From Genomics to Cancer Precision Medicine
Levi A. Garraway, M.D., Ph.D.
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Disclosure Information
Levi. A. Garraway, M.D., Ph.D.

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  Stockholder in: Foundation Medicine
Concept #1: A “Long Tail” of Cancer Genes

- Many tumors will have at least one “long tail” cancer gene as a driver of genesis or maintenance
- At a genetic/molecular level, cancer consists of many rare diseases

Melanoma Genes

The Engine of Cancer Precision Medicine

1. “G-to-P”
2. “P-to-G”
(e.g., exceptional cases)

Goal: new therapeutics and combinations directed against molecularly-defined tumors

**Cancer Precision Medicine Key Component #1:**
Systematic tumor re-biopsy capability

Fresh tumor biopsy: prior to treatment

Fresh tumor biopsy: During treatment

Fresh tumor biopsy: Upon disease progression

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**Research biopsies** for clinical trial enrollment, pharmacodynamics, or drug-resistance studies (e.g., pre-treatment, on-treatment, and post-resistance)

“Opportunistic” sampling of treatment-refractory tumor tissue obtained through routine clinical procedures (surgical resection, FNA, thoracentesis/VATS, paracentesis)

Optimization of biopsy protocols for challenging anatomic sites (e.g., bone mets)

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**Research Biopsies Performed in a Metastatic Breast Cancer Cohort (57 patients)**

<table>
<thead>
<tr>
<th>SITE OF METASTATIC BIOPSY</th>
<th>CURRENT (N=57)</th>
<th>TCGA (N=104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>14</td>
<td>25%</td>
</tr>
<tr>
<td>Liver</td>
<td>13</td>
<td>23%</td>
</tr>
<tr>
<td>Lymph Nodes</td>
<td>12</td>
<td>21%</td>
</tr>
<tr>
<td>Chest Wall / Skin</td>
<td>11</td>
<td>19%</td>
</tr>
<tr>
<td>Pleural Effusion</td>
<td>4</td>
<td>7%</td>
</tr>
<tr>
<td>Bone</td>
<td>3</td>
<td>5%</td>
</tr>
</tbody>
</table>

- Whole exome sequencing was performed successfully on research biopsies from a range of anatomic sites
- A multidisciplinary clinical infrastructure (oncology disease centers + interventional radiology, pathology, and surgery) is needed to yield research biopsies that enable high-quality genomic data

Wagle, Lin et al., ASCO (2014)

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*Compared to 104 primary, treatment-naïve HER2+ tumors sequenced in the TCGA study:
- There was no significant difference in the incidence of TP53 and PIK3CA mutations (point mutations and indels) (53% and 28%, respectively).
- The incidence of ERBB2 (HER2) mutations was significantly increased (12% vs 3%, p = 0.002).*
The genetics of resistance to RAF inhibition in BRAFV600E melanoma

Van Allen et al., Cancer Discovery (2014)
Wagle et al., Cancer Discovery (2014)

Genetic landscape of resistance to RAF/MEK inhibition

Van Allen et al., Cancer Discovery (2014)
Wagle et al., Cancer Discovery (2014)
Challenge #1: Cancer progression is *multifactorial*, and knowledge of (epi)genetic “drivers” is incomplete.

Concept #1.2: A “Long Tail” of Resistance Genes

<table>
<thead>
<tr>
<th>Mutation Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS, MEK1/2</td>
</tr>
<tr>
<td>PTEN</td>
</tr>
<tr>
<td>PIK3CA, PIK3R1</td>
</tr>
<tr>
<td>BRAF amp, MITF amp</td>
</tr>
<tr>
<td>NF1, HOXD8?</td>
</tr>
</tbody>
</table>

(RED: MAP kinase pathway reactivation)

Challenge #2: Molecular determinants of response and resistance are often *under-sampled* in the clinical arena.

Wagle, Van Allen et al., *Cancer Discovery* (2014)
Challenge #3: Response/resistance determinants may be heterogeneous

Van Allen, Wagle et al., Cancer Discovery (2014)

Intrinsic resistance to RAF/MEK inhibition in \textit{BRAF^{V600E}} melanoma

Flaherty et al., NEJM (2012)

Chapman et al., NEJM (2011)
Genomic correlates of intrinsic resistance to RAF inhibition

29 BRAF\(^{V_{600}}\)-mutant melanoma cell lines

MITF+AXL-  MITF-/AXL+

Konieczkowski et al., *Cancer Discov.* (2014) with D. Lawrence\(^{MGH}\), and K. Flaherty\(^{MGH}\)

Cancer Precision Medicine Key Component #2
Application of state-of-the-art genomic/molecular technologies to patients’ tumors

Comprehensive genomic/molecular characterization of clinical biopsy specimens

Profiling of archival (e.g., formalin-fixed, paraffin-embedded) tumor tissue

Early adoption of new technologies (e.g., single-cell RNA-seq, CTCs, cfDNA, etc.)
### Options for Translational Studies Using Massively Parallel Sequencing

<table>
<thead>
<tr>
<th>Technology</th>
<th>Percent of Genome Sequenced</th>
<th>Cost</th>
<th>Depth of Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Genome Sequencing</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcriptome Sequencing</td>
<td>2-4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Exome Sequencing</td>
<td>2% (25,000 genes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted Sequencing</td>
<td>0.005% - 0.1% (100s – 1000s of genes)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nikhil Wagle

### Targeted Panels for Clinical Sequencing at DF/HCC

**NGS Snapshot (MGH)** (Iafrate)
- Multiplex PCR based
- 190 amplicons, 43 genes
- 500X coverage
- 2 weeks
- 5-10 ng DNA
- 60 samples/week
- Parallel approach to detect 44 translocations

**OncoPanel (BWH)** (Lindeman)
- Hybrid capture based
- 300 genes
- ~300X coverage
- 3-4 weeks
- 50-100 ng DNA
- 96 samples/week
- Mutations, CNVs, fusions

**Challenge #4: Analytical validity** across diverse targeted panel based approaches.
Concept 2: Some tumor cells respond to immunotherapy and others do not.

Cancer Mutation Frequencies

Rationale for piloting Single-cell RNA-seq

Single-cell RNA-seq

Bulk RNA-seq

“Clinical” Single-Cell RNA-seq

A pipeline for single-cell RNA-seq analysis of clinical specimens:
- Surgical resections and core biopsies
- Fine-needle aspirates
- Malignant pleural effusions and ascites

with Alex Shalek and Aviv Regev

Ben Izar
Daniel Treacy
Judit Jane-Valbuena
Hierarchical clustering of tumor cells and T cells

Using single-cell RNA-seq data to infer chromosomal copy number status
Inferring the lineage and activation state of T cells by single-cell analysis

Lineage and cell cycle tracing in single ovarian cancer cells obtained from ascites
Cancer Precision Medicine Key Component #3
Clinical computational analysis to enable both discovery and clinical interpretation of omic data

Challenge #5: Clinical validity of tumor genomic/molecular information
Does it have meaning or potential value in clinical settings?

“Pillars” of clinical interpretation

- Each component will require a leader and a team
- Software solutions are critical but not sufficient: the “human touch” is also needed for each!
Different flavors of curation?

- Baseline for every case
- Bonus curation on top of this (specialized reports, etc.)
- Types of cases that need additional services (e.g., consult service)

Concept #1.3: A “Long Tail” of “Actionable” Cancer Genes

- A large proportion of cancers may contain at least one plausibly actionable genetic alteration
- The “long tail” means that the conventional clinical trial design approach may not always be feasible
Concept #3: Discovery of “driver” cancer genes/mutations is far from complete

- Many “actionable” gene mutations remain undiscovered (e.g., they are rare “VUS”)

Concept #3: Framework for “Genomics Driven” Clinical Trials

Cancer Patient Genomic Profile

Mutation A  Mutation B  Mutation C […]  No/other mutation

Drug A  Drug B  Drug C

Patient Group #1: “Targeted group”
- Mutation A + Drug A
- Mutation B + Drug B
- Mutation C + Drug C

Patient Group #2: “Empiric group”
- Randomized agnostic to mutation status but controlled for tumor type

Endpoints: tumor response rate, survival in targeted versus empiric groups

Design integrated phase I/II trials to test “genomics-driven” hypotheses
Incorporation of correlative science (pharmacodynamics, imaging, additional omics)
Plan deep characterization of relapsing tumors
Cancer Precision Medicine Key Component #4
A translational innovation platform to probe therapeutic response and resistance

New ex vivo tumor cell culture systems
Rapid characterization of somatic variants from clinical sequencing
Systematic studies of resistance to high-interest anticancer therapeutics

Concept #4: Extraordinary responders as “discoverable” rare subtypes
A 57-year-old woman with refractory metastatic anaplastic thyroid carcinoma treated on a phase II study of everolimus. The patient had a complete response lasting for 18 months.

Nikhil Wagle, Jochen Lorch, Pasi Janne
After 18 months, the patient had a recurrence. A biopsy was obtained.

Clinical mechanisms of acquired resistance to MTOR inhibitors have not been reported

Nikhil Wagle, Jochen Lorch, Pasi Janne

TSC2 Nonsense Mutations in Both Pretreatment and Resistant Tumor

Eli Van Allen, Ali Amin-Mansour, David Kwiatkowski
WES of Resistant Tumor Identifies A Likely Genomic Mechanism of Acquired Resistance to Everolimus

- Mutation in the FRB (FKBP12-Rapamycin Binding) Domain
- Not previously identified in humans
- The fission yeast homologue was identified in a mutagenesis screen for fission TOR2 mutants resistant to rapamycin

MTOR F2108L is an FRB Domain Mutation That Likely Blocks Binding of Everolimus

- Phe 2108 to Leu 2108 mutation
- No previously identified in humans
- Identified in a mutagenesis screen for fission TOR2 mutants resistant to rapamycin


Nathanael Gray
We should add crystal structures for mTOR WT and mutant with torin as well. There was also a recent paper on the crystal structure of mTOR, which may affect how we present this structure.

Nikhil Wagle, 7/28/2013
MTOR F2108L blocks inhibition of S6K phosphorylation by rapamycin

MTOR F2108L remains sensitive to direct mTOR kinase inhibitor
Example: Neoadjuvant cisplatin-based chemotherapy in bladder cancer

Van Allen et al., Cancer Discov., 2014
**ERCC2 and cisplatin sensitivity in vitro**

Van Allen et al., *Cancer Discov.*, 2014

**ERCC2 mutations in other tumor types**

Van Allen et al., *Cancer Discov.*, 2014
Summary: Biomarker Studies in Cancer Clinical Trials

- Trial design should increasingly take the “long tail” of cancer genes into account
- Profiling approaches should account for heterogeneity (but not be thwarted by it)
- Existing and emerging profiling approaches will be of great interest
- With increasing technology sophistication, analytical and clinical validity become crucial

➢ Thoughtful studies informed by biomarkers and mechanistic determinants could transform the oncology treatment landscape

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