Molecular Profiling in Advanced Cancer
Patrick J. Kiel, PharmD, BCPS, BCOP
Indiana University Simon Cancer Center
IU Health

Objectives
• Review the history and diagnostic analysis of next generation sequencing in tumor tissue.
• Discuss clinical trials that have used genotyping to guide therapy in oncologic malignancies.
• Identify the limitations and ethical issues of using next generation sequencing for determining standard of care therapy.

Getting Personal
• If 'personalized' is the future of therapy then high throughput diagnostics must foreshadow implementation at the bedside.
• NCI personalized medicine:
  – "a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease."

Genomic Alteration in Cancer

Genomic Terminology
• Genome
  – Complete set of DNA, including all of the genes
• Exome
  – Exons of all genes
• Somatic mutation
  – Alterations in DNA that occur after conception
• Germline mutation
  – Presence of an altered gene within the egg and sperm
• Driver mutation
  – Causally implicated in oncogenesis

Faculty Disclosures
• Received fees for Non-CPE services from Takeda, Genentech, and Celgene
• I will be talking about off label indications
Genomic Terminology

- **Genotyping**
  - Testing that reveals the specific alleles inherited by an individual
- **Actionable aberrations**
  - Aberrations that may impact cancer management through diagnostic, prognostic, or predictive implications
- **Druggable aberration**
  - Actionable aberration that can be targeted by a novel therapy

Historical Timeline

- 1953: Watson and Crick discover DNA structure
- 1957: Sanger Sequencing developed
- 1990: Human Genome Project initiated
- 1998: Pres. Clinton genome cannot be patented
- 2000: Human Genome Project Completed
- 2003: First complete publication of a human genome
- 2007: Pacific BioSciences enters genome race
- 2015: Genomics BCOP Lecture

The Human Genome Project

- First complete publication of a human genome
- Celera Genomics enters genome race
-Pres. Clinton, genome cannot be patented

Next Generation Sequencers (NGS)

- Hybridization based technology
- 454 Sequencing™
  - PCR amplification on beads with light signals
- First Generation
- Second
- Third
  - Pacific BioSciences: nanotechnology with molecular biology, different color light
  - Ion Torrent uses pH not light

Sequencing Logistics

- All NGS require a library obtained by amplification or ligation with custom linkers
- Each library fragment is amplified on a solid surface with covalently attached adapters that hybridize with the library adapters
- Direct step by step detection of nucleotide base incorporation
- A “digital” read from an amplified fragment of the DNA is produced

Dawn of the Sequencing Revolution

- 1990: thousand bases/day
- 2000: million bases/day
- 2014: billion bases/day

www.lifetechnologies.com, accessed 12/22/14
Non-Optical Sequence Detection

Leverages $1 Trillion investment and $50 Billion annual spend

Ion Semiconductor Chip

1M wells (1B)
165M wells (PI)
660M wells (PII)

Critical Question for the Next Ten Years:
How to Use the Genome to Improve Human Health?

$1,000 barrier for whole genome broke in September 2015 by Veritas Genetics

Genomic Alterations for Predictive Therapy

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pathway</th>
<th>Alteration</th>
<th>Disease</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT</td>
<td>PI3K/AKT</td>
<td>Mutation</td>
<td>Breast, CRC, endometrial</td>
<td>PIK inhibitors</td>
</tr>
<tr>
<td>mTOR</td>
<td>PI3K/AKT</td>
<td>Mutation</td>
<td>Bladder, breast</td>
<td>mTOR inhibitors</td>
</tr>
<tr>
<td>FGFR1, 2, 3, 4</td>
<td>FGFR</td>
<td>Mutation, amplification</td>
<td>Myeloma, leukemia, Waldenstrom, classic Hodgkin lymphoma</td>
<td>FGFR antibodies and inhibitors</td>
</tr>
<tr>
<td>MET</td>
<td>MET</td>
<td>Mutation</td>
<td>Bladder, gastric, renal</td>
<td>MET antibodies or inhibitors, Crizotinib</td>
</tr>
<tr>
<td>AMN1, 2, 3</td>
<td>AMN1</td>
<td>Mutation, rearrangement</td>
<td>Leukemia, lymphoma, myelodysplasias</td>
<td>AMN1 inhibitors, EMD 126516</td>
</tr>
<tr>
<td>ROS1</td>
<td>ROS1</td>
<td>Rearrangement</td>
<td>Bladder, gastric, colorectal</td>
<td>ROS1 inhibitors, Crizotinib</td>
</tr>
</tbody>
</table>

Cancer Causation at the Genomic Level


Direct Detection of Hydrogen Ions and Conversion to an Electrical Signal Read by a Transistor Substrate

**BRAF Pathway**

- **BRAF V600E**
  - Point mutation in 40-60% of malignant melanoma
  - Valine to glutamic acid substitution
    - Constitutive activation of extracellular signal-regulated kinase (ERK)
    - Inhibition of apoptosis and increased cell growth

**BRAF Inhibitor in Melanoma 3 (BRIM-3)**

- Positive BRAF V600E in unresectable stage IIIC/IV melanoma
- Blockade of BRAF V600E with vemurafenib 960 mg orally, BID
- Decarbazine 1,000 mg/m² IV every 3 weeks

**BRIM-3 Results**

- N=672 for OS
- Data safety monitoring board released results due to compelling efficacy
  - HR for death 0.37 (95% CI, 0.26 to 0.55)
  - HR for PFS 0.26 (95% CI, 0.2 to 0.33)
- Median PFS was 5.3 and 1.6 months

**Clinical Trial Examples**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Unique Design</th>
<th>Cancer Type</th>
<th>Standard Treatment</th>
<th>Experimental Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIVA</td>
<td>Molecular vs. conventional</td>
<td>All types, refractory</td>
<td>Per Tumor</td>
<td>Iressx, everolimus, vemurafenib, sorafenib, erlotinib, lapatinib, famotidine, abiraterone</td>
</tr>
<tr>
<td>I-SPY2</td>
<td>Adaptive model</td>
<td>Metastatic breast cancer</td>
<td>Palbociclib +/−/− trastuzumab +/−/− study drug +/−/− AC</td>
<td>AMG479, AMG386, MK-2206, MK-1775</td>
</tr>
<tr>
<td>M-RECT</td>
<td>Molecular targets vs. randomly chosen</td>
<td>All types, refractory</td>
<td>None</td>
<td>Temozolomide, everolimus, abiraterone, carboplatin, MK-1775</td>
</tr>
<tr>
<td>MATCH</td>
<td>Umbrella protocol for multiple, single arm phase II trials</td>
<td>Solid tumors +/−/− brain +/−/− metastatic +/−/− PFS +/−/− 2% +/−/− 60-100% +/−/− 100%</td>
<td>None</td>
<td>Carbons, 20 arms that target: EGFR, HER2, BRAF, MET, FGFR, GNAQ, GNA11, TSC1/2, PTEN, RAD51, ALK, ROS, FLK-3</td>
</tr>
</tbody>
</table>

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**AC, doxorubicin, cyclophosphamide**

Molecular Profiling for Refractory Cancers

- Pilot study, refractory, metastatic cancers
- Life expectancy > 3 mo
- Refractory/last line of therapy
- Investigator had to designate chemotherapy they would use if profiling was negative
- IHC and Oligonucleotide microarray on 51 genes
- Primary outcome: PFS ratio of > 1.3


Examples of Molecular Profiling

<table>
<thead>
<tr>
<th>ID</th>
<th>Primary</th>
<th>Targets Chosen</th>
<th>Profiling Treatment</th>
<th>Potential Treatment Without Profiling</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-56</td>
<td>Mesothelioma</td>
<td>RRM1, TP53</td>
<td>Gemcitabine + epirubicine</td>
<td>Gemcitabine</td>
</tr>
<tr>
<td>01-12</td>
<td>Cholangiocarcinoma</td>
<td>EGFR</td>
<td>Irinotecan + cetuximab</td>
<td>Phase I</td>
</tr>
<tr>
<td>02-13</td>
<td>Breast</td>
<td>SPARC, HER2</td>
<td>NAB paclitaxel + trastuzumab</td>
<td>Docetaxel + trastuzumab</td>
</tr>
<tr>
<td>02-14</td>
<td>Eccrine sweat gland</td>
<td>c-KIT</td>
<td>Sunitinib</td>
<td>Best supportive care</td>
</tr>
<tr>
<td>04-31</td>
<td>Ovary</td>
<td>HER2, ER</td>
<td>Lapatinib + tamoxifen</td>
<td>Bevacizumab</td>
</tr>
<tr>
<td>05-36</td>
<td>Colon</td>
<td>PDGFR, c-KIT</td>
<td>Irinotecan + sorafenib</td>
<td>Panitumumab</td>
</tr>
<tr>
<td>06-99</td>
<td>Colon</td>
<td>MGMT, VEGF, HIF1A</td>
<td>Temozolomide + sorafenib</td>
<td>Panitumumab</td>
</tr>
</tbody>
</table>


Guided by Genomics: The Clinic

- Patient referral
- Turn around time
  - Biopsy
  - Sequencing time?
- Feeding into targeted clinical trials, Phase I
- Commercially available targeted agents

Clinical Testing and Collaboration

- Advantages & Market Differentiators
  - Multi-analyte platform
  - DNA, RNA (compared to lineage matched normal controls), CNV, Fusions, and IHCs
  - Small amount of tissue required
  - Success with as little as a single-pass FNA
  - Scientific input into the platform via scientific collaboration
Tumor board
Initial visit
Patient deemed appropriate for sequencing
Targeted somatic & germline sequencing
Drug selection using a "non-standard" FDA approved drug

Tumor board

No. of Genes
Analytes
DNA, RNA, Protein
~21,000

Minimum Tissue Requirement
Core Biopsy
FNA
3-4 Weeks
3 business days
5 business days

Turnaround Time
3-4 Weeks
3 business days
10-14 days

Cost (Lab)
$17,000
$4,800
$5,000

Breast
Sarcoma
Colorectal
Neuroendocrine
Carcinoid
HCC
Gastric
Head and Neck
Renal
Other

Percent Cancer Diagnosis

Other includes < 2% of the following cancers: cervical, GCT, GBM, melanoma, mesothelioma, Merkel cell, urachal, osteosarcoma, prostate, ALL, ampulla of vater, endometrial, ileal carcinoma, neuroectodermal, pheochromocytoma, thyroid, adrenal cortical carcinoma, Ewing, esophageal carcinoma, parotid, small bowel.

Genomically-directed therapy improves PFS compared to prior line

Patients Achieving a PFS Ratio > 1.3 with Genomically Directed Therapy

Patients Achieving a PFS Ratio > 1.3 with Genomically Directed Therapy

Progression-Free Survival


Personalized Medicine, Phase I Program

- Phase I clinical trial from March 2011 to Jan 2012
- Advanced metastatic cancer, no standard
- 1,542 patients with molecular analysis required
  - 534 with targetable mutation
  - 143 with matched therapy
- Molecular profiling, PCR based sequencing:
  - PIK3CA, BRAF, KRAS, NRAS, EGFR, c-KIT, PTEN loss
  - (IHC), RET (Sanger)

Molecular Alterations


PFS and OS of Matched Targets

- Median OS 11.4 vs. 8.6 mo, p=0.04
- Median PFS 3.9 vs. 2.2 mo, p=0.001

Phase IIa Mutliple Basket Study

- 230 patients, 35 tumor types
- 52 patients with 14 tumor types with responses
  - CR=4, PR=48
- Noteworthy
  - HER2 CRC, 38% ORR
  - BRAF NSCLC, 43% ORR

Targeted Agent and Profiling Utilization Registration (TAPUR) Study

TAPUR Study Primary Objectives

- To describe the anti-tumor activity and toxicity of commercially available, targeted anti-cancer drugs prescribed for treatment of patients with advanced solid tumors, B cell NHL or MM with a genomic variant known to be a drug target.
- To facilitate patient access to commercially available, targeted anti-cancer drugs of potential efficacy for treatment of patients with an advanced solid tumor, B cell NHL or MM with a genomic variant known to be a drug target.
Implementation Process

- Team approach
- Billing
- Triageing therapy
  - Clinical Trials (local or referral)
  - Oral therapy
  - IV therapy
  - Palliative care/non-genomic guided therapy

Complex Decision/Team Approach

Barriers to Clinic Implementation

- Molecular Diagnostics
  - Choice of assay
  - Cost
  - Tissue quality
  - Tumor Content
  - Analytical validity
  - CLIA certification
  - Turnaround
  - Bioinformatic analysis

- Clinical Implementation
  - Tissue acquisition
  - Tumor heterogeneity
  - Expert interpretation
  - Pathway vs. tissue
  - Drug availability
  - Trial design and endpoints
  - Clinical validity and utility

Ethical Considerations

- American College of Medical Genetics
  - Strongest motivation to order sequencing is for patients with unclear treatment choice or poor prognosis
  - Germline exome or genome may have incidental findings
    - QT syndrome, familial hypercholesterolemia, Von Hippel Lindau
  - Patient education on:
    - Privacy, insurability, potential family member implications
    - Tumor specific guidelines need to be developed by cancer professional societies

Case #1

- 63/M with metastatic anaplastic thyroid cancer. Presented with a right neck mass and underwent a right modified neck dissection
- PET/CT revealed a 3.4 x 3 CM nodule in thyroid isthmus and activity in the right supraclavicular region
- Right upper lung nodule present consistent with metastatic spread
- First line therapy: Cisplatin/Doxorubicin
- Second line therapy: paclitaxel
- PMHx significant for ulcerative colitis that has been untreated for > 20 years
Anaplastic Thyroid Cancer

- Undifferentiated tumors of the thyroid follicular epithelium
- Incidence is 1-2 per million persons and is 1-10% of thyroid cancer globally
- Median survival in metastatic disease = 4-6 months
- Standard therapy based on anthracyline or taxane therapy
- Associated mutations
  - BRAF
  - p53
  - PIK3CA

Clinical Considerations

- Is the BRAF V600E mutation a mutation drive that is applicable amongst all cancer types?
  - BRAF alterations have been identified 20% of thyroid cancers.
  - NEJM case report of a CR on vemurafenib monotherapy
- What is the role of immunotherapy in this patient?
  - Any other markers of immunotherapy efficacy?
  - Any Contraindications?

Patient Follow-up

- Initiated on vemurafenib
- Disease regressed in right cervical node, but developed a rapidly growing new palpable midline neck mass and an FDG-avid left upper lobe nodule.
  - Mixed Response
- Developed arthralgia and myalgia that was grade 3 and intolerable

Intratumor Heterogeneity-Literature Evidence

- Genetic Drift vs. Conservative Mutations
- Four patients with Renal Cell Carcinoma
- Whole-exome multiregional spatial sequencing
- Tissue samples
  - Baseline, before temsirolimus
  - Following 6 weeks of temsirolimus (nephrectomy)
  - Continue temsirolimus, repeat biopsy

Case #1 Continued

• Given the mixed response it was decided to start nivolumab as immunotherapy. Patient in a complete response for 2 years!

Tumor Genetic Testing

• Tissue biopsy is generally considered the "gold standard" for genetic analysis
  – Includes blood or bone marrow for hematologic malignancies
• Limitations:
  – May require invasive surgery to biopsy
  – Preparation of certain tissue types (bone) can make genetic analysis challenging
  – Sampling may not be representative of tumor heterogeneity

Cell Free DNA (cfDNA)

• Small circulating fragments of DNA shed into the plasma following tumor apoptosis, necrosis or spontaneous release from cancer tissue
• Requires high level of analytical sensitivity and specificity:
  – Most circulating cfDNA is primarily from normal cells with only a small but variable fraction from the tumor
  – “Needle in the haystack”
  – Digital-PCR lower limit of detection can approach below 0.0001%

Cancer Detection: Plasma Tumor DNA (ptDNA)

• Dying cells (normal and malignant) deposit DNA into the circulation
• Each tumor has characteristic genetic aberrations
• Majority of DNA in the plasma is normal DNA, but by using next-generation sequencing, one can detect tumor DNA

Case of cfDNA Sequential Sequencing

• Mutation landscapes can change over times
• May provide insight into heterogeneity

85 yo non-smoking female diagnosed with Stage IV NSCLC, adenocarcinoma

EGFR exon 19 del
9/2015
Started erlotinib
9/2015:
D/C erlotinib
Started osimertinib
EGFR C797S
12/2015:
D/C osimertinib
Started carboplatin/pemetrexed/bevacizumab
cfDNA Limitations

- Not the assay of choice for all patients
- cfDNA results correlate the best with tumor samples in cases of advanced disease
  - Higher number of metastatic sites
  - Higher number of prior therapies
- Ideal time to obtain cfDNA analysis is at the time of progression, not while patient is responding to therapy!
- Primary site of disease matters
  - Primary gliomas have poor plasma cfDNA tumor levels secondary to the blood brain barrier
  - Cerebral spinal fluid (CSF) as a source for cfDNA:
    - Somatic alterations found in 63% of CNS metastases from solid tumors and 50% of primary brain tumors


CASE CR 029, male

- 29 y/o male with metastatic Peripheral neural ectodermal tumor
- Diagnosed 6 years ago, left orchiectomy for 100% teratoma
- Received 1 course BEP, without response, had RPLND and revealed teratoma
- Had resection of mediastinal disease in August 2012, revealed yolk sac tumor; had 1 course of VeIP
- Did well for about 2 years when had recurrence of disease in sacrum, biopsy revealed PMET
- Had 4 courses of CAV alternating with if, then resection and radiotherapy
- In November 2017 had new pulmonary mets, was started on a clinical trial, which was discontinued after developing pleural effusions
- Started CAV alternating with if January 2018

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