NOVEL TUMOR MARKERS FOR IMPROVEMENT OF CARE IN ONCOLOGY
Part I: 
Serum Based Tumor Markers
TUMOR MARKERS

- Substances that can be detected in the blood or body fluids of patients harboring an underlying malignancy
- May be synthesized by tumor or by normal host tissues in response to growth or invasion of cancer cells
- Advances in radioimmunoassays and molecular biologic methods have improved identification and utilization
CLASSIFICATION OF TUMOR MARKERS

1. Tumor antigens
   1.1 Oncofetal - produced by placental fetal complex during normal embryologic development and by tumor cells (ex. CEA, AFP, HCG)
   1.2 Defined by clonal antibodies (ex. CA125, CA19-9, PSA)

2. Enzymes/Hormones - produced in excess amounts by tumor or tumor bearing host (Ex. NSE, PAP, LDH, β-HCG)

3. Oncogenes - mRNA or DNA transcripts encoding production of antigens or proteins specific for a particular tumor
"IDEAL" TUMOR MARKER

- **Specific**- produced by cells of a particular malignancy and not by others. (cancers, normal tissues, benign condition)
- **Sensitive**- Produced by all patients harboring a specific histologic type of malignancy
- **Levels correlate directly with amount of tumor burden**
  - present early in pre-clinical tumorigenesis
  - increase with advancing stage
  - decrease with effective therapy
Laboratory Medicine Practice Guidelines

Use of Tumor Markers in Testicular, Prostate, Colorectal, Breast, and Ovarian Cancers

Edited by Catharine M. Sturgeon and Eleftherios Diamandis
CURRENTLY AVAILABLE SERUM AND TISSUE MARKERS FOR TESTICULAR TUMORS

<table>
<thead>
<tr>
<th>Marker</th>
<th>Use</th>
<th>Phase of Dev't</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP</td>
<td>Dx available</td>
<td>available</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Px/Staging</td>
<td>available</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Monitoring</td>
<td>available</td>
<td>II</td>
</tr>
<tr>
<td>hCG</td>
<td>Dx available</td>
<td>available</td>
<td>II</td>
</tr>
<tr>
<td></td>
<td>Px/Staging</td>
<td>available</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>Monitoring</td>
<td>available</td>
<td>II</td>
</tr>
<tr>
<td>LDH</td>
<td>Px/Staging</td>
<td>available</td>
<td>I</td>
</tr>
</tbody>
</table>

KEY POINTS: TUMOR MARKERS IN TESTICULAR CANCER
Tumor markers are of central importance in the diagnosis, Staging, risk assessment and monitoring of patients with Testicular cancer. AFP, hCG, and LDH have been thoroughly validated and shown to have independent prognostic value.
### THE CLINICAL USE OF PSA SERUM MARKERS IN PROSTATE CANCER

<table>
<thead>
<tr>
<th>Application</th>
<th>NACB (2008)</th>
<th>Level of Evidence</th>
<th>Strength of Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>No</td>
<td>III</td>
<td>B</td>
</tr>
<tr>
<td>Early detection (with DRE)</td>
<td>Yes</td>
<td>III</td>
<td>B</td>
</tr>
<tr>
<td>Early detection (age specific)</td>
<td>No</td>
<td>Expert opinion</td>
<td>B</td>
</tr>
<tr>
<td>Staging/prognosis</td>
<td>Yes</td>
<td>III</td>
<td>B</td>
</tr>
<tr>
<td>Surveillance/monitoring</td>
<td>Yes</td>
<td>III</td>
<td>B</td>
</tr>
</tbody>
</table>

### KEY POINTS: TUMOR MARKERS IN PROSTATE CANCER

Serum PSA levels are important in the diagnosis and treatment of patients with prostate cancer. Further improvement in understanding the natural history of the disease should enable better use of these markers in the future.
COLORECTAL CARCINOMA

CURRENTLY AVAILABLE MARKERS FOR COLORECTAL CANCER

<table>
<thead>
<tr>
<th>Marker</th>
<th>Proposed Use</th>
<th>Phase of Dev't</th>
<th>LOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>Prognosis</td>
<td>Pre-op levels establish baseline</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Surveillance after curative resection</td>
<td>In conjunction with Hx/PE/scans</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Monitoring for advanced disease</td>
<td>In conjunction with Hx/PE/scans</td>
<td>III</td>
</tr>
<tr>
<td>FOBT</td>
<td>Screening asymptomatic populations</td>
<td>Screening with FOBT reduces mortality</td>
<td>I</td>
</tr>
<tr>
<td>DNA Panels</td>
<td>Screening asymptomatic populations</td>
<td>More sensitive than FOBT in detecting</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>advanced adenomas and invasive CRC</td>
<td></td>
</tr>
</tbody>
</table>

KEY POINTS: TUMOR MARKERS IN COLORECTAL CANCER

CEA may be useful in the postoperative surveillance of patients that may be suitable for either surgical resection or systemic chemotherapy. FOBT (guaiac or fecal immunochemical test) is recommended for screening for early CRC in subjects >50 y.o. One of the most promising fecal CRC screening tests is a fecal DNA panel test. At present this is still expensive and being tried in large population-based studies.
CURRENTLY AVAILABLE SERUM MARKERS FOR OVARIAN CANCER

<table>
<thead>
<tr>
<th>Cancer Marker</th>
<th>Proposed Use</th>
<th>Phase of Dev’t</th>
<th>LOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA125</td>
<td>Ddx of pelvic masses</td>
<td>Accepted clinical use</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>Monitoring treatment</td>
<td>Accepted clinical use</td>
<td>I,II</td>
</tr>
</tbody>
</table>

KEY POINTS: TUMOR MARKERS IN OVARIAN CANCER
CA125 is the only marker for clinical use in ovarian cancer for the following indications: Early detection in combination with TVU in hereditary syndromes, Ddx in suspicious Pelvic mass, detection of recurrence, monitoring of therapy, and prognosis. IT IS NOT RECOMMENDED FOR SCREENING OF OVARIAN CANCER.
Part II:
Tissue Based Biologic Markers
How to validate a biomarker

Hypothesis

Retrospective data analysis

Biomarker candidate

Prospective validation

Choice of proper and validated test method

Statistical significance

No statistical significance

No biomarker

Biomarker
HER2: the only validated marker for predicting Herceptin response

Normal HER2 expression
- FISH negative
- HC negative (IHC 0, 1+)
- Not eligible for Herceptin

HER2 overexpression
- FISH positive
- IHC positive (IHC 3+)
- Eligible for Herceptin

HER2, human epidermal growth factor receptor 2; FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry

Bilous et al 2003
FISH Test Measures HER2 Gene Amplification

**FISH Test**

- **Chromosome 17 centromere**
- **HER2 gene**

**HER2 Normal**
- Ratio < 2.0

**HER2 Amplified**
- Ratio ≥ 2.0

IHC Test Measures HER2 Protein Overexpression

Immunohistochemistry (IHC)

IHC 0  IHC 1+  IHC 2+  IHC 3+

NCCN guidelines recommend that an IHC result of 2+ should be retested with FISH

HercepTest® package insert, 3rd edition, DAKO; NCCN, Practice Guidelines for Breast Cancer, v.3.2003
HER Signalling

lipid kinase phosphoinositide 3-kinase (PI3-K), enzyme Ak transforming factor (Akt), the mammalian homologue of the son of sevenless (SOS), rat sarcoma (RAS) enzyme; receptor activation factor (RAF); mitogen-activated protein kinase (MAPK) and mitogen extracellular signal kinase (MEK).
HER2-Positive Patients Have Lower Survival Rates

- NCCN and ASCO guidelines recommend HER2 testing for all invasive breast cancer patients, so that patients can receive appropriate treatment for their specific disease states.
  - In addition, NCCN recommends retesting IHC 2+ test results with FISH.

Herceptin MoA
EFS: HER2-positive population

- Patients: 115, Events: 36, HR: 0.56, 95% CI: 0.36-0.85, p: 0.006
- Patients: 112, Events: 52

Median follow-up is 3 years.

Unadjusted for stratification variables: adjusted HR=0.55, p=0.0062

HR, hazard ratio; CI, confidence interval; CT, chemotherapy

Overall survival: HER2-positive population

- Patients: 115, Events: 17, HR: 0.65, 95% CI: 0.34-1.23, p: 0.18
- Patients: 112, Events: 22

Median follow-up is 3 years.
<table>
<thead>
<tr>
<th>Cancer Marker</th>
<th>Proposed Use/Uses</th>
<th>Phase of Development</th>
<th>LOE*</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue-Based Markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrogen receptor (ER)</td>
<td>For predicting response to hormone therapy in both early and advanced breast cancer</td>
<td>In clinical use</td>
<td>I</td>
<td>(330, 331, 576)</td>
</tr>
<tr>
<td></td>
<td>In combination with other factors for assessing prognosis in breast cancer; ER alone is a relatively weak prognostic factor</td>
<td>In clinical use</td>
<td>III</td>
<td>(576, 577)</td>
</tr>
<tr>
<td>Progesterone receptors (PR)</td>
<td>Usually combined with ER for predicting response to hormone therapy</td>
<td>In clinical use</td>
<td>III</td>
<td>(578, 579)</td>
</tr>
<tr>
<td>HER-2</td>
<td>Determining prognosis; most useful in node-positive patients. Conflicting data in node-negative patients</td>
<td>In clinical use in some centers</td>
<td>II-III</td>
<td>(580)</td>
</tr>
<tr>
<td></td>
<td>For selecting patients with either early or metastatic breast cancer for treatment with Trastuzumab (Herceptin)</td>
<td>In clinical use</td>
<td>I</td>
<td>(581-583)</td>
</tr>
<tr>
<td></td>
<td>For predicting resistance to tamoxifen therapy in breast cancer; may be predictive of relative resistance to tamoxifen in patients with early breast cancer</td>
<td>Results conflicting, undergoing further evaluation</td>
<td>III</td>
<td>(348, 349)</td>
</tr>
<tr>
<td></td>
<td>For predicting resistance to CMF in early breast cancer; may be predictive of relative resistance to CMF in patients with early breast cancer</td>
<td>Results conflicting, undergoing further evaluation</td>
<td>III</td>
<td>(348, 349)</td>
</tr>
<tr>
<td></td>
<td>For selecting response to anthracycline-based therapy in early breast cancer, HER-2 may be associated with an enhanced response to anthracycline-based therapy**</td>
<td>Undergoing further evaluation</td>
<td>II/III</td>
<td>(348, 349, 351, 352)</td>
</tr>
<tr>
<td>Urokinase plasminogen activator (uPA)</td>
<td>For determining prognosis in breast cancer, including the subgroup with axillary node-negative disease</td>
<td>Prognostic value validated in both a prospective randomised trial and a pooled-analysis. In clinical use in parts of Europe, e.g., Germany.</td>
<td>I</td>
<td>(361-363)</td>
</tr>
<tr>
<td></td>
<td>For predicting resistance to hormone therapy in advanced breast cancer</td>
<td>Undergoing evaluation</td>
<td>III-IV</td>
<td>(584, 585)</td>
</tr>
<tr>
<td></td>
<td>For predicting enhanced response to chemotherapy in early breast cancer</td>
<td>Undergoing evaluation</td>
<td>III</td>
<td>(364, 365, 586)</td>
</tr>
</tbody>
</table>
KEY POINTS: TUMOR MARKERS IN BREAST CANCER

The best validated markers in breast cancer are all tissue based and include ER, PR, HER-2, uPA and PAI-1.

ER/PR/HER2 assays are mandatory for all newly diagnosed breast cancer patients.

UPA and PAI-1 may be helpful in determining which node negative patients do not need adjuvant chemotherapy.

Oncotype DX may also predict recurrence in this group of patients.
Image adapted from: National Human Genome Research Institute.
- analysis of mutations in **tumour DNA** encoding the EGFR gene
- It is **not**
  - Copy number of DNA of the EGFR gene (Fluorescent In-Situ Hybridisation, FISH)
  - Expression of EGFR protein in the cell (analysed by Immuno-Histochemistry, IHC)

<table>
<thead>
<tr>
<th>EGFR Measure</th>
<th>Test Material</th>
<th>Location of Test Material</th>
<th>Method of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR Mutation</td>
<td>DNA</td>
<td>Nucleus</td>
<td>Mutation Analysis e.g. Sequencing, ARMS, etc...</td>
</tr>
<tr>
<td>EGFR Copy Number</td>
<td>DNA</td>
<td>Nucleus</td>
<td>Fluorescent In-Situ Hybridisation (FISH)</td>
</tr>
<tr>
<td>EGFR Expression</td>
<td>Protein</td>
<td>Cell Membrane</td>
<td>Immunohistochemistry (IHC)</td>
</tr>
<tr>
<td>EGFR RNA Expression</td>
<td>RNA</td>
<td>Nucleus</td>
<td>RT-PCR</td>
</tr>
</tbody>
</table>
EGFR Mutation Status

EGFR wild type

EGFR mutation positive

Proliferation↑
Angiogenesis↑
Metastasis↑
Apoptosis↓

Proliferation↑
Angiogenesis↑
Metastasis↑
Apoptosis↑
Gefitinib mode of action

**EGFR** Mutation Status

**EGFR** mutation positive

- ATP
- C-fos mRNA
- Gene expression transcription
- TGF-α, FGF production
- VEGF production

- Proliferation 
- Angiogenesis 
- Apoptosis 
- Metastasis
Progression-free survival in EGFR mutation positive and negative patients

**EGFR mutation positive**

- **Gefitinib** (n=132)
- **Carboplatin / paclitaxel** (n=129)

HR (95% CI) = 0.48 (0.36, 0.64)

p<0.0001

- No. events Gefitinib, 97 (73.5%)
- No. events C / P, 111 (86.0%)

**ITT population**

Cox analysis with covariates

HR (95% CI) = 2.85 (2.05, 3.98)

p<0.0001

- No. events Gefitinib, 88 (96.7%)
- No. events C / P, 70 (82.4%)

**Treatment by subgroup interaction test, p<0.0001**

Mok et al 2009, Fukuoka et al 2009
<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Primary Site</th>
<th>Stage</th>
<th>Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR (KRAS wild type)</td>
<td>Cetuximab</td>
<td>Colon</td>
<td>metastatic</td>
<td>CRYSTAL OPUS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung</td>
<td>metastatic</td>
<td>FLEX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Head and Neck</td>
<td>Unresectable, metastatic</td>
<td>EXTREME</td>
</tr>
<tr>
<td></td>
<td>Nimotuzumab</td>
<td>Head and Neck</td>
<td>Unresectable, metastatic</td>
<td>Phase II and Phase III trials, may be combined with RT</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Bevacizumab</td>
<td>Colorectal Cancer</td>
<td>metastatic</td>
<td>E3200 trial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung Cancer</td>
<td>metastatic</td>
<td>AVAiL AVAPERL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal Cancer</td>
<td>metastatic</td>
<td>AVOREN with Interferon</td>
</tr>
<tr>
<td>CD 20+ B Cell</td>
<td>Rituximab</td>
<td>NHL</td>
<td>Metastatic and adjuvant</td>
<td>GELA LNH 98.5</td>
</tr>
</tbody>
</table>
## Tyrosine Kinase Inhibitors Being Used in Practice

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Primary Site</th>
<th>Stage</th>
<th>Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR mutated Exon 19 deletion; exon 21 L858R point mutation</td>
<td>Erlotinib</td>
<td>NSCLCA</td>
<td>Metastatic/maintenance</td>
<td>OPTIMAL (CTONG 0802)</td>
</tr>
<tr>
<td></td>
<td>Gefitinib</td>
<td>NSCLCA</td>
<td>Metastatic</td>
<td>BR 21 IPASS</td>
</tr>
<tr>
<td>CD 117/ c-kit/BCR-ABL</td>
<td>Imatinib</td>
<td>GIST</td>
<td>Metastatic, adjuvant</td>
<td>ACOSOG-Z9001</td>
</tr>
<tr>
<td>BCR ABL</td>
<td>Nilotinib</td>
<td>CML</td>
<td>Metastatic</td>
<td></td>
</tr>
<tr>
<td>Multitargetted TKIs</td>
<td>Sunitinib</td>
<td>GIST</td>
<td>Metastatic</td>
<td>Motzer, et al. NEJM 2007</td>
</tr>
<tr>
<td></td>
<td>Sorafenib</td>
<td>RCCA</td>
<td>metastatic</td>
<td>AXIS</td>
</tr>
<tr>
<td></td>
<td>Pazopanib</td>
<td>RCCA</td>
<td>metastatic</td>
<td>SHARP</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HCCA</td>
<td>metastatic</td>
<td>Phase 3</td>
</tr>
</tbody>
</table>
Summary and Conclusion

- Tumor markers are found circulating in the blood or are detected on the surface of malignant tumors.
- These can be very useful in screening, early detection, diagnosis, and in monitoring of the disease of cancer patients.
- Results of such tests, however, are rarely of value in isolation and should not be misused and misinterpreted.
- The treatment of cancer, as in the treatment of most human diseases, requires a multidisciplinary team of doctors.
- These specialists bring their wealth of experience and knowledge to the table when they meet with their colleagues in planning the best treatment approach to their patients.
Lecture 1 (Stand Alone): Derma - Phototherapy for Psoriasis

CASE ONE: Diabetic patient with poor sugar control has chest pain and needs angiography...

Lecture 2: Endo - Portland Protocol for CABG patients

Lecture 3: Cardio - Radial Approach for Coronary Angiography

CASE TWO: 30 y.o first time mom, on her 34th week of pregnancy develops stroke and needs anticoagulant...

Lecture 4: Pulmo - Thrombolism in Pregnancy

Lecture 5: Hema - Anticoagulation and the Internist

Lecture 6 (Stand Alone): IDS - Molecular Diagnosis of Infectious Diseases

CASE THREE: 78 y.o. patient with aspiration pneumonia is admitted to the ICU with sepsis. Recovers from sepsis, only to find out he has advanced lung cancer.

Lecture 7: CCM - New advances in Sepsis

Lecture 8: GI - Breaking Bad News and Decision Making in Incurable Illness

CASE FOUR: Drug dependent (alcoholic) comes in with massive GI bleeding

Lecture 9: Occult and Overt GI bleeding

Lecture 10: Wholistic approach to drug dependency

Lecture 11 (Stand Alone): Neuro - Brain Death and Organ Donation

CASE FIVE: 45 y.o female with advanced ER/PR negative, HER2+ breast cancer demands the most advanced treatment for her breast cancer

Lecture 12: Onco - Molecular therapies in malignancies

Lecture 13: Regen Med - Dendritic Stem cell vaccines for cancer