Precision Genomic Tumor Profiling and the Future of Cancer Care

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A 38-year-old man with \textit{BRAF}-mutant melanoma and subcutaneous metastatic deposits. Photographs were taken (A) before initiation of vemurafenib, a B-Raf enzyme inhibitor

\textit{Wagle et al., JCO (2011) 29, 3085-3096}
A 38-year-old man with \textit{BRAF}-mutant melanoma and subcutaneous metastatic deposits. Photographs were taken (A) before initiation of vemurafenib, a B-Raf enzyme inhibitor (B) after 15 weeks.

Wagle et al., JCO (2011) 29, 3085-3096
A 38-year-old man with BRAF-mutant melanoma and subcutaneous metastatic deposits. Photographs were taken (A) before initiation of vemurafenib, a B-Raf enzyme inhibitor, (B) after 15 weeks, and (C) after relapse, after 23 weeks of therapy.

Wagle et al., JCO (2011) 29, 3085-3096
“...an illusion of clonal dominance when assessed by single biopsies. The presence of sub clonal driver events in solid tumours may provide an explanation for the inevitable acquisition of resistance to targeted therapeutics in advanced disease.”

“Resistance is therefore a fait accompli – the time to recurrence is simply the interval required for the subclone to repopulate the lesion.”

The Sad Cancer Statistic No One is Talking About

- Efficacy of first cancer drug, on average $^{1}$
  - 3 out of 4 first line treatments fail

- Poorly defined standard of care
  - Empiric therapy

- High toxicity and multiple lines of therapy

- Poor quality of life for patient, loss of productivity, higher costs to payors/employers

$^{1}$http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/pmc_case_for_personalized_medicine.pdf
Why Have We Not Won the War Against Cancer?

- **Population** → **Patient** → **Tissues**

**Current Status**
- Standard of Care

**Single Clone**
- Clonal Dominance
- Maximum Tolerated Dose

**Multiple Clones**
- The Illusion of Clonal Dominance
- Release of Resistant Clones
Two Transformational Shifts in Oncology

Transition to Patient Centric, Value-Based Care

- $765bn of waste in the US
- Increased prevalence of value-based models such as ACOs
- Lack of coordination / interoperability amongst silos of care

Transition to Molecular Profile and Real-time Biometric-Driven Medicine

- $552bn precision medicine market
- Evolution toward comprehensive genomic and proteomic analysis
- Increased connectivity of biometric devices
- Overwhelming amounts of data and facts to arrive at a patient decision

Opportunity
To lead in a new era of healthcare

Need to rally the many powers of innovation to create singular results, where:

• **Payers** can go from being actuaries to become stewards and navigators of a complex system
• **Providers** can go from being financial managers to become caregivers again
• **Patients** can go from being anonymous and passive to become individually recognized and central to how care is given
• **Pharma** can go from developing blockbusters to developing drugs for N = 1 and transforming innovation
Agenda

• Genetics and Genomics defined
• DNA, RNA, Proteins
• Current State of Genomic and Proteomic Testing
• Precision Medicine in Oncology
Leaning a new language

- Read
- Practice
- Speak
Boundaries

- Know your job description
- Don’t go outside your boundaries, even if you might know the answer
- We are all part of a team
- Healthcare is a team activity
BACK TO BASICS
Genetics and Genomics?

- **Genetics** — the study of individual genes and their impact on single gene disorders which are relatively rare (examples: Cystic Fibrosis, Huntington’s Disease, Sickle Cell Anemia)

- **Genomics** — the study of all the genes in the human genome together, including their interactions with each other, the environment, and other factors such as how cells are regulated

- **Proteomics** — study of the human proteome, including information on protein abundances, their variations and modifications, along with their interacting partners and networks, in order to understand cellular processes
Genomics: Understanding Variation
The human body is composed of cells

How do these cells make the body function?

<table>
<thead>
<tr>
<th>When</th>
<th># of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start of life</td>
<td>One, with ½ DNA from each parent</td>
</tr>
<tr>
<td>Adult</td>
<td>37.2 Trillion, with copies of ½ DNA from each parent in each cell</td>
</tr>
</tbody>
</table>
Every cell in our body has the same DNA
What is DNA?

It's a history book - a narrative of the journey of our species through time.  
It's a shop manual, with an incredibly detailed blueprint for building every human cell.  
And it's a transformative textbook of medicine, with insights that will give health care providers immense new powers to treat, prevent and cure disease."

Francis Collins, MD  
NIH Director
DNA Transcription and Translation

Transcription (nucleus)

Translation (cytoplasm)

DNA

RNA

PROTEIN
DNA, Genes, and Chromosomes

The blueprint of every person is DNA. Each cell in your body contains the EXACT same DNA, which is ~3 BILLION bases long. Collectively, the sequence is your Genome.

DNA is composed of 2 units:
- Exons (which code for proteins → called ‘Protein Coding Regions’)
- Introns (which do NOT code for proteins, but maybe involved in regulatory activities)

Only 1% of the entire genome, or 30 million bases, are in exons.
The DNA Double Helix

- Complementary base pairing
  - A binds with T
  - C binds with G
- Duplicated strand is identical to original strand
DNA – The Book of Life
A Typical Page of the Human Instruction Book
Central dogma of molecular biology

Figure 1-2 Essential Cell Biology 3/e (© Garland Science 2016)
The making of proteins

A codon is made of three base pairs
64 codons total

1 codon (AUG) encodes methionine and starts translation of all proteins

61 codons encode 20 amino acids (redundant code)

3 codons stop protein translation

# Amino Acid Codon Table

<table>
<thead>
<tr>
<th>1st base</th>
<th>2nd base</th>
<th>3rd base</th>
<th>Standard genetic code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td><strong>U</strong></td>
<td>UUU</td>
<td>(Phe/F) Phenylalanine</td>
<td>UCU</td>
</tr>
<tr>
<td></td>
<td>UUC</td>
<td></td>
<td>UCC</td>
</tr>
<tr>
<td></td>
<td>UUA</td>
<td></td>
<td>UCA</td>
</tr>
<tr>
<td></td>
<td>UUG</td>
<td></td>
<td>UCG</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>CUU</td>
<td>(Leu/L) Leucine</td>
<td>CCU</td>
</tr>
<tr>
<td></td>
<td>CUC</td>
<td></td>
<td>CCC</td>
</tr>
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<td></td>
<td>CUA</td>
<td></td>
<td>CCA</td>
</tr>
<tr>
<td></td>
<td>CUG</td>
<td></td>
<td>CGG</td>
</tr>
<tr>
<td><strong>A</strong></td>
<td>AUU</td>
<td>(Ile/I) Isoleucine</td>
<td>ACU</td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td></td>
<td>ACC</td>
</tr>
<tr>
<td></td>
<td>AUA</td>
<td></td>
<td>ACA</td>
</tr>
<tr>
<td></td>
<td>AUG[A]</td>
<td>(Met/M) Methionine</td>
<td>ACG</td>
</tr>
<tr>
<td><strong>G</strong></td>
<td>GUU</td>
<td>(Val/V) Valine</td>
<td>GCU</td>
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<td></td>
<td>GUC</td>
<td></td>
<td>GCC</td>
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<td></td>
<td>GUG</td>
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<td>GCG</td>
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</tbody>
</table>
Proteins

Triose phosphate isomerase
Protein function

• Roles in cells:
• Structural (cytoskeleton)
• Mechanical (muscle)
• Biochemical (enzymes)
• Cell signaling (hormones)
From DNA to Proteins – Gene Expression

Whole Genome 3 billion bases
Whole Exome 180,000 exons = 30 million bases
Whole Transcriptome = **47,000** RNA transcripts/variants
Whole Proteome 20,000 – 25,000 proteins
What are the unique challenges for proteomics compared to genomics?

- One gene can encode more than one protein (even up to 1,000)
- Proteins are dynamic (genome is relatively static)
- Proteins are co- and post-translationally modified
- Proteins exist in a wide range of concentrations in the body - difficult to detect low abundance proteins and most important proteins for cancer may be those found in the lowest concentrations
THE CURRENT STATE OF GENOMIC AND PROTEOMIC TESTING
Molecular Testing Technologies of DNA, RNA and **Protein** Analytes  (slide 1 of 2)

**Immunohistochemistry (IHC)**
- uses antibody interactions to measure protein expression
- serves as a surrogate for direct protein expression

**Protein microarrays**
- a high-throughput method used to track the interactions and activities of proteins
- determines protein function
Molecular Testing Technologies of DNA, RNA and Protein Analytes (slide 2 of 2)

Flow cytometry

- provides rapid quantitative and qualitative analysis of cell size, cytoplasmic complexity, DNA/RNA content, membrane-bound and intracellular proteins
- used for transplantation rejection testing, leukemia/lymphoma phenotyping, measurement of proliferation markers, etc.

Mass Spectrometry (MS)

- a primary analytical technique used to identify and precisely quantify target proteins
- a protein expression analysis
Molecular Testing Technologies of DNA, RNA and Protein Analytes (slide 1 of 4)

**Fluorescence/Chromogenic in situ hybridization (FISH/CISH)**
- uses fluorescent/chromogenic probes to measure # of DNA copies
- detects DNA rearrangements/RNA translocations, as well as RNA expression levels

**Polymerase chain reaction (PCR)**
- used to exponentially amplify a single or a number of target DNA sequences
Reverse transcription PCR (RT-PCR)

- used to reverse transcribe the RNA of interest into its DNA complement through the use of reverse transcriptase
- commonly applied to qualitatively detect RNA expression
Molecular Testing Technologies of DNA, RNA and Protein Analytes (slide 3 of 4)

**Sanger sequencing by capillary electrophoresis**

- uses chemically modified bases (A, G, C, T) to cause PCR termination each time a base is incorporated into the growing DNA chain
- subsequently the resulting PCR products of varying lengths are separated on a gel-filled capillary and pieced together to reveal the original DNA sequence
Molecular Testing Technologies of DNA, RNA and Protein Analytes (slide 4 of 4)

Microarrays
• used to measure the expression levels of large numbers of genes simultaneously
• or to genotype multiple regions of a genome

Next Generation Sequencing (NGS)
• a high throughput sequencing technology used for genome and transcriptome sequencing
• massively parallel sequencing
NGS – Next Generation Sequencing

1977-2005 Sanger sequencing
- Read length of 650 - 800 bp
- 96 sequence reads
- Capillary gel electrophoresis
- Single investigator, departmental or university core facility

2005 NGS
- AKA – High throughput sequencing
- Read length of 15 – 600 Gb
- Millions of sequence reads in parallel

Whole Genome Sequencing (WGS)

- Hypothesis-free approach
- Gives the *most complete picture* of variants across the whole genome
- All 3 billion bases at 30x read depth (which means each base is read 30 times for accuracy)
- Therapeutically relevant alterations can reside within intronic regions of splice sites
- Data can be difficult to interpret
- Most expensive
Exome Sequencing (All genes)

- Targets coding regions of genome
- 1 – 2% of genome, only 30 million bases at 60x read depth
- Greater sequencing depth; more coverage means greater sensitivity
- 8 – 10x cheaper than WGS
Targeted Sequencing (Many genes / gene panels / single genes)

- Hypothesis-driven approach targets genes of interest
- May miss key mutations
- Most sensitive approach as very high coverage is possible
- Selection of genes: 1 – 500 at 100X – 1000X read depth
- Sequencing of exons only or specific regions of exons plus introns
- Data is easier to interpret
- Least expensive
RNA analysis approaches

Whole Transcriptome Sequencing or RNA sequencing

• This provides Sequence Analysis of all the RNA messages
• This ALSO provides quantitation of all the different RNA messages

Quantitative PCR (qPCR or RT-PCR)

• Provides RNA expression quantitation
Limitations of traditional molecular testing
IHC, CISH/FISH, DNA and RNA sequencing (slide 1 of 2)

Genomic (CISH/FISH, hot spot and limited gene panels)

- High tissue consumption
- Limited number of genes = limited number alterations = limited actionability
- Presence of gene alterations does not always correlate with altered protein expression = high potential for false positives
Limitations of traditional molecular testing
IHC, CISH/FISH, DNA and RNA sequencing (slide 2 of 2)

Proteomic (IHC)

- High tissue consumption
- High potential for false positives or negatives
  - Pre-analytical variability
  - Antibody sensitivity, specificity, cross reactivity problems
  - Subjective interpretation
  - Reproducibility

Challenging access to off-label therapies/clinical trials
Potential pitfalls of acting on incomplete data from not measuring everything
Laboratory Quality Control

CLIA (Clinical Laboratory Improvements Amendments of 1988)
- Compliance required for all clinical labs
- Does not require proficiency testing for genetic tests

CAP/ACMG (College of American Pathologists and American College of Medical Genetics)
- Voluntary proficiency testing program for gene testing labs
PRECISION MEDICINE IN ONCOLOGY
Uses of Genetic and Genomic Tests in Oncology

Diagnosis
- Confirm the specific diagnosis
- Molecular classification of specific types of cancer

Prognosis
- Identify genetic markers predictive of aggressive disease and response to treatment

Risk Assessment and Management
- Provide predictive testing for cancer risk and treatment response
- Allow preventive risk management
- Provide intranatal information

Treatment decision making
- Markers for targeted therapy
From DNA to RNA to Protein to Drug

By combining proteomics with genomic interpretation, it allows a holistic understanding of the progression of disease and universe of options for individualized care.

Identifying the “Molecular Address” of Cancer
Breast Cancer

H & E staining
# Breast Cancer

## TNM Staging

<table>
<thead>
<tr>
<th>ANATOMIC STAGE/PROGNOSTIC GROUPS</th>
</tr>
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<tbody>
<tr>
<td>Stage 0</td>
</tr>
<tr>
<td>Stage IA</td>
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<tr>
<td>Stage IB</td>
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<tr>
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<td>Stage IIA</td>
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<tr>
<td>Stage IIIC</td>
</tr>
<tr>
<td>Stage IV</td>
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</tbody>
</table>

A New Taxonomy of Cancer: From Organs to Molecules

- Cancers are currently classified by the organ or tissue, with therapies geared to those specific areas.
- As more is learned about the basic biological processes in cancers, a new perspective has emerged.
- The shift from an organ-focused to a gene-focused approach to cancer is already having a profound effect on the way cancer is treated.

Interview with Edward J. Benz, Jr., MD www.standup2cancer.org/innovations_in_science/view/genomics_and_the_future_of_cancer_treatment
Grouping Patients by Molecular Profile
A New Taxonomy of Cancer: From Organs to Molecules

From DNA to RNA to Protein to Drug

By combining proteomics with genomic interpretation, it allows a holistic understanding of the progression of disease and universe of options for individualized care.

Identifying the “Molecular Address” of Cancer

Whole Genome Seq + RNA Seq + Protein Quantification

PROTEINS
Questions?