Excellence in Immunohistochemistry: The Diagnostic Challenge

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Immunohistochemistry: The Challenges

Immunohistochemistry: The Challenge is US!

- Where we have been...
- Where we are now...
- Where do we need to be? Will IHC be replaced by molecular tests, like the electron microscope was replaced with IHC?
- Who will LEAD?

Where we have been…

NCI sponsored workshop May 1977

- Direct and indirect peroxidse conjugate methods
- Tissue preparation
- Controls
- Background staining
- Evaluation of results

NCI sponsored workshop May 1977

- The “danger of false positive and false negative results”
- Results from different laboratories should be reliable and reproducible.
- For IHC to be used in laboratories, methods of evaluation, development and standardization are required.
- Comparative studies of the same material by different laboratories should be encouraged.

DeLellis R et al. Report of a workshop sponsored by the NCI. Am J Clin Pathol 1979. 71:483-88

- May 1977 the role of IPX in diagnostic pathology
- Identify tumor types
- Demonstrate Ig in lymphoid tumors
- Identify hormones in endocrine tumors

Battifora H. The multitumor (sausage) tissue block. A novel method for IHC testing. Lab Invest 1986 55:244-8

- The need for monoclonal antibody testing.
- Sensitivity studies.
- Interlaboratory QC.
- The first TMAs.
- Also: *Miller RT, Groothuis. Multitumor "sausage" blocks in IHC: simplified method, uses and role in QA. Am J Clin Pathol. 1991;96:228.*

Special Report: Quality Control in Immunohistochemistry Am J Clin Pathol 1989. 92:836-43.

- Manufacturer's testing program-a standardized antibody specificity, performance, test certification, and product information sheet.
- Ab specificity~Fixation/sausage block tissue specificity/optimization.

Special Report: Quality Control in Immunohistochemistry Am J Clin Pathol 1989. 92:836-43.

- IHC manual
- To address: performance criteria, quality assurance, technical pitfalls, interpretation.

Special Report: Quality Control in Immunohistochemistry Am J Clin Pathol 1989. 92:836-43.

- Standardization of package inserts.
- Clone, species, lot# and concentration, immunoglobulin class, total protein/antibody concentration, diluent, immunogen

Special Report: Quality Control in Immunohistochemistry Am J Clin Pathol 1989. 92:836-43.

- Antibody operating chracteristics: fixative reactivities, optimal processing, antigen retreival.
- Positive control
- Storage requirements
- Shelf life.

Special Report: Quality Control in Immunohistochemistry Am J Clin Pathol 1989. 92:836-43.

- Published Reports-materials/methods
  - Fixative/processing/IHC technique/ab titer,clone & lot, source, AR, chromogen, QC, interpretation

Taylor CR Quality Assurance and Standardization in IHC. A proposal for the BSC annual meeting. Biotechnic & Histochem 67(2) 1992.

Phase 1: Guidelines & Standards

- Antibodies/Reagents: (pkge insert)
- Technical Procedures: (establish general guidelines, minimum controls)
- Interpretation and Reports: (guidelines for format and content of IHC report).

Taylor CR Quality Assurance and Standardization in IHC. A proposal for the BSC annual meeting. Biotechnic & Histochem 67(2) 1992.

Phase 2: Testing & Proficiency programs

- Antibodies/reagents: uniform internal testing for mfgrs/sausage block.
- Technical procedures: using standard test substrates to test effectiveness of stain protocols.
- Interpretation & reports: designed to test interpretive abilities.

Taylor CR Quality Assurance and Standardization in IHC. A proposal for the BSC annual meeting. Biotechnic & Histochem 67(2) 1992.

Phase 3: External reference testing laboratory qualifications.

- Antibodies/reagents:submit reagents to refernce lab for certification.
- Technical procedures: training programs for technologists.
- Interpretation & reports: qualifications, expereince, utilization for pathologists.

Editorial: The Total Test Approach to Standardization of Immunohistochemistry

- Taylor C. Arch Pathol Lab Med 2000; 124:945.

The Total Test-Immunohistochemistry

Elements of testing	QA issues	Responsibility
Clinical question;test selection	Indications;stain selection; specimen collection fixation etc	Pathologist;clinician; tecnologists.
Technology/methodology	Reagents;protocols;sensitivity, specificity, qual, prof testing.	Pathologist/technologist
Results:validation/reporting	Criteria (+, -);report content; tat.	Pathologist/tech
Interpretation	Qualifications;prof testing integration of report	Pathologist/clinican

THE IMMUNOHISTOCHEMISTRY REPORT (ADASP)

- The diagnostic problem.
- Type of specimen analyzed. Fixation.
- Antibodies used, state specificity & clone where appropriate.
- Both positive & negative results; patterns & intensity.
- Integrate into final surgical pathology report.

In your Report:

- Fixative and length of fixation.
- Processing mode (paraffin embedded, alcohol-fixed, frozen,cell block, etc).
- Ab clone, & target:
- + and (-) control status.
- Extent and distribution of staining.

Reporting:Quantitation

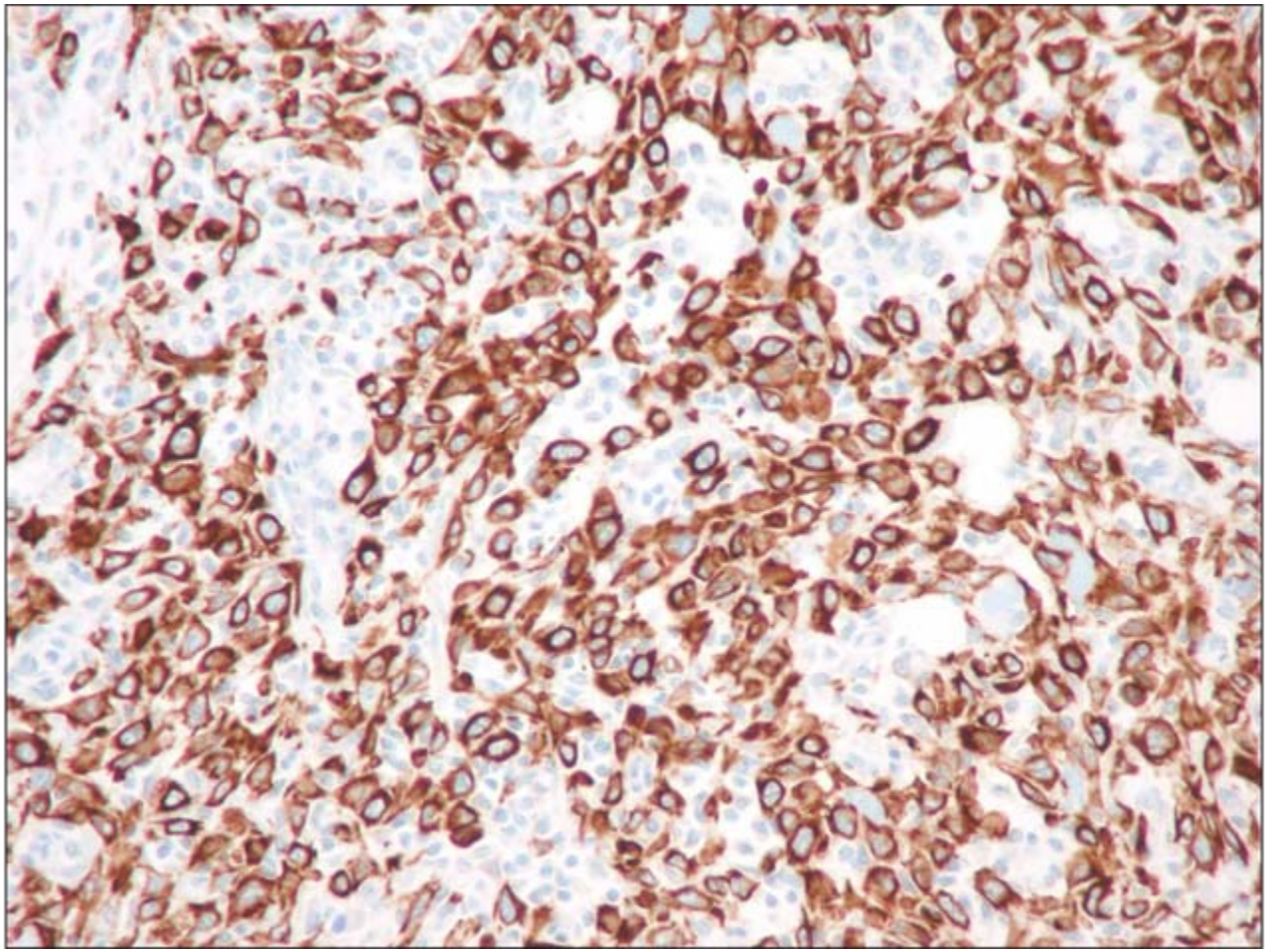
- Visual semi-quantitation (state percent cells +, semi-quantitate intensity.
- Instrument assisted-use a template that mirrors the device, and state the instrument and vendor. State if FDA approved.

Where we are now…

“Shake & Bake” Immunohistochemists

- Immunohistochemistry is sometimes referred to as analogous to “cooking in a kitchen”
- The right mix of ingredients
- The right temperatures
- The right “incubation”

Voilà



- 1980'S: development of IHC diagnostic use
- Predictive markers-hormone receptor testing
- 1990's: further addition of menu of diagnostic antibodies: carcinoma, melanoma, lymphoma, sarcoma, germ cell tumors
- September 1998-Trastuzumab gets FDA approval-Her2 testing comes on the scene.
- 2007 ASCO-CAP Guidelines for Her2 testing-first time fixation time was dictated for a pathology specimen-generated by disgruntled oncologists.

To Be Proactive…missed opportunity?

- Newfoundland debacle…40% false negative ER rate 1997-2005.
- ASCO-CAP Guidelines 20 years after initiation of ER/PR testing…

- ASCO's involvement in predictive/prognostic IHC testing is a **symptom**, that not all is well!
- The vendors of molecular tests preach to clients that pathologists cannot do well in quantitating anything (IHC) or grading tumors (e.g. breast cancer).
- …Pathologists cannot fix tissues appropriately
- …Do not have standards for IHC

DANGER!

- Poorly validated molecular tests.
- “Nearest neighbor analysis” yields gaps in databases…
- Can give completely erroneous results…

CTTR (December 2008)

The Difficult Diagnosis in Surgical Pathology:

The Hunt for the Elusive Primary Site

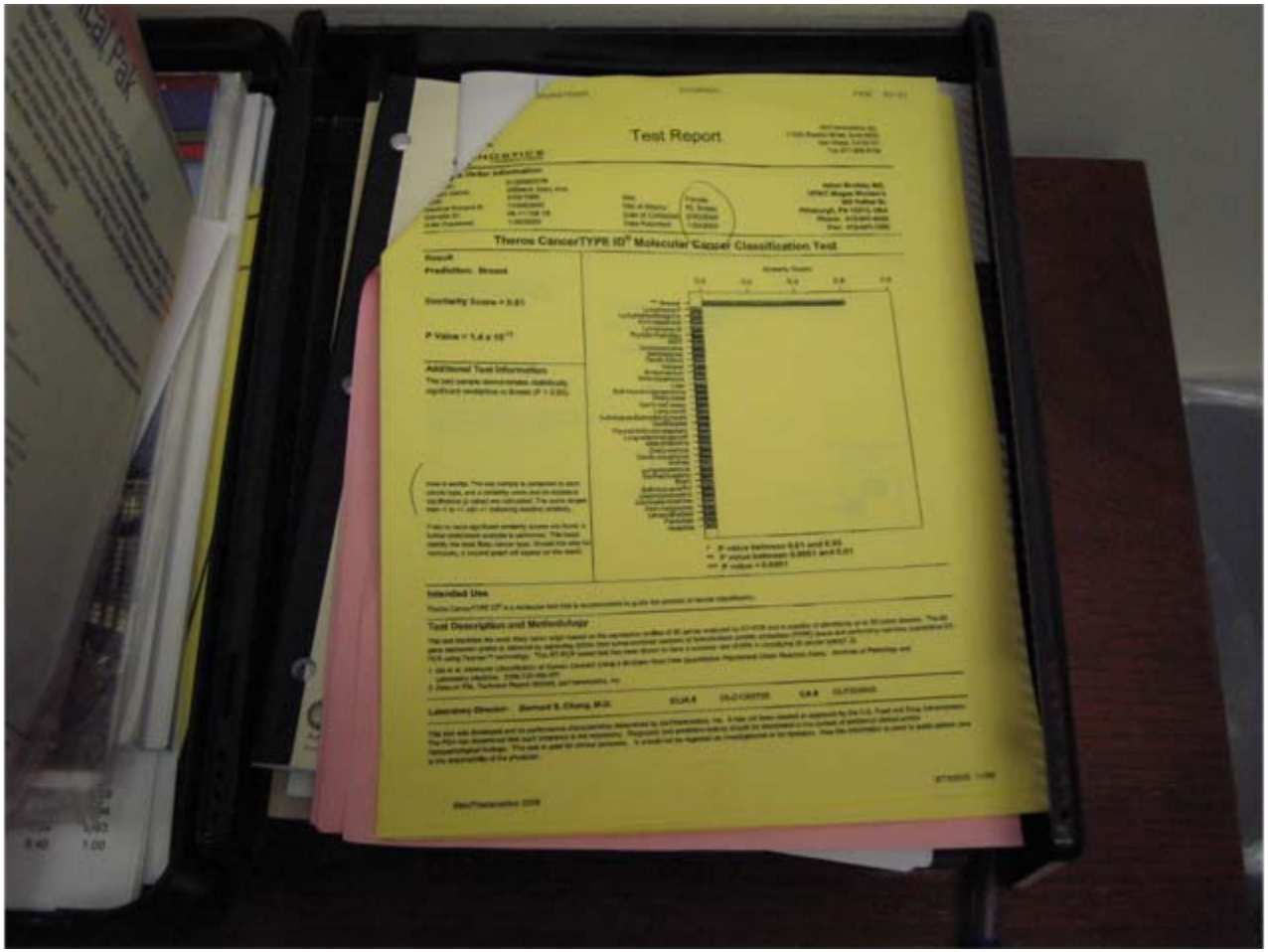
Noel Weidner, M.D.

Team "Traditional" vs. Team "RNA"
Who Won? Who's Right? You Decide.

- **For these 11, *Cancer TYPE ID*™ classified 6 (55%) as they were called by CTTR/Weidner.**

Team "Traditional" vs. Team "RNA"
Who Won? Who's Right? You Decide.

Final Diagnosis (Case #):	
3. Urothelial Carcinoma	Bladder Carcinoma
4. Prostate Carcinoma	Prostate Carcinoma
5. Hepatocellular Carcinoma	Hepatocellular Carcinoma
6. GI Tract Carcinoma	Germ-cell Tumor
7. GI Tract Carcinoma (Appendix)	Appendix Adenocarcinoma
9. Merkel-cell Carcinoma	Synovial Sarcoma
10. Papillary Thyroid Carcinoma	Thyroid Carcinoma
14. Adult Granulosa-cell Tumor	Meningioma
15. Sarcoma (High-grade)	Squamous Carcinoma
16. Leiomyosarcoma	Leiomyosarcoma
17. Giant-cell Tumor	Mesothelioma



Test Report

Theros CancerTYPE ID® Molecular Cancer Classification Test

Result
Prediction: Breast

Similarity Score = 0.61

P Value = 1.4×10^{-11}

Additional Test Information

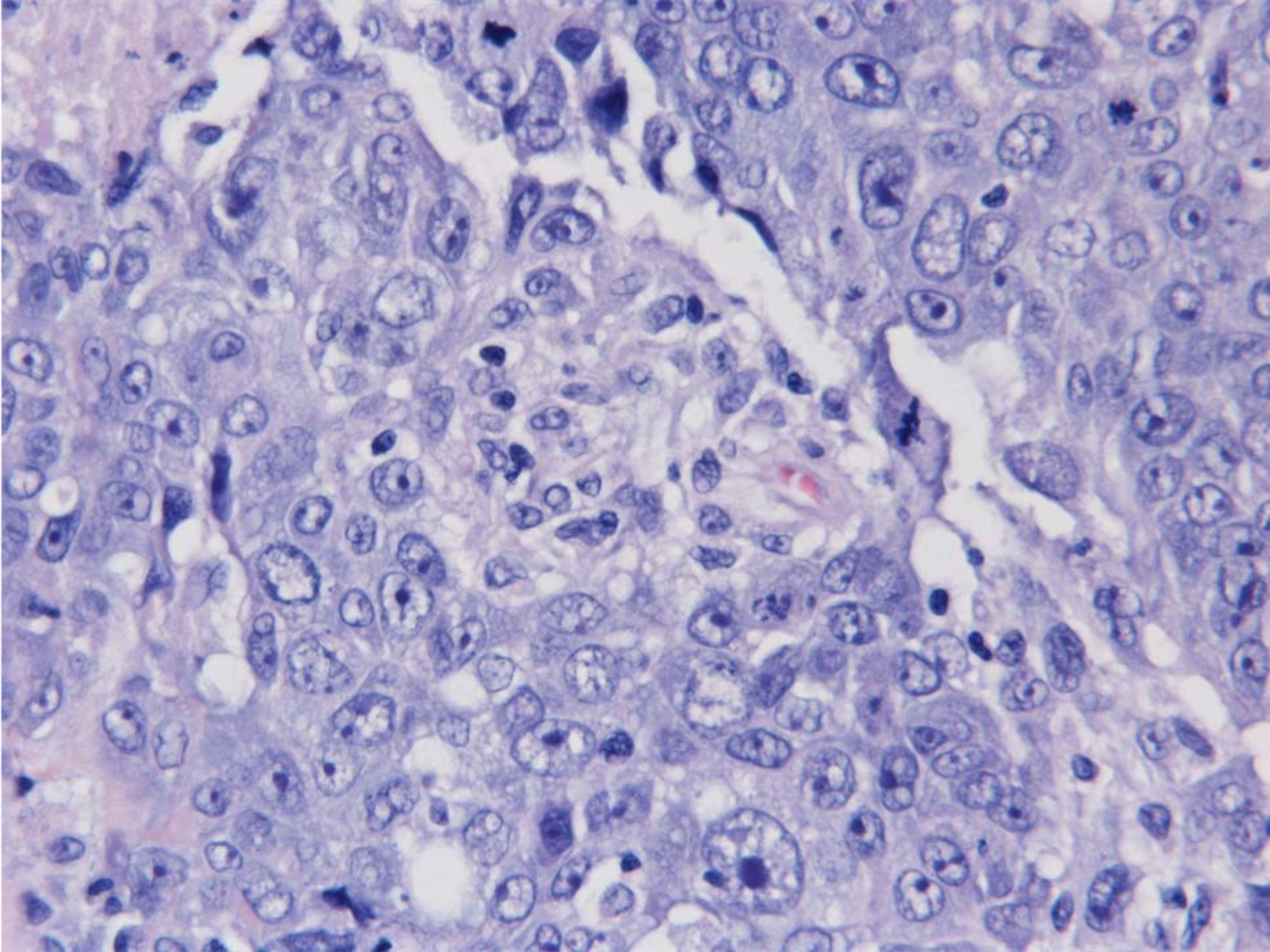
Intended Use

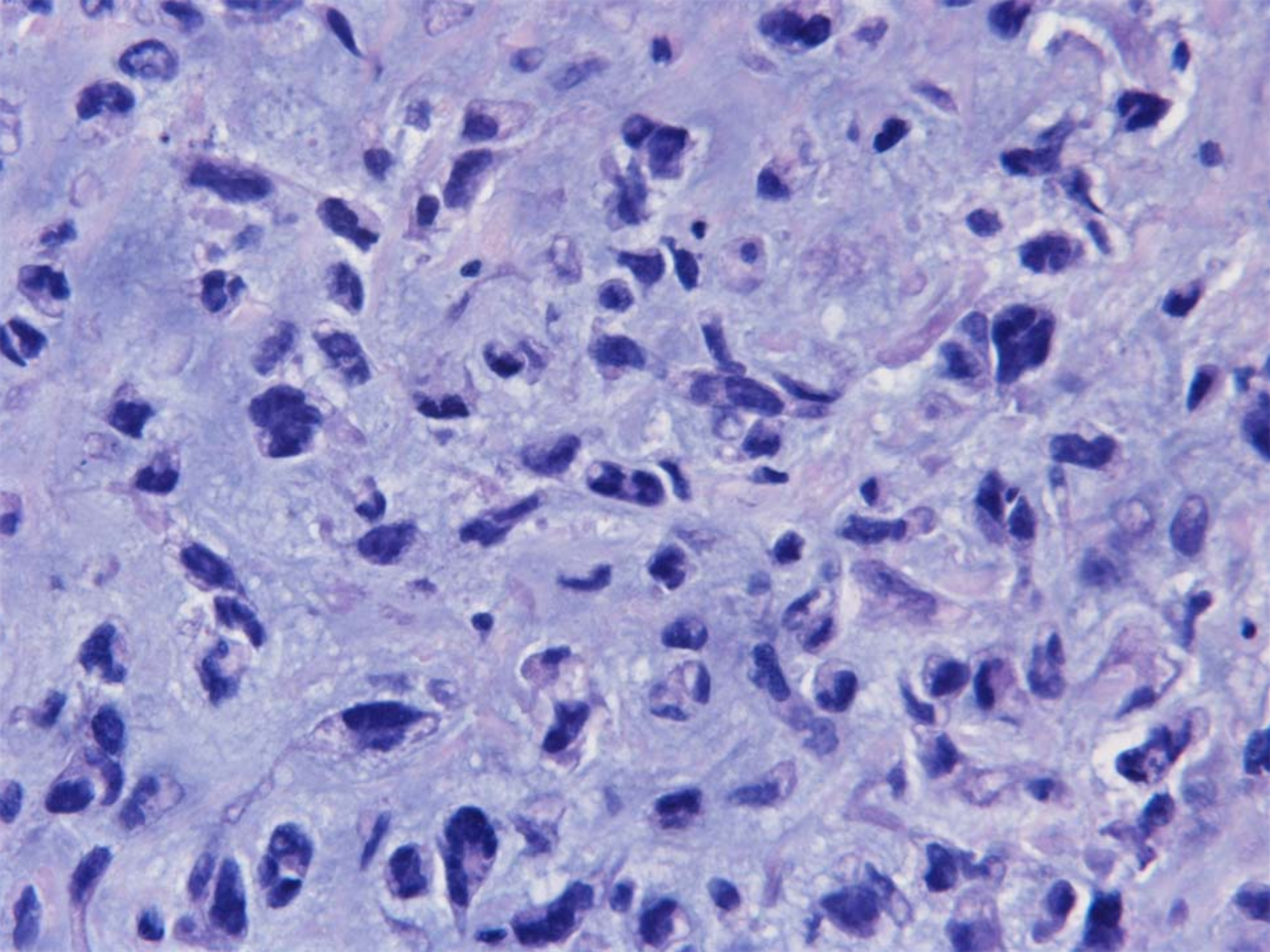
Test Description and Methodology

Laboratory Director: Bernard S. Chang, M.D.

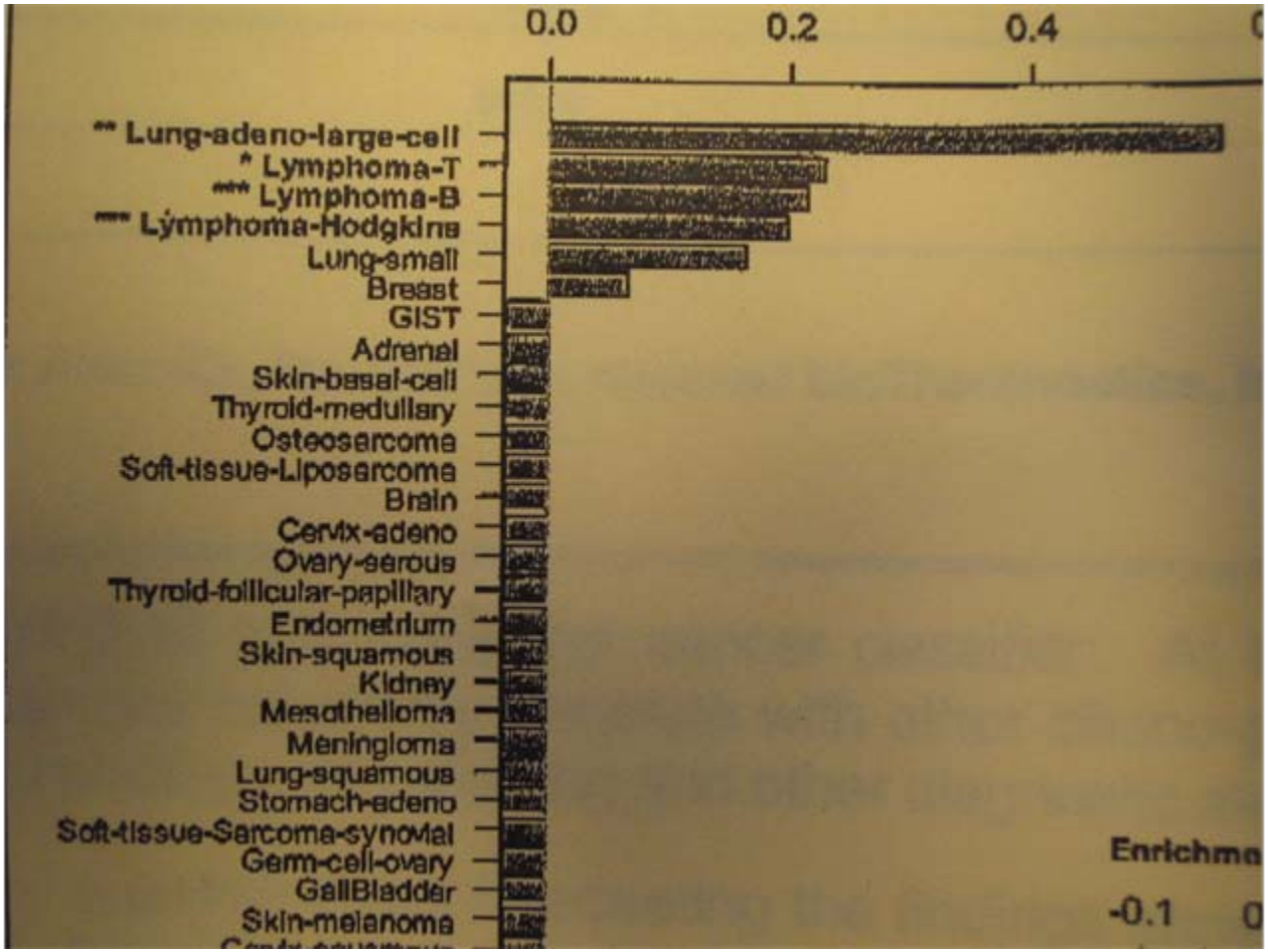
- 11/30/2007: 9, 8:30 and 10:30 o’clock: Invasive ductal carcinoma, NG 3.
- ER: H Score =5.0
- PR/Her 2 = Negative

- 5/30/08: s/p neoadj Rx R Total Mastectomy
- 3 nodules of metaplastic carcinoma, 2.0, 3.0, 1.5 cm
- Angiolymphatic invasion
- 0.2 cm deep margin
- L Breast: No tumor.





- 12/11/08: L Axillary Node Core Biopsy- metastatic high grade adenocarcinoma c/w prior right sided breast cancer.
- 1/14 axillary l. nodes positive


0.0
0.2
0.4
** Lung-adeno-large-cell
* Lymphoma-T
*** Lymphoma-B
**** Lymphoma-Hodgkins
Lung-small
Breast
GIST
Adrenal
Skin-basal-cell
Thyroid-medullary
Osteosarcoma
Soft-tissue-Liposarcoma
Brain
Cervix-adeno
Ovary-serous
Thyroid-follicular-papillary
Endometrium
Skin-squamous
Kidney
Mesothelioma
Meningioma
Lung-squamous
Stomach-adeno
Soft-tissue-Sarcoma-synovial
Germ-cell-ovary
GallBladder
Skin-melanoma
Enrichme
-0.1

Molecular testing for TUO

- Consultation with pathologist is *essential*
- Pros/cons of each molecular test: database have tumor type? How many? Contamination?
- Final correlations: does the molecular test result *make sense* in the context of all other data? (clinical, imaging, H/E, IHC, EM?)
- Vital importance of pathologist review of molecular testing for patient safety

IHC: Where we need to be…

- Theranostic applications (individualized patient therapy)..ER,PR,Her2, EGFR, Ki-67,…
- Genomic applications-colorectal MSI/mismatch repair proteins; basal-like breast tumors-BRCA implications…
- Standardization-preanalytic, analytic, postanalytic..
- Quantitation…

- Pathologists hold the future of IHC
- Molecular morphology
- Will IHC go the way of the electron microscope? or be **replaced** by molecular testing?

Guidance…Leadership for Patient Safety

- UKNEQAS (1969/1985) >evidence based
- NordiQC >evidence based..1999/2003
- ..the principal advantage of external quality assessment (EQA) is the ability to detect differences of quality between laboratories and provide guidance on how to achieve acceptable standards.
- Canada>national standards committee
- CAP..

WE have MANY challenges…

THANK YOU!