Cancer Genomics & Precision Medicine in the 21st Century

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Outline

• Define terms
• Describe vision for how genetic characterization of tumors will change treatment paradigms for cancer in the future
• Describe ongoing clinical trials currently using this approach
• Describe potential difficulties/pitfalls
Terms/Vocabulary

- **Single Nucleotide Polymorphisms (SNP)**
  - Variation in single base in DNA in germline, most common variants in genome (over 50 million identified)
  - SNP arrays interrogate the entire genome—uses DNA from germ-line (blood)

- **Used in Genome Wide Association Studies (GWAS)**
  - Typically uses SNP arrays to compare populations (with disease or not)
  - Determines risk or susceptibility to some state
Terms (con’t)

• RNA expression profiles-determines global messenger RNA expression in a sample-using hybridization of mRNA to a Chip

• Methylation arrays-determines global methylation of the genome-an epigenetic change typically inserts a methyl group at CpG islands in DNA and alters transcription-using hybridization of DNA to a Chip

• Massively parallel sequencing-allows for rapid sequencing of entire exome (WES) on whole genome (WGS) or cDNA (RNA-seq)
**GWAS**

Region 2
Prostate only
- rs979200
- rs1456310
- rs6993569
- rs6470494

Region 2
Breast only
- rs13281615
- rs16902124
- rs16902126

Region 3
Prostate & Colon
- rs6983267
- rs10505476
- rs7837328

Region 1
Prostate only
- rs1447295
- rs7837688

Possible Master Cancer Susceptibility Region 8q24
Genetic Predisposition to Breast Cancer European Population

Population genotype relative risk vs. Population risk-allele frequency

Uncommon
Rare

New Loci
CGEMS
CGEMS/BCAC

5p12
5q11.2
16q12.1
8q24
10q26
2q35
Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell Lymphoma

- Activated B Cell-like DLBCL (ABC)
- Germinal Center B Cell-like DLBCL (GCB)
- Primary Mediastinal B Cell Lymphoma

Genes

Lymphoma Biopsies

High

Low

Gene Expression

IRF4
PIM2
CCND2
FOXP1
IL16
CD44
IGHM
MME
CR2
KCNN3
LRMP
LMO2
MYBL1
SLAM
TNFSF4
CCL17
PDL2
MAL
IL4R1
Dissecting Cancer into Molecularly and Clinically Distinct Subgroups by Gene Expression Profiling

Diffuse Large B Cell Lymphoma

Probability of survival

Progression-free survival (yrs) (R-CHOP Rx)

GCB DLBCL  75%
ABC DLBCL  40%

3-year progression-free survival

P = 2.27 x 10^{-8}
Different Therapeutic Strategies for Subsets of ABC DLBCL Based on Characteristics of Mutations Activating NF-κB

**Mutant CARD11**
- Coiled-coil mutation
- CARD11-on

**Wild type CARD11**
- CARD11-on

**Treatment prospect**
- IKKβ inhibitor
- Proteasome inhibitor
- Neddylation inhibitor

**IKK activation**
- IKKγ
- IKKβ
- IKKα

**NF-κB**

**mTOR**

**Chronic active BCR signaling**

**ITAM mutation**

**Treatment prospect**
- BTK inhibitor
- SRC-family inhibitor
- SYK inhibitor
- PKCβ inhibitor
- PI(3) kinase / mTOR inhibitor
Two images of a breast cancer
Expression profile Identification of Breast Tumor Intrinsic Subtypes

SDH Deficient GIST Have Global Hypermethylation
Massively Parallel Sequencing (Next-Generation Sequencing)

1. **Genomic DNA or RNA**
   - Fragmentation
2. **Fragment**
   - Size Selection
3. **DNA Fragments of Similar Sizes**
   - Adaptors Ligation
4. **Genomic DNA Library**
   - Amplification and Sequencing

**Ref. Genome**

- Align (Map) Reads to Ref. Genome

**Genome Sequence**

- AGCTGCTGTCGCGAAACTCCGATCGACTGCTGATCGACTCGATCACTCGATCGTAGTCGAGAGTACTCGATGCT
Types of Alterations that can be Detected using

Reference sequence
Chr 1

Non-human sequence
Chr 5

Point mutation  Indel

Homozygous deletion  Hemizygous deletion

Gain  Translocation breakpoint

Copy number alterations

Matthew Meyerson, Stacey Gabriel and Gad Getz
NATURE REVIEWS GENETICS VOLUME 11 | OCTOBER 2010
Next Generation Sequencing will Identify Other Driver Mutations and Enable Individualized Therapy for Cancer Therapy

- Roche / 454 Genome Sequencer FLX Titanium
- Illumina / GAII/HQ 2500 Whole Genome 48 hrs
- PacBio RS Ion Torrent
- Life Technologies SOLiD v4
- Life Technologies 5500 XL
- Life Technologies Ion Torrent
- Life Technologies Proton 1 Genome 2 hrs
- Helicos HeliScope
Comprehensive Analysis of the Rhabdomyosarcoma genome: Study Design

**Discovery set**
Whole Genome Sequencing (WGS)
(Complete Genomics Platform)
46 RMS
(22 ARMS)

**Validation set**
Whole Exome (Agilent, SOLiD, Illumina)
133 RMS (52 ARMS)
30 Overlap with CG WGS

**SNP Array**
HumanOmni2.5-8 BeadChip
134 RMS (38 ARMS)
30 Overlap with CG WGS
2/46 Samples had Aberrant Fusions

Incorrect Diagnosis

1. Sample 1 – Original Histology: Sarcoma, Undifferentiated RMS, Not Otherwise Specified. Had ALK-NPM1 fusion by whole genome sequencing; Review of Histology = Hematological malignancy-most likely misdiagnosed ALCL

2. Sample 2 - Original Histology: Consistent with RMS Presence of RET-NCO4 fusion by whole genome sequencing which has been reported in papillary thyroid carcinoma. Likely misdiagnosed or sample label error from source
Typical Fusion Positive ARMS; Fusion Gene, Low Aneuploidy, Low Somatic Mutation rate

t(2;13)

11pLOH
Typical ERMS; Higher Aneuploidy, Higher Somatic Mutation rate
ARMS-Fusion Gene Detection:

- 22-ARMS by Histology
- 14-PAX3-FOXO1
- 3-PAX7-FOXO1
- 1-PAX3-NCOA1
- 1-Novel
- 3-Fusion Negative
Fusion Negative ARMS Shows massive 2q Rearrangement with in-Frame \textit{PAX3-IN080D}

\textbf{Purple:} tail-to-head  \textbf{Green:} head-to-tail junction (possibly tandem duplication)  
\textbf{Orange:} tail-to-tail junction or head-to-head junction (inversion)
RNAseq Confirms Expression of *PAX3-INO80D* Novel Fusion Transcript
HOW HAS THIS CHANGED CANCER TREATMENT?
Change in View of Lung Cancer

- 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
## Shifting the Paradigm

<table>
<thead>
<tr>
<th>Previous Approach</th>
<th>New Practices</th>
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<tbody>
<tr>
<td>Descriptive medicine</td>
<td>Understanding of disease mechanisms</td>
</tr>
<tr>
<td>Empirical diagnosis</td>
<td>Mechanism-based diagnosis/treatment</td>
</tr>
<tr>
<td>Grouped by Organ Site</td>
<td>Sub-grouped by molecular/biological classification</td>
</tr>
<tr>
<td>Uniform treatment</td>
<td>Individualized treatment</td>
</tr>
<tr>
<td>Retrospectively diagnose disease</td>
<td>Prospectively evaluate relative disease risk</td>
</tr>
<tr>
<td>Acute care</td>
<td>Early detection and intervention</td>
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Toward Precision Medicine

Put more science into clinical trials

Pharmaco dynamic measurements

Investigational drug

Molecular diagnostics: candidate approach

Molecular diagnostics: unbiased approach

Responders

Non-responders

Modified from American Association for Cancer Research
Precision Medicine

Breast cancer

Prostate cancer

Lung cancer

Molecular diagnostics

Treatment A

Treatment B

Treatment C

Standard TX

Modified from American Association for Cancer Research
Cancer is a disease of the genome

• Therefore, if we precisely define the cancer genome, we will understand and cure cancer
  • Why we must be cautious about such statements

• Definitions

• Founder mutations—first genomic mutation
  • These are often lesions that lead to genomic/chromosomal instability (p53, RB, etc.) and are often not fully transforming

• Driver mutations—these are mutations that are required for expression of fully transformed phenotype
• Driver mutations are the mutations we would like to target and inhibit their function

• Passenger mutations-these mutations are “collateral damage” resulting from genomic instability and are not required for maintaining the transformed phenotype, therefore are “noise” in the system

• Since most cancers are rapidly evolving biologic entities, it is a major task to sort out “drivers” from “passengers”, and these may change over time
Mutation Rates across Cancers are not Uniform

Courtesy of Gad Getz
Clonal Evolution

Founder RB1 and p53 mutations followed by additional mutations

• Driver mutations in signaling pathways (kinases) are components of highly integrated “wiring” that is not a one way flow of information
  • Because these are critically important for normal cell functions, these are highly regulated pathways
• Perturbation of a single component of will lead to activation of other components due to feedback activation or loss of feedback repression
Kinase oncogene dependence and principles of drug resistance.

Wagle N et al. JCO 2011;29:3085-3096
Example of Vemurafenib

- 50-60% of melanoma patients have driver mutations in BRAF (V600E)
- At doses of vemurafenib that inhibit 90% of B-RAF activity, most patients respond rapidly with tumor shrinkage
- Median duration of response is less than 12 months due to resistance
- What are the mechanisms of resistance?
Example-BRAF (V600E) mutations in colon cancer

• **Unresponsiveness of colon cancer to BRAF (V600E) inhibition through feedback activation of EGFR** Prahallad A, et al. Nature Jan 26 2012

• **Mechanism**-appears to be inhibition of BRAF leads to inhibition of MEK and ERK, leading to reduced phosphatase activity of CDC25C, leading to reduced dephosphorylation of EGFR, leading to increased activation and EGFR signaling
Neuroblastoma

- One of the SRBCT
- Derived from primordial neural crest cells destined to become sympathetic ganglia in the peripheral nervous system not CNS

Incidence:
- 1 per 100,000 in children < 15 yrs in US (650 cases per year)
- The most common extra cranial solid tumor for children
- 7-10% of cancers of childhood

Survival Rates:
- 95-70% for low stage tumors (1,2,3)
- ~50% patients present with advanced disease
- < 30% of children over 1 year old with advanced disease and/or MYCN amplification despite aggressive therapy
Patient (19yr) with High-risk Neuroblastoma

**Diagnosis**

- 4 cycles Induction
- A3973

**Surgery**

- 8 cycles of Salvage Therapy
- 7 cycles of RA
- Radiation multiple sites
- Low dose MIIBG

**Met1-BM:**
- Bone marrow biopsy at diagnosis. >80% tumor

**Met2-Liver:**
- Primary: tumor removed by surgery viable margin
- Whole genome seq of liver Met2 & RNAseq of Met1, primary and Met2

**Death**

~4 Months
Forty-four (44) non-synonymous mutations found in index sample (Liver Met)
Chromothripsis was evident by massive complex rearrangements detected at chromosomes 4q and 13p
Ion Torrent: Deep Re-sequencing (1000x) of primary (bone marrow) and 4 primaries (adrenal): 14/44 (32%) small variants were present in all samples, 30 unique to liver met
RNAseq of Met1, Primary, Liver Met2 to identify expressed driver mutations:

- 14/44 commonly mutated in all 3 tumors
- 12 the gene is expressed
- 9 variant allele expressed
- 3 genes ($\text{NUFIP1}$, $\text{GATA2}$, and $\text{LPAR1}$) high variant allele fraction $>30\%$
30/44 Somatic Mutations Unique to Liver Met2

- 11/30 the variant was expressed in Liver Met2
- *De-Novo* mutations in liver arising during the course of disease but absent in primary (4 regions) and bone marrow met
Summary

1. Neuroblastoma is marked by aneuploidy in recurrent regions but lack frequently recurring mutations
2. Possible that classic mutations may not drive tumorigenesisis
3. Possible that each individual tumor has its own set of driver mutations
4. Ongoing efforts including RNAseq underway and will identify key onocogenic drivers and targets for therapy
Many Novel Drivers Epigenetic (red)
Many Not Currently Druggable

**PBRM1** – Renal cell carcinomas
**EZH2, MEF2B** – Lymphomas
**KCNJ5** – Adrenal adenomas
**DNMT3A, SF3B1, SRSF2, U2AF35** – Leukemias
**MLL2, MLL3, DDX3X** – Medulloblastomas
**ARID1A, PPP2R1A** – Ovarian cancers
**DAXX, ATRX** – Pancreatic endocrine tumors
**BAP1, TTRAP, PREX2** – Melanomas
**IDH1, 2** – Gliomas
**CIC, FUBP1** – Oligodendrogliomas
**MED12** - Leiomyomas
**H3F3A, HIST1H3B**- Diffuse intrinsic pontine glioma
**ATRX, ARID1A, ARID1B, PTPN11** - Neuroblastoma

Adapted from Vogelstein
M-PACT: Molecular Profiling based Assignment of Cancer Therapeutics

Pilot Trial to Assess the Utility of Genetic Sequencing to Determine Therapy and Improve Patient Outcome in Early Phase Trials

NCI-Sponsored Clinical Trial
Objective

• Assess whether the response rate (CR+PR) and/or 4-month PFS is improved following treatment with agents chosen based on the presence of specific mutations in patient tumors.
  – Only patients with pre-defined mutations of interest will be eligible
  – Study treatments, regardless of cohort, will be chosen from the list of regimens defined in the protocol
  – Arm A: Receive treatment based on an study agent prospectively identified to work on that mutation/pathway
  – Arm B: Receive treatment with one of the study agents in the complementary set (identified to not work on one of the detected mutations/pathways)
Patient Population

- Patients with refractory solid tumors that have progressed on at least one line of standard therapy or for which no standard treatment is available that has been shown to improve survival.
- Adequate organ function (AST/ALT < 3xULN, Bil < 1.5 xULN, S. Cr < 1.5 x ULN, platelets > 100K, ANC > 1500)
- Study regimens: As long as the same set of protocols are offered to a given set of patients, the number and actual treatments regimens can vary over time

<table>
<thead>
<tr>
<th>Mutations in DNA repair pathways</th>
<th>Veliparib + Temozolomide</th>
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<tbody>
<tr>
<td></td>
<td>MK1775 + carboplatin</td>
</tr>
<tr>
<td>Mutations in the PI3K pathway; loss of</td>
<td></td>
</tr>
<tr>
<td>PTEN, Akt amplification</td>
<td>mTOR inhibitor -Everolimus</td>
</tr>
<tr>
<td>Mutations in the RAS pathway</td>
<td>GSK 1120212 (MEK inhibitor)</td>
</tr>
</tbody>
</table>
Study Design

Sequence fresh biopsy tissue from all patients.

Biopsy

Mutation detected

Randomly assign pt to Arm A or B if actionable mutation identified (Clinical team blinded to the specific mutation data).

Arm A: Targeted therapy based on the patient’s mutational data

Arm B: Therapy not corresponding to the patient’s mutational data

Mutation not detected

Assign protocol (allow cross over at progression to the targeted agent)

Off-Study
Statistical Design

- Patients will be randomized 2:1 to Arm A (experimental) versus Arm B (control)
- Within Arm A, up to 30 patients will be treated within each of the treatment cohorts. Within each treatment cohort of Arm A, discriminate between tumor response rates of 20% vs. 5% and, as a secondary endpoint, 4-month PFS rates of 50% vs. 25%
- The two Arms will be compared with respect to both objective response rate and PFS – this is a randomized comparison.
<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Pathways/Function</th>
<th>Gain or Loss of Function?</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>RAS/RAF/ERK/MEK</td>
<td>Gain</td>
</tr>
<tr>
<td>NF1</td>
<td>RAS</td>
<td>Loss</td>
</tr>
<tr>
<td>Kras</td>
<td>RAS/RAF/ERK/MEK</td>
<td>Gain</td>
</tr>
<tr>
<td></td>
<td>AKT/PI3K</td>
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<td>Gain</td>
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<tr>
<td></td>
<td>AKT/PI3K</td>
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<tr>
<td>Hras</td>
<td>RAS/RAF/ERK/MEK</td>
<td>Gain</td>
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<td></td>
<td>AKT/PI3K</td>
<td></td>
</tr>
<tr>
<td>AKT1</td>
<td>AKT/PI3K</td>
<td>Gain</td>
</tr>
<tr>
<td>AKT2</td>
<td>AKT/PI3K</td>
<td>Gain</td>
</tr>
<tr>
<td>AKT3</td>
<td>AKT/PI3K</td>
<td>Gain</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>AKT/PI3K/RAS/RAF/ERK/MEK</td>
<td>Gain</td>
</tr>
<tr>
<td>PTEN</td>
<td>AKT/PI3K/RAS/RAF/ERK/MEK</td>
<td>Loss</td>
</tr>
<tr>
<td>P53</td>
<td>DNA Repair</td>
<td>Loss</td>
</tr>
<tr>
<td>FBXW7</td>
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<tr>
<td>ATM</td>
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<tr>
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<tr>
<td>NBN</td>
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<tr>
<td>ATR</td>
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</tr>
<tr>
<td>MGMT</td>
<td>DNA repair</td>
<td>Loss</td>
</tr>
</tbody>
</table>
Conclusions

- The ability to obtain full genomic data on a given tumor will allow us to make rational choices for therapy
- Functional genomics may provide help in choosing combination therapy
  - Combinations will not be easy due to enhanced toxicities
- Cancer as a chronic disease is not a bad thing as long as we recognize rapid development of resistance and clonal evolution