The Impact of Molecular Diagnostics on Contemporary Cancer Management

James J. Stark, MD, FACP

Professor of Clinical Internal Medicine, EVMS
Medical Director, Cancer Program
Director of Palliative Care
Maryview Medical Center

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Case Presentation

- 54 y.o. man presented in February, 2009, with widespread lytic lesions of bone – referred as possible multiple myeloma
- Only relevant history is strong family history of prostate cancer
- CBC and all chemistries normal
- While waiting for initial lab workup to return he developed symptoms of impending spinal-cord compression with mild leg weakness and urinary retention
- Admitted to the hospital for emergency decompression and tissue diagnosis
CT scan of spine

Punched out lytic lesion
Pathology – Low Power
Pathology – High Power
Additional Work-Up

- CT showed many small lung nodules
- Tissue stained for β-subunit HCG and α-fetoprotein as well as PSA – all negative
- Serum markers including CEA, CA27-29, CA 19-9 and above tests all negative
- Myeloma workup looking for paraprotein negative
- Rapidly developed new bone lesions
- Specimen of paraffin-embedded tissue block sent to reference lab for “Cancer Type ID” assay (m-RNA analysis) in hopes of finding a primary
- Details of assay to follow….
Molecular Work-Up of Our Patients’ Tumor

• Concluded with 99% confidence that he had renal cell carcinoma
• New CT scan showed new small suspicious lesion in left kidney and increase in size of previously noted lung lesions…
New Renal Lesion: Is this the Primary?
Lung Lesions and Hilar Mass
Case, continued

- On the basis of the m-RNA analysis he was started on Sunitinib
- By the time this was started he was almost bed-ridden; he pursued a downhill course and died before we could ascertain whether this drug really did him any good
- No “empiric” chemotherapy was ever given
A Brief History of Molecular Diagnostics

• For a long time H&E staining was the mainstay of histologic diagnosis of cancer
• Pattern recognition differentiated:
  – Carcinoma
    • Adeno, Squamous, Small Cell, etc.
  – Sarcoma
  – Myeloid Malignancies
  – Lymphoid Malignancies
  – Neuro-endocrine Malignancies
Classic Colonic Adenocarcinoma
Oral Cavity Squamous Cell Carcinoma
Small Cell Lung Cancer
Small Cell Carcinoma or Lymphoma?

By immunoperoxidase staining this is B-cell lymphoma.
The Impact of Molecular Diagnostics on Contemporary Cancer Management

• The following presentation gives highlights from a burgeoning field, with significant advances occurring on a daily basis
• It cannot be encyclopedic in its approach
• Rather I hope to hit the high points in a very active and exciting area of basic and clinical research
• First…some relevant history
First Breakthroughs in Solid Tumors – the 1970’s

- The Hormone Receptor
- Immunoperoxidase staining looking for proteins expressed on the surface or in the cytoplasm of cells – as in the last example (small cell carcinoma vs. lymphoma)
The Hormone Receptor Story

- Marc Lippman at NCI and others recognized that breast and other cancers expressed hormone receptors in their cytoplasm.
- Those receptors bind steroid hormones; that complex then migrates into the nucleus of the cell and affects genes within the cell that control cell growth.
The Significance of the Presence of the Hormone Receptor

- Lends credence to the site of origin of the tumor as breast if the tumor assayed is in a metastatic site rather than the breast
- Guides therapy towards the use of hormonal manipulation rather than cytotoxic chemotherapy
The Significance of the Hormone Receptor in the History of this Field

- Applied technology already known in the 70’s to solving a new problem
- Only recently have newer techniques (Northern Blot and Polymerase Chain Reaction) been used investigationally to improve the accuracy of this test
- Represented a benchmark in going beyond conventional histology in determining structure and function of a cancer
Further significance of the Estrogen Receptor: Disease-Free Survival with Adjuvant Tamoxifen versus concentration of quantitative ER and PR in women over 50 (NSABP B09)

The Importance of Surface Immunoglobulins to this Story

• Lymphomas until the 1970’s were classified purely morphologically – nodular vs. diffuse, large vs. small cells
• The discovery of monoclonal surface immunoglobulins on lymphoid tissue changed forever the approach to, and treatment of, non-Hodgkin lymphoma
• The therapy of B-cell neoplasms (lymphomas, multiple myeloma) was changed substantially with the development of an antibody to the CD20 surface molecule expressed on B cells preferentially – i.e., Rituximab
• Molecular diagnostics drove the development of novel therapy
Monoclonal Antibodies in Lymphoid Diseases, continued

• Next important antibody was OKT3 used therapeutically in the treatment of allograft rejection
• Directed against the T-cell and its role in graft rejection
• As a marker can help distinguish T- from B-cell lymphoma in difficult cases
• Being investigated in the treatment of T-cell malignancies
Other Monoclonal Antibodies in the Treatment of Lymphoid Malignancies

- Alemtuzumab (Campath) directed against CD-52 in refractory CLL
- Ofatumumab picks up patients refractory to chemotherapy and Alemtuzumab – just approved by FDA and marketed as Arzerra
- All of these therapies are based on utilizing unique molecular aspects of lymphoid cells as targets for novel therapies
Newer targets for the Treatment of Cancer: the Her-2/neu Oncogene

- 185 KD transmembrane glycoprotein receptor with intracellular tyrosine kinase activity
- Overexpressed in 18-20% of breast cancers
- Presence predicts for more virulent disease
The Her-2/neu Oncogene
Her-2, continued

• Patients who are “Her-2+” are so by virtue of making numerous copies of this transmembrane protein as part of the defect in regulation associated with the mutation

• Such patients historically (prior to the development of Trastuzumab) had a much higher overall mortality
Her-2, continued

- The addition of Trastuzumab to conventional chemo therapy in the adjuvant and metastatic setting has resulted in marked improvement in the outcome of such patients
- The classic adjuvant trial...the HERA trial
Disease-Free survival in patients getting adjuvant chemotherapy with or without subsequent Trastuzumab

Adjuvant Herceptin, continued

- These seminal observations have been extended to other groups of patients
- This treatment became the overnight standard of care for patients with Her-2 positive breast cancer, either primary or metastatic
- The original observation about a unique gene on the surface of breast cancer cells started the cascade of developments which has led to revolutionary new treatment of breast cancer
The Philadelphia Chromosome in the Era of Molecular Biology

• For decades the exchange of genetic material between chromosomes 9 and 22 in CML has been well known...
Schematic of BCR-ABL
For decades the exchange of genetic material between chromosomes 9 and 22 in CML has been well known.

The ability to find a single cell with the translocation in a sea of normal cells is a relatively new development: Fluorescence in-situ hybridization (FISH).
FISH

• Uses short pieces of DNA which are complementary to a genetic sequence of interest (a probe)
• Probe binds specifically to target DNA sequence
• Probe is linked to a fluorescent compound for visualization
• 200 cells typically scored
• Always targeted to a specific mutation; Not a hunt for any mutation
FISH: When you know what you are looking for…
In this case the novel BCR-ABL sequence
How Imatinib Works
Outcomes in Patients with CML treated with Imatinib

• A high percentage of patients with convert to FISH-negative in marrow and peripheral blood

• A smaller number will have complete disappearance of disease by Polymerase Chain Reaction techniques
Approximate relationship between response, the putative number of leukemic cells, and the level of BCR-ABL transcripts

- Diagnosis, pretreatment, or hematologic relapse
- Complete hematologic response
- Complete cytogenetic response
- Major molecular response
- Undetectable transcript (complete molecular response)
Long-Term Results

- The greater the log reduction in tumor burden the more likely that the patient will stay in morphologic remission.
- After ten years of experience with Imatinib, median survival of original group of patients has not yet been reached.
- Previously median survival of patients with newly diagnosed CML receiving best therapy was 3-4 years.
The Role of the Epidermal Growth Factor Receptor in Lung Cancer

- Required for tumor growth
- Mutated about 15-20% of the time
- After several years of research on Erlotinib (Tarceva), investigators determined that this mutation was critical to success with this drug
The Many Targets of Tyrosine Kinase Inhibition

Diagram showing various targets and pathways affected by tyrosine kinase inhibition, including Trastuzumab for HER2/neu, EGFR, and Pertuzumab. Other targets like RAS activation, PI3K/AKT, MAPK, HIF1α, VEGF, and PTEN LOF are also depicted.
Time to treatment failure of patients treated with Erlotinib grouped by EGFR mutational status

The next step in this adventure: Gene-Expression Profiling
Using Gene-Expression Profiling to Create Prognosis in Primary Breast Cancer: the Oncotype DX test

By regression analysis 21 genes were picked which, if mutated, alter prognosis. Those genes are analyzed in this test and a risk-of-recurrence score is derived based on the types of mutations seen.
Probability That Patients Would Remain Free of Distant Metastases among 151 Patients with Lymph-Node-Negative Breast Cancer with the Use of Gene-Expression Profiling, the St. Gallen Criteria, and the National Institutes of Health (NIH) Consensus Criteria

Using Recently Acquired Technology to Address the Problem of the Unknown Primary

- 4-5% of cancers present as unknown primaries with metastasis being the first evidence of cancer
- Until now there have been only a limited number of ways to analyze their tumors ante-mortem
- A variety of genes can now be sequenced and compared to a library of genetic mutations compiled for a wide variety of tumors
- Genes looked at include genes for transcription factors, trans-membrane proteins and tumor-specific genes (e.g., TTF-1 for lung cancer)
Commercial Test Addresses Problem:

The CancerTYPE ID® Gene Characteristics

• 92 genes not normally measured by routine laboratory testing:
  – Transcription factors (e.g., HOX-A9, HOX-B8)
  – Plasma membrane proteins (e.g., HTR3A, CHRM3)
  – Uncommonly measured tumor-specific markers (e.g., ESR1 for breast, PRAME for melanoma)
  – Compared genetic sequence in these genes of the unknown tumor against a library of 2000+ tumors whose site of origin was known

### Impact of Diagnostic Procedures on Healthcare Systems

<table>
<thead>
<tr>
<th>Misclassifications</th>
<th>In a retrospective study reviewing the frequency and impact of errors in ~24000 cases, 45% of gynecologic errors and 39% of non-gynecologic errors were associated with harm&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost</td>
<td>Traditional diagnostic methods often fail to diagnose hard-to-identify cancers, even after extensive work-ups that average nearly $18,000&lt;sup&gt;2,3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

There is an unmet need for standardized assays to support diagnostic evaluation and reduce diagnostic uncertainty

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<sup>1</sup>Raab SS, et al. *Cancer* 2005;104:2205-2213;  
<sup>3</sup>Schapira DV, Jarrett AR. *Arch Int Med*. 1995;155:2050-2054
## Applying this technology to a series of twenty unknown primary tumors

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Biopsy Site</th>
<th>Light Microscopic Histology</th>
<th>Molecular Assay Diagnosis</th>
<th>Actual Primary Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>59 F</td>
<td>Axillary node</td>
<td>PDC</td>
<td>Breast</td>
<td>Breast</td>
</tr>
<tr>
<td>65 F</td>
<td>Axillary node</td>
<td>PDA</td>
<td>Breast</td>
<td>Breast</td>
</tr>
<tr>
<td>51 F</td>
<td>Bone</td>
<td>PDC</td>
<td>Breast</td>
<td>Breast</td>
</tr>
<tr>
<td>64 F</td>
<td>Supraventricular node</td>
<td>PDA</td>
<td>Breast</td>
<td>Breast</td>
</tr>
<tr>
<td>85 F</td>
<td>Chest wall mass</td>
<td>PDA</td>
<td>Ovary</td>
<td>Primary peritoneal</td>
</tr>
<tr>
<td>69 F</td>
<td>Inguinal node</td>
<td>Adenocarcinoma</td>
<td>Ovary</td>
<td>Primary peritoneal</td>
</tr>
<tr>
<td>87 F</td>
<td>Omentum</td>
<td>PDA</td>
<td>Ovary</td>
<td>Primary peritoneal</td>
</tr>
<tr>
<td>87 F</td>
<td>Paratracheal mass</td>
<td>PDC</td>
<td>Ovary</td>
<td>Ovary</td>
</tr>
<tr>
<td>68 F</td>
<td>Spleen</td>
<td>PDA</td>
<td>Intestine</td>
<td>Colon</td>
</tr>
<tr>
<td>61 M</td>
<td>Liver</td>
<td>PDA</td>
<td>Intestine</td>
<td>Colon</td>
</tr>
<tr>
<td>42 F</td>
<td>Brain</td>
<td>PDA</td>
<td>NSCLC</td>
<td>NSCLC</td>
</tr>
<tr>
<td>67 M</td>
<td>Subcutaneous mass</td>
<td>Squamous carcinoma</td>
<td>NSCLC</td>
<td>NSCLC</td>
</tr>
<tr>
<td>59 M</td>
<td>Brain</td>
<td>PDA</td>
<td>NSCLC</td>
<td>NSCLC</td>
</tr>
<tr>
<td>74 M</td>
<td>Bones</td>
<td>Adenocarcinoma</td>
<td>Gastric</td>
<td>Gastric</td>
</tr>
<tr>
<td>76 M</td>
<td>Axillary node</td>
<td>PDC</td>
<td>Melanoma</td>
<td>Melanoma</td>
</tr>
<tr>
<td>60 M</td>
<td>Small intestine</td>
<td>PDC</td>
<td>Unclassifiable</td>
<td>NSCLC</td>
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<tr>
<td>38 M</td>
<td>Mediastinal node</td>
<td>PDA</td>
<td>Unclassifiable</td>
<td>NSCLC</td>
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<tr>
<td>61 M</td>
<td>Supraclavicular node</td>
<td>PDC</td>
<td>Testis</td>
<td>Pancreas</td>
</tr>
<tr>
<td>62 M</td>
<td>Retroperitoneal node</td>
<td>PDA</td>
<td>Colorectal</td>
<td>Gastric</td>
</tr>
<tr>
<td>75 F</td>
<td>Chest wall mass</td>
<td>PDC</td>
<td>Soft tissue sarcoma</td>
<td>NSCLC</td>
</tr>
</tbody>
</table>

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Greco A et al. *J Clinical Oncology* 27:15s, 2009 (Abstract 11070)
Results from the 92-gene molecular assay had the potential to change treatment in 53% of CUP cases
A Phase II Study of Chemotherapy Treatment Based on Molecular Profiling Diagnosis for Patients with CUP

First-Line Treatments Administered for Specific Assay Diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell lung cancer</td>
<td>Platinum-based doublet +/- bevacizumab</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Paclitaxel/bevacizumab</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Paclitaxel/carboplatin +/- bevacizumab</td>
</tr>
<tr>
<td>Pancreas cancer</td>
<td>Gemcitabine/erlotinib</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>FOLFOX (or FOLFIRI) + bevacizumab</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>Sunitinib or bevacizumab</td>
</tr>
<tr>
<td>Other specific diagnoses</td>
<td>Standard first-line treatment per treating MD</td>
</tr>
</tbody>
</table>

No primary site after standard clinical, pathological evaluations

CancerTYPE ID Assay

No Specific Diagnosis

Empiric CUP treatment (paclitaxel/carboplatin/bevacizumab/erlotinib)

Specific Diagnosis

Treatment based on assay diagnosis
Interim Results

• Current regimens for CUP patients have a median survival of 7-11 months

• CTID provided a prediction in 98 of 110 patients (89%)

• 61 patients received assay directed therapy

The Current State of the Unknown Primary and Genetic Variance

• A number of competing technologies are being developed to look at a variety of ways of comparing sequences in RNA versus an established library of tumors
• The winning technology has not yet emerged
• All of the technologies represent an advance over what was previously available
Summary

• In the last forty years cancer diagnostics has advanced beyond morphologic analysis.
• The understanding of the relationship between abnormal structure and function has progressed rapidly.
• Therapies designed to exploit the differences between normal and abnormal structure have advanced in number and sophistication.
• All the current advances in Medical Oncology are coming in the area of “targeted” therapy with few new all-purpose chemotherapy drugs coming on line in the last few years.
• As our understanding of structure and function of normal versus abnormal becomes more advanced, cancer therapy will progress accordingly.