Declaration of Conflict

- Sponsored Research Agreements
  - Amgen
  - Illumina
  - ImClone/Bristol Myers-Squibb
  - Millennium Pharmaceuticals
  - Merck Pharmaceuticals
  - NanoOncology/PepTMed
  - Novartis

- Consultant
  - Coriell
  - Pfizer
  - TetraLogic

- Other Support
  - NCICHQ, Komen, GOG, RTOG, Mary Kay Foundation
  - Kansas Bioscience Authority
  - Philanthropy

Outline: Identifying Targeted Pathways to Personalize Therapies

- Biomarkers and Personalized Cancer Medicine
- DNA Sequencing and the Human Genome
- The Cancer Genome Atlas (TCGA) Initiative
- Examples of Companion Diagnostics
- Barriers Towards Identifying Effective Biomarkers

“Omics”-Based Medicine: A New Era of Health Care

Molecular (Genomic) Medicine
Personalize Medicine
Precision Medicine
- Advances in science and technology, and an increased understanding of health and disease at the molecular level.
Gene Splicing Alterations

Adopted from Cameron Brennan

Gen"omics” Approaches

- Anisokaryosis
- Rearrangement
- Translocation
- Copy number alterations
- Somatic mutations
- Methylation or epigenetic modification
- Altered expression

Personalized Medicine

- Refers to the tailoring of medical treatments to the individual characteristics of each patient.
- It does not mean the creation of drugs or medical devices that are unique to a patient.
- The ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment.

Personalized Cancer Medicine Aims To Deliver

- The right drug or treatment
- At the right time
- To the right patient
- At the right cost

But this can only be achieved when we have an armory of accurate clinical tests and companion drugs at our disposal

clinical test = biomarker evaluation

Precision Medicine

- “The use of genomic, epigenomic, exposure, and other data to define individual patterns of disease, potentially leading to better individual treatment.” - National Academy of Sciences-2011

What is a Biomarker?

- Biomarker: “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

Many Uses of Cancer Biomarkers

- Cancer risk and early detection
- Differential diagnosis and staging
- Prognosis, prediction of therapy response, therapy monitoring, early detection of recurrence

Types of Cancer Biomarkers

- Genomics
- Transcriptomics
- Proteomic
- Metabolomics

Sources of Biomarkers

<table>
<thead>
<tr>
<th>Genomics</th>
<th>Transcriptomics</th>
<th>Proteomic</th>
<th>Metabolomics</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>RNA</td>
<td>Polypeptides</td>
<td>Small Molecules</td>
</tr>
<tr>
<td>Genes</td>
<td>Transcripts</td>
<td>Proteins</td>
<td>Metabolites</td>
</tr>
</tbody>
</table>

- ~22,000 | 250,000 to 1,000,000,000 | 600

Genetic Factors | Environmental Influences

- Electrophoresis 1995 16(7): 1090-4

Why DNA?

“DNA makes RNA, RNA makes protein, and protein makes us.”
Francis Crick, ca. 1957
Types of Cancer Biomarkers

- DNA mutations (point mutations, indels, SNPs, gene copy, gene fusions, etc.)
- RNA expression patterns
- Gene methylation patterns (hyper- and hypomethylation)
- Protein levels or protein state
- Circulating tumor cells
- Blood platelets
- Exosomes (RNA/DNA/Protein filled microvesicles)
- Antibodies (autoantibodies)
- Tumor infiltrating lymphocytes (TIL)
- Metabolites
- Molecular imaging compounds

Tumor Biomarkers-Where Do They Come From and Where Do They Go?

- Tumor markers are substances that are associated with a malignancy.
- Produced by tumor or by the body in response to tumor growth.
- Released into the circulation (and thus measured in the blood), and could be subsequently filtered through the kidneys (and thus measured in the urine).

Translational Medicine

Translational Research Initiatives

- Basic Discoveries
- Clinical Trials

Bench ➔ Bedside ➔ Practice

Biomarkers: Accelerate scientific discovery and improve patient care
- Biobanking: Highly annotated biospecimens
- Biomedical Informatics: Convert data into actionable information

James Watson & Francis Crick in 1953

Human Genome Project

February, 2001 (launched 1990)

http://sgugenetics.pbworks.com/f/1301513883/genome_project_cartoon.png
The Human Genome

- Each human cell contains ~3 billion bases
- A human cell's DNA totals about 3 meters in length
- There are ~22,000 genes
- ~1% of the sequence codes for proteins
- ~25% makes up genes and their regulator regions
- The majority of DNA has unknown functions

Sequencing Cost vs Throughput

Sequencing Technologies

- First generation sequencing technology
  - Sanger Sequencing
- Second generation sequencing technology
  - Roche - 454
  - Illumina – GA II
  - Applied Biosystems - SOLiD
- Third generation sequencing technology
  - Helicos
  - PacBio
  - Oxford Nanopore

Sanger Sequencing

Technology has driven the success of genome sequencing
The 100 human genomes to be sequenced in this competition will be donated by 100 centenarians (ages 100 or older) from all over the world, known as the 100 Over 100.

http://genomics.xprize.org

Cracking the Cancer Code

“It won’t be long before that will dip to $1,000. In fact, it may soon cost more to store and analyze the data extracted from tumor genomes than it will to generate the maps.”

June 13, 2011 | Vol. 177 No. 24

DNA Sequencing Caught in Deluge of Data

• DNA sequencing is becoming faster and cheaper at a pace far outstripping Moore’s law (the rate at which computing gets faster and cheaper—the number of transistors on integrated circuits doubles approximately every two years).
DNA Sequencing Caught in Deluge of Data

- The lower cost, along with increasing speed, has led to a huge increase in how much sequencing data is being produced.
- World capacity is now 13 quadrillion DNA bases a year, an amount that would fill a stack of DVDs two miles high.

By ANDREW POLLACK

Is DNA Sequencing Making a Difference in Cancer Medicine?

First Example – Beth McDaniel

- In 2005, Mrs. McDaniel, then 62 was diagnosed with Sezary syndrome, a rare T cell lymphoma, in which white blood cells become cancerous and migrate to the skin.
- All her doctors could tell her was that the disease was incurable, that there was no standard treatment, and that on average patients at her stage die within a few years.
- For five years chemotherapy held her disease at bay. But in the summer of 2010, she much worse, with hundreds of tumors popping up under her skin. “Some grew as large as kiwi fruits and split open”.

First Example (continued…)

- A newly approved drug for melanoma, ipilimumab, was known to interfere with CTLA4.
- The drug seemed to suppress her cancer for two months, but then it returned.
- Her doctors searched for other targets, but Mrs. McDaniel died soon afterward.
First Example (continued…)

- Did sequencing the patient’s tumor genome make a difference in survival, quality of life, etc.?
- The team that tried to save her was heartbroken too, and was left with a long list of what-ifs. “If you really look at it, what did we buy her?” Dr. de Castro asked. Mrs. McDaniel was dying last January. Yet would she have survived as long even without the sequencing or the drugs? Did the team make a difference?
- “I hope we did,” Dr. de Castro said, “but it’s hard to know.”.

Second Example

- Dr. Wartman, 25 was finishing medical school when he was diagnosed with acute lymphoblastic leukemia (ALL).
- Nine months of intensive chemotherapy, followed by 15 months of maintenance chemotherapy the cancer seemed to be gone.
- Five years later it came back and he received intensive chemotherapy and a bone-marrow transplant from his younger brother.
- After years of treatment and two relapses of ALL, Dr. Wartman had exhausted all conventional approaches to his disease.

Second Example - Dr. Lukas Wartman

In Treatment for Leukemia, Glimpses of the Future

Second Example …continued

- An expensive drug called sunitinib, typically used to treat kidney cancer, was known to block FLT3 receptors.
- Two weeks after Dr. Wartman began taking the drug, tests revealed that his leukemia was in remission.
Second Example ...continued

• Did sequencing the patient’s tumor genome make a difference in survival, quality of life, etc.?  
• His colleagues want to look for the same mutation in the cancer cells of other patients with his cancer. And they would like to start a clinical trial testing Sutent to discover whether the drug can help others with leukemia, or whether the solution they found was unique to Lukas Wartman.  
• Dr. Wartman himself is left with nagging uncertainties. He knows how lucky he is, but what does the future hold? Can he plan a life? Is he cured?  
• “It’s a hard feeling to describe,” he said. “I am in uncharted waters.”

Some Ethical, Legal, Policy Issues of DNA Sequencing

• Revelation of “off-target” mutations  
• Many revealed disorders will have no prevention or treatment  
• Revelation of nonpaternity, consanguinity, incest  
• Costs of genetic counseling and follow-up  
• Possible forensic uses of data  
• Data storage and privacy  
• Huge number of novel missense variants  
• Who should have access - disparity within the US healthcare system

Impact on Healthcare

• Limited access and noncompetitive pricing  
• Increased healthcare costs  
• Lack of peer review and comparison  
• Hampered quality assurance  
• Potential undetected systematic errors  
• Interference with medical training  
• Restricted opportunity and incentive for test improvements and advancement of the field

The Cancer Genome Atlas (TCGA)

• The Cancer Genome Atlas (TCGA) - the molecular basis of cancer through the application of genome analysis technologies, including large-scale genome sequencing.  
• TCGA began as a three-year pilot in 2006 with an investment of $50 million each from the National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI).  
• The success of the pilot with three initial tumor types led the National Institutes of Health to commit major resources to TCGA to collect and characterize more than 20 additional tumor types.

TCGA*

• Big, “comprehensive”, quality controlled science funded by the NCI  
  - DNA sequencing (exons and entire genome)  
  - DNA copy number  
  - RNA profiling (mRNA and miRNA)  
  - Promoter methylation  
• Allocated funding = $275M  
• All cancer types welcome  
• Analysis platforms have evolved and continue to do so  
• There is pivotal clinical and demographic data to go along with the genomic

*Understanding genomics to improve cancer care
Not All Serous Ovarian Cancers are Alike

Subtype and survival signature are independently related to outcome
Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network

We analyzed primary breast cancers by genome-wide copy number arrays, DNA methylation, exome sequencing, messenger RNA sequencing, and mRNA sequencing and found the following results. Our ability to interrogate information across multiple modalities is a key to predicting genes with potential for therapeutic targets. Our analyses identified 30,626 somatic mutations in 510 breast tumours, of which 30,318 are point mutations, 4 dinucleotide mutations, and 2,302 insertions/deletions.

Molecular Targets and Drugs

Why are Biomarkers Important?

• One in 5,000 to 10,000 compounds become an approved drug;
• Costs to discover, develop and commercialize a new drug today exceeds $1.2B;
• Reimbursement environment is becoming increasingly restrictive;
• Days of “blockbuster” drugs to treat all cancer patients is likely over;
• Pharma can’t afford to have a drug fail when it reaches the clinic: identify patients most likely to benefit.

The Pharmaceutical Industry
Personalized Healthcare and Why Biomarkers are Important

<table>
<thead>
<tr>
<th>CML Patients</th>
<th>All Breast CA</th>
<th>HER2+ Breast CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imatinib</td>
<td>Herceptin</td>
<td>Herceptin</td>
</tr>
<tr>
<td>90% Response</td>
<td>&lt;10% Response</td>
<td>35-50% Response</td>
</tr>
</tbody>
</table>

Companion Diagnostics

Using Predictive Biomarkers to Enable the Clinical Development of Candidate Oncology Medicines

Some Examples

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2/neu receptor</td>
<td>Select Herceptin (trastuzumab) for breast cancer</td>
</tr>
<tr>
<td>BRCA1/2</td>
<td>Breast and ovarian cancer inherited risk, prophylactic tamoxifen and surgery, and PARP inhibitors (?)</td>
</tr>
<tr>
<td>KRAS</td>
<td>FDA approved the KRAS companion diagnostic for ERBITUX in metastatic colorectal cancer</td>
</tr>
<tr>
<td>CYP2D6/CYP2D19</td>
<td>Guide prescribing/adjust dose of ~25% of commonly used drugs</td>
</tr>
<tr>
<td>VKOR/CYP2C9</td>
<td>Dosing of warfarin</td>
</tr>
</tbody>
</table>

KRAS Mutation Became a Required Test; however...

- The occurrence of KRAS mutation is predictive of nonresponse and shorter survival in patients treated by anti-EGFR antibody for metastatic colorectal cancer, leading to limit its use to patients with wild-type KRAS tumors.
- However, only half of these patients show benefit from treatment.
- BRAF mutation status, EGFR amplification, and cytoplasmic expression of PTEN were associated with outcome measures in KRAS wild-type patients treated with a cetuximab-based regimen (Laurent-Puig, et al, JCO, 2009).

Biomarker Can Reduce Healthcare Costs

<table>
<thead>
<tr>
<th>Agent</th>
<th>Manufacturer</th>
<th>Cancer</th>
<th>Cost/month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erbitux</td>
<td>Imclone</td>
<td>Colon</td>
<td>$9,600</td>
</tr>
<tr>
<td>Avastin</td>
<td>Genentech</td>
<td>Lung</td>
<td>$8,800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breast</td>
<td>$7,700</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colon</td>
<td>$4,400</td>
</tr>
<tr>
<td>Gleevec</td>
<td>Novartis</td>
<td>GIST</td>
<td>$3,816</td>
</tr>
<tr>
<td>Herceptin</td>
<td>Genentech</td>
<td>Breast</td>
<td>$3,195</td>
</tr>
<tr>
<td>Tarceva</td>
<td>OSI Pharma</td>
<td>Lung</td>
<td>$2,679</td>
</tr>
<tr>
<td></td>
<td>Genentech</td>
<td>Genentech</td>
<td></td>
</tr>
</tbody>
</table>

Source: Public company disclosures, Rodman & Renshaw
KRAS and BRAF mutations

Phase 1 Study of Crizotinib: Expansion Cohort of ALK Translocation Positive Lung Cancers

- ALK-EML4 translocations occur in ~2-7% of NSCLC, resulting in the constitutive activation of the Anaplastic Lymphoma Kinase (ALK).
- Patients with this gene inversion are typically non-smokers who do not have mutations in the EGFR or KRAS genes.
- Recent data from a Phase 1 study evaluating crizotinib (PF-02341066) in translocation positive NSCLC tumors (n = 82 of 1500) showed a dramatic response rate—57% at 6 months, including one CR.

Discussion Points

- A negative predictive biomarker (e.g., KRAS) can be just as useful as a positive predictive biomarker (e.g., BRAF).
- Biomarkers don’t need to be perfectly predictive, but need to enrich the population of patients who are most likely to benefit.

What are the Limitations to Identifying Effective Biomarkers?
We now know the real situation is much more complicated...

The EGFR “Interactome” (simple version)

The Integrated Cell Signaling Circuit

Tumor Resiliency
Delivering tailored therapeutics: Bench-to-Bedside-to-Bench learning enables translational oncology

“...it is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.”
- Charles Darwin

Complexity of Targeting