Importancia del papel del patólogo en la validación de nuevos biomarcadores en cáncer: Ejemplos de éxito y retos futuros

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Biomarkers Used in Cancer Management: Questions to be addressed by Cancer Biomarkers

All patients with same diagnosis

Diagnostic

What type of cancer is it?

Prognostic

Will the cancer return?

Predictive

Is this the optimal drug for my cancer?

Pharmacodynamics

What’s the optimal dose for my body?

Adapted from McShane, LM. SABCS 2010
Biomarkers Used in Cancer Management

All patients with same diagnosis

- High risk of disease recurrence
- Low risk of disease recurrence
- No benefit to specific treatment
- Benefit to specific treatment

Prognostic

Predictive and Pharmacogenetic
# Biomarkers Used in Cancer Management

<table>
<thead>
<tr>
<th>Type of biomarker</th>
<th>Uses in management and clinical trials</th>
<th>Identification</th>
<th>Validation</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prognostic biomarker</td>
<td>Treatment choice, patient selection and stratification</td>
<td>Easy, but often flawed or biased</td>
<td>Frequent, but often inadequate because of regression to the mean or flaws in the initial identification study</td>
<td>Poor performance status, elevated hepatic enzymes, multi-site metastases in advanced colorectal cancer. Lymph node status, tumor size, tumor grade, proliferation, cyclin D1 in breast cancer.</td>
</tr>
</tbody>
</table>
Potential role for biomarker-based diagnostics

Past

Drugs

Which patients respond best?

Current and future

Determine molecular profile of the patient’s tumour

Determine which drugs are most appropriate
Histological grade: prognosis in breast cancer

Old Nottingham series (1977-89)

Recent Nottingham series (1990-2002)

Breast cancer prognostic classification in the molecular era: the role of histological grade

Emad A Rakha1, Jorge S Reis-Filho2, Frederick Baehner3, David J Dabbs4, Thomas Decker5, Vincenzo Eusebi6, Stephen B Fox2, Shu Ichihara4, Jocelyne Jacquemier7, Sunil R Lakhani8, José Palacios1, Andrea L Richardson2, Stuart J Schnitt9, Fernando C Schmitt10, Puay-Hoon Tan10, Gary M Tse11, Sunil Badve12 and Ian O’Ellis11

Rakha, EA et al. Breast Can Res 2010
Biomarkers Used in Cancer Management

Biomarkers in cancer research, global. Cancer Biomarkers Market Revenues ($bn), 2007-16

Cancer predictive biomarkers published literature

Alymani, NA. et al. Eur J Can 2010
What is a clinically useful prognostic biomarker?

Correlation with outcome not necessary sufficient to impact clinical decisions

Does the biomarker provide information beyond standard prognostic factors?

**Good prognosis group may forego additional therapy**

**This prognostic information is not helpful**

- Hazard ratio = .18
- Hazard ratio = .56
What is a clinically useful predictive biomarker?

What is a treatment by marker interaction, and are they all created equal?

**Qualitative interaction**
- New drug better for M+ (HR=0.44)
- Control drug better for M- (HR=1.31)
- Interaction = 0.44/1.31 = 0.33

**Qualitative interaction**
- New drug better for M+ (HR=0.44)
- Control drug better for M- (HR=0.76)
- Interaction = 0.44/0.76 = 0.58
Biomarker study deficiencies: Convenience samples

- Retrospective collections
- Biased
- Heterogeneous patient characteristics
- Heterogeneous or unknown treatments and follow up care
- Insufficient sample size
- Uncertain specimen and data quality

What can we do with our marker on these 89 specimens?
Diagnostics and biomarker development

AACR Cancer Progress Report, Clin Cancer Res 2012
Taube, SE. Et al. JNCI 2009
Diagnostics and biomarker development

- Biology well-understood
- Plausible link to agent activity
- Prevalence in target populations sufficient
- Assessable on available specimens

- Specimens available and annotated
- Clear specification of technical protocol
- Defined assay validation criteria
- Assessment of cost and feasibility in clinical setting
- Potential impact on therapeutical market

- Reference lab
- Biomarker-related data collection plan
- Quality monitoring of biomarker assessment
- Monitoring testing failures
- Monitoring impact on therapeutical usage
The genetic basis for cancer treatment decisions

- **Lung Adenocarcinoma**
  - Other?
  - KRAS
  - EGFR
  - ALK
  - MET
  - NRAS
  - MEK1
  - BRAF
  - RET
  - ROS
- **Lung Squamous Cancer**
  - Other?
  - EGFR
  - FGFR
  - PI3K
  - MAPK
  - TOR
- **Breast Cancer**
  - Other?
  - ERBB2
  - PIK3CA
  - FGFR1
  - PTEN
  - AKT
- **Colorectal Cancer**
  - Other?
  - KRAS
  - NRAS
  - ERBB2/3
  - PIK3CA
  - BRAF
  - PTEN
- **Melanoma**
  - Other?
  - NF1
  - KRAS
  - NRAS
  - BRAF
- **Head and Neck Squamous Cancer**
  - Other?
  - CDKN2A
  - CCND1
  - EGFR, ERBB2
  - HRAS
  - PIK3CA
  - PTEN

(PTEN and CDKN2A are frequently inactivated)
Drug and Biomarker Codevelopment: HER2 case study

Basic biology

- HER2/neu is a member of the human ErbB family of receptors, a group of transmembrane receptors with intracellular tyrosine kinase activity and extracellular binding domain.
- HER2 receptor does not appear to have a specific ligand but can signal by forming heterodimers with other members of the ErbB family.
- Amplification of the HER2 gene produces overexpression of this cell membrane receptor protein and activation of several downstream signal transduction pathways.
- Studies in HER2-transfected cells, as well as transgenic animals, support the hypothesis that amplification and/or overexpression of this proto-oncogene contributes to the pathogenesis and clinical aggressiveness of tumors (1,2).
- HER2 is overexpressed in approximately 20%-30% of human breast cancers (3-5). Overexpression rarely occurs in the absence of gene amplification in breast cancer (i.e., in approximately 3% of cases).

Clinical observations

- HER2 overexpression identifies a subgroup of breast cancer patients with aggressive disease, frequently hormone receptor-negative with poor prognosis (3).
- HER2 gene amplification has been associated with resistance to a variety of cytotoxic agents and endocrine therapies (6,7).

Agent description

- Trastuzumab (Herceptin) is a humanized monoclonal antibody with high specificity for the HER2 protein.
- Trastuzumab has demonstrated antitumor activity when used as a single agent in first- or second-line treatment of HER2-amplified or HER2-overexpressing metastatic breast cancer (8,9).
Drug and Biomarker Codevelopment: HER2 case study

Development path and clinical trial design(s)

- Given strong laboratory and clinical data supporting the importance of the target and antitumor activity noted from target inhibition, clinical development of trastuzumab was initially focused on testing the agent in breast cancer patients with HER2 overexpression and/or HER2 amplification in their tumors.
- Trastuzumab received Food and Drug Administration approval in 1998 for the treatment of HER2-overexpressing metastatic breast cancer, as a single agent or in combination with paclitaxel, in patients who have received one or more chemotherapy regimens.
- In 2006, trastuzumab was approved for adjuvant treatment of HER2-overexpressing breast cancer, either in combination with doxorubicin, cyclophosphamide, and paclitaxel or as a single agent following chemotherapy based on striking results from pivotal phase III trials (10–14).
- Detection of HER2 protein overexpression by immunohistochemistry (IHC) or HER2 gene amplification by fluorescence in situ hybridization (FISH) was advised for selection of patients for trastuzumab therapy.

Assays (trade name of assay, what was measured, method, manufacturer)

- HercepTest, HER2 protein (A085 polyclonal antibody), IHC; Dako, Carpinteria, CA.
- Pathway, HER2 protein (CB11 monoclonal antibody), IHC; Ventana Medical Systems, Tucson, AZ.
- PathVysion, HER2 gene, FISH; Abbott Laboratories, Abbott Park, IL.
- INFORM, HER2 gene, FISH; Ventana Medical Systems, Tucson, AZ.
- SPoT-Light, HER2 gene, chromogenic in situ hybridization (ISH); Invitrogen, Carlsbad, CA.
- EnzMet GenePro, HER2 gene, silver-enhanced ISH; Ventana Medical Systems, Tucson, AZ.

Issues

- Debate continues about the optimal assay methodology and potential efficacy of trastuzumab in patients who do not express HER2 (15–19).
Basic biology

- EGFR is a member of the human ErbB family of receptors.
- Upon ligand binding, EGFR homodimerizes or heterodimerizes with another member of the ErbB receptor family, activating its protein tyrosine kinase domain and initiating downstream signaling via cellular pathways controlling proliferation, survival, motility, and angiogenesis (20–22).
- EGFR is expressed in a variety of malignancies, and experimental evidence suggests that its inhibition can induce tumor stasis or, less commonly, regression (20,21).
- Most frequent EGFR abnormality reported in human cancers is receptor overexpression, but unlike HER2, which is in the same family of receptors, high concordance between overexpression and gene amplification has not been well demonstrated.
- At the time of initial clinical trials evaluating EGFR inhibitors, gene amplification and/or mutations were known to occur in glioblastoma but not in other tumor types, and markers of sensitivity or resistance were not known.
- After antibodies and small molecules were commercially available, additional potential predictive biomarkers emerged, including EGFR gene amplification or increased copy number by fluorescence in situ hybridization (FISH), activating EGFR mutations in lung cancer patients and KRAS mutations that predicted lack of response to EGFR inhibition in colorectal and lung carcinomas.

Clinical observations

- EGFR overexpression determined by immunohistochemistry has been associated with poorer clinical outcomes in some settings (20).
- High EGFR gene copy number identified by FISH might be a better predictor for survival in tyrosine kinase inhibitor (TKI)–treated non–small cell lung cancer (NSCLC) patients (23,24).
- Clinical evidence suggests that EGFR inhibition can induce tumor stasis or regression (20,25–27).
- Specific somatic mutations, small deletions, insertions, or point missense mutations in the EGFR tyrosine kinase correlate with better prognosis and increased objective response rate in NSCLC patients treated with small molecule TKIs (25–28) but not with cetuximab (29).
- KRAS mutations appear to predict for insensitivity of tumors to both antibodies and small molecules (30–34).

Agent description

- Monoclonal antibodies, such as cetuximab and panitumumab, and TKIs, such as erlotinib and gefitinib, targeting EGFR have been developed.
Drug and Biomarker Codevelopment: EGFR case study

Development path and clinical trial design(s)

- Given the paucity of biological information about markers of sensitivity and/or resistance, initial clinical trials focused on cancers that frequently express EGFR, but did not exclude patients with tumors that did not express EGFR.
- Monoclonal antibodies were initially evaluated in colorectal and head and neck carcinoma patients, and the initial development of small molecule TKIs focused on non-small cell lung cancer as initial signals of activity were seen in these settings.
- Evaluations of monoclonal antibodies in colorectal carcinoma have generally required EGFR protein expression as detected by immunohistochemistry for eligibility; however, EGFR expression has generally not been required for enrollment into trials of inhibitors of EGFR tyrosine kinases.

Assays (trade name of assay, what was measured, method, and manufacturer)

- pharmDx, EGFR protein, IHC; Dako, Carpinteria, CA.
- HTScan EGFR-phosphorylated protein, IHC; Cell Signaling Technology, Beverly, MA.
- CONFIRM EGFR, EGFR protein, IHC; Ventana Medical Systems, Tucson, AZ.
- PathVysion, EGFR gene (Locus Specific Identifier for EGFR labeled with Spectrum Orange and Chromosome Enumeration Probe 7 labeled with Spectrum Green), FISH, Abbott Laboratories, Abbott Park, IL.

Issues

- Detectable EGFR (high expression not necessarily critical) appears to correlate with clinical benefit from EGFR inhibitors in some cases but fails to provide predictive information in others. It is unclear whether these differences are due to test methodologies, the biology of the disease being evaluated, or a combination of both (35,36).
- EGFR mutations that occur more frequently in East Asian patients and never smokers are associated with improved response rate and outcome and rarely occur with KRAS mutations (37–39). Patients with EGFR mutations may develop resistance through emergence of secondary mutations or c-MET amplification (40).
- KRAS mutations are associated with lack of activity of EGFR antibodies in colorectal carcinoma and perhaps in NSCLC although data are less definitive because of limited samples analyzed retrospectively from randomized clinical trials (30–34).
Drug and Biomarker Codevelopment: BRAF case study
Survival in BRAF V600–Mutant Advanced Melanoma Treated with Vemurafenib

Drug and Biomarker Codevelopment: BRAF case study
A new paradigm in cancer management: massive target profiling

Unselected tumor samples

Identification of tumor classes

Selected tumor samples

A-type: marker genes q, r, s are high
B-type: marker genes x, y, z are high

Index with prognostic or predictive information
Oncotype Dx Recurrence Score in ER+, N-, Tam+ patients

A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer

Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang, Ph.D., Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin, Ph.D., Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew Watson, Ph.D., Taesung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher, M.D., D. Lawrence Wickerham, M.D., John Bryant, Ph.D., and Norman Wolmark, M.D.

Lower likelihood of recurrence
Greater magnitude of TAM benefit
Minimal, if any, chemotherapy benefit

Greater likelihood of recurrence
Lower magnitude of TAM benefit
Clear chemotherapy benefit

The New England Journal of Medicine

Original Article

Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*
Molecular profiling in cancer

### nCounter® GX Human Cancer Reference Kit

<table>
<thead>
<tr>
<th>Genes List</th>
<th>Description</th>
<th>Specifications</th>
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</thead>
<tbody>
<tr>
<td>ABC1, CDC25C, CDC25B, EFRB2</td>
<td>Level of multiplexing</td>
<td>230 genes known to be differentially expressed in human cancer</td>
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<tr>
<td>ABL1, CDC25C, CDC25B, EFRB3</td>
<td>Recommended amount of starting material</td>
<td>100ng of total RNA, or lysate from ~10,000 cells</td>
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<tr>
<td>AKT1, CDC25C, CDC25B, EFRB4</td>
<td>Sample types supported</td>
<td>Total RNA, cell lysates in GITC, FFPE-derived total RNA and PAXgene lysed whole blood, amplified RNA</td>
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<tr>
<td>AKT2, CDC25C, CDC25B, ERCC2</td>
<td>Reaction volume</td>
<td>30 μL</td>
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<tr>
<td>APC, CDC2, ERCC4, ERSR1</td>
<td>Limit of detection</td>
<td>0.5fM spike-in control (~1 copy per cell); 90% of the time</td>
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<tr>
<td>AR, CDK4, ESR1</td>
<td>Fold change sensitivity</td>
<td>&gt; 1.5 fold (&gt; 5 copies per cell)</td>
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<tr>
<td>AREG, CDK6, ET31, IFNIR1</td>
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<td>&gt; 2 fold change (&gt; 1 copy per cell)</td>
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<td>ATM, CDKN1A, ETS2</td>
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<td>BRAF, CTGF, FGF4</td>
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<td>BRCA2, CXXC9, FLT1</td>
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<td>CASP10, CYP1A1, FLT3</td>
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<td>CDK4, EGFR, GATA1</td>
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<td>CDK5, EGR1, GNAT</td>
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<tr>
<td>CDC2, EPSh1, GPR87</td>
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</tbody>
</table>

### Specifications

- **Level of multiplexing**: 230 genes known to be differentially expressed in human cancer
- **Recommended amount of starting material**: 100ng of total RNA, or lysate from ~10,000 cells
- **Sample types supported**: Total RNA, cell lysates in GITC, FFPE-derived total RNA and PAXgene lysed whole blood, amplified RNA
- **Reaction volume**: 30 μL
- **Limit of detection**: 0.5fM spike-in control (~1 copy per cell); 90% of the time
- **Fold change sensitivity**: > 1.5 fold (> 5 copies per cell)
- **Spike correlation**: R² ≥ 0.95
- **Linear dynamic range**: 7 × 10⁶ total counts
- **Controls**: 6 positive and 8 negative in each reaction
Prediction Analysis of Microarray (PAM) 50 in breast cancer

N=761

Parker, JS et al. J Clin Oncol 2009
Foundation Medicine: targetable molecular landscape in cancer
Performance characteristics include:
- Next-generation sequencing platform on FFPE
- Depth of coverage at a median of 500x, up to >1000x
- Sensitivity of 99% with alterations of 10% frequency
- Ability to identify alterations with frequencies of <1%
- Requirements of 50 ng of DNA or < 40 microns of tissue
Trastuzumab is effective in a subset of ERBB2 amplified breast cancer.

Coexistent genomic alterations can provide explanations for resistance and rationale for study of select combinations.
Case Report: EGFR mutant lung adenocarcinoma

2nd gen. EGFR TKI, possibly with cetuximab
PARP inhib./Plat-based chemo
Nuttins/MDM2 inhib.

Genomic alterations detected in acquired resistance to EGFR-TKIs are diverse and may explain the largely disappointing results seen to date with second generation agents

Use of clinical grade NGS may identify one or more tumor specific treatment options
Case Report: Non-small cell lung cancer

Test Results

Non-small cell lung cancer

Genomic Alterations
- EML4-ALK fusion
- CDKN2A loss
- CDKN2B loss

Additional disease relevant genes with no reportable alterations detected
- KRAS
- EGFR

Although crizotinib is efficacious in ALK rearranged NSCLC, little is known to explain variability in response magnitude and duration
Foundation Medicine: targetable molecular landscape in cancer

NSCLC: Actionable Genomic Alterations

- Genes with Actionable Alterations
- Genes with Alterations, Actionability Unknown
Mensajes finales

La nueva medicina personalizada requiere de los patólogos,

1. Un uso de biomarcadores (predictivos) (co-) validados para la toma de decisiones terapéuticas

2. Conocimientos de las alteraciones moleculares propias de las células tumorales

3. Asumir el reto de incorporar y conocer las nuevas tecnologías en nuestra práctica