NET gene expression (mRNA) quantification in blood

Classification Algorithms for multianalyte algorithm analysis

Pancreatic NET Transcriptomes

Molecular delineation of small intestinal NETs

NET gene expression (mRNA) quantification in blood

Classification Algorithms for multianalyte algorithm analysis

Pancreatic NET Transcriptomes

Molecular delineation of small intestinal NETs

Tumor Tissue Transcriptome Arrays

2005

2006

2009

2010

2011

2012

2013

2014

2015

2016

2017

2018

2019

2020

Guidelines

PRRT Predictor

Monitoring Progression

Minimum Residual Disease

Diagnostic Accuracy

Clinical Risk quantification of signature

Molecular signatures in blood of small intestinal NETs

NET gene expression (mRNA) quantification in blood

Pancreatic NET Transcriptomes

Molecular delineation of small intestinal NETs

Tumor Tissue Transcriptome Arrays

Platform Steps

Developmental Timeline
Artificial Intelligence-based Mathematical Modelling
Blood-based tumor-related gene signatures

22,000 genes

51 Canonical NET Markers
Correlation of Blood mRNA with Tumor ($R^2$)

TUMOR transcriptome

BLOOD signature

TUMOR:BLOOD

$R^2 > 0.8$

Blood mRNA quantification directly reflects tumor gene expression

NETest functions as a liquid biopsy
Circulating (blood-based) mRNA measurements reflect tissue gene expression and provide a surrogate marker or "liquid biopsy" for tumor biology.
Whole Blood Sample

mRNA isolation

cDNA synthesis

qPCR: Multi Gene

Circulating Gene Expression Fingerprint

Diagnostic Analysis
“Tumor Score”

Analysis 2
“Omic Index”

NETest Risk Index

0% - 40% Low

41% - 79% Intermediate

>80% High

NETest Score

RISK OF NET

0 20 40 60 80 100

0 HRS - 8 HRS
NETest is a circulating fingerprint that captures GEP-NET pathobiology and provides a risk scale (0-100%).

Risk scale captures tumor activity (including “omes”) and provides a metric of tumor behavior.

Modlin I et al. PlosOne 2013;e63364
Kidd M et al. ERC 2015; 22:561-75
NETest: Performance Metrics

Accurate, Robust, Reproducible

Blood Multi Gene PCR Assays

- Sensitivity >90%
- Specificity >90%
- Negative Predictive Value >90%
- Positive Predictive Value >90%
- Accuracy >93%
- AUROC >90%
- Inter-assay: 0.5-2%
- Intra-assay: 0.4-1.5%
Multi Gene Expression Assay vs CgA

**AUROC: NETs vs controls**

- **CgA ELISA Protein Marker:**
  - AUC < 0.65

- **Blood Gene Expression:**
  - AUC > 0.90

- **Z-statistic:**
  - > 6.0, \( p < 0.0001 \)

**NETest:**
- Sensitivity (85-98%) and specificity (93-97%) (\( n = 1,020 \))

Modlin I et al. PlosOne 2013;e63364
Numerous validation studies have demonstrated greater accuracy and reliability of the NETest over currently used one-dimensional biomarkers.

NETest outperforms single tumor markers in all analytical metrics.
PCR Blood Test Accuracy – Limit of Detection
Comparison with imaging

CT Scan

10mm³ = 1,000,000,000 cells

PCR Blood Test

Limit of Detection = 1 Tumor cell/ml

5.5 liters of blood (70kg male)

Blood PCR Tests = ~125,000 x more sensitive than imagery

Modlin I et al. Endocrinol Metab Clin North Am. 2018
Defining Disease Progression

GEP-NETs (n=34)
Follow-up: median 4 years (2.2-5.4)
Grade I: n=15, Grade II: n=17; 31 (91%): stage IV.
Baseline, longitudinal imaging and biomarkers available
Progression defined (RECIST 1.0).

Baseline NETest

NETest vs. Imaging

NETest (>80%) predicts PFS and is clinically actionable

Pavel et al., Neuroendocrinology 2017
Demonstration of Clinical Utility

Assessment of NETTest Clinical Utility in a U.S. Registry-Based Study

Eric Liu, a Scott Paulson, b Anthony Gulati, c Jon Freudman, d William Grosh, e Sheldon Kafir, f Prasana C. Wickremesinghe, g Ronald R. Salem, h Lisa Bodei i

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Key Words. Biomarker • Carcinoid • Liquid biopsy • NET transcripts • Neuroendocrine • Registry
Registry Assessment of Clinical Utility

- The utility of the NETest in the clinical management of NETs is evident
- **Diagnostic accuracy** of the NETest was ≥96%
- **Reproducibility**: NETest scores were highly (97%)
- **Clinical status correlation**: There was a significant with initial NETest levels and (stable vs. progressive disease, 93%)
- **Clinical outcomes**: NETest levels were strongly associated with outcome (93%–100%) in both watch-and-wait programs and treatment protocols
- **Multivariate analyses** identified the NETest as the only variable (including clinical characteristics) significantly related to PFS
- **A high NETest** (≥80%) identified disease progression or treatment failure and was associated with a shorter PFS
- **A low NETest** (≤40%) indicated stable disease and prompted a reduction in imaging in 40%

Using the NETest values even at the simplest levels (low or high) identifies disease stabilization or disease progression. Scores can be used to recommend intervention or treatment modification.
NETest allows appropriate treatment intervention

CgA had no clinical utility

LOW = 1-2xULN
20-40%

HIGH = 4-5xULN
80-100%

Liu et al. The Oncologist 2018
Validated Role in Somatostatin Analog Therapy

**USA REGISTRY STUDY 2018**

**NET test**

- **Low** (1-2xULN)  
  - n=32  
  - 100%

  - **Documented Disease Stability**

  - **Continue on SSAs**

- **High** (4-5xULN)  
  - n=14  
  - 100%

  - **Documented Disease Progression**

  - **Increase dose and/or Additional therapy**

Liu et al. The Oncologist 2018
“Low scores also associated with decreased imaging frequency in ~40% of patients. The authors were comfortable using the NETest level to postpone imaging in these patients.”

In our study, a low NETest score was associated with a decreased imaging frequency in ~40% of patients, irrespective of whether they were in the watch-and-wait or treatment group. It seems likely that using the NETest as an adjunctive diagnostic has cost utilization implications and radiation exposure considerations.
In our study, a low NETest score was associated with a decreased imaging frequency in ~40% of patients, irrespective of whether they were in the watch-and-wait or treatment group. It seems likely that using the NETest as an adjunctive diagnostic has cost utilization implications and radiation exposure considerations.

“Low scores also associated with decreased imaging frequency in ~40% of patients. The authors were comfortable using the NETest level to postpone imaging in these patients.”
Outcomes – Survival and NET Disease

National Cancer Institute’s Neuroendocrine Tumor Clinical Trials Planning Meeting 2011

- PFS should be the primary endpoint for any clinical trials for NETs.
- Long overall survival durations would effectively preclude trials being performed.
- PFS subsequently validated as surrogate for OS in NETs.

Kulke et al. JCO 2011, The Oncologist 2017
Imaoka et al. ERC 2017

Validation of the NETest to predict PFS

**NETest Validation of the NETest to predict PFS**

**Low NETest (green)** = extended PFS
**High NETest (red)** = verified rapid disease progression

![Graphs showing survival rates with NETest categories](image)
A meta-analysis of the accuracy of a neuroendocrine tumor mRNA genomic biomarker (NETest) in blood

K. Öberg¹, A. Califano², J. R. Strosberg³, S. Ma⁴, U. Pape⁵, L. Bodei⁶, G. Kaltsas⁷, C. Toumpanakis⁸, J. R. Goldenring⁹, A. Frilling¹⁰ & S. Paulson¹¹

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Available online 6 January 2020
How accurate is the NETest?

What can the NETest be used for?

NETest – Meta-Analysis

In vitro Diagnostic

Disease Status (SD/PD)

Meta-Analysis

Monitor Therapy

Natural History

Oberg et al. Annals Oncology 2020
The NETest is an accurate biomarker suitable for clinical use in NET disease management.

The meta-analysis supports the utility of the NETest as an IVD to establish a diagnosis and monitor therapeutic efficacy.

The use of this as a biomarker provides information relevant to NET management consistent with observations regarding utility of liquid biopsies in other oncological disciplines.
NETest cases
Patient #1 Surgery - Am I cured?

- 60 yr man, presented with unexplained abdominal pain.
- Underwent ERCP and MRI.
- Tumor detected in pancreas.
- Surgery undertaken.

Disease removal

Is surgery effective?
Molecular signature demonstrates that surgery was not effective for disease control (residual/microscopic disease left in abdomen)

Liquid Biopsy can be used to identify efficacy of the surgery and thereafter monitor disease status and the need for treatment.
Patient #2: Surgery Am I cured?

Molecular signature confirms complete resection
Surgery was effective for disease control (tumor removal)

Post-operatively follow up with the liquid biopsy (Molecular tool) can now be used to accurately monitor that patient remains disease free.....
Patient #3

- 64 yr woman
- Lung cancer
- Atypical/neuroendocrine
- Diagnosis 11/2012
- Surgery 1/2013
- Lower left lobectomy

**IMAGING**

No evidence of disease

**2015**

**HORMONE LEVELS**

<table>
<thead>
<tr>
<th>CGA</th>
<th>Date</th>
<th>Value</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGA</td>
<td>8/10/2015</td>
<td>63.3</td>
<td>&lt; 108</td>
</tr>
</tbody>
</table>
Patient #3

HORMONE LEVELS

| CGA  | 5/20/2016 | 85 ng/mL | Normal | < 108 |

IMAGING

-ve

2016

MULTIGENE MOLECULAR SIGNATURE

NET test Percentage (%)

NET test
- High
- Intermediate
- Low
- Normal

POS

No evidence of disease

Molecular Evidence of disease
MULTIGENE MOLECULAR SIGNATURE

PREDICTING THERAPY “COMPANION” DIAGNOSTIC

DIAGNOSED DISEASE METASTASES

WHAT TREATMENT?

Molecular tool predicts patient will respond (99%)
Patient #3

Effective metastatic disease control through molecular-based diagnosis, treatment prediction and monitoring.
How to accurately predict response to PRRT?

If high uptake at Ga-DOTATATE predicts response to PRRT, why do pts with same high update get different results???

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Grade</th>
<th>Ki67</th>
<th>FDG</th>
<th>ECOG</th>
<th>Krenning Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-NET</td>
<td>G2</td>
<td>4%</td>
<td>neg</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>R-NET</td>
<td>G3</td>
<td>20%</td>
<td>neg</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>SI-NET</td>
<td>G2</td>
<td>19%</td>
<td>neg</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Only 60% of pts with high uptake at Ga-DOTATATE (Krenning Grade 4) respond to PRRT!
Predicting PRRT

Current clinical factors cannot predict PRRT response!

Critical unmet need:
Biomarker to predict PRRT
Development of a biomarker to predict response to Lu-PRRT

Circulating NET Gene Expression

Nine "Omic" Clusters

Pre-PRRT Blood signature: High vs. Low

Grade: Low vs. High grade

Predict PRRT response

\[ P = \frac{e^{a+bX}}{1 + e^{a+bX}} \]

Logistic Regression Model

Binary Output

R

NR

Ki67 index

Bodei L et al. EJNMMI 2016
PRRT Predictive Biomarker - PPQ

89-97% prediction accuracy for therapeutic response to targeted radiation
PRRT genomic signature in blood for prediction of $^{177}$Lu-octreotate efficacy

Lisa Bodei$^{1,2}$ · Mark S. Kidd$^3$ · Aviral Singh$^4$ · Wouter A. van der Zwan$^5$ · Stefano Severi$^6$ · Ignat A. Drozdov$^3$ · Jaroslaw Cwikla$^7$ · Richard P. Baum$^{2,4}$ · Dik J. Kwekkeboom$^{2,5}$ · Giovanni Paganelli$^8$ · Eric P. Krenning$^{2,8}$ · Irvin M. Modlin$^{2,9}$

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$n=158$

Specific Transcript Genes + Histopathology Grading
PPQ Multi Gene Assay vs. Monoanalyte CgA
Three Prospective Cohorts ($n=158$)

PPQ >93% prediction accuracy for response vs CgA <50%
Utility of the NETest as a monitor of PRRT

European Journal of Nuclear Medicine and Molecular Imaging
https://doi.org/10.1007/s00259-019-04601-3

ORIGINAL ARTICLE

PRRT neuroendocrine tumor response monitored using circulating transcript analysis: the NETest

Lisa Bodei$^{1,2,3,4,5}$ · Mark S. Kidd$^6$ · Aviral Singh$^7$ · Wouter A. van der Zwan$^8$ · Stefano Severi$^9$ · Ignat A. Drozdov$^6$ · Anna Malczewska$^{10}$ · Richard P. Baum$^{2,3,4,5,7}$ · Dik J. Kwekkeboom$^{2,3,4,5,8}$ · Giovanni Paganelli$^9$ · Eric P. Krenning$^{2,3,4,5,8,11}$ · Irvin M. Modlin$^{2,3,4,5,12}$

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Utility of the NETest as a monitor of PRRT

NETest levels accurately reflect response to PRRT

Increases in non-responders

NETest decreases in PRRT responders

Bodei et al. EJNMMI 2019
Utility of the NETest as a monitor of PRRT

Changes in the NETest can be used to monitor PRRT response

Bodei et al. EJNMMI 2019