

Association of Molecular Pathology:
companion meeting at USCAP

The Clinical Significance of
Genomic Classifications of Breast Cancer

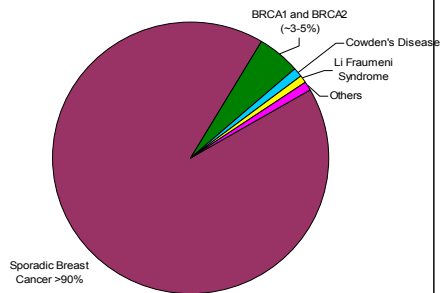
San Diego, CA
March 2007

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Objectives

- Understand how genomic (expression-based) classifications can be applied to risk stratifying breast cancer patients
 - Compare/contrast different analysis methods
- Learn what genes are commonly identified using “unsupervised” methods to define biological subgroups
- Understand the current challenges in clinically implementing genomic classifications

Familial Breast Cancer



Clinical Breast Cancer Facts

- More than 210,000 women are diagnosed with breast cancer each year in the US
- Approximately 40,000 women die of metastatic breast cancer in the US each year
- Approximately 70% of breast cancers are ER α -positive and receive tamoxifen or an aromatase inhibitor
- Approximately 30% of women with ER α -positive breast cancer fail hormone therapy
- Chemotherapy improves outcome (10 yr survival) in early breast cancer in young women (<50 yrs) by 71-78% for lymph node negative and from 42%-53% for lymph node positive disease
- Molecular Challenge: Develop prognostic classification schemes that accurately identify those at risk and not at risk for relapse and identify those likely to respond or not respond to current therapies

Barriers to Developing Molecular Diagnostics for Solid Tumors

- There is often a profusion of small studies done in the early phases of discovery and all claim novelty and superiority without adequate validation and comparison
- Many studies lack statistical significance because they are not initially designed with the power to address the hypotheses posed
- Lack of well defined and clinically annotated cases/specimens
- Lack of specimens with long periods of follow-up
- There is a need for standardization of reagents and assay procedures to facilitate comparison of data from multiple studies
- Intellectual properties issues complicate the development of assays/tests that can be performed for acceptable costs

General Analytical Strategies to Developing Expression-Based Classifications of Cancer

- **Unsupervised Analyses and Classification**
 - Hierarchical cluster genes with differential expression across tumors and generate a “biological” classifications based on sample-associated dendrogram
 - Limitation: Clustering is for discovery, not classification
- **Supervised Analyses**
 - Select genes that associate with a particular clinical phenotype (eg, relapse, node status, etc)
 - Limitation: Gene selection is “overtrained” and not reproducible when testing on independent cohorts
- **Hypothesis**
 - Select genes based on physiology and functional mechanism shown in model system (eg, serum response in fibroblasts)
 - Limitation: Mechanism may not be translatable to humans

Discovery and Validation of Transcript Biomarkers

- Independent validation of microarray signatures using "pure" training and test sets
- Transitioning from discovery to clinical implementation
 - Arriving at a diagnostic gene set
 - Minimizing microarray gene sets
 - Method of sample classification
 - Biomarker validation across platforms
 - Microarray to qRT-PCR to IHC
 - Developing a clinical diagnostic assay
 - Rigorous validation of each biomarker on the final platform
 - Specimen collection, transport, and stability
 - Fresh frozen, FFPE, etc
 - Clinical interpretation

Biomarker Assessment

- What makes a "good" biomarker
 - Accuracy and reproducibility of classification
 - Robustness within and across platforms and conditions
- How do we know a biomarker is behaving the same across different conditions?
 - Are we gleaning the same information across different platforms and different methods of procurement?
 - Comparison to "gold standard"
 - Comparison to outcome

Estrogen Receptor – Need we know more?

- The estrogen receptor consists 2 subunits (α and β) that hetero- and homo- dimerize causing direct and indirect activation of nuclear transcription and cell proliferation
- There are at least 3 mechanisms for estrogen dependent growth:
 - 1) Canonical - direct DNA contact of estrogen responsive elements (ERE) in the nucleus
 - 2) Indirect stabilization of transcription factor complexes that bind promoter and enhancing elements
 - 3) "Non-genomic" activation of signal transduction pathways (e.g., MAPK) in the cytosol through localization outside the nucleus

The Estrogen Receptor Cluster

- Estrogen induced genes cluster together
- 17 β -estradiol (E2) differentially regulates over 400 genes, the majority of which are down-regulated (*Frasor J et al, Endocrinology 2003*)
- ER- α binds over 150 promoters in response to E2
- FOXA1 knockout blocks E2 dependent activation and inhibits G1 re-entry (*Laganieri J, PNAS 2005*)

SUBTYPE IDENTIFICATION and DEVELOPING CLASSIFICATION TOOL



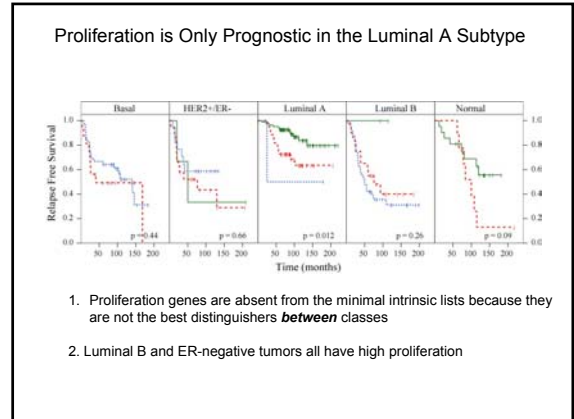
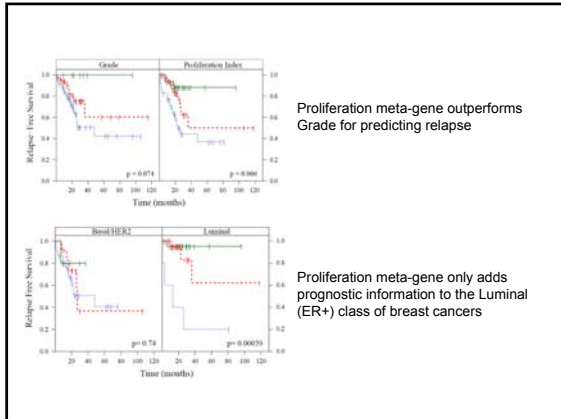
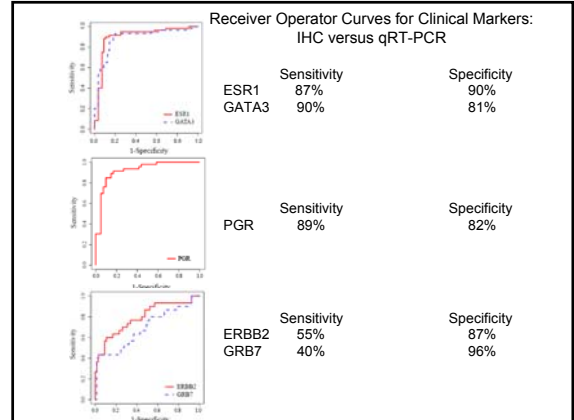
Differences Identified Between Stanford "Intrinsic" Set and More Recent UNC/Utah Set

- Stanford dataset (Perou, Nature 2000; Sorlie, PNAS 2001&2003) contained a LumB (ER+) group with poor prognosis
 - Artifact of hierarchical clustering?
 - No LumB Samples in the training set?
 - Lacking "intrinsic" genes necessary to identify the LumB class?
- UNC/Utah "intrinsic" list contained "proliferation" genes
 - Stanford "intrinsic" list based on before and after chemo treated pairs and UNC/Utah "intrinsic" list based on pairs receiving same treatment
 - Are genes of proliferation turned-off after chemo treatment?
- UNC/Utah "intrinsic" set has approximately 3X more genes (1300 versus 550)
 - Stanford arrays 8,000 genes versus UNC/Utah arrays 17,000 genes

Agreement Between “Intrinsic” Classification, IHC, and Real-Time qRT-PCR

- 117/126 (93% concordance) classified the same between microarray (402 gene intrinsic set) and qRT-PCR (37 gene minimal intrinsic set)
- 49/55 (89%) Luminal tumors were ER-positive by IHC
- 46/55 (85%) HER2+/ER- or Basal-like were ER-negative by IHC

Perreard et al, Breast Cancer Research 2006

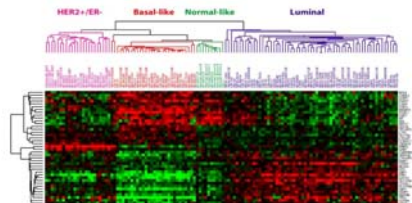


Validation of Biological Class Prediction from FFPE

- Microarray data as training set
 - Large intrinsic gene set (1393 genes)
 - Minimized intrinsic gene set (40 genes)
- Developed centroids and classified a test set of 35 samples using microarray and qRT-PCR data
 - Minimized intrinsic gene set (40 genes)
 - Both FFPE and fresh frozen preparations
- Compared to 3 marker IHC classification
 - ER+ => LUMINAL
 - ER-, HER2+ => HER2+/ER-
 - ER-, PR-, HER2- => BASAL (triple negative)

Microarray Training Set Large (1393) and Minimized (40)

124 samples: Luminal (64 samples), HER2+/ER- (23 samples), Basal-like (28 samples), and Normal-like (9 samples)



Analytical Tools

- Single Sample Predictor (SSP)
 - Centroid Based Prediction Algorithm
 - Hu, Z. et al, BMC Genomics 2006
- Predictive Analysis of Microarrays (PAM)
 - Nearest Shrunken Centroids Algorithm
 - Tibshirani, R. et al, PNAS, 2002
- Distance Weighted Discrimination (DWD)
 - Identification and adjustment of systematic biases
 - Benito, M et al., Bioinformatics, 2004

Prediction on Test Samples by PAM and SSP

- Predictive Analysis of Microarray (PAM)
 - 33/35 (94%) of the test samples classified the same across different platforms (microarray and PCR) and preparations (fresh frozen and FFPE)
 - 34/35 (97%) of the test samples classified the same class between fresh frozen and FFPE qRT-PCR
- Single Sample Predictor
 - 30/35 (86%) of the test samples classified the same across different platforms (microarray and PCR) and preparations (fresh frozen and FFPE)
 - 32/35 (91%) of the test samples classified in the same class between fresh frozen and FFPE qRT-PCR

Discrepancies between PAM, SSP, and IHC for Classification of Test Samples:

Sample	Centroid Predictors				IHC		
	MA (1393)	MA (40)	PCR-FF (40)	PCR-FFPE (40)	ER	PR	HER2
BR00-0572	BASAL	BASAL	BASAL	BASAL	NEG	NEG	POS
PB205	BASAL	BASAL	BASAL	BASAL	NEG	NEG	POS
PB362	NORMAL-LIKE	NORMAL-LIKE	NORMAL-LIKE	LUMINAL	POS	POS	NEG
PB376	HER2+ER-	BASAL	BASAL	BASAL	NEG	NEG	POS
PB311	HER2+ER-	HER2+ER-	HER2+ER-	LUMINAL	POS	POS	NEG
PB149	LUMINAL	NORMAL-LIKE	NORMAL-LIKE	LUMINAL	POS	POS	POS
UB60	LUMINAL	HER2+ER-	HER2+ER-	HER2+ER-	NEG	NEG	POS

- Basal-like by PAM and SSP but HER2+ER- by IHC
- Agreement by PAM and SSP but inconsistent across methods
- Discrepancies only within SSP Classification

NOTE: PAM and SSP showed 100% agreement from FFPE tissues

HER2 Gene Amplification Corroborates Genomic Classification

Sample	Centroid Predictors				IHC			PCR
	MA (1393)	MA (40)	PCR-FF (40)	PCR-FFPE (40)	ER	PR	HER2	HER2 AMP
PB205	BASAL	BASAL	BASAL	BASAL	NEG	NEG	POS	NEG
PB376	HER2+ER-	BASAL	BASAL	BASAL	NEG	NEG	POS	NEG
BR00-0284	HER2+ER-	HER2+ER-	HER2+ER-	HER2+ER-	NEG	NEG	POS	NEG
PB455	HER2+ER-	HER2+ER-	HER2+ER-	HER2+ER-	NEG	NEG	POS	POS
PB314	HER2+ER-	HER2+ER-	HER2+ER-	HER2+ER-	NEG	NEG	POS	POS
UB37	HER2+ER-	HER2+ER-	HER2+ER-	HER2+ER-	NEG	POS	POS	POS
UB60	LUMINAL	HER2+ER-	HER2+ER-	HER2+ER-	NEG	NEG	POS	POS

- Primarily Basal-like by PAM and SSP and not HER2 amplified
- Definitely HER2+ER- by all methods but not HER2 amplified
- All HER2+ER- by PAM and HER2 amplified

Genomic Health Inc 21 Gene Predictor

- ER Status
 - ESR1, PgR, BCL2, SCUBE2
- HER2
 - HER2, GRB7
- Proliferation
 - MIB1(Ki-67), STK6/15, MYBL2, Survivin, Cyclin B1
- Invasion
 - Stomolysin 3, Cathepsin L2
- Misc
 - GSTM1, CD68, BAG1
- Housekeepers
 - B-actin, GAPDH, RPLPO, GUS, TFRC



- RT-PCR assay (21 genes) to predict relative risk of distant metastasis in women with node-negative (stage I or II), ER positive tumors treated with tamoxifen
- Validation using 668 formalin-fixed, paraffin-embedded samples obtained from patients enrolled in B14 NSABP clinical trial from 1982-1988
- High Price Tag

A Multi-gene Assay to Predict Recurrence of Tamoxifen-Treated Node-Negative Breast Cancer, Paik et al., The New England Journal of Medicine, 351:2817-26 (2004)

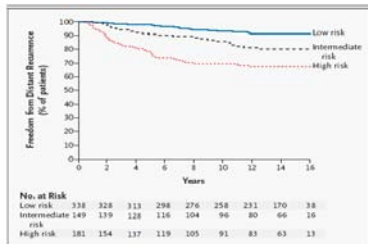


Figure 2. Likelihood of Distant Recurrence, According to Recurrence-Score Categories.
 A low risk was defined as a recurrence score of less than 18, an intermediate risk as a score of 18 or higher but less than 31, and a high risk as a score of 31 or higher. There were 28 recurrences in the low-risk group, 25 in the intermediate-risk group, and 56 in the high-risk group. The difference among the groups is significant (P<0.001).

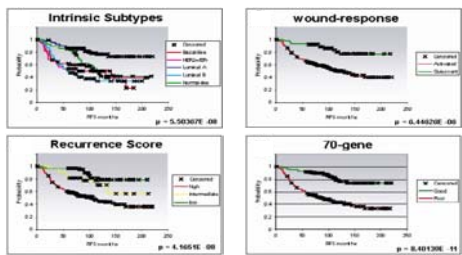
Head-to-Head Comparison of Four Expression-Based Assays For Prognosis in Breast Cancer

Classification Results from 295 Tumors from NKI (Chang et al, PNAS 2005)

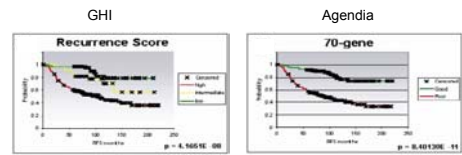
Intrinsic Subtype	GHI Recurrence Scores		Agendia 70-Genes		Wound-Response	
	Low	High	Good	Poor	Quiescent	Activated
Basal-like	53	0	53	0	53	3
Luminal A	Low	62	62	87	87	46
	High	25	26	36	78	
Luminal B	Low	1	1	9	4	
	High	4	50	48	51	
HER2+/ER-	Low	0	0	3	0	
	High	35	35	32	35	
Normal-like	Low	7	7	16		
	High	4	18	13	14	

Fan C et al, NEJM 2006

All Four Expression-Based Assays are Significant Predictors of Survival using a Multivariate Cox Proportional Hazards Model (Model Included: ER Status, Node Status, Age, Tumor Size and Treatment)



Head-to-Head Comparison of Two Commercial Expression-Based Assays For Prognosis in Breast Cancer



Overall concordance for all tumors (ER-positive and ER-negative) for evaluating risk was 85% (221/262). There were 63 out of 70 (90%) classified as low risk and good prognosis
 Within ER-positive alone there was 79% agreement (163/207). There Were 73 out of 87 (84%) classified as low risk and good prognosis

Conclusions

- Breast cancer can be risk-stratified using expression-based methods. Different gene sets and analytical techniques can provide the same information for prognosis and treatment
- Microarray data can be used as a training set to predict classification on samples profiled by qRT-PCR and many of the tools used for quantification can be applied across platforms
- Centroid-based predictors can robustly identify biological subtypes of breast cancer across platforms (microarray and qRT-PCR) and procurement methods (fresh frozen and FFPE)

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:

1. Ma, X.-J., Patel, R., Wang, X, et al. Molecular classification of human cancers using a 52-gene real-time quantitative polymerase chain reaction assay. *Arch. Path. Lab. Med.*, 2006; 130:465-473
2. Ismael, G., de Azambuja, E., Awada, A., Molecular Profiling of a Tumor of Unknown Origin. *New England J. Med.*, 2006, 355:1071-1072
3. Talantov, D. Baden, J., Jatkoa, T. et al., A Quantitative Reverse Transcriptase-Polymerase Chain Reaction Assay to Identify Metastatic Carcinoma Tissue of Origin. *J. Mol. Diag.* 2006, 8:320-9
4. Tothill RW, Kowalczyk A, Rischin D, et al. An expression-based site of origin diagnostic method designed for clinical application to cancer of unknown origin. *Cancer Res.* 2005;65:4031-4040.
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9. Giordano TJ, Shedden KA, Schwartz DR, et al. Organ-specific molecular classification of primary lung, colon, and ovarian adenocarcinomas using gene expression profiles. *Am J Pathol.* 2001; 159:1231-1238.
10. Ramaswamy S, Tamayo P, Rifkin R, et al. Multiclass cancer diagnosis using tumor gene expression signatures. *Proc Natl Acad Sci U S A.* 2001; 98:15149-15154.
11. Golub et al., *Molecular Classification of Cancer: Class Discovery and Class Prediction by Gene Expression Monitoring.* Science, 1999, 286:531-537.

Molecular Classification of Cancer: Implications for Carcinoma of Unknown Primary

USCAP/AMP
March 25th, 2007

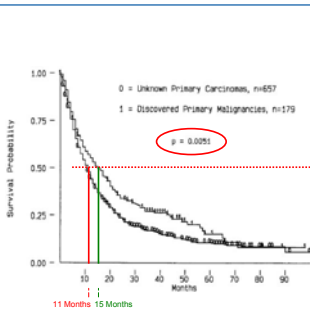
Mark G. Erlander, Ph.D.
Chief Scientific Officer
AviaraDx

Classifying Metastatic Cancer: A Continuum

- 3-5% of diagnosed cancers have an unknown primary¹
- 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 classification¹
 - 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

¹Pavlidis et al., Eur. J. Cancer 39, 1990-2005, 2003

Knowledge of Primary Site Improves Survival¹

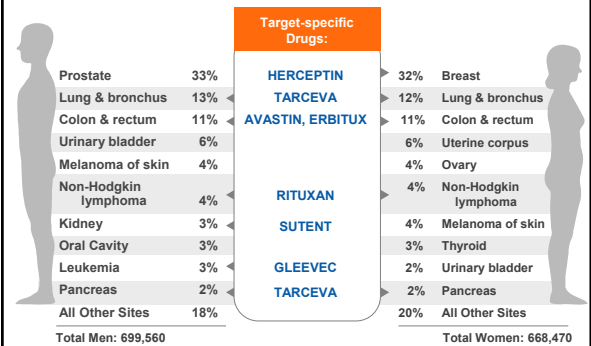


Cancers with favorable treatments²:

- 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
² Pavlidis et al, Eur. J. Cancer, 39, 1990-2005, 2003

Knowing Cancer Origin Informs Therapy



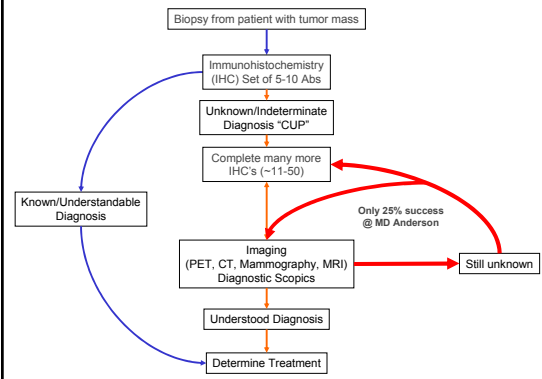
Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.
Source: American Cancer Society, 2004.

Current Approach To CUP¹

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

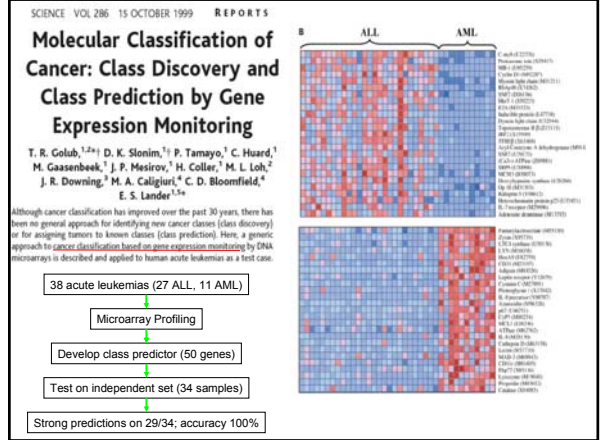
¹Pavlidis et al., Eur. J. Cancer 39, 1990-2005, 2003

Current Routine for Cancer Classification

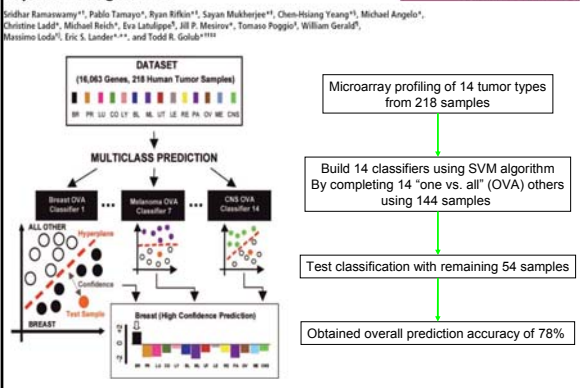


Tumor Classification via Gene Expression is Established

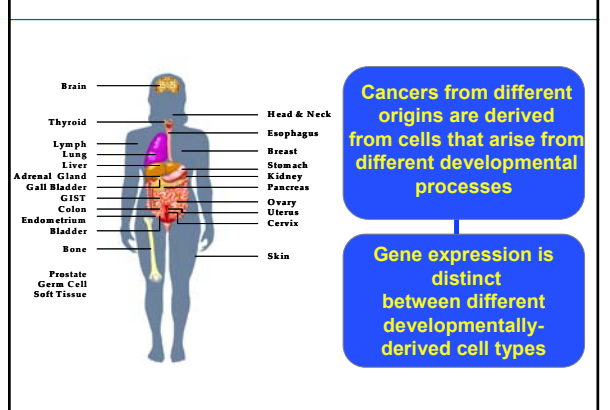
1. Ma, X.-J., Patel, R., Wang, X. et al. Molecular classification of human cancers using a 92-gene real-time quantitative polymerase chain reaction assay. *Arch. Path. Lab. Med.*, 2006; 130:465-473
2. Ismael, G., de Azavedo, E., Awada, A. Molecular Profiling of a Tumor of Unknown Origin. *New England J. Med.*, 355:1071-1072
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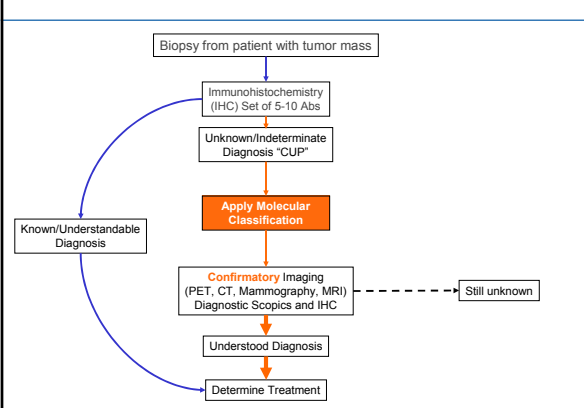
Multiclass cancer diagnosis using tumor gene expression signatures



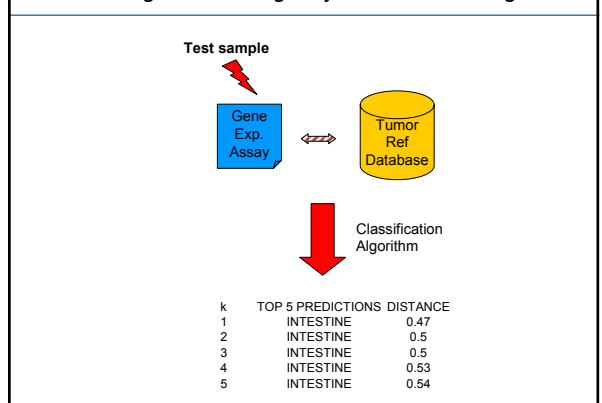
Why Gene Expression Profiling Can Classify Cancers Types



Clinical Use of Molecular Classification of Cancer



Predicting Cancer Origin by Classification Algorithm



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**

Molecular Profiling of a Tumor of Unknown Origin

TO THE EDITOR: A 20-year-old woman had abdominal pain since the beginning of her pregnancy. Biopsy of the esophagus, stomach, and duodenum and abdominal ultrasonography showed only gastroesophageal reflux. Two months later, gynecological examination revealed a palpable left suprapubic lump and the ultrasonography showed a 4-cm, well-circumscribed, hypoechoic, solid mass. The patient underwent an exploratory laparotomy during the 34th week of gestation, during which her baby was delivered by cesarean section. The infant was subsequently admitted to the neonatology ward. During the surgery, no primary tumor was identified. Hepatic biopsy showed a metastatic, well-differentiated mucinous adenocarcinoma. Microscopic, frozen ultrasonography, and colonoscopy revealed no adenomatous, and ¹⁸F-fluorodeoxyglucose-labeled positron-emission tomography showed uptake foci in the pancreas and in the cervical, left supraclavicular, paratracheal, and retroperitoneal lymph nodes, beyond the primary area excision. Magnetic resonance imaging (MRI) revealed multiple hepatic and duodenal vertebral metastases. Serum tumor markers were as follows: carcinoembryonic antigen, 10.6 ng per milliliter (normal range, 0 to 2.5); cancer antigen 19-9, 43.0 U per milliliter (normal range, 0 to 35); alpha-fetoprotein, 49.3 ng per milliliter (normal range, 0 to 9.8); neuron-specific enolase, 42.4 ng per milliliter (normal range, 0 to 10); cancer antigen 15-9, less than 0.1 U per milliliter (normal range, 0 to 20); and human chorionic gonadotropin, less than 1.4 ng per milliliter (normal range, 0 to 0.1).

Immunohistochemical analysis of the biopsy specimens revealed that three markers were positive: carcinoembryonic antigen, cytokeratin 15, and cytokeratin 7. The following markers were negative: estrogen receptor, progesterone receptor, thyroid transcription factor 1, caudal-type homeobox 2, cytokeratin 20, thyroglobulin, and alpha-fetoprotein. The immunohistochemical report suggested a primary tumor of upper gastrointestinal origin.

Molecular profiling was performed with the use of the CupPrint[®] algorithm. The data from a gene-expression database of 647 cases of cancer, a 30-gene signature algorithm was designed for the discrimination of 51 tumor types, being 9 in this case. (The nearest neighbor algorithm assigns the 5 nearest neighbors to the sample size being analyzed.) This top-5 grouping indicated that the tumor had an upper gastrointestinal origin. The final weighted prediction from this grouping pointed to the gallbladder as the primary organ.

MRI cholangiopancreatography revealed an anastomotic stricture of the gallbladder (Fig. 1). Multiple frozen sections were examined, the largest of which was in concert with the gallbladder wall. This finding also suggested that the gallbladder was the probable primary site. In such cases, immunohistochemical staining is useful in determining the origin of an unknown primary tumor.^{1,2}

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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

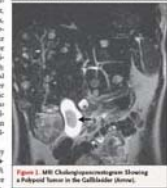


Figure 1. MRI cholangiopancreatography showing an anastomotic stricture of the gallbladder (Fig. 1).

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 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

Dimitri Talarov,* Jonathan Baden,* Tim Jatkoo,* Kristina Hahn,* Jack Yu,* Yoshoda Rajpurich,* Yiqiu Jiang,* Chang Choi,* Jeffrey S. Ross,* David Adams,* Yubin Wang,* and Abhinav Mazumder*

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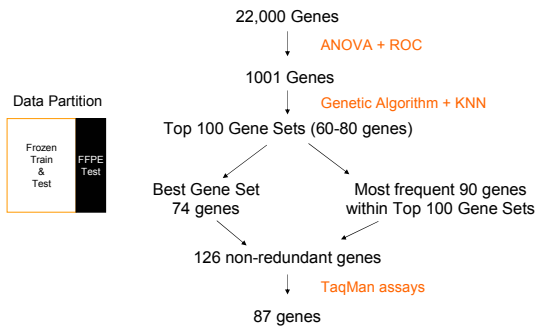
- RT-PCR assay for 10 genes + 2 reference
- Classifies 6 cancer types + other
- Database = 260 FFPE samples
- Overall accuracy = 78%
- Assay Performance of Independent set of 48 samples including metastatic carcinoma of known origin and CUPs

Known mets: 11/15 or 73.3%
Resolved CUP: 17/22 or 77.3%

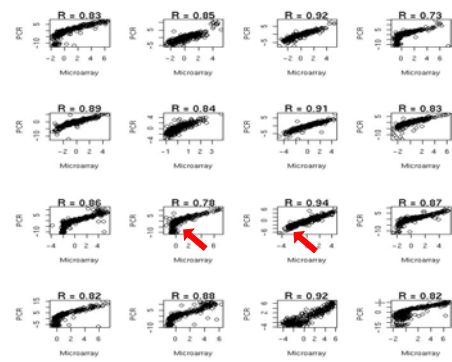
92 gene assay for classifying 39 cancer types

- 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



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

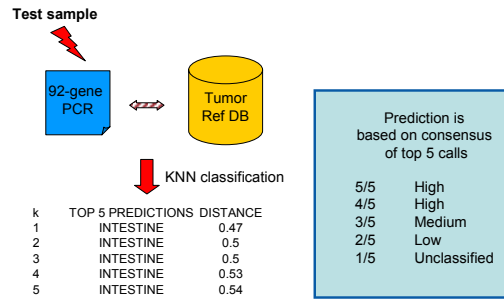
Xianjun Ma, PhD, Rajesh Patel, PhD, Kunqun Wang, PhD, Renelle Solberg, BS, Jui-Ming, BS, Rupal Desai, BS, J. Sall Rajji, BS, Hui Wang, PhD, Shihy Chu, BS, Kimberly Stocker, BS, Raju Raju, PhD, Howard Rubin, MD, Max Moore, PhD, David Eisenbach, PhD, Dennis Spruiell, MD, Heidi Ehrhardt, PhD

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 pathology 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 technology to a lab by developing a real-time quantitative polymerase chain reaction (RT-PCR) assay. **Design**—We constructed a microarray database of 44k 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 multiclass 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 12 different tumor classes in the validation set of 119 FFPE tumor samples. **Conclusions**—The RT-PCR-based expression assay involving 92 genes represents a general tool for accurately and efficiently identifying the site of origin for metastatic tumors, especially in the cases of cancer of unknown primary. The assay uses RT-PCR and real-time FFPE samples, making it suitable for rapid clinical diagnosis. (Arch Pathol Lab Med. 2006;110:455-473)

PCR-BASED ASSAY

- Classification of 39 cancer types
- Overall success rate of 87% for 32 tumor classes from formalin-fixed paraffin-embedded samples
- Prediction based on k-nearest neighbor algorithm

Predicting Cancer Origin by K Nearest Neighbor



Classifying 39 Cancer Types

Adrenal	Pheochromocytoma
Brain	Astrocytoma anaplastic, Astrocytoma fibrillary, Glioblastoma, Glioblastoma multiforme, Oligoastrocytoma, Oligodendroglioma
Breast	Adenocarcinoma of breast, ductal, Adenocarcinoma of breast, lobular
Carcinoid-intestine	Tumor of duodenum, carcinoid, Tumor of ileum, carcinoid, Tumor of small intestine, carcinoid
Cervix-adeno	Carcinoma of cervix
Cervix-squamous	Carcinoma of cervix, squamous cell
Endometrium	Adenocarcinoma of endometrium, endometrioid, Adenocarcinoma of endometrium, papillary serous
Gallbladder	Adenocarcinoma of gall bladder
Germ-cell-ovary	Teratoma, immature, Teratoma of ovary, mature, cystic, Tumor of ovary, yolk sac
GIST	Gastrointestinal stromal tumor of stomach
Kidney	Carcinoma of kidney, renal cell, chromophyll, renal cell clear cell, oncocytoma
Leiomyosarcoma	Carcinoma of liver, hepatocellular
Liver	Adenocarcinoma of lung
Lung-adeno-large-cell	Carcinoma of lung, small cell
Lung-small	Carcinoma of lung, squamous cell
Lung-squamous	Lymphoma, large B-cell, diffuse
Lymphoma-B	Lymphoma, Hodgkins
Lymphoma-Hodgkins	Lymphoma, peripheral T
Lymphoma-T	Meningioma, atypical, recurrent, fibroblastic, meningothelial, secretory
Mesothelioma	Mesothelioma of pleura
Osteosarcoma	Osteosarcoma
Ovary-clear	Adenocarcinoma of ovary, clear cell
Ovary-serous	Adenocarcinoma of ovary, serous
Pancreas	Pancreas—Adenocarcinoma of pancreas, ductal, mucinous, Carcinoma of pancreas, acinar cell
Prostate	Adenocarcinoma of prostate
Skin-basal-cell	Carcinoma of skin, basal cell
Skin-melanoma	Malignant melanoma
Skin-squamous	Carcinoma of skin, squamous cell
Soft-tissue-Liposarcoma	Adenocarcinoma of colon, duodenum, rectum, small intestine
Soft-tissue-MFH	Liposarcoma of lower extremity
Soft-tissue-Sarcoma-synovial	Histiocytoma, malignant fibrous, malignant fibrous, metastatic, Myxofibrosarcoma
Stomach-adeno	Sarcoma, spindle, epithelial
Testis-other	Adenocarcinoma of stomach, signet ring cell
Testis-Seminoma	Carcinoma of testis, embryonal, Teratoma, of testis, metastatic, Tumor of testis, mixed germ cell
Thyroid-follicular-papillary	Seminoma of testis
Thyroid-medullary	Carcinoma of thyroid, follicular, Carcinoma of thyroid, papillary
UrinaryBladder	Carcinoma of thyroid, medullary
	Carcinoma of bladder, transitional cell, Carcinoma of bladder, urothelial

Performance of 92 gene assay for 39 types

Tumor Types	Sensitivity	Specificity	PPV	NPV	Tumor Types	Sensitivity	Specificity	PPV	NPV
Adrenal	1.000	1.000	1.000	1.000	Mesothelioma	1.000	1.000	1.000	1.000
Brain	1.000	0.996	0.905	1.000	Osteosarcoma	0.833	0.998	0.833	0.998
Breast	1.000	0.998	0.974	1.000	Ovary-clear	0.813	0.998	0.829	0.994
Carcinoid-intestine	0.700	0.998	0.875	0.994	Ovary-serous	1.000	0.987	0.682	1.000
Cervix-adeno	0.376	0.996	0.600	0.991	Pancreas	1.000	0.983	0.719	1.000
Cervix-squamous	0.733	0.991	0.688	0.992	Prostate	1.000	0.996	0.905	1.000
Endometrium	0.583	0.992	0.692	0.987	Skin-basal-cell	0.857	0.998	0.857	0.998
Gallbladder	0.222	0.996	0.500	0.987	Skin-melanoma	1.000	1.000	1.000	1.000
Germ-cell-ovary	0.200	0.994	0.400	0.985	Skin-squamous	0.692	0.989	0.600	0.993
GIST	0.917	0.998	0.917	0.998	Small-and-large-bowel	0.974	0.977	0.755	0.998
Kidney	1.000	1.000	1.000	1.000	Soft-tissue-Liposarcoma	0.825	0.994	0.825	0.994
Leiomyosarcoma	0.813	0.992	0.765	0.994	Soft-tissue-MFH	0.429	0.989	0.500	0.985
Liver	0.938	1.000	1.000	0.998	Stomach-adeno	0.909	1.000	1.000	0.998
Lung-adeno-large-cell	0.926	0.996	0.900	0.985	Stomach-squamous	0.091	1.000	1.000	0.982
Lung-small	0.909	0.993	0.714	0.998	Testis-other	0.846	0.994	0.786	0.996
Lung-squamous	0.692	0.994	0.750	0.993	Testis-Seminoma	0.933	0.996	0.875	0.998
Lymphoma-B	0.727	0.996	0.800	0.994	Thyroid-follicular-papillary	1.000	0.994	0.913	1.000
Lymphoma-Hodgkins	1.000	0.989	0.625	1.000	Thyroid-medullary	0.875	1.000	1.000	0.998
Lymphoma-T	0.333	0.998	0.667	0.993	UrinaryBladder	0.792	0.996	0.900	0.991
Meningioma	0.909	0.996	0.909	0.998	Overall	0.916	0.995	0.916	0.995

- 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).

Input Normalization: Housekeeping Genes?

- **Are currently used genes robust?**

- e.g., **GAPDH**

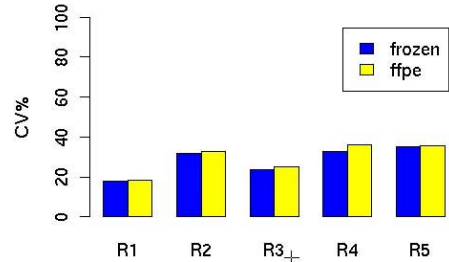
Barber et al., GAPDH as a housekeeping gene: Analysis of GAPDH mRNA expression in a panel of 72 human tissues. *Physiol. Genomics* (March 15, 2005).

"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

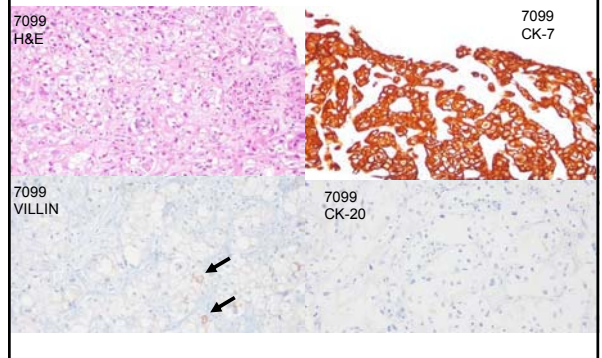
Five Reference Genes: Similar variation in FFPE and Frozen samples



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



Work up Results

Assay Result

K1 - PANCREAS
K2 - PANCREAS
K3 - LUNG
K4 - PANCREAS
K5 - PANCREAS

Prediction:
PANCREAS

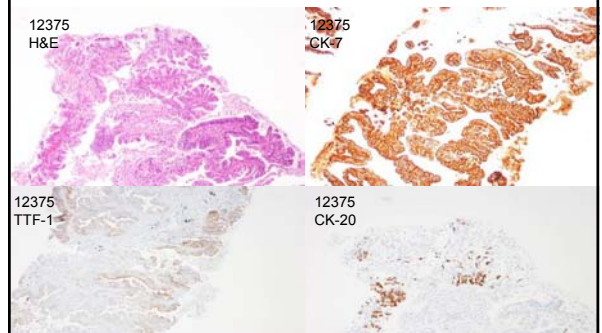
Original Pathology diagnosis

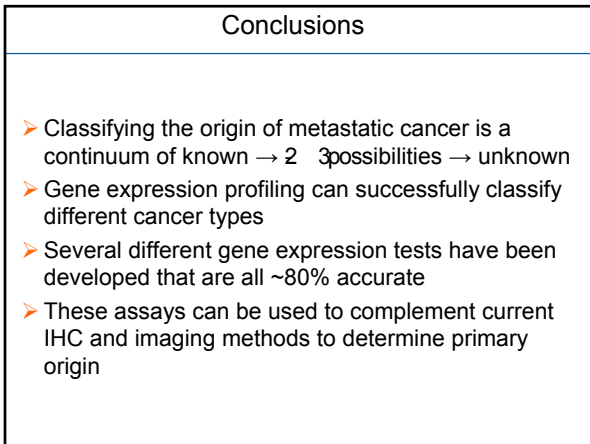
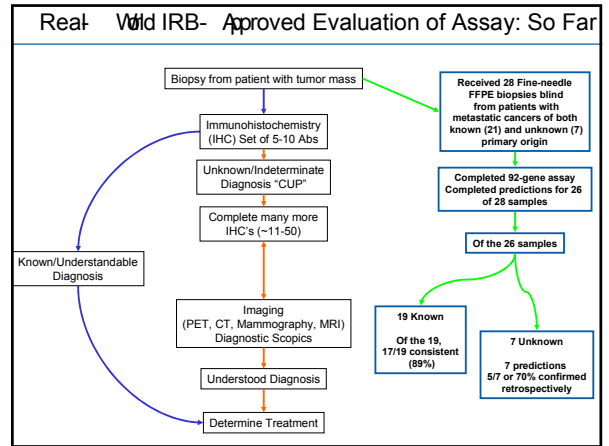
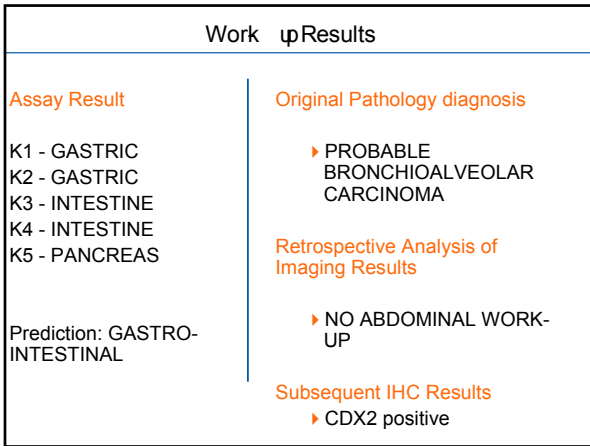
➢ MUCINOUS
ADENOCARCINOMA

Retrospective Analysis of Imaging Results

➢ PANCREATIC TUMOR

94 year old man with multiple masses in lung





Microsatellite Instability Testing in Colon Cancer: The Role of the Pathologist

USCAP 2007 Annual Meeting

Companion Meeting: Association for Molecular Pathology

Antonia R. Sepulveda M.D., Ph.D.

Associate Professor of Pathology, University of Pittsburgh

The goals of this lecture are:

- 1: Introduction to microsatellite instability (MSI) testing in colorectal cancer (CRC), test interpretation, review the criteria for MSI testing in CRC and the role of the pathologist in requesting MSI tests
- 2: Discuss the use of integrated diagnostic/molecular pathology reports: reporting MSI test results and DNA mismatch repair protein immunohistochemistry (IHC) in CRC
- 3: Discuss the clinical applications of MSI testing:
 - a) Widely accepted clinical indication: To help identify patients who might have HNPCC
 - b) Possible indications in the future: To select patients for specific chemotherapy (such as 5FU) and to assess prognosis.

Microsatellite Instability Testing in CRC and Interpretation

Microsatellite instability is a type of mutation that occurs in short DNA segments known as microsatellite regions. Mutations that occur in the microsatellite repeats result in increased size (by addition of nucleotides) or reduced size (by loss of nucleotides) in the repeat. These changes in size of the microsatellite repeat are known as microsatellite instability.

Microsatellite instability occurs because of deficient/loss of DNA mismatch repair (MMR), which requires the function of several DNA mismatch repair proteins (hMLH1, hMSH2, hMSH6, hPMS2, hMSH3 and hMLH3) (reviewed in ¹). Loss of expression of these proteins in tumors can be detected by IHC performed on paraffin sections. **Microsatellite instability is the hallmark mutation in cancers of patients with hereditary non-polyposis colorectal cancer (HNPCC) and is currently used in clinical practice primarily to help identify patients who might have HNPCC.** Patients with HNPCC almost always show high levels of MSI in their CRC tissue. However, we need to be aware that approximately 15% sporadic colorectal cancers and other sporadic adenocarcinomas of the spectrum of HNPCC cancers can have MSI-positive status ². Therefore, confirmation of HNPCC requires identification of germline mutations (detected in peripheral blood DNA) in one of the DNA mismatch repair genes (reviewed in ¹), whereas somatic hypermethylation of the hMLH1 promoter leading to loss of hMLH1 expression is the underlying abnormality causing MSI in sporadic tumor tissues ³.

For the MSI test DNA is extracted from unstained sections from formalin fixed and paraffin embedded tissue specimens. PCR amplification is performed with sets of primers that amplify five microsatellite markers (BAT25, BAT26, D2S123, D5S346 and D17S250), known as the NCI panel ⁴. Using the NCI five microsatellite marker panel, tumors can be classified as: 1) **MSI-High level (MSI-H)** when MSI is detected in at least two of the five markers (30% or greater markers positive for MSI); 2) **MSI-Low (MSI-L)** if MSI is detected at only one marker (less than 30% of the markers positive for MSI); 3) **Microsatellite stable (MSS)** if none of the five markers shows MSI.

Alternative panels of microsatellite markers have been proposed and may be used in clinical practice, based on local laboratory preference ⁵. If a tumor is MSI-H the patient might have HNPCC, and it is important that the MSI test report states that there are potential genetic implications of the test results and genetic counseling should be recommended. If a patient has an MSI-positive tumor, most specifically MSI-H, together with a detailed family history of cancer, germline mutations are required to confirm a diagnosis of HNPCC, and consent by the patient for testing is necessary for germline mutation testing (but not for MSI testing). If HNPCC is ruled out, MSI-H identifies a sub-group of sporadic adenocarcinomas that have distinct clinical pathological features namely better survival and resistance to 5-fluorouracil (5FU). Germline mutations of DNA MMR genes in HNPCC affect hMLH1 (approximately 40%), hMSH2 (approximately 40%), hMSH6 (approximately 10%), and PMS2 (approximately 5%) (reviewed in ¹). In HNPCC, the specific MMR genes affected by germline mutation show loss of expression by IHC in the tumor tissue.

If no loss of expression of hMSH2 or hMLH1 is seen in MSI-H tumors by IHC or if the tumor is MSI-L or MSS but there is clinical suspicion of HNPCC, evaluation of other MMR genes, in particular hMSH6 and hPMS2 may be performed, by immunohistochemical stains and/or germline mutational analyses ^{6,7}.

Correlation of MSI Test Results and DNA Mismatch Repair Protein IHC

The DNA mismatch repair proteins are normally present in the cell nucleus, therefore loss of nuclear expression is the pattern observed in MSI-H tumors. Most laboratories routinely testing for DNA mismatch repair proteins currently offer IHC for the 3 main repair proteins (hMLH1, hMSH2 and hMSH6). Interpretation of IHC when the 3 stains are done in the same tumor usually permits the successful identification of the DNA mismatch repair gene that underlies microsatellite instability. Note that when hMLH1 expression is lost hPMS2 is also lost because hPMS2 requires hMLH1 for stability through heterodimerization; in contrast, if hPMS2 is lost hMLH1 expression is preserved because hMLH1 also forms heterodimers with other proteins, thus being protected from degradation. Similarly, when hMSH2 expression is lost, hMSH6 is also lost because hMSH6 requires hMSH2 for stabilization through heterodimerization; in contrast, if hMSH6 is lost hMSH2 expression is preserved because hMSH2 also forms heterodimers and is stabilized by other proteins. The usual patterns of immunoreactivity are summarized in the table below.

	IHC MLH1	IHC PMS2	IHC MSH2	IHC MSH6
MLH1 Mutation	Loss	Loss	Preserved	Preserved
MSH2 Mutation	Preserved	Preserved	Loss	Loss
MSH6 Mutation	Preserved	Preserved	Preserved	Loss
PMS2 Mutation	Preserved	Loss	Preserved	Preserved

Clinical Indications for Microsatellite Instability Testing in Colorectal Cancer

The MSI test is currently used primarily to help identify patients who might have HNPCC. The most recent guidelines to help decide whether a patient should undergo molecular testing to rule out HNPCC, known as the revised Bethesda criteria ^{7,8}, are as follows:

- 1) **Patient is diagnosed with colorectal cancer before the age of 50 years.**
- 2) **Presence of synchronous or metachronous colorectal or other HNPCC related tumors** (stomach, urinary bladder, ureter and renal pelvis, biliary tract, brain (glioblastoma), sebaceous gland adenomas, keratoacanthomas and small bowel), regardless of age.
- 3) **Colorectal cancers with a high-microsatellite instability morphology** (Tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous or signet-ring cell differentiation, poorly differentiated carcinomas with medullary growth pattern), diagnosed before the age of 60 years.
- 4) Colorectal cancer with one or more first degree relatives with colorectal cancer or other HNPCC-related tumors. One of the cancers must have been diagnosed before the age of 50 years (including adenomas, which must have been diagnosed before the age of 40 years).
- 5) Colorectal cancer with two or more relatives with colorectal cancer or other HNPCC-related tumors, regardless of age.

As can be seen from the above criteria, the pathologist can easily identify patients with criteria 1 and 2, and criterion 3 specifically depends on the identification by the pathologist of tumors that demonstrate MSI-suggestive histology. Using criterion 3 alone, in patients between 50 and 60 years of age we identified MSI-High in 57% of CRC tested ⁹. In various Centers MSI reflex testing is requested by the pathologist in the cases identified by the pathologist as fulfilling one of the above criteria, and the results of the MSI test are then integrated with the surgical pathology report. While some Centers perform IHC for the main DNA mismatch repair proteins (hMLH1, hMSH2, and hMSH6) along with the MSI test, reporting the IHC results in the same final pathology report along with the MSI test results, others perform IHC and MSI tests sequentially, only in tumors that are found to be MSI-High, depending on local preference.

MSI and Prediction of Tumor Prognosis and of Response to Chemotherapy

Several studies have reported unique clinicopathological features of tumors related to their MSI status, namely a relationship with response to chemotherapy ¹⁰ and an improved prognosis in tumors that are MSI-H.

Chemotherapy of stage II and III CRC with 5-fluorouracil did not improve survival if the tumor was MSI-High. In contrast, patients with microsatellite stable tumors treated with 5-FU had better survival compared with patients who were not treated. Therefore, treatment selection for colorectal cancer may be optimized by combining molecular testing of the tumor for MSI in addition to clinicopathological stage ^{10,11}.

Studies have shown that MSI-H colorectal cancers show less lymph node metastases burden and have better survival ^{11,12}. The relationship between MSI and improved prognosis was independent of stage, site, tumor grade, and age and was associated with a 60% decrease in death attributable to colon cancer ¹². Other recent studies support these data ¹³.

The application of MSI testing of colorectal cancers with the purpose of evaluating prognosis and selection of treatment is not currently part of routine practice but may receive support in the near future, as more studies continue to confirm the findings reported in the past few years.

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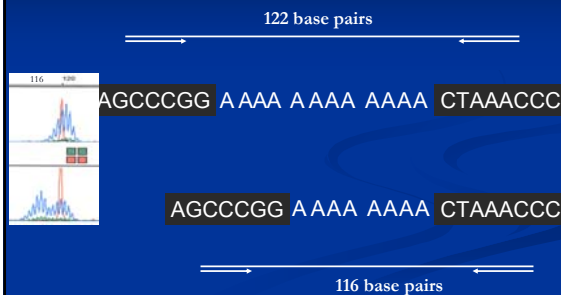
Microsatellite Instability Testing in Colon Cancer: The Role of the Pathologist

Antonia R. Sepulveda M.D., Ph.D.
University of Pittsburgh

2007 USCAP Annual Meeting

- Introduction to microsatellite instability (MSI)
- MSI test for colorectal cancer (CRC) and test interpretation
- Review the criteria for MSI testing in CRC and the role of the pathologist in requesting MSI tests and reporting test results
- Discuss the use of integrated diagnostic/molecular pathology reports:
 - reporting MSI test results and DNA mismatch repair protein immunohistochemistry (IHC) in CRC

Microsatellite Instability



DNA Mismatch Repair (MMR) Proteins

- MutS: MSH2, MSH6, MSH3
- MutL: MLH1, PMS2, MLH3

MMR proteins function as heterodimers
The heterodimers are required for protein stability

MutS

MSH2-MSH6
MSH2-MSH3

MutL

MLH1-PMS2
MLH1-MLH3

TESTING MSI IN TUMORS PROTOCOL

- Unstained sections from formalin fixed paraffin embedded tissue
- Scrape selected areas for DNA extraction
- PCR amplification with MSI panel
- DNA fragment characterization by electrophoresis



MSI TEST PANEL OF MICROSATELLITE MARKERS

- NCI 5 microsatellite marker panel
 - Mononucleotide repeat: BAT 25 and BAT 26
 - Dinucleotide repeat: D2S123, D5S346 and D17S250

MSI SUB-TYPES

- High level MSI (MSI-H)
 - MSI in $\geq 30\%$ of markers tested (at least 2 markers out of five)
- Low level MSI (MSI-L)
 - MSI in $<30\%$ of markers tested (only 1 MSI-positive marker)
- Microsatellite stable (MSS)
 - No markers show MSI

CLINICAL IMPLICATIONS OF MSI-H

- MSI-H might indicate that the patient has HNPCC. Family history/genetic testing required to rule-out this possibility.
- If HNPCC is ruled out:
 - MSI-H in sporadic carcinoma
 - Associated with loss of MLH1 in tumor cells by IHC
 - Associated with MLH1 promoter CpG hypermethylation

CLINICAL IMPLICATIONS OF MSI-H

- MSI-H sporadic tumors are associated with improved survival.
- Nodal involvement is less prevalent in MSI-positive tumors.
- Tumors with microsatellite instability may show increased resistance to chemotherapeutic agents.

Hereditary non-polyposis colorectal cancer (HNPCC)

- $>90\%$ germline mutations in **MLH1** and **MSH2** MMR genes with loss of protein in cancer tissue
- Few cases are associated with **MSH6**, **PMS2**, and rare **MSH3** mutation
- $>90\%$ CRC with MMR protein loss show MSI-H in cancer tissues, but there are exceptions
 - Cases of DNA repair protein loss by IHC (eg. MSH6) that are MSI-L or MSS
- Almost all MSI-H CRC show loss of main DNA repair proteins, but there are exceptions
 - Cases with DNA repair protein mutations without loss by IHC (but tumor is MSI-H)

Terdiman, Gastroenterology 2001 (121) 4

Revised Bethesda Guidelines for HNPCC and MSI Testing

Umar, A. et al. JNCI 96: 261-268, 2004

- 1-CRC diagnosed in a patient less than 50 years of age.
- 2-CRC with MSI-H histology diagnosed in a patient less than 60 years of age.

REGARDLESS OF AGE OF PATIENT :

- 3-Synchronous, metachronous CRC, or other HNPCC-associated tumors.
- 4-Individual with CRC and at least one first degree relative with CRC/HNPCC tumor less than 50 years of age.
- 5-Patient with CRC and two or more first- or second-degree relatives with CRC/HNPCC-related tumors.

Comparison Between Initial vs. Revised Bethesda Guidelines for HNPCC Testing

Number of cases: 75	Bethesda	Revised Bethesda
MSI-H	8	17
MSS	37	58

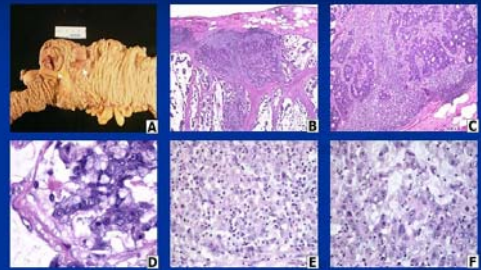
- More MSS cases were identified
- The revised guidelines identified 9 additional MSI-H cases.

Gologan & Sepulveda et al.
Arch Pathol Lab Med 2005; 129: 1390-1397

HNPCC Related Tumors

- Colorectal
- Small bowel
- Stomach
- biliary tract
- pancreas
- sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome
- Endometrial
- ovarian
- Bladder, ureter and renal pelvis
- brain (usually glioblastoma as seen in Turcot syndrome)

Gross and Morphologic Features of MSI-H CRC

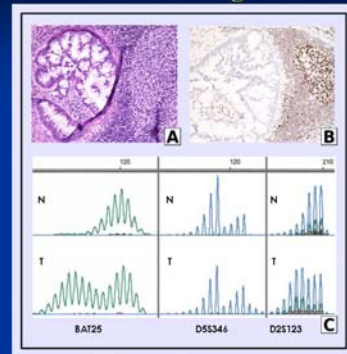


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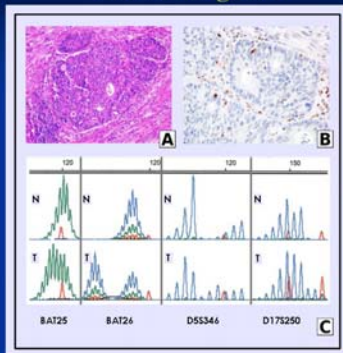
Microsatellite Instability Test Report

- High-level microsatellite instability detected (MSI-H).
- Low-Level microsatellite instability detected (MSI-L).
- No microsatellite instability detected (MSS).
- Immunohistochemistry for MLH1, MSH2 and MSH6 showed preserved /or loss of expression of....DNA repair proteins.

MSI-H COLORECTAL CARCINOMA hMSH2-Negative



MSI-H COLORECTAL CARCINOMA hMLH1-Negative



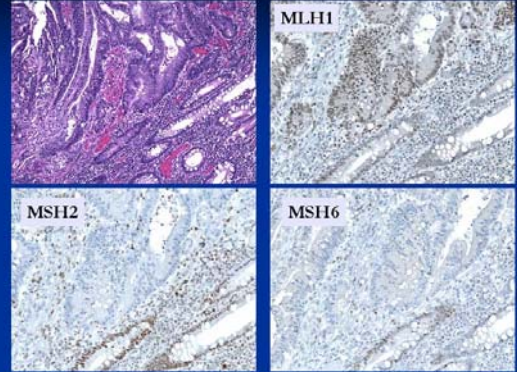
Immunohistochemical Patterns in MSI-H CRC

	IHC MLH1	IHC PMS2	IHC MSH2	IHC MSH6
MLH1 Mutation	Loss	Loss	Preserved	Preserved
MSH2 Mutation	Preserved	Preserved	Loss	Loss
MSH6 Mutation	Preserved	Preserved	Preserved	Loss
PMS2 Mutation	Preserved	Loss	Preserved	Preserved

Integrated Pathology Report: Example Case

- 45 year old man
- Transverse colon obstructing mass
- Right hemicolectomy: 10 cm exophytic mass (T3N0MX)
- MSI and IHC for MLH1, MSH2 and MSH6 performed-reflex test by pathologist

Morphology and IHC



Microsatellite Instability Test Report

- High-level microsatellite instability detected (MSI-H).
- Immunohistochemistry for MLH1, MSH2 and MSH6 showed loss of expression of MSH2 and MSH6 in tumor cell nuclei.
- Note: MSH2 is most likely to represent the underlying primary genetic defect in this MSI-H tumor.

CONCLUSIONS

- Microsatellite instability testing of CRC is currently indicated to help identify patients with HNPCC
- Pathologists can play a significant role in identifying patients for MSI test using the Bethesda guidelines
 - -Reflex testing-
- Important to establish a protocol for handling CRC specimens that may have MSI test performed in consensus with the local team of GI, Oncologists and Surgeons, and to have available genetic counseling
- Combined vs. sequential or alternative MSI and IHC for DNA repair proteins depends on local preferences