Cancer Research and the rise of clinical genomics

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Medical Director, Institute for Translational Oncology Research
Greenville Health System Cancer Institute
Overview

- The basics of Genomics
- Clinical Applications in cancer genomics
- Molecular profiling of tumors
- Next steps for moving genomics into the daily treatment planning for cancer patients
Genomics Primer

• **Genetics:** the study of single genes and their effects

• **Genome:** refers to all of the genetic material (DNA) within an organism

• **Genomics:** the study of how information flows from the genome to the phenotype
Central Dogma

DNA → RNA → Protein

Genotype
- RNAs function & structure
- Protein sequence
- Protein structure
- Protein Function
- Phenotype
Genomics Primer

The other -omics / -omes

- **Transcriptome**: the set of all RNA molecules
- **Exome**: that part of the genome formed by exons and transcribed into RNA
- **Proteomics**: study of structure and function proteins
- **Metabolome**: study of all small molecules and reflects the interaction of the organism’s genome and the environment.
All DNA is a polymer with a non-variable backbone of the sugar deoxyribose, a phosphate residue and either a purine or pyrimidine base.
Pyrimidines and Purines

- Backbone forms rightward staircase
- Major groove and minor groove are important regulatory sites
- Early target for cancer therapeutics
Each Chromosome:

- a single double stranded DNA molecule plus
- structural DNA binding proteins (e.g. histones) plus
- specialized segments, the centromeres and telomeres
Chromosome Structure

- Telomere
- Satellite
- Secondary constriction
- Nucleolar organizer (NOR)
- Primary constriction
- Centromere
- Kinetochore for attachment of spindle fibres
- Heterochromatin
- Euchromatin
- Chromatin fibre
Genomics Primer

• **Exon:** the coding region of a gene that dictates a functional product, either protein or ncRNA

  only 1-2% of the genome but responsible for 85% of disease causing mutations

• **Introns:** segments of genes situated between exons that are removed before transcription into mRNA and do not code for proteins
Genomics Primer

Non-Coding RNA’s (non-mRNA)

- rRNA
- tRNA
- miRNA
  - micro RNA’s
  - 21-24 nucleotide long
  - modulate gene expressions at post-transcriptional level
  - regulate cellular proliferation and cell cycle transport
- siRNA
  - small interfering RNA’s
  - modulate gene expression
- tiRNA
  - transcription initiation RNA
- long nc RNA
The Human Genome Project, 2003

http://www.genome.gov/
Human Genome Project

• 1989 → 2000
• Multinational
• 3 Billion Dollars
• “Complete Sequence of the Human Genome”
• 3.3 Billion Nucleotides – 25,000 Genes
• Template from anonymous donors
“The real problem is not a science problem anymore, but rather a problem of math.”

George Sledge
2010 William L. McGuire Memorial Lecture
SABCS
Impact of Genomics on the practice of Medicine

- Characterizing or even defining new diseases.
- New treatment discoveries with greater efficiency.
- Individualized treatments with greater precision.
There are known knowns; there are things we know we know. We also know there are known unknowns; that is to say we know there are some things we don’t know. But there are also unknown unknowns -- the one we don’t know we don’t know.

- Donald Rumsfeld
Basics of Gene Sequencing
**Sanger Sequencing**

- **Pro:** can do long sequences of DNA this way
- **Con:**
  - Expensive
  - Need a lot of DNA to start with
  - Can only do one sequence at a time
  - Time intensive

Image by the National Human Genome Research Institute
Enter Next Generation Sequencing methods
Next Generation Sequencing

Genome Machine

- Standardized chips can assess for specific gene mutations
- Takes hours rather than days
- $ rather than $$$$$$
Next Generation Sequencing (NGS)

• Pros:
  – Fast
  – Cheap (<$0.01/Mbase)
  – Generates many terabytes of data
  – Many sequences simultaneously

• Cons:
  – Fewer reads of each individual base so less accurate (but improving)
  – In general, can read relatively short segments (but improving)
## Comparison Of NGS Platforms

<table>
<thead>
<tr>
<th>Platform</th>
<th>Chemistry</th>
<th>Read Length</th>
<th>Run Time</th>
<th>Gb/Run</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 GS Junior</td>
<td>Pyro-sequencing</td>
<td>500</td>
<td>8 hrs.</td>
<td>0.04</td>
<td>Long Read Length</td>
<td>High error rate in homopolymer</td>
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<tr>
<td>(Roche)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>454 GS FLX+</td>
<td>Pyro-sequencing</td>
<td>700</td>
<td>23 hrs.</td>
<td>0.7</td>
<td>Long Read Length</td>
<td>High error rate in homopolymer</td>
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<tr>
<td>(Roche)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HiSeq (Illumina)</td>
<td>Reversible</td>
<td>2*100</td>
<td>2 days</td>
<td>120</td>
<td>High-throughput / cost</td>
<td>Short reads</td>
</tr>
<tr>
<td>Terminator</td>
<td></td>
<td>(rapid mode)</td>
<td>(rapid mode)</td>
<td></td>
<td></td>
<td>Long run time</td>
</tr>
<tr>
<td>SOLiD (Life)</td>
<td>Ligation</td>
<td>85</td>
<td>8 days</td>
<td>150</td>
<td>Low Error Rate</td>
<td>Short reads</td>
</tr>
<tr>
<td>(Life)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Long run time</td>
</tr>
<tr>
<td>Ion Proton</td>
<td>Proton Detection</td>
<td>200</td>
<td>2 hrs.</td>
<td>100</td>
<td>Short Run times</td>
<td>New*</td>
</tr>
<tr>
<td>(Life)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PacBio RS</td>
<td>Real-time</td>
<td>3000 (up to</td>
<td>20 min</td>
<td>3</td>
<td>No PCR Longest Read Length</td>
<td>High Error Rate</td>
</tr>
<tr>
<td>Sequencing</td>
<td></td>
<td>15,000)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MS Kim, 2013
Comparison of NGS platforms

Table 1: Price comparison of benchtop instruments and sequencing runs

<table>
<thead>
<tr>
<th>Platform</th>
<th>List price</th>
<th>Approximate cost per run</th>
<th>Minimum throughput (read length)</th>
<th>Run time</th>
<th>Cost/Mb</th>
<th>Mb/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>454 GS Junior</td>
<td>$108,000</td>
<td>$1,100</td>
<td>35 Mb (400 bases)</td>
<td>8 h</td>
<td>$31</td>
<td>4.4</td>
</tr>
<tr>
<td>Ion Torrent PGM (314 chip)</td>
<td>$80,490a,b</td>
<td>$225c</td>
<td>10 Mb (100 bases)</td>
<td>3 h</td>
<td>$22.5</td>
<td>3.3</td>
</tr>
<tr>
<td>(316 chip)</td>
<td>$425</td>
<td></td>
<td>100 Mb (100 bases)</td>
<td>3 h</td>
<td>$4.25</td>
<td>33.3</td>
</tr>
<tr>
<td>(318 chip)</td>
<td>$625</td>
<td></td>
<td>1,000 Mb (100 bases)</td>
<td>3 h</td>
<td>$0.63</td>
<td>333.3</td>
</tr>
<tr>
<td>MiSeq</td>
<td>$125,000</td>
<td>$750</td>
<td>1,500 Mb (2 × 150 bases)</td>
<td>27 h</td>
<td>$0.5</td>
<td>55.5</td>
</tr>
</tbody>
</table>

Note: Pricing may vary between countries and/or sales territories. Instrument prices do not include service contracts. Sample prices do not include the cost of generating the initial fragmented genomic DNA library with adaptors (an additional cost of between $75–200 depending on method used). Cost per megabase assumes one sample and one sample sequencing kit per run. Unless stated, pricing information is from the online supplement of ref. 3.

Declining cost of progress

B Mole, www.sciencenews.org, 2/6/14
Genomic applications in the clinic
Two distinctly different mutations

- **Germline**
  - Present in every cell of the body
  - Inheritable
  - Identifies families at risk for malignancy or other disease
  - Typically single gene

- **Somatic**
  - Present only in the disease specific cells
  - Not inherited (although the tendency to acquire them may be)
  - Often identifies the “driver” mutation for the disease
  - May be (often) multiple
• **Washington**, Wed., May 21 2008 — The President has signed into law the Genetic Information Nondiscrimination Act (GINA) that will protect Americans against discrimination based on their genetic information when it comes to health insurance and employment. The bill had passed the Senate unanimously and the House by a vote of 414 to 1. The long-awaited measure, which has been debated in Congress for 13 years, will pave the way for people to take full advantage of the promise of personalized medicine without fear of discrimination.

*Reuters, 2008*
Germline mutations

- Familiar with BRCA1 & 2 story as exemplified by high profile cases recently in the news.
- Confers extremely high lifetime risk of breast cancer and ovarian cancer (BRCA1).
- Other cancers, including pancreatic and prostate, in the BRCA2 kindreds.
- Lynch syndrome (hereditary non-polyposis colon cancer) also important germline mutation.

- Both of these mutations affect the ability to correct errors of DNA duplication or to repair DNA damage.
Lifetime risk associated with BRCA mutations

## BRCA and Cancer

Although the risk of cancer is greater for women than men with BRCA 1/2 gene mutations, both sexes face elevated lifetime chances of several types of cancer. *Risk of cancer as a percentage, by gender.*

<table>
<thead>
<tr>
<th>MEN</th>
<th>Cancer type</th>
<th>U.S. white</th>
<th>BRCA1 mutation carriers</th>
<th>BRCA2 mutation carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>0.1%</td>
<td>1-5%</td>
<td>7%</td>
</tr>
<tr>
<td></td>
<td>Prostate</td>
<td>16</td>
<td>*</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>2</td>
<td>N.S.</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>1</td>
<td>Up to 3</td>
<td>3-5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WOMEN</th>
<th>Cancer type</th>
<th>U.S. white</th>
<th>BRCA1 mutation carriers</th>
<th>BRCA2 mutation carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>13%</td>
<td>60-80%</td>
<td>50-70%</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>1-2</td>
<td>20-45</td>
<td>10-20</td>
</tr>
<tr>
<td></td>
<td>Melanoma</td>
<td>2</td>
<td>N.S.</td>
<td>Up to 5</td>
</tr>
<tr>
<td></td>
<td>Pancreas</td>
<td>1</td>
<td>Up to 3</td>
<td>3-5</td>
</tr>
</tbody>
</table>

N.S. = Not significant; *Some evidence of an increased risk for men younger than 65

SOURCE: Penn Medicine’s Basser Research Center for BRCA

MIKE PLACENTRA / Staff Artist
# Lynch syndrome risks

## Cancer Risk in Individuals with Lynch syndrome (HNPCC) to Age 70 Compared to General Population

<table>
<thead>
<tr>
<th>Cancer</th>
<th>General Population Risk</th>
<th>Lynch syn. Risk</th>
<th>Mean Age of Onset in Lynch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>7 %</td>
<td>80%</td>
<td>45 years</td>
</tr>
<tr>
<td>Endometrium</td>
<td>2.7%</td>
<td>20-60%</td>
<td>46 years</td>
</tr>
<tr>
<td>Stomach</td>
<td>&lt;1%</td>
<td>11-19%</td>
<td>56 years</td>
</tr>
<tr>
<td>Ovary</td>
<td>1.5%</td>
<td>9-12%</td>
<td>42.5 years</td>
</tr>
<tr>
<td>Hepatobiliary tract</td>
<td>&lt;1%</td>
<td>2-7%</td>
<td>54 years</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>&lt;1%</td>
<td>4-5%</td>
<td>~55 years</td>
</tr>
<tr>
<td>Small Bowel</td>
<td>&lt;1%</td>
<td>1-4%</td>
<td>49 years</td>
</tr>
<tr>
<td>Brain / CNS</td>
<td>&lt;1%</td>
<td>1-3%</td>
<td>50 years</td>
</tr>
</tbody>
</table>

*from: [http://www.genetests.org](http://www.genetests.org)*
DPD deficiency

• Germline mutation in enzyme needed to catabolize thymidine and uracil.
• Deficiency leads to extreme toxicity from 5FU.

Somatic mutations in cancer

The hope for personalized therapy
“Using information about a person’s genetic makeup to tailor strategies for the detection, treatment, or prevention of disease.”

Francis Collins
Head of the Human Genome Project
Mutations in Cancer

• Amplifications: the HER2 gene in breast cancer
• Point mutations: EGFR in lung cancer and KRAS in colon cancer
• Fusion genes: EML-Alk4 in lung cancer and BCR-abl in chronic myeloid leukemia
Some cancers appear to depend on a single switch.

When the switch is on, the cancer cells are actively dividing. When the switch is turned off, the cells become quiescent or die.
The revolution in targeted treatment: Chronic Myeloid Leukemia

The History of CML

- 1845: Identification of Ph chromosome link to CML
- 1960: A-MuLV transforms NIH and lymphoid cells
- 1970: A-MuLV generates distinct disease from M-MuLV
- 1975: A-MuLV encodes gag-abl
- 1980: Characterization of Ph chromosome in BCR-ABL with ABL tyrosine kinase activity
- 1982: ABL oncproteins transform 1st cells and generate leukemia in vivo
- 1984: Imatinib mesylate leads to remission in >90% of CML chronic phase patients
- 1996: CML patients resistant to inhibitor express BCR-ABL mutants
- 2000: Structure of ABL bound to inhibitors reveal mechanism of ABL and drug actions
- 2002: Imatinib mesylate suppresses ABL oncprotein tyrosine kinase and cellular transformation

References:
- 23, 25, 26
- 29-33
- 51-66
- 100, 101, 263, 264
- 155, 161, 162, 165, 166
- 249, 250
- 254, 255, 258
One of the few cancers that appears to be a single switch problem.

A gene translocation occurs as an accident of cell division and a new protein is made. It is always in the “on” position.

Gleevec (imatinib) is a molecule specifically designed to turn off the switch.

Because of its specificity, this pill has less side effects than traditional therapy.
Philadelphia Chromosome

Chromosome 9

Chromosome 22

q11

c-BCR

reciprocal translocation

q34

c-ABL

Philadelphia Chromosome

t(9;22) (q34;q11)

BCR/ABL

BCR/ABL (210 kDa)

c-BCR

c-ABL

OD

Ser/Thr kinase

DBL

SH3 SH2

Tyr kinase

NLS

DNA BD

actin BD
Gleevec: HOW IT WORKS

CML Enzyme > ATP > Cancer Protein > CML

Gleevec blocks the ATP-Cancer Protein pathway, preventing CML.
Gleevec and CML

- 95% of patients are progression free at 5 years
- Side effects are generally mild
- Many of these patients have no measurable cancer (…can we stop the drug?)
Somatic mutations: Amplification

- Her2 (ERBB-2) amplification occurs in 20-25% of breast cancers.
- HER2 is a signaling protein that activates many cellular pathways such as proliferation.
- Her2 amplification historically associated with highly aggressive, poor prognosis breast cancer.
- Discovery of the mutation, led to therapeutics which have dramatically lessened the risk of dying of this type of breast cancer.
Targeting Her2 in breast cancer

Joint Analysis: Disease-Free Survival

**B-31**
- **AC → TH**: 87%, 85%
- **AC → T**: 74%, 66%
- **N**: 872, 171
- **Events**: 864, 83
- **HR = 0.45, 2P = 1 \times 10^{-9}**

**N9831**
- **AC → TH**: 87%, 86%
- **AC → T**: 78%, 68%
- **N**: 807, 90
- **Events**: 808, 51
- **HR = 0.55, 2P = 0.0005**

Years From Randomization
Point Mutations which alter function

- EGFR mutations predict sensitivity to kinase inhibitors in lung cancer
- BRAF mutations present in 60% of metastatic melanoma and these mutations can be targeted
EGFR and TKI sensitivity

EGFR TKI sensitive

G719X
Deletion / insertion

Exon 18 → Exon 19 → Exon 20 → Exon 21

L858R L861Q

Exon 20 T790M insertion

EGFR TKI resistant
EGFR

Ligand (EGF, TGF-alpha)

Ligand-binding domain

EGFR homodimer or EGFR heterodimer with ERBB2 or ERBB3

PI3K

Grb-2

SOS

RAS

RAF

MEK

MAPK

PTEN

AKT

mTOR

STAT 3/5

Survival

Proliferation
Erlotinib (Tarceva™) properties

- Small-molecule inhibitor of HER1/EGFR tyrosine-kinase (TK)
- Chemical class: quinazoline
- Orally available
Tarceva

Case history of response to erlotinib

July 23, 2002

Pretreatment

July 23, 2002

Post-treatment with erlotinib

May 6, 2003

Gefitinib vs. Standard Chemo in EGFR Mutation Positive Patients

Progression-Free Survival

Kobayashi, Proc ASCO, Abstr #8016
Now more than 50% of lung cancers harbor specific molecular targets.

Traditional view based on microscopic appearance

Promise of tailored therapy

- Mutations associated with drug sensitivity
  EGFR Gly719X, exon 19 deletion, Leu858Arg, Leu861Gln
- Mutations associated with primary drug resistance
  EGFR exon 20 insertions
- Mutations associated with acquired drug resistance
  EGFR Thr790Met, Asp761Tyr, Leu747Ser, Thr854Ala
Genetic Profiles by Histologic Subtype

Oncogenic drivers differ between adenocarcinomas and squamous cell carcinomas

Mutant BRAF in melanoma

• Targeting mutant BRAF when present, results in rapid responses.
• Teaches us about resistance and escaped mechanisms.
Application of clinical genomics to new drug development,

…and to the appropriate use of old drugs
Current Status of Drug Development

**PhRMA 2004 R&D expenditures**

- **$9.6 Billion**
  - 5,000-10,000 Compounds
  - **Drug Discovery**
  - Safety Focus: Modify compound to reduce side effects

- **$15.9 Billion**
  - 250 Compounds
  - **Pre-Clinical**
  - Safety Focus: Lab and animal testing performed to test for potential adverse effects

- **$3.4 Billion**
  - 5 Compounds
  - **Clinical Trials**
  - Phases I and II: Find safe dose and side effects
  - Phase III: Check for adverse effects and efficacy

- **$4.9** Billion
  - 1 FDA Approved Drug
  - Safety Focus: Strong evidence of safety needed for approval
  - FDA inspects manufacturing safety; ongoing studies of approved drugs' safety

- **LARGE-SCALE MANUFACTURING/PHASE IV**
  - FDA Review
Decreasing Costs of Drug Development with appropriate target and patients

- Target – EGFR
- Agent – Iressa
- Development Methodology – cytotoxic model using randomized trials
- Results: < 10% RR in NSCLC (over 10 years ago)
- Development Costs - $1.7 billion

- Target – RAF
- Agent – PLX 4032 (now called Vemurafenib)
- Development Methodology Companion Diagnostic – BRAF mutations
- Results: ESMO 2009 RR 77% TTP > 6 months
- Development Costs: < $100 million
<table>
<thead>
<tr>
<th>Drug</th>
<th>Cost</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab</td>
<td>&gt;$100,000</td>
<td>iv</td>
</tr>
<tr>
<td>Avastin</td>
<td>&gt;$100,000</td>
<td>iv</td>
</tr>
<tr>
<td>Cetuximab</td>
<td>&gt;$100,000</td>
<td>iv</td>
</tr>
<tr>
<td>Revlimid</td>
<td>&gt; $90,000</td>
<td>po</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>&gt; $70,000</td>
<td>po</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>&gt; $36,000</td>
<td>po</td>
</tr>
<tr>
<td>Tarceva</td>
<td>&gt; $20,000</td>
<td>po</td>
</tr>
</tbody>
</table>
New Frontiers

Molecular characterization of tumor cells may lead to accurate (and sometimes unexpected) treatment choice.
Molecular Profiling results at ITOR
Molecular Profiling

• Study performed at 6 centers nationwide; Greenville Health System Cancer Institute was one of the sites.
• Tumor tissue is submitted for exhaustive molecular characterization; hopefully predicts which medications likely to be helpful.
• Patient treatment ensues with unheard of understanding of the tumor biology and against unsuspected targets.
Patients with PFS ratio ≥ 1.3

Results of Bisgrove Trial
Temozolamide and colon cancer
MD Anderson experience

- Of 955 patients studied, profiling was successful in 852 (89%).
- 41.5% of patient had more than one mutation.
- For those patients with 1 mutation, 29% achieved response to guided therapy and the duration of response was nearly double the response to their previous treatment.

Next steps at ITOR

Rare Tumor Clinic
• As defined by WHO, ultra-rare tumors occur in less than 6 per 100,000 persons.
• Individually they are uncommon, but in aggregate these cancers account for 15-20% of adult malignancies.
• Poorly served by randomized trials, progress is very slow.
• Ideal situation for genomic assessment and personalized care.
Rare tumor program

• Patients meeting criteria per WHO guidelines
• All offered state of the art genomic interrogation
• Success measurements:
  – Target with known drug available
  – Target identified with drug in development
• Of the first 39 patients, 61% with identified actionable targets. To be presented at ASCO in June 2015.
• Importantly, 3 potential germ line mutations identified.
Thank you for the time today