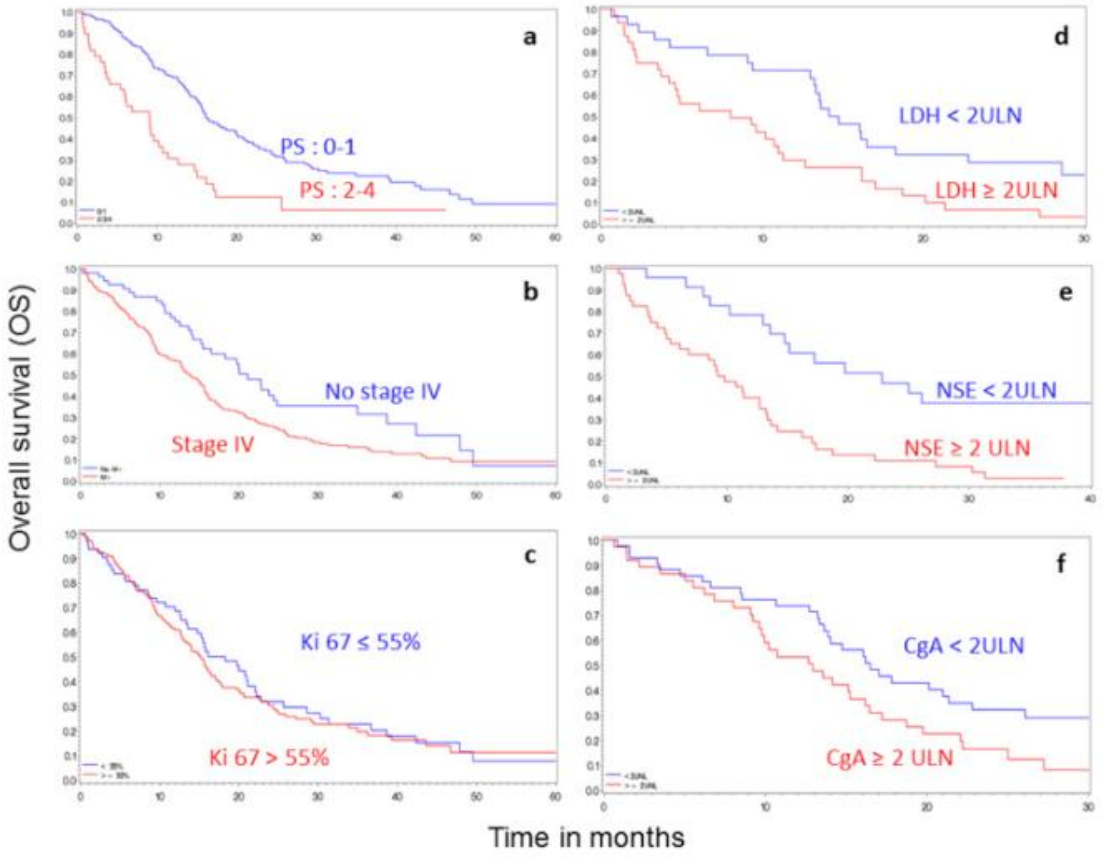



Chromogranin A is a good biomarker for G1-G2 NETs while NSE is better for high-grade tumors (G2 and NEC)

Korse CM et al. Eur J Cancer2012 Mar;48(5):662-71.

# Comparison of NSE and chromogranin A as predictors of survival in GEP-NEC




p<0.001

p<0.04

Walter T el al. Eur J Cancer 2017 Jul;79:158-165.

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# Causes for non-malignant increase in Chromogranin A

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- Impaired renal function
- Chronic Atrophic Gastritis
- Proton Pump Inhibitor treatment
- Impaired liver function
- Stress (increase adrenal medulla activity)
- Inflammatory bowl disease

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# General vs. Specific Marker

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- A general marker can be found in many different (neuroendocrine) cells– indicates that the cell is of neuroendocrine type

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# Neuroendocrine cells in the GI-tract
# Specific markers

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| Cell type | Peptide hormone | Amine |
|---|---|---|
| Alpha | Glucagon | |
| Beta | Insulin | |
| Delta | Somatostatin | |
| PP | Pancreatic polypeptide | |
| Enterochromaffin (EC) | Tachykinins | Serotonin |
| Enterochromaffin-like (ECL) | | Histamin |
| Gr | Ghrelin/obestatin | |
| G | Gastrin | |
| I | Cholecystokinin | |
| K | GIP | |
| N | Neurotensin | |
| S | Secretin | |
| VIP | Vasoactive intestinal peptide | |
| X | Amylin | |

# Analytical and preanalytical validation of a new mass spectrometric serum 5-hydroxyindoleacetic acid assay as neuroendocrine tumor marker

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Niina Tohmola [a,*], Outi Itkonen [b], Timo Sane [c], Helene Markkanen [b], Sakari Joenväärä [a], Risto Renkonen [a,b], Esa Hämäläinen [b]

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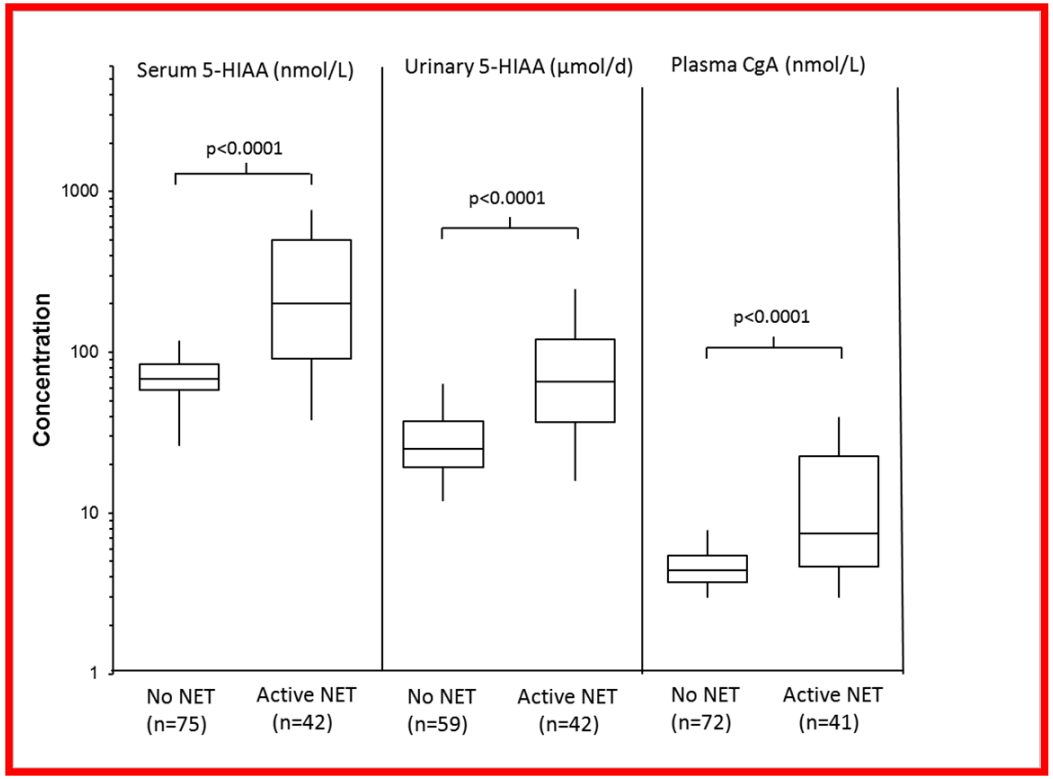
| Character | Mean (range) |
|---|---|
| **Sex, M/F** | 44/44 |
| **Age, years** | 64 (20-85) |
| **Origin of NET, n** | |
| Foregut | 4 |
| Midgut | 82 |
| Hindgut | 2 |
| **Clinical status of NET, n** | |
| Clinical remission | 46 |
| Stable or progressive disease | 42 |
| **Time from diagnosis of NET, years** | |
| Patients in remission | 4.6 (1-12) |
| Patients with stable or progressive disease | 5.2 (0.5-22) |

The assay for serum 5-HIAA discriminates between healthy individuals and patients with NET and is well suited for the diagnosis and follow-up of NETs

Clinica Chimica Acta 428 (2014) 38–43



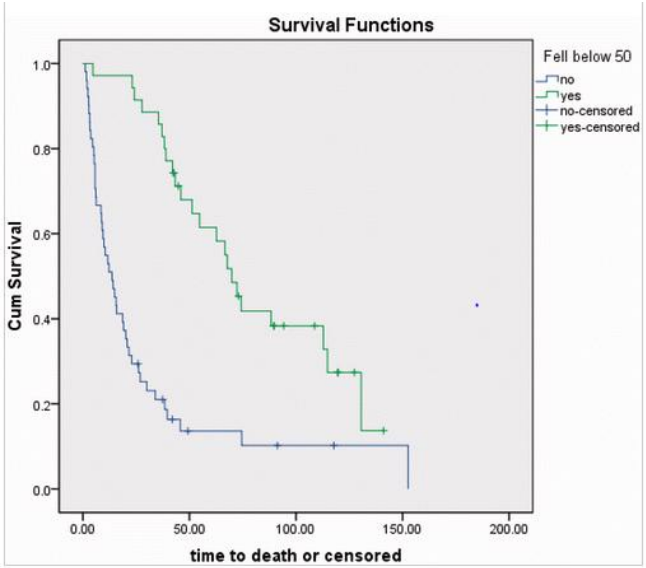

Comparison betweenNET patients and healthy subjects

# Tachykinins

- Tachykinins have had a renaissance during the last few years – produced in EC-cells (SI-NET specific biomarker)
- Tachykinins (NKA) can discriminate patients with severe carcinoid heart disease and is a specific marker for SI-NETs (Ardill et al, QJM 2016)

SI-NET patients who had a reduction in NKA from increased levels down to 50 ng/L or less during treatment had a significantly longer overall survival

| | Number of patients | First NKA ⩾ 50 ng/L median (range) | Median (range) survival in months from first NKA ⩾ 50 ng/L | Significance between early and late |
|---|---|---|---|---|
| Total group NKA ⩾ 50 ng/L | 86 | 91 (50–1250) | 28.8 (2.0–152.6) | |
| Earlier period | 35 | 116 (50–1250) | 20.2 (2.1–152.6) | P = 0.019 |
| Later period | 51 | 75 (52–1176) | 39.1 (2.0–143.8) | |
| Earlier period (clinic patients) | 32 | 101 (50–1250) | 23.2 (2.0–152.6) | P = 0.016 |
| Later period (clinic patients) | 43 | 75 (52–1176) | 52.1 (21.0–143.8) | |




Published in: Joy ES Ardill; David R McCance; Wendy V Stronge; Brian T Johnston; *Ann Clin Biochem* 53, 259-264.
DOI: 10.1177/0004563215592021

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# Do we need new/more biomarkers?

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- Biomarkers can be used to
  - Set a correct diagnosis
  - Estimate tumor burden
  - Identify tumor recurrence
  - Foresee prognosis
  - Predict therapy response

- So – we further biomarkers – but are those that we have any good?

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# Which are the latest news?

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- New proteins measurable in blood and in tumor tissue
- Circulating tumor cells
- miRNAs
- Genetic markers
- The NETest

# Multiplex proximity ligation/extension assay

## *Novel Serum Biomarkers in Small Intestinal Neuroendocrine Tumors*

Edfeldt et al Neuroendocrinology 2017

96 patients with metastasized SI-NETs + 23 controls were included

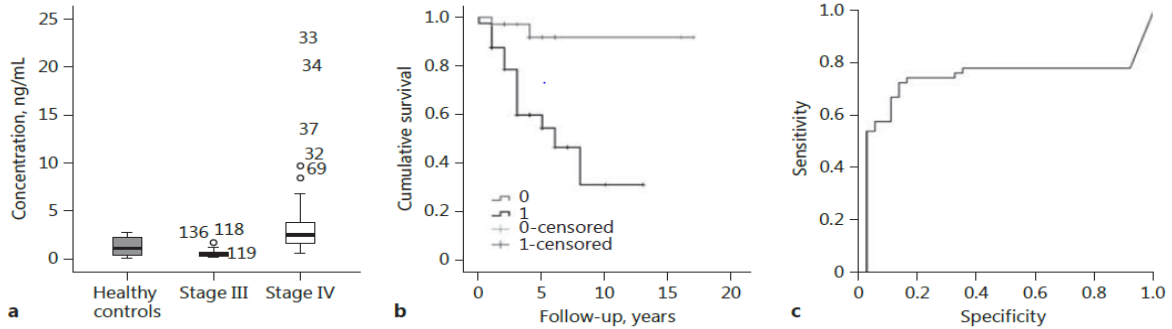
69 biomarkers were screened in serum using multiplex PLA

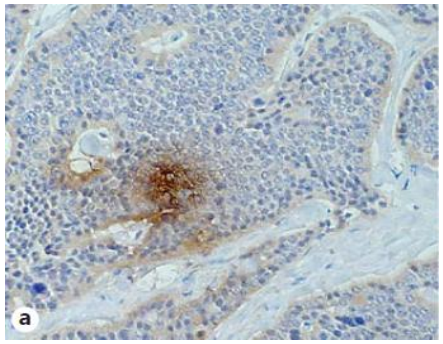
76 further biomarkers were analyzed by multiplex PEA

Results were confirmed in an extended cohort using IHC and ELISA

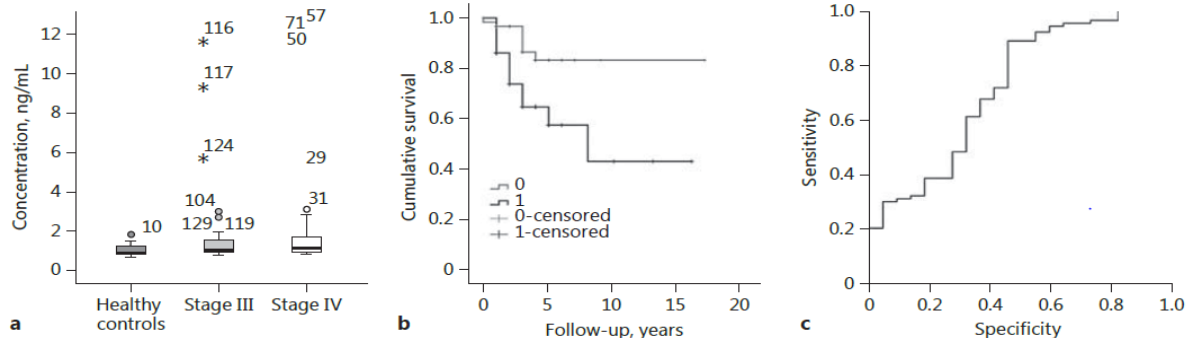
# Three proteins were of interest
# DcR3, TTF3 and Midkine

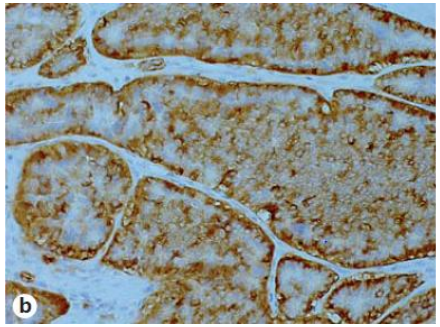
DcR3



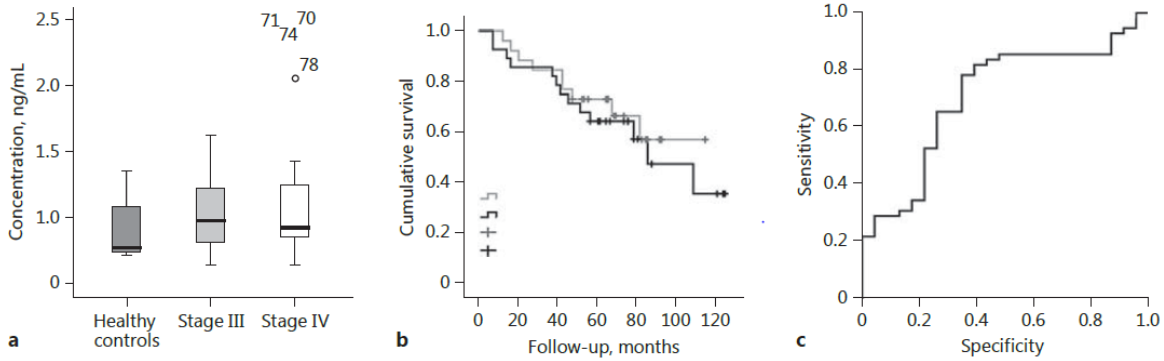



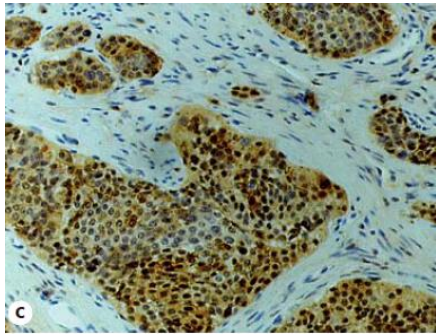

TTF3



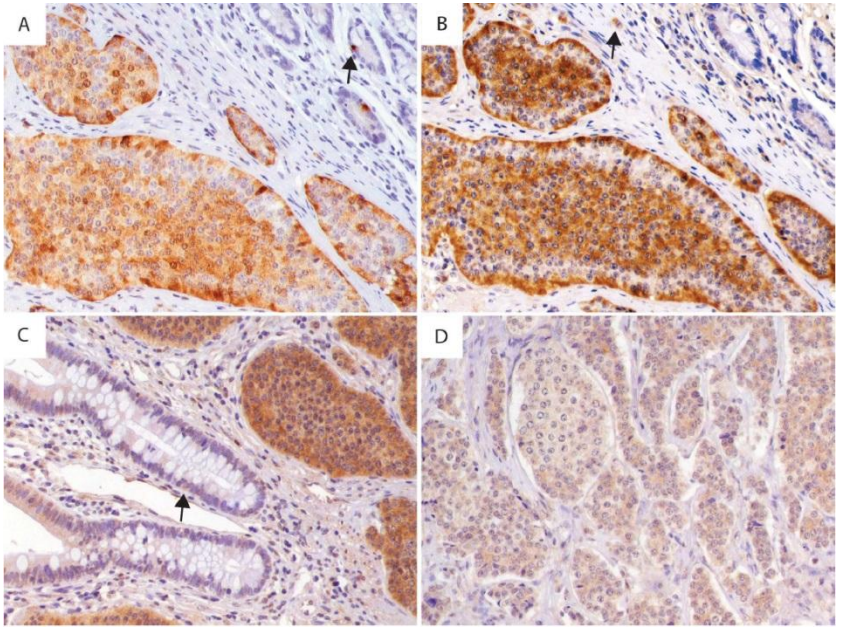

Midkine



# Melatonin in SI-NETs

- Melatonin IR was found in all 26 SI-NET patients examined
- Melatonin receptor MT1 was low or absent in all tumors
- Melatonin receptor MT2 was highly expressed in primary tumors but low in metastases
- Plasma levels ranged between 4.5 – 220.0 pg/L
- High plasma levels were associated with nausea (p= 0.027) and flush (p=0.020)




Melatonin Receptor 2

Primary Tumor Liver Metastases

Söderquist F et al, PLoS One 2016

# So, where are we as far as proteins takes us?

- DcR3
- TFF3
- Midkine
- Melatonin
- …and many moore

- Several new potential protein biomarkers have been suggested
- Pros – proteins are relatively easy to measure
- Cons – none of the candidates have shown true clinical benefit yet

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# Circulating Tumor Cells

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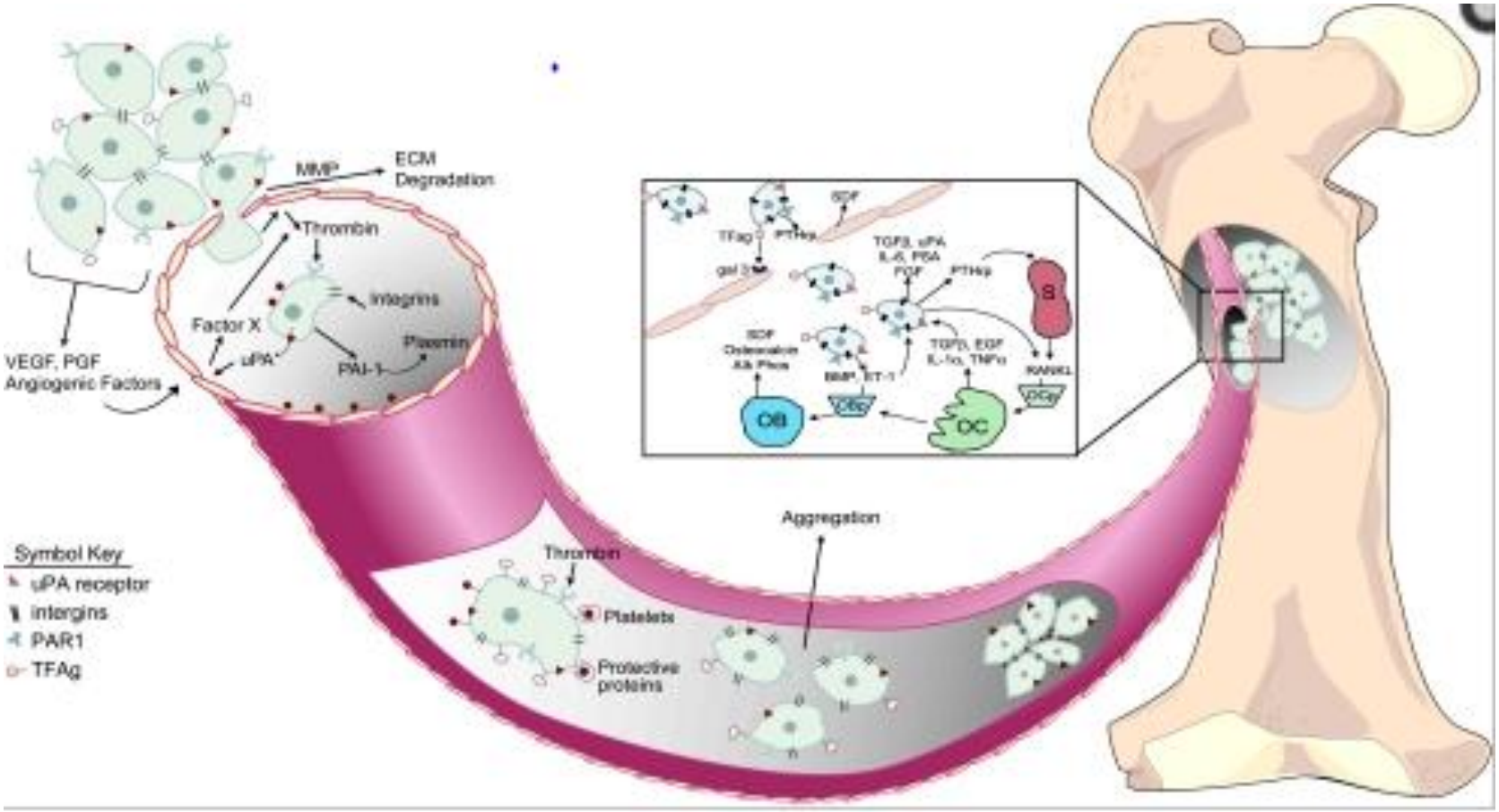


Loberg et al. Neoplasia. 2004 Jul; 6(4): 302–309

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# Should we measure CTCs in NETs?

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- CTCs can be correlated to PFS and OS in breast cancer (and other cancers)
- The CellSearch platform can detect CTCs in a 10 ml blood sample
- NETs express EpCAM which is required for detection of CTCs by this platform

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# The number of CTCs which can be found in blood is correlated to survival

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## PFS and OS in patients with NET (n 175)

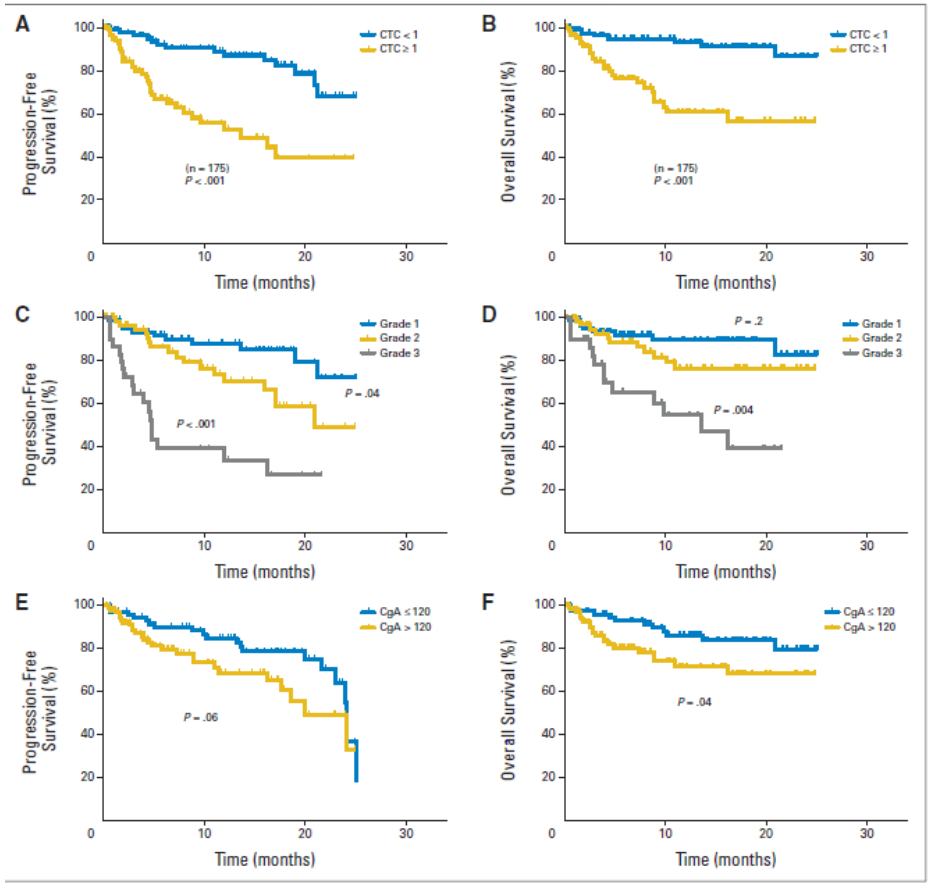



**Table 4.** Univariate Analysis for Prognostic Factors in Patients With Grade 1 NETs (n = 83)

| Risk Factor | No. of Patients | PFS HR | PFS 95% CI | PFS *P* | OS HR | OS 95% CI | OS *P* |
|---|---|---|---|---|---|---|---|
| Baseline CTC count | | | | | | | |
| < 1 | 50 | 1.0 | | | 1.0 | | |
| ≥ 1 | 33 | 5.0 | 1.3 to 18.5 | .017 | 7.2 | 1.3 to 39.4 | .023 |
| Baseline CgA, pmol/L | | | | | | | |
| ≤ 120 | 29 | 1.0 | | | 1.0 | | |
| > 120 | 54 | 2.4 | 0.6 to 9.4 | .200 | 1.3 | 0.3 to 5.6 | .724 |
| Burden, % | | | | | | | |
| < 25 | 44 | 1.0 | | | 1.0 | | |
| ≥ 25 | 39 | 2.8 | 0.8 to 9.8 | .098 | 2.6 | 0.6 to 10.8 | .197 |
| ECOG PS | | | | | | | |
| 0-1 | 78 | 1.0 | | | 1.0 | | |
| ≥ 2 | 5 | 2.2 | 0.3 to 18.0 | .449 | 3.0 | 0.4 to 25.0 | .311 |
| Age for every 10 years | | 1.2 | 0.7 to 2.0 | .562 | 1.2 | 0.7 to 2.2 | .559 |

Abbreviations: CgA, chromogranin A; CTC, circulating tumor cell; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; NET, neuroendocrine tumor; OS, overall survival; PFS, progression-free survival.

Survival curves according to (A, B) presence of circulating tumor cells (CTCs), (C, D) grade, and (E, F) chromogranin A (CgA) demonstrating differences in (A, C, E) progression-free and (B, D, F) overall survival.

Khan et al JCO 2012

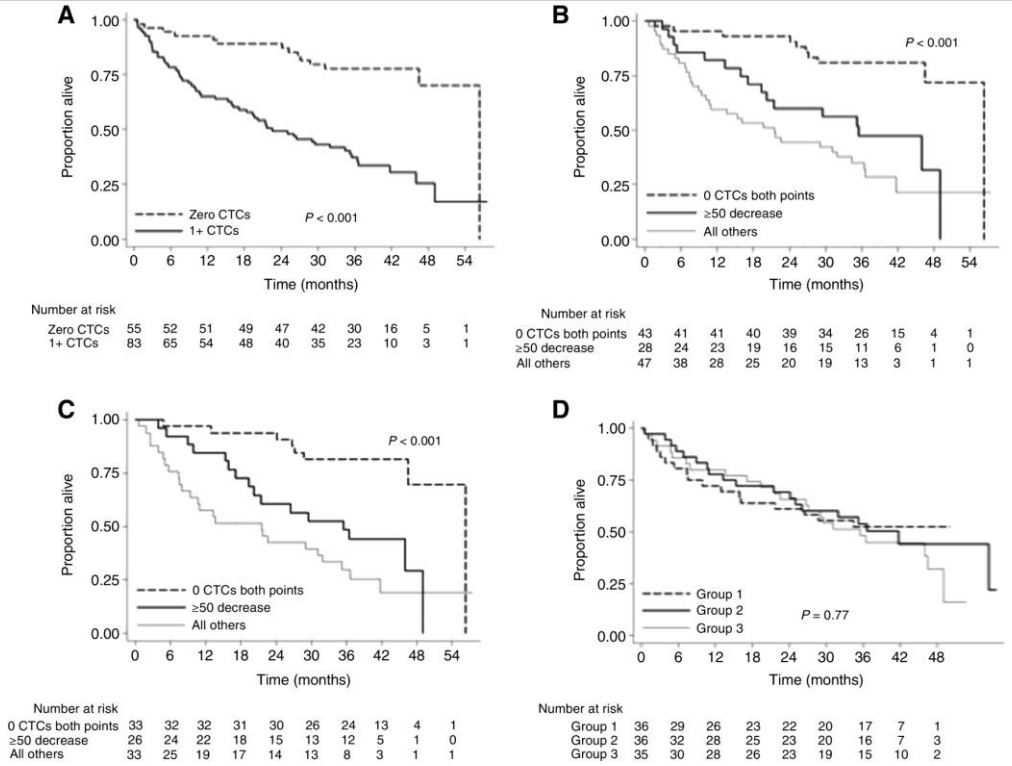
# CTCs as predictive markers for response

Table 2.

Association between changes in CTCs and CgA and response to therapy

| | Disease control* | Disease progression | |
|---|---|---|---|
| First posttreatment CTC | | | |
| 0 | 47/49 | 2/49 | $P < 0.001$ |
| 1–8 | 15/25 | 10/25 | |
| >8 | 9/26 | 17/26 | |
| Changes in CTCs | | | |
| Group A 0–0 CTCs | 35/36 | 1/36 | $P < 0.001$ |
| Group B ≥50% reduction | 20/24 | 4/24 | |
| Group C All others | 16/40 | 24/40 | |
| First posttreatment CgA | | | |
| CgA ≤ 120 | 24/35 | 11/35 | $P = 0.53$ |
| CgA > 120 | 44/59 | 15/59 | |
| Changes in CgA | | | |
| Group 1 >27% reduction | 19/29 | 10/29 | $P = 0.61$ |
| Group 2 ≤27% reduction or <12% increase | 24/32 | 8/32 | |
| Group 3 ≥12% increase | 24/32 | 8/32 | |

*Stable disease or partial response.




**The Kaplan–Meier survival curves demonstrating**
**(A) effect of the presence of baseline CTCs on OS**
**(B) OS dependent on changes in CTCs at first posttreatment time point (3–5 weeks) compared with baseline CTC**
**(C), OS dependent on changes in CTCs at second posttreatment time point (10–15 weeks) compared with baseline CTC in groups**
**(D), OS dependent on changes in CgA at first posttreatment time point**

**Mohid S. Khan et al. Clin Cancer Res 2016;22:79-85**

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# So, what is the status for CTCs?

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## Pros

- Useful in other kinds of malignancies
- Seems to be interesting for NETs (of any origin?)
- Quite easy to take a sample

## Cons

- Not a routine assay – the CellSearch instrument is not available everywhere
- Further studies are needed to verify the results

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# MicroRNAs

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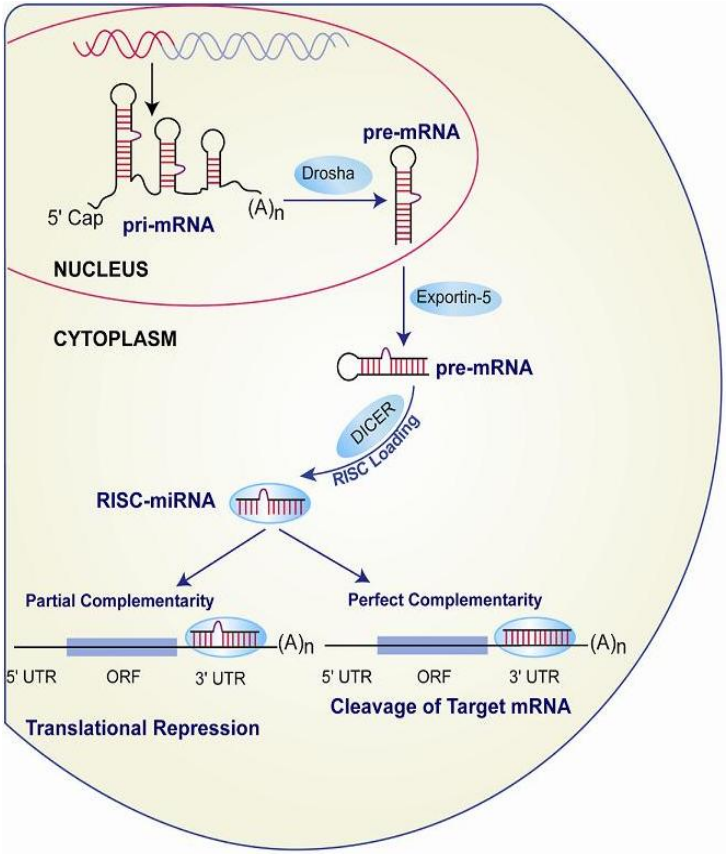


miRNAs are expressed by non-coding parts of the genome and are post-transcriptional regulators, which control cell proliferation, differentiation and apoptosis in a variety of cells by degradation or translation inhibition of specific mRNAs

miRNAs have been identified as potential oncogenes or tumor suppressors

From, Bioinformation, 2010; 5(6): 271-276

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# miRNAs in SI-NETs and P-NETs

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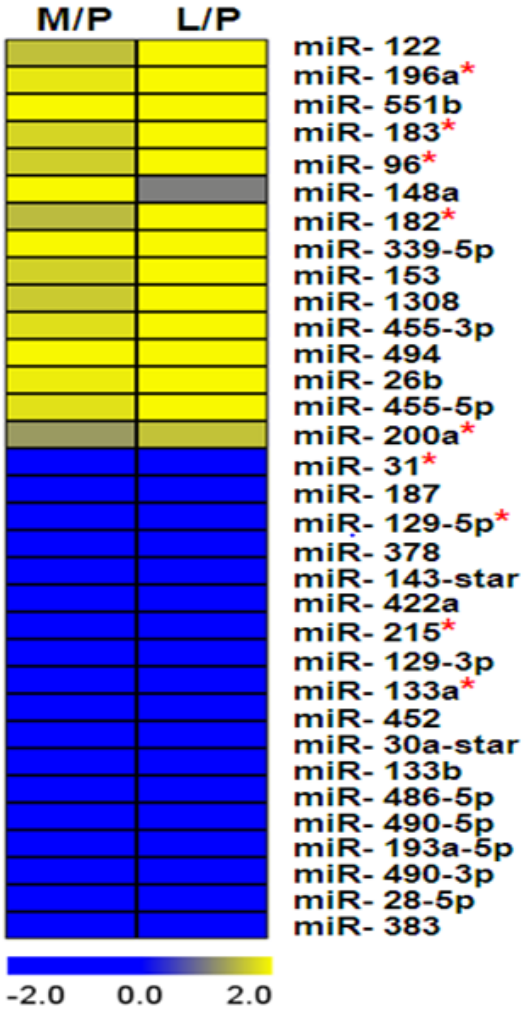
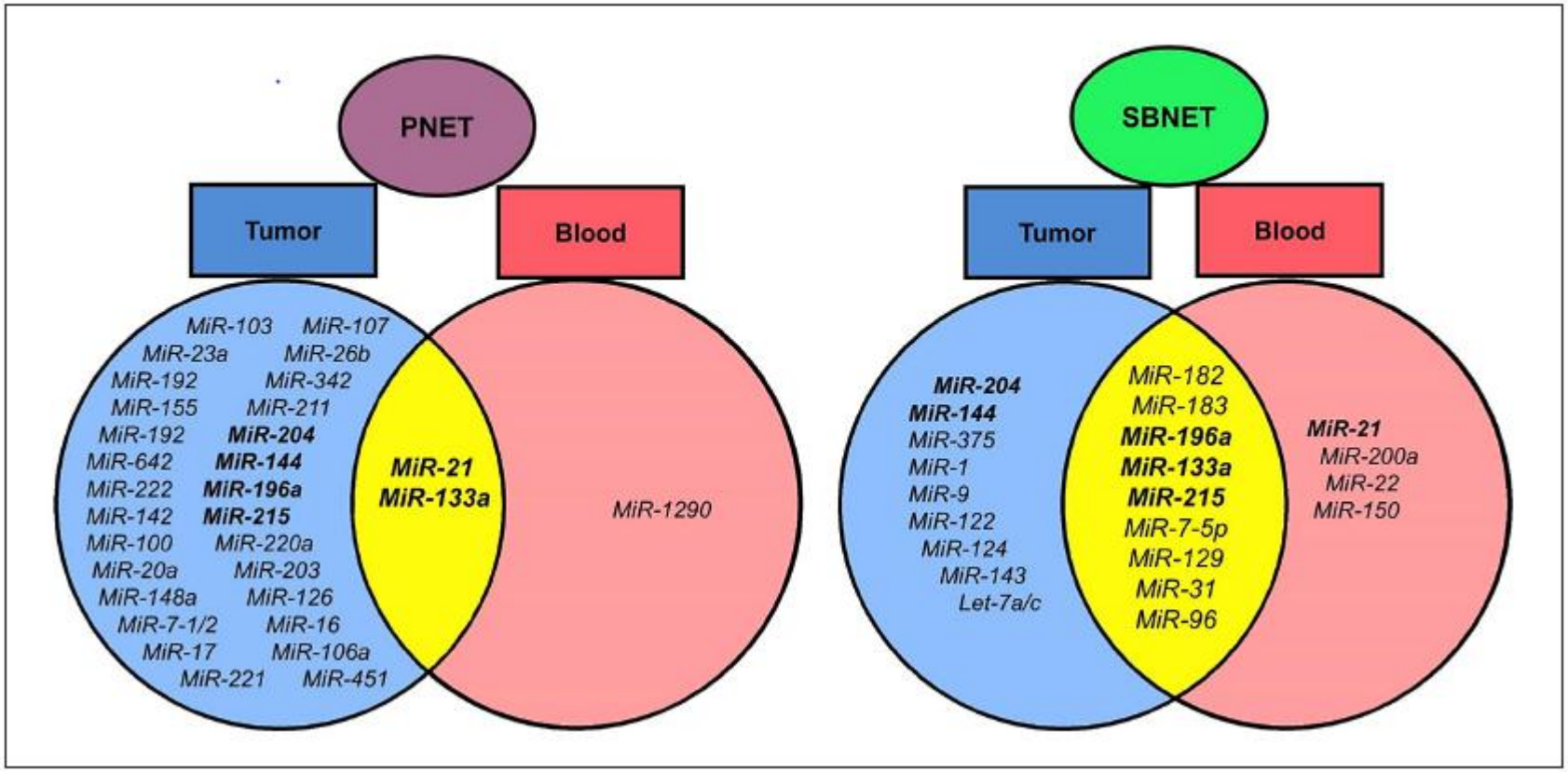



- The expression of miR-96, -182, -183, -196a and -200a is significantly upregulated in **SI-NET** cells compared to normal EC cells
- The expression of miR-31, -129-5p, -133a and -215 revealed significant down-regulation in **SI-NET** cells compared to normal EC cells
- Comparison of miRNA expression between **pNET**, normal pancreatic islets and serum samples
  - miR-624 correlated with Ki67 index
  - miR-201 correlated with metastatic disease
  - miR-193b was higher in neoplastic than in normal pancreatic islets, and also increased in serum

**Difficult to find any predictive or prognostic miRNAs**

# Systematic overview of miRNA expression in NET




Malczewska A et al, Neuroendocrinology 2018

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# And, the status for microRNAs?

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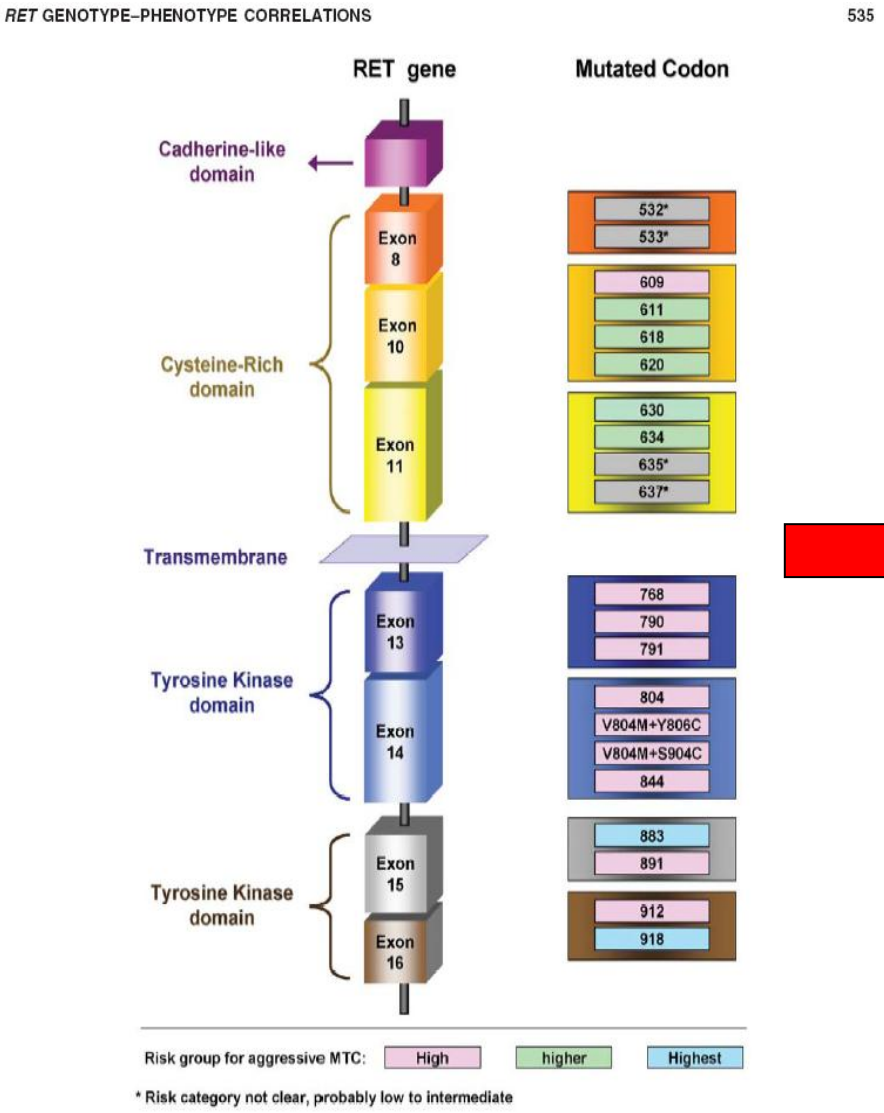
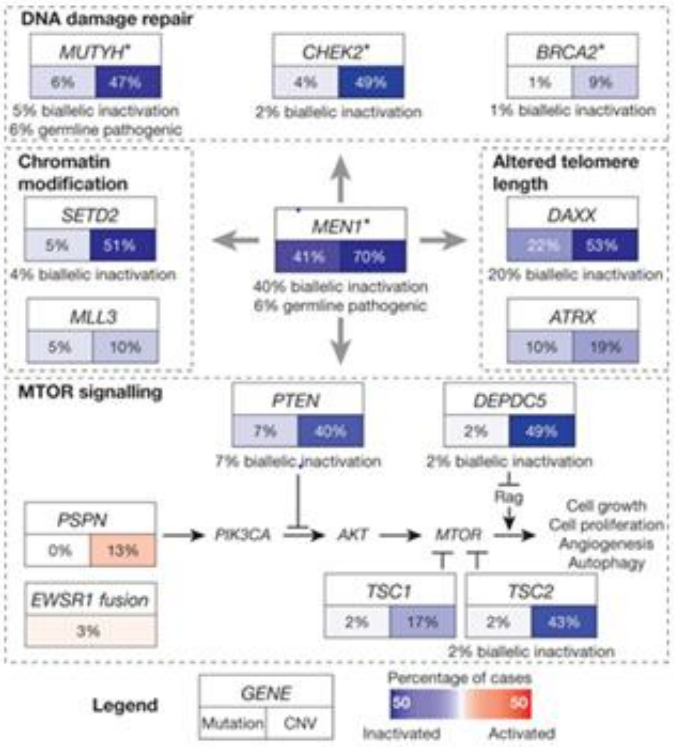
- An enormous amount of effort has been put into miRNAs to define their role in tumor development
- Their place in the diagnostic work-up of NETs is still quite uncertain

# Genetic testing




- Some inherited NETs have specific genetic changes and should be tested
  - MEN 1; *MENIN*, no phenotype/genotype correlation
  - MEN 2; *RET*, with phenotype/genotype correlation
  - Von Hipple-Lindau; no phenotype/genotype correlation

# The genetic landscape of Pan-NETs




- *MENIN* often mutated in both hereditary and sporadic cases
- *DAXX* and *ATRX* have been identified as predictors of prognosis
- mTOR pathway mutations may be of importance for treatment
- Mutations in DNA-repair pathways genes a new finding

A Scarpa *et al. Nature* 1–7 (2016) doi:10.1038/nature21063

# Loss of DAXX and ATRX Are Associated With Chromosome Instability and Reduced Survival of Patients With Pancreatic Neuroendocrine Tumors

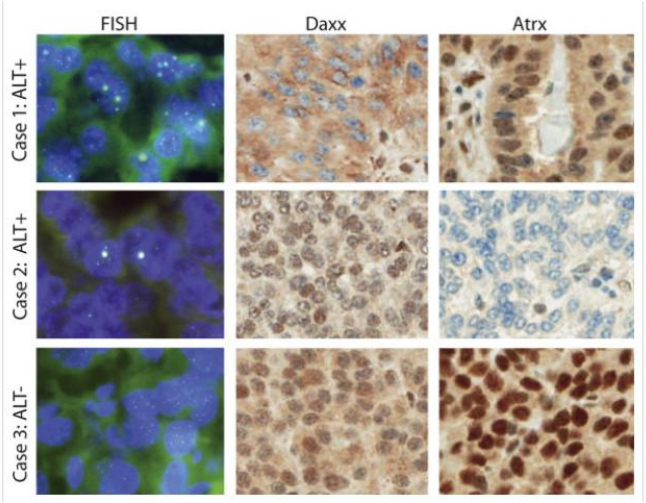
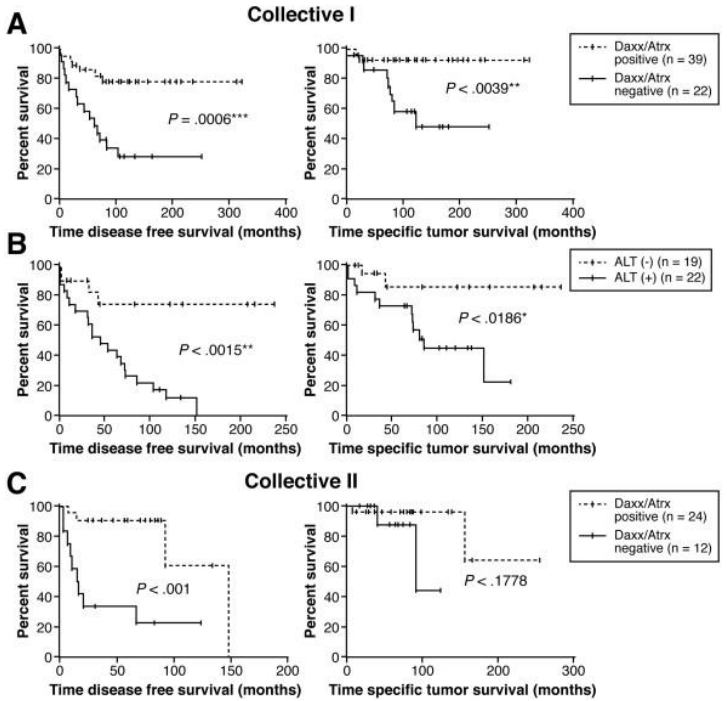



Table 2.

Correlation Between ALT Activation, CIN, and DAXX and ATRX Nuclear Expression

| | CIN- | CIN+ | *P* value | ALT + | ALT - | *P* value |
|---|---|---|---|---|---|---|
| ALT | | | | | | |
| Positive | 4 | 16 | .0095 | | | |
| Negative | 13 | 7 | | | | |
| DAXX/ATRX nuclear staining | | | | | | |
| Positive | 14 | 9 | .0361 | 3 | 17 | .0003 |
| Negative | 6 | 16 | | 17 | 7 | |

Figure 2 ( *A* ) Kaplan–Meier curves of collective I depicting a significantly shorter relapse-free survival and a shorter tumor-specific survival in DAXX- or ATRX-negative pNETs compared with DAXX- and ATRX-positive pNETs.




*DAXX/ATRX* and Alternative Lengthening of Teolmeres (ALT) associate with Chromosomal Instability (CIN) in turn associating with worse outcome

**Marinoni et al**
**Gastroenterology 2014**

# SI-NETs
# Sporadic and inherited

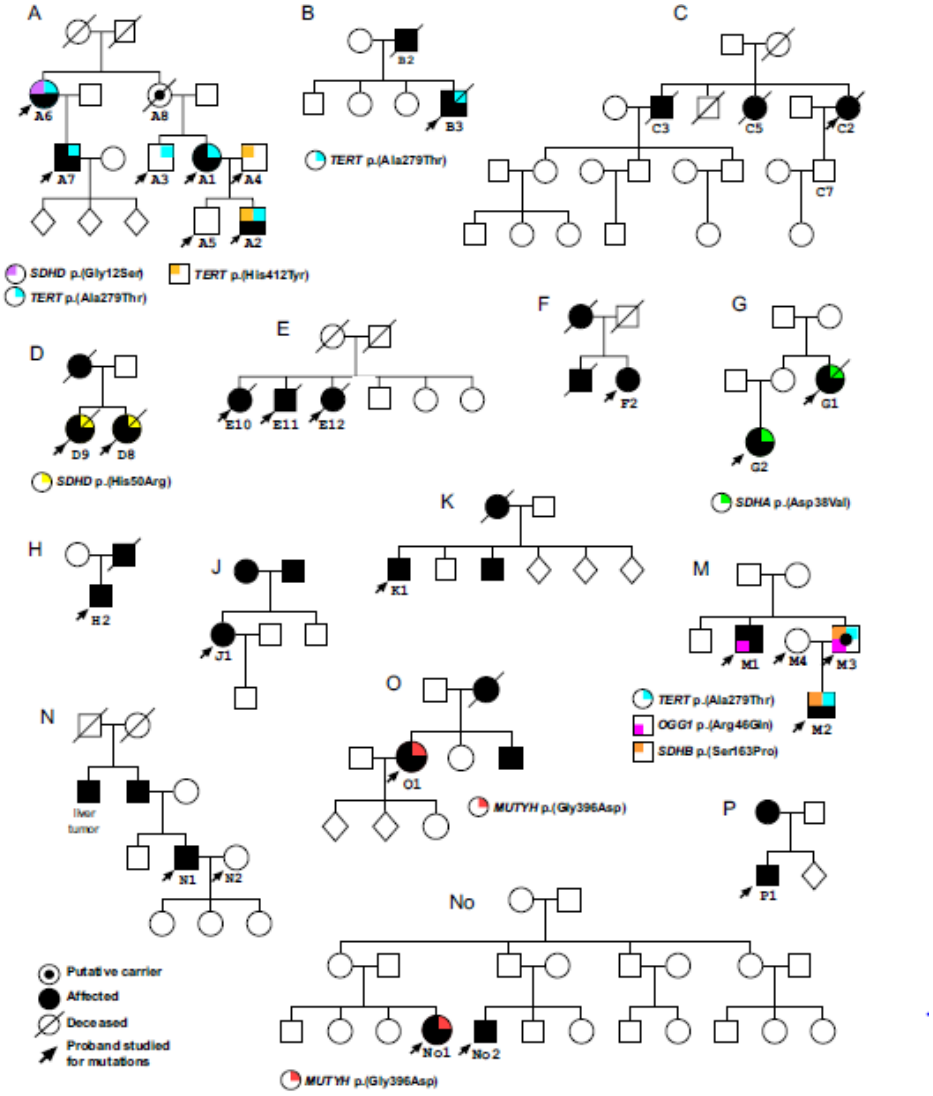
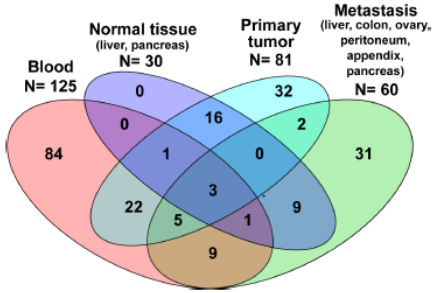



**Table 1** Summary of clinical data comparing hereditary and sporadic patients.

| Patients | Hereditary ($n=26$) | Sporadic ($n=215$) |
|---|---|---|
| WHO grade 1[a] | 10 | 115 |
| WHO grade 2[a] | 8 | 45 |
| Unknown tumor grade | 8 | 55 |
| TxNxM0[b] | 5 | 61 |
| TxNxM1[b] | 20 | 147 |
| Unknown tumor stage | 1 | 7 |
| Dead with disease | 13 | 111 |
| Alive with disease | 11 | 96 |
| No follow-up information | 2 | 8 |
| Median age at diagnosis | 57 (34–68) | 61 (23–90) |
| Median survival (months) | 83 (40–348) | 92.5 (2–348) |




Germline mutations
TERT
SDHA
SDHD
MUTYH
OGG1

Loss of chromosome 18
CDKN1B mutations
DNA repair – ***MUTYH, OGG1***

Dumanski JP et. al Endocrine-Related Cancer 2017

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# Genetic tests can be used in the clinic (?)

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- Clinical sequencing of tumors are becoming increasingly used
- The challenge is to know how to interpret the results
- In some cases, mutations may be useful for prognosis
- For patients with inherited syndromes, genetic testing is valuable for relatives
- More research is needed to define how mutation screening (or whole genome sequencing) can be included in the clinical practice
  - The problem with data storage capacity is fundamental and needs a solution

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# The NETest

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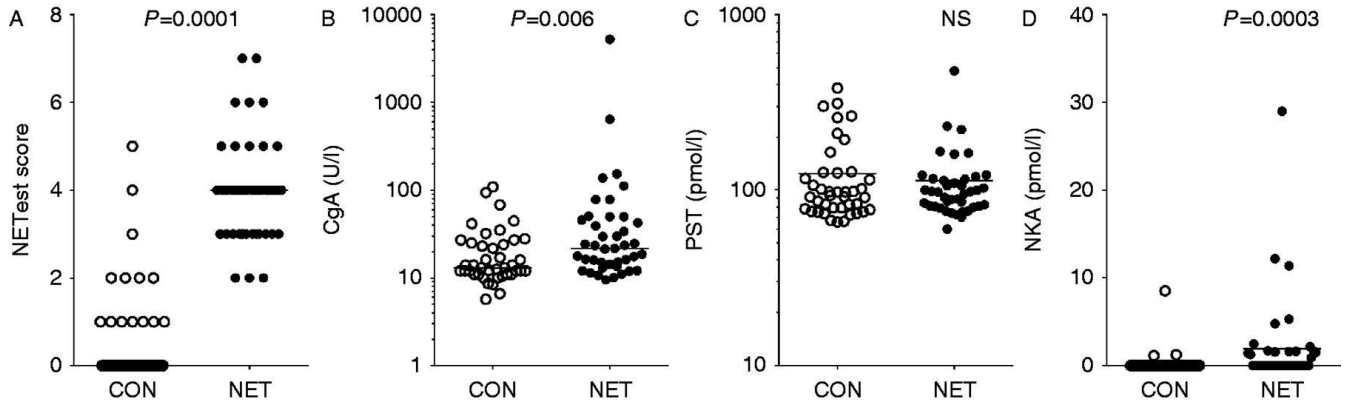
- A PCR based test containing 51 neuroendocrine tumor gene transcripts
- Can be used for both SI-NETs and P-NETs
- Can define stable and progressive disease
- Can identify NET disease recurrence prior to imaging




Irvin M Modlin et al. Endocr Relat Cancer 2014;21:615-628

# Can the NETest be used to predict response?

- A study of 49 NET patients undergoing $^{68}$Ga-DOTA PET/CT
- $SUV_{max}$, CgA, Ki67 and NETest were analyzed
- A combination of circulating transcript levels, particularly MORF4L2, and imaging effectively differentiated progressive from stable disease.

Bodei et al. Eur J Nucl Med Mol Imaging 2015

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# What about the NETest

AKADEMISKA SJUKHUSET

- Still few published studies
- We don’t really know what is being measured
- However, the test is already commercially available – patients may ask for it so we need to understand it’s usefulness and clinical value

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# Conclusions

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There are several new potential biomarkers, but data is missing on their usefulness in the clinic

When we are evaluating new potential candidates special attention should be given to:

- How easy is the sampling?
- How reproducible is the analysis?
- How sensitive and specific is it?
- The cost/benefit of the analysis.

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# Thank you for your attention

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