HER2 FISH and IHC Testing

Gregory J. Tsongalis, Ph.D.
Professor of Pathology
Director, Molecular Pathology
Dartmouth Medical School
Dartmouth Hitchcock Medical Center
Norris Cotton Cancer Center
Lebanon, NH
Mt. Lafayette, White Mountains, NH
Overview of FISH: Human Chromosomes

- Chromosome means “colored body”.
- Contain most of the human DNA
- Chromosomes reside in the nucleus
Chromosome Structure

Key Points

• A chromosome is formed from a single DNA strand.

• After replication (S-phase), the strand is duplicated, two sister chromatids formed.

• Each chromosome undergoes a series of compression and compaction steps to form the metaphase chromosome.
Chromosome Parts

• **Centromere**
  - Consists of highly repetitive DNA
  - Contains the kinetochore and the $\alpha$-satellite DNA
  - Interacts with microtubule proteins to move chromosomes during mitosis and meiosis

• **Telomere**
  - Maintains the discreetness of chromosomes and prevents them from sticking together
**Terminology**

- **Karyogram**: display of mitotic chromosomes of an individual, lined up from the largest to the smallest and according to location of centromere
- **Karyotype**: is the use of nomenclature to describe the chromosomal complement
- **Idiogram**: diagrammatic representation of a chromosome
Karyotype Nomenclature

- **Arms of the chromosome**
  - Short arm = p
  - Long arm = q

- **Nomenclature**
  - 46,XX – normal female karyotype
  - 46,XY – normal male karyotype
  - 47,XXY – compatible with Klinefelter syndrome
  - 47,XX,+21 – compatible with a female with Down syndrome
  - 46,XY,t(1;2)(q21;q22) – A male with a translocation between the long arms of chromosomes 1 and 2
Chromosome Abnormalities

- **Numerical abnormalities**
  - Mitotic or meiotic non-disjunction
  - Inheritance of a marker chromosome
  - Anaphase lag
  - Irregular cell cycle/abnormal spindle proteins

- **Structural Abnormalities**
  - Inherited abnormality
  - Chromosome instability
  - Abnormal DNA repair/breaks abnormal reunion
Fluorescence *in situ* hybridization (FISH)

- Initiated in 1990
- “Southern on a glass slide” - non radioactive probes
- Multiple probe types
- Multiple tissue applications
FISH: What has changed? (TARGET)

Cell culture
  ▼
  colchicine
  ▼
  arrest in metaphase
  ▼
  cells lysed on slide
  ▼
  metaphase spread

Non-dividing Interphase cells
FISH: What has changed? (CHEMISTRIES)

1. Direct vs indirect detection.

2. Development of non-radioactive fluorescent labeled probes.

3. Dual color detection (simultaneous targets)

4. Commercial availability of probes (paints, α-satellites, LSI) and FDA approval.
Fluorescence In Situ Hybridization

- Labeling with fluorescent dye
- Denature & Hybridize
Types of FISH Probes

- **Centromere probes**
  - Specific for given chromosome.

- **Locus specific probes**
  - Specific chromosome regions known to be involved in genetic syndromes or cancers.

- **Subtelomeric probes**
  - Used to identify cryptic rearrangements in the subtelomeric regions of chromosomes.

- **Whole chromosome paints**
  - Specific for chromosomes 1 through 22, plus X,Y.
FISH on Tissues

- Amniotic fluid / CVS
- Non-neoplastic Tissue
- Stimulated peripheral blood
- Bone marrow or unstimulated PB cultured/uncultured, smears,
- BM clot
- Tumors- biopsy or tissue section
  - Fresh, cultured, paraffin-embedded (formalin-fixed) tissue
Types of Probe Strategies

- Dual/Triple color; dual fusion
- Dual color; single fusion
- Extra signal rearrangement
- Break-apart rearrangement
- Multi-color FISH
FISH in CML

Arrows indicate breakpoints within the two involved genes.

Chromosome 9

Chromosome 22

ABL1 gene

BCR gene

Dual Color, Single Fusion

Dual Color, Extra Signal

Dual Color, Dual Fusion
BCR/ABL1 Probes
FISH Applications

• Prenatal Lab
  • Detection of specific trisomies (13, 18, 21) and numerical sex abnormalities (XY), follow with G-band to confirm.

• Leukocyte Lab
  • Detection of interstitial or terminal deletions (submicroscopic) or additional unknown material, and marker chromosomes.

• Cancer Lab
  • Detect numerical and structural anomalies, diagnosis, remission, minimal residual disease, relapse, and transplant engraftment.
Abnormal Gene Copy Number Is Common Finding In Human Cancers

46 chromosomes (diploid)

85 chromosomes (aneuploid)
Gene Amplification

• Three “chromosome” structures associated with amplification include:
  – Homogenously staining region (HSR)
  – Integrated region of amplification
    • Double minute chromosome (DM)
      • Small, paired, acentric extra-chromosomal body
  – Episomes
    • Submicroscopic extra-chromosomal body
HER2: A Target For Therapy

• HER2 refers to Human Epidermal growth factor Receptor 2
  – Also referred to as
    • neu
    • HER-2/neu
    • c-erbB-2

• HER2 is a proto-oncogene that encodes a transmembrane glycoprotein, p185.
HER2

• HER2 is a member of the type I growth factor receptor gene family.
  – EGFR (erbB-1)
  – HER-3 (erbB-3)
  – HER-4 (erbB-4)

• No known ligand
Transmembrane structure of HER2 monomer

- Extracellular domain (632 amino acids)
  - Ligand-binding site
- Transmembrane domain (22 amino acids)
- Intracellular domain (580 amino acids)
  - Tyrosine kinase activity

Plasma membrane

Cytoplasm
HER2 In Breast and Gastric Cancer

- 25% - 30% of breast cancers and 7-34% of gastric cancers are HER2 amplified or overexpressed
  - Higher cell proliferation rate
  - Metastasize faster
  - Greater tumor burden
  - Patients exhibit a poor prognosis
Indicators of increased HER2 production

1 = gene copy number
2 = mRNA transcription
3 = cell surface receptor protein expression
4 = release of receptor extracellular domain
Methods for Determination of HER2 Status

<table>
<thead>
<tr>
<th>Detection Method</th>
<th>HER-2/neu Molecule Detected</th>
<th>Assay Detects</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>Cell-membrane protein</td>
<td>Protein overexpression</td>
</tr>
<tr>
<td>FISH</td>
<td>DNA</td>
<td>Gene amplification</td>
</tr>
<tr>
<td>ELISA</td>
<td>Extracellular Domain (ECD)</td>
<td>Quantitates ECD in the serum or p185 in tissue</td>
</tr>
</tbody>
</table>
What is IHC? (ImmunoHistoChemistry)

- Familiar and well accepted technology
- Inexpensive
- Subjective scoring
- Semi-quantitative
- Protein target may be affected by tissue processing
HER-2 Analysis by IHC

Normal 0  Normal 1+  Abnormal 2+  Abnormal 3+
PathVysion™ Assay Overview

Chromosome Map

17

CEP17

LSI HER2

Ratio = 1.0

Normal

Ratio = 14

Amplified
HER-2 Analysis by FISH

- Normal
- Normal
- Abnormal low amplification
- Abnormal high amplification
<table>
<thead>
<tr>
<th>Detection Method</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC</td>
<td>cell localization, ease of use, comfort factor, cost</td>
<td>reproducibility, heterogeneity, endogenous enzymes, fixation issues, epitope specific</td>
</tr>
<tr>
<td>FISH</td>
<td>cell localization, direct detection, reproducibility, accuracy</td>
<td>cost, fluorescent microscopy</td>
</tr>
<tr>
<td>ELISA</td>
<td>specimen type, TAT</td>
<td>no cell localization, heterogeneity</td>
</tr>
</tbody>
</table>

**Which of these processes is tumor specific?**
HER2 In Breast And Gastric Cancer
Fixation and Paraffin Embedding Result in Decreased Antigenicity

2 to 5-fold Amplified and Overexpressed

Slamon et al., *Science* 244: 707-712, 1989
Percent of Breast Cancers in Various Expression Categories Identified by Immunostaining with 28 Different Antibodies.

< 2.0 Not Amplified (FISH-)

≥ 2.0 Amplified (FISH+)
## Comparison of FISH and IHC Assay Results in Breast Cancers with Known HER2 Gene Status

<table>
<thead>
<tr>
<th>Assay</th>
<th>#Amp/#Non-Amp</th>
<th>Sensit</th>
<th>Specif</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH (Vysis)</td>
<td>41/76</td>
<td>95%</td>
<td>99%</td>
<td>97%</td>
</tr>
<tr>
<td>FISH (Ventana)</td>
<td>44/73</td>
<td>95%</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>IHC-R60</td>
<td>39/78</td>
<td>91%</td>
<td>100%</td>
<td>97%</td>
</tr>
<tr>
<td>IHC-10H8</td>
<td>38/79</td>
<td>88%</td>
<td>100%</td>
<td>96%</td>
</tr>
<tr>
<td>IHC-DAKO</td>
<td>30/87</td>
<td>70%</td>
<td>100%</td>
<td>89%</td>
</tr>
<tr>
<td>IHC-Ventana</td>
<td>31/86</td>
<td>72%</td>
<td>100%</td>
<td>90%</td>
</tr>
</tbody>
</table>

43 amplified breast cancers and 74 non-amplified breast cancers.

*Press et al., J Clin Oncol 20: 3095-3105, 2002*
HER2 Detection: Why Should It Matter?

1. Ultimately, patient outcome based on accurate therapeutic decision making is at risk.

2. Therapeutics are extremely expensive and should be used on the patients most likely to respond.
Summary of HER2 Testing In Breast Cancer

• 95% concordance between IHC and FISH is seldom achieved for IHC 0, 1+ and 3+.

• Misclassification of 5% of cases, either false-positive or false-negative, is important.

• Inherent technical problems related to IHC in FFPE tissues strongly argue for FISH.

• No data to support need for a FISH “equivocal zone” or a change to a FISH ratio of 2.2 for gene amplification.

• FISH is more accurate in FFPE tissues.
HER2 in Gastric Cancer

• Gastric cancer is the 4th most common cancer diagnosed worldwide

• 5-year survival is 36% in patients with operable disease

• 5-year survival is 5-20% in patients with metastatic disease

• Is HER2 a good or poor prognostic indicator in gastric cancer???????
Highlights of the ToGA Trial

- Trastuzumab for gastric cancer (ToGA)
- 122 centers, 24 countries
- 594 patients
- Eligibility criteria tumor had overexpression or amplification of HER2
- IHC or FISH – central lab
- Chemo vs chemo plus trastuzumab
Overall survival

Progression free survival

Bang et al. Lancet 2010;376:687-697
Conclusions of the ToGA Trial

- Trastuzumab plus chemotherapy can be a therapeutic option for patients with HER2 positive advanced gastric and gastro-esophageal junction cancer.

- Overall survival was longer in patients with high expression (2+/FISH+ or 3+) of HER2 versus low expression (0/1+ or FISH negative)
Conclusions of the ToGA Trial

- Overall HER2-positivity rate of 22.1%
- HER2-positivity rate was similar between Europe (23.6%) and Asia (23.5%).
- HER2-positivity rates were higher in gastro-oesophageal junction (GEJ) than stomach cancer (33.2% vs 20.9%; p<0.001)
- Intestinal than diffuse/mixed cancer (32.2% vs 6.1%/20.4%; p<0.001).

Bang et al., ASCO, 2009, Abstract #4556
HER2 Testing and the ToGA Trial

• Human cancers are not the same disease

• One would not expect HER2 to be expressed or amplified at a similar rate in gastric cancer versus breast cancer

• A new set of IHC scoring was developed for this trial due to the “biological differences between breast and gastric tumors”
  – tumor heterogeneity, basolateral membrane staining
HER2 Testing and the ToGA Trial

• Correlation not the same as in breast cancer
  – High expression: IHC 2+/FISH+ and IHC 3+ (had better OS)
  – Low expression: IHC 0 or 1+, FISH positive

• No description of scoring for FISH positives
  – Pos/neg
  – Ratio of LSI HER2:CEP17
  – Why does this matter?
    • HER2 FISH is considered a quantitative molecular test.
# HER2 Testing and the ToGA Trial

<table>
<thead>
<tr>
<th>HER2 Status</th>
<th>%Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH pos/IHC 0</td>
<td>10</td>
</tr>
<tr>
<td>FISH pos/IHC 1+</td>
<td>12</td>
</tr>
<tr>
<td>FISH pos/IHC 2+</td>
<td>27</td>
</tr>
<tr>
<td>FISH pos/IHC 3+</td>
<td>44</td>
</tr>
<tr>
<td>FISH neg/IHC 3+</td>
<td>3</td>
</tr>
<tr>
<td>FISH pos/IHC no result</td>
<td>1</td>
</tr>
<tr>
<td>FISH no result/IHC 3+</td>
<td>3</td>
</tr>
</tbody>
</table>

Was this low level amplification? Good response

Table 2 - Bang et al. Lancet 2010;376:687-697
Multicenter Study

- Comparison of HER2FISH and IHC in gastric cancer
- Dartmouth (NH), Baystate Medical Center (MA), St. Barnabas Medical Center (NJ)
- 60 cases (biopsy and resected tumors)
- IHC at core lab and interp by all 3 labs
- FISH at all 3 labs
- Technical reproducibility and Scoring criteria?
Summary

• Cancer diagnostics is beginning to experience the molecular revolution.

• We better understand the disease and can offer better therapeutic options.

• FISH analysis is a definitive tool for the anatomic pathologist and should be considered the new special stain.
DHMC Molecular Pathology Laboratory and Translational Research Program

Samantha Allen
Claudine Bartels, Ph.D.
Heather Bentley
Betty Dokus
Susan Gallagher
Carol Hart
Arnold Hawk
Joel Lefferts, Ph.D.
Rebecca O’Meara
Elizabeth Reader
Mary Schwab
Laura Tafe, M.D.
Brian Ward
Brendan Wood