Cancer, Genes and Comprehensive Genomic Profiling

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Upstate Medical University
Genetic Aberrations in Cancer

- **Proto-oncogene**
  - e.g. growth inducer
  - Dominant
  - Growth without regulation
  - Abnormal growth

- **Tumor suppressor gene**
  - e.g. growth inhibitor
  - Recessive
  - Loss of growth inhibition
  - Abnormal growth

Slide modified from Dr. Terri McVeigh, Royal Marsden Hospital
“Genetic Testing”

- Genomes
  - Mutations/rearrangements

- Epigenomes
  - Epigenetic modifications
  - “MethyloMe”

- Transcriptomes
  - Gene expression
  - Non-coding RNA expression

- Proteomes
  - Protein expression

Slide modified from Dr. Terri McVeigh, Royal Marsden Hospital
Analysing Genetic Sequence Variation

Targeted analysis
- Only specified genes of interest are analysed

“Open” Analysis
- All genes are analysed

- Increasing data
- Increasing cost-efficiency
- Increasing uncertainty?

Specific mutation testing (hotspot)
Single gene testing
Multigene Panel Testing
Whole Exome Testing
Whole Genome Testing

Slide modified from Dr. Terri McVeigh, Royal Marsden Hospital
Cancer: The Battle of Two Genomes

Germline

Somatic
# Germline vs Somatic Variation

<table>
<thead>
<tr>
<th>Germline</th>
<th>Somatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited*</td>
<td>Acquired</td>
</tr>
<tr>
<td>Present in all cells</td>
<td>Present in only some cells</td>
</tr>
<tr>
<td>Present from conception</td>
<td>Occur after conception</td>
</tr>
<tr>
<td>Heritable</td>
<td>Not heritable</td>
</tr>
</tbody>
</table>

- Increased risk of somatic variants
  - Exposure to Carcinogenic substances (cigarette, UV)
  - Age
  - Germline variant causing genomic instability

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Slide modified from Dr. Terri McVeigh, Royal Marsden Hospital
Cancer as a Genetic Disorder

Cancer Evolution: Drivers and Passengers

Driver mutation: confers growth/survival advantage

Passenger mutation: does NOT confer growth/survival advantage

Occurs as a consequence of genomic instability

Slide modified from Dr. Terri McVeigh, Royal Marsden Hospital
Actionability Of Somatic Variants

Chakravarty, D et al, OncoKB: A Precision Oncology Knowledge Base, JCO Precision Oncology 2017 :1, 1-16
Personalized Approach Improves Cancer Treatment Outcomes

Genomics-matched targeted therapy = BEST OUTCOME

Targeted therapy w/o mutation matching = Worst outcome

(Ref: Schwaederle et al., JCO 2015)
## Barriers to Precision Oncology

### Clinical, Technical and Access Challenges

<table>
<thead>
<tr>
<th></th>
<th>Cancer Genome Complexity</th>
<th>Limitations of Current Panels</th>
<th>Limitations of Real World Samples</th>
<th>Lack of Access to Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>- Hundreds of genes, millions of alterations driving tumor growth</td>
<td>- Most of the “tumor type” and “hot spot” panels only look at frequently altered genes and only at commonly altered areas of the gene</td>
<td>- Low tumor purity frequent in metastatic/recurrent/post-treatment samples</td>
<td>- Limited approved targeted therapies in many tumor types</td>
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<td></td>
<td>- Every patient’s tumor genomic profile is unique</td>
<td></td>
<td>- Less invasive sampling leads to smaller specimen sizes from work-ups</td>
<td>- Challenge to access off-label therapies</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Difficulty to access and enroll in clinical trials</td>
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</table>
Classes of Genomic Alterations in Cancer

Testing for each alteration type can exhaust small clinical samples with limited amounts of DNA available for testing.

**Base Pair Substitutions**
(Mutations)
e.g. BRAF, EGFR

**Insertions and Deletions**
e.g. EGFR, ERBB2 (HER2)

**Copy Number Alterations**
(Amplifications, Homozygous deletions)
e.g. HER2, MET
e.g. PTEN, TSC1/2

**Rearrangements**
(Fusions)
e.g. ALK, RET

**Capillary Sequencing, Mass Spectrometry**

**Tumor**

**Blood**

**Capillary Sequencing, Gel Size Shift Assays**

**IHC (overexpression), Fluorescence In Situ Hybridization (FISH)**

**RT-PCR, FISH**
Advantages of Hybrid Capture Based Comprehensive Genomic Profiling

• Single Hotspot or Mutiplex HotSpot Tests not sensitive enough especially for INDELs and fusions
• Multiple HotSpot Tests consume the specimen
• HotSpot tests typically do not cover the entire exomes of cancer genes
• Only a focused targeted NGS-based assay can detect all 5 classes of genomic alterations on the same clinical sample simultaneously
• Hybrid capture method can enhance coverage depth (>1,000X) to reduce false negative risk
• WES/WGS creates too much unimportant data, requires fresh tissue, requires a matched normal tissue sample, confounds what information to send to the clinician, is too costly and takes too long to complete
Foundation Medicine

- Founded in 2010 by Scientists and Clinicians from the Broad Institute of MIT and Third Rock Ventures
- Began patient testing in 2011
- Has issued >350,000 clinical reports
- CLIA, CAP, NYSDOH certified and accredited
- Offers multiple tests to assist in the care of cancer patients
- In US, it is covered by Medicare and numerous national health insurance providers
- Roche acquired majority stake in 2015 and full ownership in 2018
- Achieved FDA Approval and CMS National Coverage as a Clinical Diagnostic Test and Universal Companion Diagnostic in 2017 by Parallel Review
Analytical Validation

Demonstration of high accuracy and reproducibility required for clinical use

**Base Substitutions**  
(MAF 5-100%)

Sensitivity: >99.9%  Specificity: >99.9%

**Insertions/Deletions**  
(1-40bp, MAF 10-100%)

Sensitivity: 98%  Specificity: >99%

**Copy Number Alterations**  
(>20% tumor content, zero or ≥8 copies)

Sensitivity: >95%  Specificity: >99%

**Gene Fusions**  
(>20% tumor content, select introns)

Sensitivity: >99%  Specificity: >99%

Controlled validation studies:  
Cell-line pools with known alterations:
- 2056 subs  227 indels
- 210 CNAs  32 fusions

Concordance studies with existing platforms on clinical samples:
- 118 subs/indels: Sequenom, PCR
- 185 CNAs: FISH, IHC
- 43 fusions: break-apart FISH

Frampton et al, *Nature Biotechnology* 2013
False Negatives

Insensitive Or Narrow Genomic Testing Misses Key Mutations

- Key driver alterations missed by insensitive tests on low tumor cell purity clinical grade specimens
- Key driver alterations missed by narrow tests that cannot accurately detect all 4 classes of clinically relevant alterations
- Patients treated with cytotoxic drugs instead of targeted therapies
- Patients either not treated with immunotherapies at the right time or not treated at all
## Foundation Medicine Tests

<table>
<thead>
<tr>
<th>Target Tumor Types</th>
<th>Specimen†</th>
<th>Number of Genes Includes</th>
<th>Cancer Immunotherapy Biomarkers</th>
<th>Companion Diagnostic</th>
</tr>
</thead>
<tbody>
<tr>
<td>All solid tumours</td>
<td>FFPE tissue</td>
<td>324 (DNA)</td>
<td>MSI and TMB</td>
<td>FDA-approved CDx for 17 targeted therapies</td>
</tr>
<tr>
<td>All solid tumours</td>
<td>Peripheral whole blood</td>
<td>70 (DNA)</td>
<td>MSI and TMB</td>
<td></td>
</tr>
<tr>
<td>FFPE tissue</td>
<td></td>
<td></td>
<td>MSI and TMB in late 2019</td>
<td></td>
</tr>
<tr>
<td>Haematologic malignancies, sarcomas*</td>
<td>FFPE tissue, bone marrow aspirate, or peripheral whole blood</td>
<td>406 (DNA) 265 (RNA)</td>
<td>MSI and TMB</td>
<td></td>
</tr>
</tbody>
</table>

* Soft tissue and bone; † For full details, refer to specimen instructions at www.foundationmedicine.com.

ctDNA: circulating tumour DNA; FFPE: formalin-fixed paraffin-embedded tissue; IHC: immunohistochemistry;
MSI: microsatellite instability; TMB: tumor mutational burden.
1. Foundation Medicine, Inc. (2018) FoundationOne CDx Technical Specifications;
**Indication** | **Genomic Finding** | **Therapy**
--- | --- | ---
Non-Small Cell Lung Cancer (NSCLC) | *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations, *EGFR* exon 20 T790M alterations | Gilotrif® (afatinib), Iressa® (gefitinib) or Tarceva® (erlotinib), Tagrisso® (osimertinib)
ALK rearrangements | | Alecensa® (alectinib), Xalkori® (crizotinib) or Zykadia® (ceritinib)
*BRAF* V600E | | Tafinlar® (dabrafenib) + Mekinist® (trametinib)
Melanoma | *BRAF* V600E and V600K | Mekinist® (trametinib) or Cotellc® (cobimetinib) + Zelboraf® (vemurafenib)
Colorectal Cancer (CRC) | *KRAS* wildtype (absence of mutations in codons 12 & 13), *KRAS* and *NRAS* wildtype (absence of mutations in exons 2, 3, and 4) | Erbitux® (cetuximab), Vectibix® (panitumumab)
Breast Cancer | *ERBB2* (HER2) amplification | Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab emtansine) or Perjeta® (pertuzumab)
Ovarian Cancer | *BRCA1/2* alterations | Rubraca® (rucaparib)
Foundation Medicine has (Co-)Authored over 375 Publications Between 2011 and 2019

Publications covering almost all tumour types, some rare, include:

- Assay validations
- Biomarker validations
- Publications supporting the clinical validity and utility of CGP
- Case reports
- Review articles

Key Foundation Medicine publications reporting assay and biomarker validations across the publication portfolio

bTMB: blood-based tumour mutational burden; CGP: comprehensive genomic profiling; Q: quarter; TMB: tumour mutational burden.

**FOUNDATIONONE CDx™ In Clinical Practice**

**TURNAROUND TIME 10-12 DAYS**

FDA-approved, CLIA-certified, CAP-accredited laboratory, NYS-approved

**Sample requirements:**
- Surface area >25 mm²
- Sample volume ≥1mm³
- Tumor content >20%

**Laboratory process:**
- >50 ng dsDNA
- Library construction
- Hybridization capture
- Illumina HiSeq platform

**Analysis methods:**
- Customized computational biology algorithms
- Manual secondary review

**Report curation:**
- Clinically relevant genomic alterations
- FDA-approved therapies in patient tumor type
- FDA-approved therapies in other tumor types
- Available clinical trials
EML4-ALK FISH Negative NSCLC: Positive for Novel ALK Fusion On CGP and Confirmed by ALK IHC (Jan 2011)

Effective Crizotinib Schedule for Brain Metastases in ALK Rearrangement Metastatic Non–Small-Cell Lung Cancer

Nir Peled, MD, PhD,* Leor Zach, MD,† Ori Liran, MSc,* Maya Irouze, PhD,* Paul A. Bunn Jr., MD,‡ and Fred R. Hirsch, MD, PhD‡
Response to Cabozantinib* in Patients with RET Fusion-Positive Lung Adenocarcinomas

Note papillary architecture and nuclear clearing. If psammoma bodies are present it is highly predictive of RET fusion.


*Loxo-292 Selpercatinib nearing regulatory approval.
Sarcomatoid NSCLC and Exon 14 \textit{MET} Splice Site Mutation

\textbf{Figure 1.} The genomic position of \textit{MET} Exon 14 alterations. Genome coordinates are human genome build GRCh37/hg19. Genomic positions with alterations occurring in more than one case are indicated with \textasteriskcentered for two and the number of cases for greater than two. A, chr7:116,411,600-116,412,200. B, chr7:116,411,300-116,415,300.

Frampton et al Cancer Disc 2015
**ERBB2 Sequence Mutations Enriched in Mricropapillary Variant of Urothelial Carcinoma**

- The micropapillary variant of urothelial carcinoma, a known clinically aggressive subtype of urinary bladder cancer, harbors an unprecedented high frequency of ERBB2 mutations especially in the extracellular regulatory domain of the ERBB2 gene.

- IHC and FISH will not identify UC that harbors a non-amplification ERBB2 sequence alteration.

- The well-documented aggressive clinical course attributed to MPUC has also been linked to micropapillary carcinomas of the endometrium, breast and lung, however, no association with ERBB2 mutations in these other aggressive types of micropapillary carcinomas has been reported to date.

- Preliminary evidence suggests that ERBB2-mutated MPUC may respond to anti-ERBB2 targeting agent.

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**ERBB2 Mutations in 6 Cases of Micropapillary Urothelial Carcinomas**

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ERBB2: S310F, TP53: R282W, FBXW7: G423V</td>
</tr>
<tr>
<td>3</td>
<td>ERBB2: S310Y, ARID1A: V663fs*15, BAP1: loss, MCL1: amplification</td>
</tr>
<tr>
<td>7</td>
<td>ERBB2: R157W</td>
</tr>
<tr>
<td>9</td>
<td>ERBB2: S310F, TP53: Q244S, R81: Q262*, ARID1A: E1176F*, NF2: E463K</td>
</tr>
</tbody>
</table>

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Response to anti-HER2 Targeted Therapy in a Patient with \textit{ERBB2} Sequence Mutations in TNBC

\textbf{Pre-therapy:} extensive active disease

\textbf{Post-therapy:} good response with lower/less activity

Response of a HER2 FISH/IHC Negative Cutaneous Adnexal Carcinoma with an ERBB2 S310F Mutation to anti-HER2 Targeted Therapy
Complete Regression In A Pediatric Glioblastoma

Molecularly matched targeted therapy against *BRAF* V600E

- Male patient diagnosed with glioblastoma at 9 years old
- Treated with radiation/vorinostat (6-wks) and chemotherapy (vorinostat, bevacizumab, and topotecan; 24 mos)
- Tumor recurred 8 months after completion of therapy
- Comprehensive genomic profiling identified *BRAF* V600E mutation and the patient was treated with vemurafenib
- Recurrent tumor was no longer detectable after 4 months on therapy; response is ongoing for 7 months
Response of Spitzoid Melanoma with BRAF Fusion to Trametinib-based MEK Inhibition over 1.5 Months

Ross et al Int J Cancer Feb 2016
ALK-Fusion Positive
Metastatic Inflammatory
Myofibroblastic Tumor

Lovely et al Cancer Disc 2014
Identified Genomic Alterations And Corresponding Treatment Options In The CUPISCO Study

<table>
<thead>
<tr>
<th>CUPISCO arm</th>
<th>% Genomic alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any precision therapy</td>
<td>32%</td>
</tr>
<tr>
<td>Subcutaneous trastuzumab + pertuzumab + chemotherapy (<em>HER2 actionable alterations</em>)</td>
<td>9%</td>
</tr>
<tr>
<td>Atezolizumab (TMB-high [≥16 mutations/ Mb), MSI-high])</td>
<td>9%</td>
</tr>
<tr>
<td>Ipatasertib (<em>AKT1, PI3K actionable alterations</em>)</td>
<td>8%</td>
</tr>
<tr>
<td>Olaparib (<em>BRCA1, BRCA2 or homologous recombination deficiency based on loss of heterozygosity</em>)</td>
<td>6%</td>
</tr>
<tr>
<td>Vemurafenib + cobimetinib (<em>BRAF^{V600} alterations</em>)</td>
<td>3%</td>
</tr>
<tr>
<td>Erlotinib + bevacizumab (<em>EGFR actionable alterations</em>)</td>
<td>2%</td>
</tr>
<tr>
<td>Vismodegib (inactivating <em>PTCH1</em>, activating <em>SMO</em> alterations)</td>
<td>1%</td>
</tr>
<tr>
<td>Alectinib (<em>ALK, RET rearrangements</em>)</td>
<td>1%</td>
</tr>
<tr>
<td>Entrectinib (Approved globally per protocol version 6, local regulatory review and approval process ongoing; NTRK, ALK and ROS1 alterations)</td>
<td>0.33%</td>
</tr>
</tbody>
</table>

Samples can be eligible for more than one arm, since the sum of the % Genomic alterations column adds up to more than “Any precision therapy”. Some patients have *ERBB3* genomic alterations which are also considered as actionable for anti-HER2 therapies. Not all *EGFR* alterations were considered actionable for erlotinib. MSI, microsatellite instability; TMB, tumour mutational burden.

Ross JS et al. Presented at ESMO 2019 Barcelona, ESP
Long tail plot of genomic alterations in 303 cases of CUP. In all, there were 223 genes in the FoundationOne® CDx bait set that were altered in at least one of the 303 pts.

Genes shown have an alteration frequency of 1% or higher. CUP, carcinoma of unknown primary; pts, patients.

Ross JS et al. Presented at ESMO 2019 Barcelona, ESP
CUP Example

- A 59-year old white female with a 60 pack-year history of smoking presented with new onset seizures
- MRI revealed a solitary 2.6 x 1.9-cm mass in the right frontal lobe of the brain and a 2 x 4 cm left mid-abdominal mass
- Thoracic imaging was negative
- Pathologic examination of the resected brain metastasis revealed a poorly differentiated carcinoma
- Treatment with carboplatin and docetaxel revealed no interval change in scans
Comprehensive genomic profiling was conducted on FFPE resected cerebral metastasis. *MET* copy number status (16x) was determined by modeling copy variation and aneuploidy across the genome.
Case 1 Response to anti-MET Targeted Therapy

-1 month

18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET)/computed tomography (CT) fusion image of transaxial left mid abdominal mesenteric mass before starting crizotinib and 3 and 7 months after starting crizotinib, respectively.

Durable Response to Crizotinib in a MET-Amplified, KRAS-Mutated Carcinoma of Unknown Primary

Norma A. Palma1, Siraj M. Ali2,3, Jamie O’Connor3, Deepa Dutta3, Kai Wang2, Said Somaifar2, Gary A. Palmer2, Deborah Morosini2, Jeffrey S. Ross1, Daron Lipson1, Phil J. Stephens1, Mayur Patel4, Vincent A. Miller5, Nicholas Koutrelakos6

1Foundation Medicine, Cambridge, Mass., 2Genentech, Inc., 3Stanford University School of Medicine, Palo Alto, Calif., and 4American Radiology Services, and 5Maryland Oncology Hematology, Columbia, MD, USA
### CUP Today and Tomorrow

<table>
<thead>
<tr>
<th>Today: Untargeted Cytotoxic Chemotherapy</th>
<th>Tomorrow: Targeted Therapy</th>
</tr>
</thead>
</table>

![Diagram showing current and future approaches to CUP therapy](image)
Immuno-Oncology

Nivolumab (anti-PD-1)
Pembrolizumab (anti PD-1)
Atezolizumab (Anti-PD-L1)

Pharmaceutical Journal.com
Modulating Response To Cancer Immunotherapy

- PD-L1 Expression by IHC
- Tumor Infiltrating Lymphocytes
- $\gamma$-Interferon Signature
- $PD-L1$ ($CD274$) Amplification
- MSI Status
- Tumor Mutational Burden
- Tumor Neo-antigen Load
- Single Gene Predictors
  - Efficacy
  - Resistance
  - Hyper-progression
Tumor Mutational Burden And Response To ICPI

PD-1 Inhibition In NSCLC

High Total Mutational And Neoantigen Burden Predicts Benefit
Current Issues Involving TMB

• Most predictive studies are retrospective and feature IO drugs only
• Prospective trials are combining chemotherapy + IO and TMB may be less predictive in this setting especially when low TMB cut-offs are used
• Prospective studies are emerging
• Prognostic vs predictive significance
• How and where to set cut-offs for a continuous variable
• How can TMB be combined with other ICPI biomarkers such as PD-L1 expression, MSI status, etc. to enhance clinical outcome?
• How can TMB influence the use of neo-adjuvant ICPI treatments?
• Can pre-ICPI treatment with radiation increase the TMB?
• Will liquid biopsy (blood) TMB prove useful in deciding when and if ICPI therapy should be used?
Conclusions

• There is no more exciting time than now to be involved in cancer genomics and translational research

• This “marrying” of clinical and genomic data are continuing to drive a new wave of discovery and improve outcomes for patients with cancer