Molecular Classification of Cancer: 
Implications for Carcinoma of Unknown Primary

USCAP/AMP
March 25th, 2007

Mark G. Erlander, Ph.D.

Summary of Talk:

- 3-5% of diagnosed cancers have an unknown primary
- Classifying the origin of metastatic cancer is a continuum of known → 2-3 possibilities → unknown
- Knowledge of primary site improves patient survival
- Knowing cancer origin informs therapy
- Gene expression profiling can successfully classify different cancer types
- Gene expression assays have been developed that are “friendly” to routine formalin-fixed paraffin-embedded fine-needle biopsies
- These assays can be used to complement current IHC and imaging methods to classify metastatic cancers

References:


Molecular Classification of Cancer: Implications for Carcinoma of Unknown Primary

USCAP/AMP
March 25th, 2007

Mark G. Erlander, Ph.D.
Chief Scientific Officer
AviaraDx
Classifying Metastatic Cancer: A Continuum

- 3-5% of diagnosed cancers have an unknown primary\(^1\)
- Numbers vary: cancer classification of metastatic cancer is a continuum of known → differential diagnosis of 2-3 → CUP
- A CUP at hospital “X” may not be a CUP at hospital “Y”
- CUP histological classification\(^1\)
  - 50% well to moderately differentiated adenocarcinomas
  - 30% poorly or undifferentiated adenocarcinomas
  - 15% squamous cell carcinomas
  - 5% undifferentiated neoplasms
- Metastases to liver (25%) and bone (25%)\(^1\)

Knowledge of Primary Site Improves Survival

Cancers with favorable treatments:
- Germ cell carcinomas
- Ovarian cancer
- Breast cancer
- Cervical squamous cancer
- Neuroendocrine cancers
- Prostate cancer

1 Abbruzzese et al, JCO, Vol 13, No 8 (August), 1995
# Knowing Cancer Origin Informs Therapy

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>33%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>13%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>11%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>6%</td>
</tr>
<tr>
<td>Melanoma of skin</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
</tr>
<tr>
<td>Kidney</td>
<td>3%</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>3%</td>
</tr>
<tr>
<td>Leukemia</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2%</td>
</tr>
<tr>
<td>All Other Sites</td>
<td>18%</td>
</tr>
<tr>
<td><strong>Total Men:</strong></td>
<td><strong>699,560</strong></td>
</tr>
</tbody>
</table>

Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.


### Target-specific Drugs:

- HERCEPTIN
- TARCEVA
- AVASTIN, ERBITUX
- RITUXAN
- SUTENT
- GLEEVEC
- TARCEVA

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Breast</td>
<td>32%</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>12%</td>
</tr>
<tr>
<td>Colon &amp; rectum</td>
<td>11%</td>
</tr>
<tr>
<td>Uterine corpus</td>
<td>6%</td>
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<tr>
<td>Ovary</td>
<td>4%</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>4%</td>
</tr>
<tr>
<td>Melanoma of skin</td>
<td>4%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>3%</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>2%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2%</td>
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<tr>
<td>All Other Sites</td>
<td>20%</td>
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<tr>
<td><strong>Total Women:</strong></td>
<td><strong>668,470</strong></td>
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</tbody>
</table>

Current Approach To CUP

- Histologically-confirmed metastatic cancer
- Medical history
- Physical examination
- Biochemistry
- Urinalysis
- FOBT
- Chest radiograph
- CT of abdomen and pelvis
- Mammography
- Pathology consultation
- Immunohistochemistry

Current Routine for Cancer Classification

1. Biopsy from patient with tumor mass

2. Immunohistochemistry (IHC) Set of 5-10 Abs

   - Unknown/Indeterminate Diagnosis “CUP”

   - Complete many more IHC’s (~11-50)

   - Imaging (PET, CT, Mammography, MRI) Diagnostic Scopics

   - Understood Diagnosis

   - Determine Treatment

   - Still unknown

- Only 25% success @ MD Anderson
Tumor Classification via Gene Expression is Established


Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring

T. R. Golub,1,2,* D. K. Slonim,1† P. Tamayo,1 C. Huard,1 M. Gaasenbeek,1 J. P. Mesirov,1 H. Coller,1 M. L. Loh,2 J. R. Downing,3 M. A. Caliguri,4 C. D. Bloomfield,4 E. S. Lander1,5,*

Although cancer classification has improved over the past 30 years, there has been no general approach for identifying new cancer classes (class discovery) or for assigning tumors to known classes (class prediction). Here, a generic approach to cancer classification based on gene expression monitoring by DNA microarrays is described and applied to human acute leukemias as a test case.

38 acute leukemias (27 ALL, 11 AML) → Microarray Profiling → Develop class predictor (50 genes) → Test on independent set (34 samples) → Strong predictions on 29/34; accuracy 100%
Multiclass cancer diagnosis using tumor gene expression signatures


Microarray profiling of 14 tumor types from 218 samples

Build 14 classifiers using SVM algorithm
By completing 14 “one vs. all” (OVA) others using 144 samples

Test classification with remaining 54 samples

Obtained overall prediction accuracy of 78%
Why Gene Expression Profiling Can Classify Cancers Types

Cancers from different origins are derived from cells that arise from different developmental processes.

Gene expression is distinct between different developmentally-derived cell types.
Clinical Use of Molecular Classification of Cancer

Biopsy from patient with tumor mass

Immunohistochemistry (IHC) Set of 5-10 Abs

Unknown/Indeterminate Diagnosis “CUP”

Apply Molecular Classification

Confirmatory Imaging (PET, CT, Mammography, MRI)
Diagnostic Scopics and IHC

Still unknown

Known/Understandable Diagnosis

Understood Diagnosis

Determine Treatment

Determine Treatment
Predicting Cancer Origin by Classification Algorithm

Test sample

Gene Exp. Assay

Tumor Ref Database

Classification Algorithm

k | TOP 5 PREDICTIONS DISTANCE
---|------------------------
1  | INTESTINE 0.47
2  | INTESTINE 0.5
3  | INTESTINE 0.5
4  | INTESTINE 0.53
5  | INTESTINE 0.54
Commercial and In-Development Tests for Molecular Cancer Classification

- 92 gene PCR test for 39 cancer types
  - Quest
  - LabCorp

- Microarray-based test for 43 cancer types
  - Agendia (Europe)

- Microarray-based test for 15 tissue types representing 60 different morphologies (in-development)
  - Pathwork Diagnostics

- PCR-based test for 6 cancer types (in development)
  - Veridex
Agendia’s CupPrint®

- 600 individual samples representing 43 different tumor types
- 88% accuracy for a blinded 94 sample validation study, using formalin fixed paraffin embedded tissue
Pathwork™ Tissue of Origin Test

A microarray-based test
Measures the expression of >1600 genes
Helps identify Uncertain Primary Cancers
Currently, 15 tissue types representing 60 different morphologies
Test in development; now under review at the FDA
Pathwork™ Tissue of Origin Test: Case Study

- Biopsy from the neck mass of a patient previously diagnosed with thyroid cancer revealed metastatic poorly differentiated carcinoma.
- Pathologist suspected possible thyroid metastasis; IHC positive for TTF1.
- Patient treated for presumptive thyroid cancer.
- Patient later developed multiple neck, chest wall, and pleural recurrences.
- No response to thyroid cancer therapy observed.
- Pathwork™ Tissue of Origin Test reveals “non-small cell lung” as tissue of origin while no thyroid signature identified.
- TTF1 known to cross-react with lung.

Test under FDA review — not for clinical diagnostic use.

Platform Session (Section G)
Clinical Applications of Gene Expression Microarrays: Reproducibility of a Tissue of Origin Test for Metastatic Tumors of Unknown Origin
FA Monzon et al
Monday, March 26
2:15 PM
A Quantitative Reverse Transcriptase-Polymerase Chain Reaction Assay to Identify Metastatic Carcinoma Tissue of Origin

Veridex’s Assay

- RT-PCR assay for 10 genes + 2 reference
- Classifies 6 cancer types + other
- Database = 260 FFPE samples
- Overall accuracy = 78%
- Assay Performance of Independent set of 48 samples including metastatic carcinoma of known origin and CUPs

Known mets: 11/15 or 73.3%
Resolved CUP: 17/22 or 77.3%
92-gene assay for classifying 39 cancer types

- Classifies a large number of different tumor types
  
  Completed gene expression profiling of 466 frozen and 112 FFPE tumor samples w/ whole-genome microarray
  
  ✓ 25% were metastatic cancers of known origin
  ✓ IRB approved
  ✓ Two independent pathology reviews

  Result: can classify 39 tumor types

- Need an assay that is robust and sensitive for fine-needle biopsies
  
  Used bioinformatic approach to reduce whole genome to small gene set
  
  ✓ Genetic Algorithms

  Converted microarray to TaqMan RT-PCR assay
  
  ✓ Assay is compatible with RNA extracted from formalin-fixed tissues
  ✓ Assay is compatible with fine-needle biopsies
  ✓ Assay uses reference genes to normalize different sample inputs
  ✓ Assay has quality control cut-offs

  Use proven method for making predictions – K-Nearest Neighbor (KNN)
  
  ✓ Use top-five consensus for prediction

- Published in Archives of Pathology and Laboratory Medicine
Translating Microarray to Real-time RT-PCR Assay

22,000 Genes → ANOVA + ROC

1001 Genes → Genetic Algorithm + KNN

Top 100 Gene Sets (60-80 genes)

Best Gene Set: 74 genes

Most frequent 90 genes within Top 100 Gene Sets

126 non-redundant genes → TaqMan assays

87 genes
PCR has Greater Dynamic Range
PCR-based Assay for Classifying 39 Cancer Types

Molecular Classification of Human Cancers Using a 92-Gene Real-Time Quantitative Polymerase Chain Reaction Assay

Xiao-Jun Ma, PhD; Rajesh Patel, PhD; Xianqun Wang, PhD; Ranelle Salunga, BSc; Jaiji Murage, BSc; Rupal Desai, BSc; J. Todd Tuggle, BSc; Wei Wang, PhD; Shirley Chu, BSc; Kimberly Stecker, BSc; Rajiv Raja, PhD; Howard Robin, MD; Mat Moore, PhD; David Baunoch, PhD; Dennis Sgroi, MD; Mark Erlander, PhD

- **Context.**—Correct diagnosis of the tissue origin of a metastatic cancer is the first step in disease management, but it is frequently difficult using standard pathologic methods. Microarray-based gene expression profiling has shown great promise as a new tool to address this challenge.

- **Objective.**—Adoption of microarray technologies in the clinic remains limited. We aimed to bridge this technological gap by developing a real-time quantitative polymerase chain reaction (RT-PCR) assay.

- **Design.**—We constructed a microarray database of 466 frozen and 112 formalin-fixed, paraffin-embedded (FFPE) samples of both primary and metastatic tumors, measuring expression of 22,000 genes. From the microarray database, we used a genetic algorithm to search for gene combinations optimal for multitumor classification. A 92-gene RT-PCR assay was then designed and used to generate a database for 481 frozen and 119 FFPE tumor samples.

- **Results.**—The microarray-based k-nearest neighbor classifier demonstrated 84% accuracy in classifying 39 tumor types via cross-validation and 82% accuracy in predicting 112 independent FFPE samples. We successfully translated the microarray database to the RT-PCR platform, which allowed an overall success rate of 87% in classifying 32 different tumor classes in the validation set of 119 FFPE tumor samples.

- **Conclusions.**—The RT-PCR-based expression assay involving 92 genes represents a powerful tool for accurately and objectively identifying the site of origin for metastatic tumors, especially in the cases of cancer of unknown primary. The assay uses RT-PCR and routine FFPE samples, making it suitable for rapid clinical adoption.

*(Arch Pathol Lab Med. 2006;130:465–473)*
Predicting Cancer Origin by K-Nearest Neighbor

Test sample

92-gene PCR ➔ Tumor Ref DB

KNN classification

<table>
<thead>
<tr>
<th>k</th>
<th>TOP 5 PREDICTIONS</th>
<th>DISTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTESTINE</td>
<td>0.47</td>
</tr>
<tr>
<td>2</td>
<td>INTESTINE</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>INTESTINE</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>INTESTINE</td>
<td>0.53</td>
</tr>
<tr>
<td>5</td>
<td>INTESTINE</td>
<td>0.54</td>
</tr>
</tbody>
</table>

Prediction is based on consensus of top 5 calls:
- 5/5 High
- 4/5 High
- 3/5 Medium
- 2/5 Low
- 1/5 Unclassified
### Classifying 39 Cancer Types

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cancer Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>Pheochromocytoma</td>
</tr>
<tr>
<td>Brain</td>
<td>Astrocytoma anaplastic, Astrocytoma fibrillary, Glioblastoma, Glioblastoma multiforme, Oligoastrocytoma, Oligodendroglioma</td>
</tr>
<tr>
<td>Breast</td>
<td>Adenocarcinoma of breast ductal, Adenocarcinoma of breast lobular</td>
</tr>
<tr>
<td>Carcinoid-intestine</td>
<td>Tumor of duodenum carcinoid, Tumor of ileum carcinoid, Tumor of small intestine carcinoid</td>
</tr>
<tr>
<td>Cervix-adeno</td>
<td>Adenocarcinoma of cervix</td>
</tr>
<tr>
<td>Cervix-squamous</td>
<td>Carcinoma of cervix squamous cell</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Adenocarcinoma of endometrium endometrioid, Adenocarcinoma of endometrium papillary serous</td>
</tr>
<tr>
<td>GallBladder</td>
<td>Adenocarcinoma of gall bladder</td>
</tr>
<tr>
<td>Germ-cell-ovary</td>
<td>Teratoma immature, Teratoma of ovary mature cystic, Tumor of ovary yolk sac</td>
</tr>
<tr>
<td>GIST</td>
<td>Gastrointestinal stromal tumor of stomach</td>
</tr>
<tr>
<td>Kidney</td>
<td>Carcinoma of kidney renal cell chromophil, renal cell clear cell</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>Liver</td>
<td>Carcinoma of liver hepatocellular</td>
</tr>
<tr>
<td>Lung-adeno-large-cell</td>
<td>Adenocarcinoma of lung</td>
</tr>
<tr>
<td>Lung-small</td>
<td>Carcinoma of lung small cell</td>
</tr>
<tr>
<td>Lung-squamous</td>
<td>Carcinoma of lung squamous cell</td>
</tr>
<tr>
<td>Lymphoma-B</td>
<td>Lymphoma large B-cell diffuse</td>
</tr>
<tr>
<td>Lymphoma-Hodgkins</td>
<td>Lymphoma Hodgkins</td>
</tr>
<tr>
<td>Lymphoma-T</td>
<td>Lymphoma peripheral T-cell</td>
</tr>
<tr>
<td>Meningioma</td>
<td>Meningioma atypical recurrent, fibroblastic, meningothelial, secretory</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>Mesothelioma of pleura</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>Osteosarcoma</td>
</tr>
<tr>
<td>Ovary-clear</td>
<td>Adenocarcinoma of ovary clear cell</td>
</tr>
<tr>
<td>Ovary-serous</td>
<td>Adenocarcinoma of ovary serous</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Pancreas--Adenocarcinoma of pancreas ductal, mucinous, Carcinoma of pancreas acinar cell</td>
</tr>
<tr>
<td>Prostate</td>
<td>Adenocarcinoma of prostate</td>
</tr>
<tr>
<td>Skin-basal-cell</td>
<td>Carcinoma of skin basal cell</td>
</tr>
<tr>
<td>Skin-melanoma</td>
<td>Malignant melanoma</td>
</tr>
<tr>
<td>Skin-squamous</td>
<td>Carcinoma of skin squamous cell</td>
</tr>
<tr>
<td>Small-and-large-bowel</td>
<td>Adenocarcinoma of colon, duodenum, rectum, small intestine</td>
</tr>
<tr>
<td>Soft-tissue-Liposarcoma</td>
<td>Liposarcoma of lower extremity</td>
</tr>
<tr>
<td>Soft-tissue-MFH</td>
<td>Histiocytoma malignant fibrous, malignant fibrous metastatic, Myxofibrosarcoma</td>
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<td>Soft-tissue-Sarcoma-synovial</td>
<td>Sarcoma synovial biphasic</td>
</tr>
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<td>Stomach-adeno</td>
<td>Adenocarcinoma of stomach mucinous, Adenocarcinoma of stomach signet ring cell</td>
</tr>
<tr>
<td>Testis-other</td>
<td>Carcinoma of testis embryonal, Teratoma of testis metastatic, Tumor of testis mixed germ cell</td>
</tr>
<tr>
<td>Testis-Seminoma</td>
<td>Seminoma of testis</td>
</tr>
<tr>
<td>Thyroid-follicular-papillary</td>
<td>Carcinoma of thyroid follicular, Carcinoma of thyroid papillary</td>
</tr>
<tr>
<td>Thyroid-medullary</td>
<td>Carcinoma of thyroid medullary</td>
</tr>
<tr>
<td>UrinaryBladder</td>
<td>Carcinoma of bladder transitional cell, Carcinoma of bladder urothelial</td>
</tr>
</tbody>
</table>
## Performance of 92-gene assay for 39 types

<table>
<thead>
<tr>
<th>Tumor Types</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Tumor Types</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
<td>Mesothelioma</td>
<td>1.000</td>
<td>1.000</td>
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<td>1.000</td>
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<tr>
<td>Brain</td>
<td>1.000</td>
<td>0.996</td>
<td>0.905</td>
<td>1.000</td>
<td>Osteosarcoma</td>
<td>0.833</td>
<td>0.998</td>
<td>0.833</td>
<td>0.998</td>
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<td>Breast</td>
<td>1.000</td>
<td>0.998</td>
<td>0.974</td>
<td>1.000</td>
<td>Ovary-clear</td>
<td>0.813</td>
<td>0.998</td>
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<td>0.991</td>
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<td>1.000</td>
<td>0.983</td>
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<td>0.688</td>
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<td>Prostate</td>
<td>1.000</td>
<td>0.996</td>
<td>0.905</td>
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<td>Endometrium</td>
<td>0.563</td>
<td>0.992</td>
<td>0.692</td>
<td>0.987</td>
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<td>0.857</td>
<td>0.998</td>
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<td>GallBladder</td>
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<td>0.996</td>
<td>0.500</td>
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<td>0.400</td>
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<td>Skin-squamous</td>
<td>0.692</td>
<td>0.989</td>
<td>0.600</td>
<td>0.993</td>
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<td>GIST</td>
<td>0.917</td>
<td>0.998</td>
<td>0.917</td>
<td>0.998</td>
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<td>1.000</td>
<td>Soft-tissue-Liposarcoma</td>
<td>0.625</td>
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<td>0.989</td>
<td>0.500</td>
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<td>Soft-tissue-Sarcoma-synov</td>
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<td>1.000</td>
<td>1.000</td>
<td>0.986</td>
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<td>0.900</td>
<td>0.985</td>
<td>Stomach-adeno</td>
<td>0.091</td>
<td>1.000</td>
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<td>0.993</td>
<td>0.714</td>
<td>0.998</td>
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<td>0.846</td>
<td>0.994</td>
<td>0.786</td>
<td>0.996</td>
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<td>Lung-squamous</td>
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<td>0.994</td>
<td>0.750</td>
<td>0.993</td>
<td>Testis-Seminoma</td>
<td>0.933</td>
<td>0.996</td>
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<td>0.800</td>
<td>0.994</td>
<td>Thyroid-follicular-papillary</td>
<td>1.000</td>
<td>0.994</td>
<td>0.813</td>
<td>1.000</td>
</tr>
<tr>
<td>Lymphoma-Hodgkins</td>
<td>1.000</td>
<td>0.989</td>
<td>0.625</td>
<td>1.000</td>
<td>Thyroid-medullary</td>
<td>0.875</td>
<td>1.000</td>
<td>1.000</td>
<td>0.998</td>
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<td>Lymphoma-T</td>
<td>0.333</td>
<td>0.998</td>
<td>0.667</td>
<td>0.993</td>
<td>UrinaryBladder</td>
<td>0.792</td>
<td>0.996</td>
<td>0.905</td>
<td>0.991</td>
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<tr>
<td>Meningioma</td>
<td>0.909</td>
<td>0.998</td>
<td>0.909</td>
<td>0.998</td>
<td>Overall</td>
<td>0.816</td>
<td>0.995</td>
<td>0.816</td>
<td>0.995</td>
</tr>
</tbody>
</table>

- **Sensitivity**: the ability to predict true positives. = true positives / total observed positives. TP/(TP+FN).
- **Specificity**: the ability to predict true negatives = true negatives / total observed negatives. TN/(TN+FP).
- **PPV (Positive Predictive Value)**: fraction of true positives among the predictive positives = true positives / total number of predicted positives. TP/(TP+FP).
- **NPV (Negative Predictive Value)**: fraction of true negatives among the predictive negatives = true negatives / total number of predicted negatives. TN/(TN+FN).
• Are currently used genes robust?
  • e.g., GAPDH


  “These data provide an extensive analysis of GAPDH mRNA expression in human tissues, and confirm previous reports of the marked variability of GAPDH expression between tissue types.”

• Search our large tumor database
  • Find stable set of genes in frozen set
  • Validate in FFPE set
  • Pre-determined that requirement is 3-5 genes
  • Chose 5 genes
Five Reference Genes: Similar variation in FFPE and Frozen samples
Real-world Evaluation of Cancer Classification Assay

- Collaboration with Sharp Memorial Hospital (H. Robin, M.D., IRB approved)

- Retrospective set of blinded samples representing known and unknown primary origin were analyzed (ongoing study)

- All samples were CT-guided fine-needle biopsies that are formalin-fixed paraffin-embedded from lymph nodes, liver, bone, lung and pleura

- Cancer area(s) on section was demarcated by Sharp Hospital pathologist for macro-enrichment of sample

- 92-gene Cancer Classification Assay was completed on samples

- Determined concordance of assay result with known

- For Unknown: Completed retrospective analysis of imaging results and/or conducted subsequent IHC
65 year old woman with liver and bone metastases
<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Original Pathology diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1 - PANCREAS</td>
<td>▶ MUCINOUS ADENOCARCINOMA</td>
</tr>
<tr>
<td>K2 - PANCREAS</td>
<td></td>
</tr>
<tr>
<td>K3 - LUNG</td>
<td></td>
</tr>
<tr>
<td>K4 - PANCREAS</td>
<td></td>
</tr>
<tr>
<td>K5 - PANCREAS</td>
<td></td>
</tr>
</tbody>
</table>

Prediction: PANCREAS

Retrospective Analysis of Imaging Results

▶ PANCREATIC TUMOR
94 year old man with multiple masses in lung
Work-up Results

Assay Result

K1 - GASTRIC
K2 - GASTRIC
K3 - INTESTINE
K4 - INTESTINE
K5 - PANCREAS

Prediction: GASTRO-INTESTINAL

Original Pathology diagnosis

› PROBABLE BRONCHIOALVEOLAR CARCINOMA

Retrospective Analysis of Imaging Results

› NO ABDOMINAL WORK-UP

Subsequent IHC Results

› CDX2 positive
Real-World IRB-Approved Evaluation of Assay: So Far

- Biopsy from patient with tumor mass

  - Immunohistochemistry (IHC) Set of 5-10 Abs

  - Unknown/Indeterminate Diagnosis “CUP”

  - Complete many more IHC’s (~11-50)

- Known/Understandable Diagnosis

- Imaging (PET, CT, Mammography, MRI) Diagnostic Scopics

  - Understood Diagnosis

  - Determine Treatment

- Received 28 Fine-needle FFPE biopsies blind from patients with metastatic cancers of both known (21) and unknown (7) primary origin

  - Completed 92-gene assay
  - Completed predictions for 26 of 28 samples

- Of the 26 samples

  - 19 Known
    - Of the 19, 17/19 consistent (89%)

  - 7 Unknown
    - 7 predictions 5/7 or 70% confirmed retrospectively
Classifying the origin of metastatic cancer is a continuum of known $\rightarrow$ 2-3 possibilities $\rightarrow$ unknown

Gene expression profiling can successfully classify different cancer types

Several different gene expression tests have been developed that are all $\sim$80% accurate

These assays can be used to complement current IHC and imaging methods to determine primary origin