Biological and Therapeutic Insights from the Cancer Genome

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Dana-Farber Cancer Institute
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Cancer is a disease of the genome

- Theodor Boveri (1914)
- Chromosomal defects lead to abnormal cell proliferation

Concerning the origin of malignant tumors. T. Boveri
(Translated 2008)
Cancer arises from alterations in normal cellular genes

• Transforming src sequences from the Rous Sarcoma Virus are present in the DNA from normal cells.

Major Categories of Tumor Genomic Alterations

- **Point mutations**
  - AGT: Arg
  - CGT: Cys
  - GGT: Gly
  - TGT: Ser
  - GAT: Asp
  - GCT: Ala
  - GTT

  - Activation of oncogenes-RAS in many cancers
  - Inactivation of TS genes-TP53 in many cancers

- **Copy number alterations**
  - Amplification
  - Activation of oncogenes-ErbB2 in breast cancer
  - Deletion
  - Inactivation of TS genes-Rb in retinoblastoma

- **Translocations**
  - Activation of many genes-Bcr-ABL in CML

Cancer Genome Insights

- Insights into biology
- Insights into precision medicine
Fundamental insights from cancer genome sequencing - 1

Recurrent *IDH1/2* mutations in GBM and AML link genetics to cancer metabolism

Mutations that disrupt chromatin remodeling and DNA methylation occur in many cancers

Fundamental insights from cancer genome sequencing - 3

- Mutations that disrupt mRNA splicing occur in multiple cancer types

Many mutations (~30%) disrupt Notch signaling and squamous differentiation in head/neck cancer (Stransky et al., Science 2011)

Mutations that may dysregulate squamous differentiation occur in 44% of lung squamous cancer (TCGA, Nature 2012)
“Chains” of rearrangements in prostate cancer genomes

Berger et al., Nature, 2011
Generation of “closed chains” in ETS-positive prostate cancers
Generation of “closed chains” in ETS-positive prostate cancers
Generation of “closed chains” in ETS-positive prostate cancers
Chromosomal deletions reveal additional chains

Site 1
Site 2
Site 3

Anisotropic dependencies

Sylvan Baca
ChainFinder Algorithm

- Create a graph representation of rearrangement breakpoints (nodes) and chromosomal deletion segments (edges)

- Search the graph for sets of connected breakpoint nodes that are statistically unlikely to have arisen independently

Baca et al., *Cell* (2013)
Chained rearrangements are common in prostate cancer ("chromoplexy")

84% of tumors have ≥ 1 chain
65% of tumors have ≥ 2 chains
Some chains exhibit subclonality

Baca at al., Cell (2013)
Cancer genes are often disrupted by chromoplexy

Two sections of chromosome 10q

Baca at al., *Cell* (2013)
Cancer genes are often disrupted by chromoplexy

<table>
<thead>
<tr>
<th>Gene</th>
<th># tumors with disruption by chromoplexy</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERG (fusion with TMPRSS2)</td>
<td>15 (of 26 fusion-positive cases)</td>
</tr>
<tr>
<td>PTEN</td>
<td>10</td>
</tr>
<tr>
<td>NKX3-1</td>
<td>4</td>
</tr>
<tr>
<td>TP53</td>
<td>3</td>
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<tr>
<td>CDKN1B</td>
<td>2</td>
</tr>
<tr>
<td>RB1</td>
<td>2</td>
</tr>
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</table>

Baca at al., Cell (2013)
Chromoplexy in other tumor lineages

Head and neck squamous
Lung adenocarcinoma
Breast
Lung adenocarcinoma
Melanoma
Fundamental insights from cancer genome sequencing

A continuum model for tumor evolution

- Gradual progression
- Punctuated progression
- Catastrophe

- Point mutations, singleton translocations and SCNAs
- "closed chains"
- Chromothripsis

- Genomic rearrangement

- Time


Baca at al., *Cell* (2013)
The “dark matter” of the cancer genome

• Regions of the genome that we cannot easily interpret

• Examples:
  – regulatory regions
  – intergenic regions
  – repeat-rich DNA
  – “non-focal” copy number alterations
Identification of two recurrent mutations in the \textit{TERT} promoter

- 17 of 19 (89\%) melanomas had one of two mutations within 100bp of the transcription start site of the \textit{TERT} promoter
- Both are C to T transitions (indicative of UV damage)
- Mutations were mutually exclusive

\begin{align*}
\text{-100} & \quad \text{(1,295,250)} & \quad \text{(1,295,228)} \\
\text{C250T} & \quad \text{C228T} & \quad +1 \\
\end{align*}

\text{TERT} promoter
50 of 70 (71%) harbor TERT promoter mutations

Huang, Hodis et al., Science (2013)
C228T and C250T create consensus ETS sites (GGAA/T)

TERT promoter

C228T

CCCCTTCCGGG
GGGGGAAGGCCC

C250T

CCCCTTCCGGG
GGGGGAAGGCCC

Huang, Hodis et al., Science (2013)
C228T and C250T mutations augment transcriptional activity from the TERT promoter.

* p< 0.01

* p< 0.05

Mary Jue Xu
Recurrent TERT promoter mutations in cancer cell lines

Cell Lines
\( n = 150 \)

- **WT**
- **C228T**
- **C250T**

![Bar graph showing the number of cell lines with different mutations across various cancer types.](image-url)
Cancer Genome Insights

Insights into biology

Insights into precision medicine
Genomics-Driven Cancer Medicine: Guiding Principles

**Principle #1:** Molecular pathways involved in tumor survival and progression are often activated by genetic alterations.

In several major tumor types, ~40-60% harbor at least one genomic alteration affecting an “actionable” proliferation or survival mechanism.
Genomics-Driven Cancer Medicine: Guiding Principles

Principle #1: Molecular pathways involved in tumor survival and progression are often activated by genetic alterations.

Principle #2: Anticancer agents targeting many oncogenic pathways have entered clinical trials.

Spectrum of Targeted Anticancer Agents in Clinical Development
**GENOMIC STUDIES**

**TUMOR DEPENDENCY**

- *ERBB2* (breast cancer)
- *KIT* (GIST)
- *EGFR* (lung cancer)
- *SMO* (BCC)
- *ALK* (lung cancer)
- *BRAF* (melanoma)

... 

**RATIONAL THERAPEUTICS**

- Trastuzumab, Lapatinib
- Imatinib
- Erlotinib
- GDC0449, LDE225
- Crixotinib
- Vemurafenib, dabrafenib, trametinib

... 

**TUMOR RESPONSE (advanced disease)**

- weeks-months
- months-years
- months
- months
- Months-1 yr
- ...
Genomics-Driven Cancer Medicine: Guiding Principles

**Principle #1:** Molecular pathways involved in tumor survival and progression are often activated by genetic alterations.

**Principle #2:** Anticancer agents targeting many oncogenic pathways have entered clinical trials.

**Principle #3:** Genomics technologies enable robust tumor genomic profiling in the clinical arena.
CanSeq: Prospective Whole Exome Sequencing

Prospective whole-exome sequencing on patients at DFCI/BWH with return of clinically actionable results to clinical care team

- Metastatic Lung Adenocarcinoma
  - Prior to 1st line systemic therapy
  - 200 Patients

- Metastatic Colorectal Adenocarcinoma
  - Prior to 2nd line systemic therapy
  - 200 Patients

- Metastatic Castrate-Resistant Prostate Cancer
  - At progression on hormonal therapy
  - 150 Patients

- Metastatic Her2+ or ER+ Breast Adenocarcinoma
  - Progression on trastuzumab/endocrine Rx
  - 100 Patients
Big Data in Oncology

Source: NHGRI
**Precision Heuristics for Interpreting the Alteration Landscape (PHIAL)**

Mutations

- \(\ldots\text{ACC}\ldots\)
- \(\ldots\text{TAG}\ldots\)

Insertion/Deletions

- \(\ldots\text{TCG}\ldots\)
- \(\ldots\text{AACC}\ldots\)

Copy Number Alterations

Rearrangements

Investigate

- Clinical Actionability
- Investigate Biological Relevance
- Cancer Genes
- Cancer Pathways
- COSMIC
- Synonymous Variants

“May it be a light to you in dark places, when all other lights go out.”

Galadriel, *The Fellowship of the Ring* (Tolkein)

Eli Van Allen
Evaluating Actionable Alterations
Evaluating Actionable Alterations

Table 4. Actionable findings with details, sorted by actionability score

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>Variant</th>
<th>Coverage</th>
<th>Allelic_faction</th>
<th>Tier</th>
<th>Trials</th>
</tr>
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<tbody>
<tr>
<td>KRAS</td>
<td>p.A146V</td>
<td>Missense_Mutation</td>
<td>248</td>
<td>0.61</td>
<td>Actionable: Tier 2-A, Plausibly Actionable, Tier 1-B(R), Prognostic/Diagnostic-B</td>
<td>Click here</td>
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<tr>
<td>STK11</td>
<td>p.G279fs</td>
<td>Frame_Shift_Del</td>
<td>23</td>
<td>0.48</td>
<td>Plausibly Actionable: Tier 1-C, 1-D, and 2-B</td>
<td>Click here</td>
</tr>
<tr>
<td>ATM</td>
<td>p.K208fs</td>
<td>Frame_Shift_Inc</td>
<td>39</td>
<td>0.36</td>
<td>Plausibly Actionable: Tier 2-B</td>
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<td>BCL6</td>
<td>p.E419V</td>
<td>Missense_Mutation</td>
<td>112</td>
<td>0.53</td>
<td>Theoretically Actionable: Tier 2-E</td>
<td>Click here</td>
</tr>
</tbody>
</table>

**KRAS p.A146V:** Activating mutations in KRAS are among the most common genetic alterations in human tumors. KRAS mutations play a central role in tumor progression in multiple cancer types, and have been implicated in poor prognosis and resistance to therapy. KRAS alterations are common across numerous malignancies. Activating KRAS mutations are found in 15 to 30% of all patients with non-small cell lung cancer (NSCLC).

This alteration has rarely been found in other cancer types. This alteration has only been reported in 15 colorectal cancer cases in the COSMIC database. An additional 68 cases of A146T have been reported in colorectal cancer in the COSMIC database. However, one systematic study of exon 4 mutations in colorectal cancer demonstrated the presence of A146 mutations in 5% of colon cancers.

This alteration is a known activating mutation, though may be less potent than the more common codon 12 and 13 mutations.

Activating mutations in KRAS predict poor survival in patients with NSCLC, though these studies have generally only included codon 12 and 13 mutations. Activating mutations in KRAS may predict sensitivity to inhibitors of the RAS/RAF/MEK/ERK pathway. Preclinical studies have shown that MEK inhibitors, in particular, may be effective for KRAS mutant tumors, and these agents are in clinical trials for patients with KRAS mutant cancers. Activating KRAS mutations may also predict resistance to anti-EGFR therapies.

**STK11 p.G279fs:** STK11 is a well-known tumor suppressor (also known as LKB1) that is commonly inactivated in several cancers. Germline mutations in STK11 cause Peutz-Jeghers Syndrome (PJS).

This gene has been implicated in NSCLC. In addition, it is commonly seen in conjunction with KRAS mutations.

This gene has been implicated in NSCLC. This specific alteration has not been reported in the COSMIC database for NSCLC, though inactivating mutations in STK11 are common in this tumor type, occurring at a rate of 5-15% of NSCLC. They commonly co-occur with KRAS mutations.

This alteration is likely inactivating, since it is a frameshift mutation that occurs at codon 279 out of 434.

Loss of STK11 activates the MTOR pathway and therefore may predict sensitivity to inhibitors of this pathway. Preclinical evidence suggests that MTOR
Cancer Genome Evaluation Committee (CGEC)

- Judy Garber, Co-chair
- Pasi Janne, Co-chair
- George Demetri
- Matthew Freedman
- Charles Fuchs
- Levi Garraway
- Gad Getz
- Monica Giovanni
- Stacy Gray
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- Steven Joffe
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- Neal Lindeman
- Jeffrey Meyerhardt
- Cynthia Morton
- Michael Murray
- Giovanni Parmigiani
- Mark Pomerantz
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- Scott Rodig
- Barrett Rollins
- Geoffrey Shapiro
- Sapna Syngal
- Eliezer Van Allen
- Nikhil Wagle
- Brian Wolpin
- Matthew Yurgelun
Reporting Results to Clinicians

CanSeq Cancer Genome Report

Patient ID: xxxxxxx
DOB: xxxx
Diagnosis: Lung Adenocarcinoma

<table>
<thead>
<tr>
<th>ACTIONABLE SOMATIC ALTERATIONS</th>
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</thead>
<tbody>
<tr>
<td>Alteration</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>KRAS A146V</td>
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<td></td>
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<tr>
<td></td>
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<tr>
<td>STK21 G279fs</td>
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<td>ATM K208fs</td>
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KRAS A146V

- Activating mutations in KRAS are among the most common genetic alterations in human tumors. KRAS mutations play a central role in tumor progression in multiple cancer types, and have been implicated in poor prognosis and resistance to therapy.
- KRAS alterations are common across numerous malignancies. Activating KRAS mutations are found in 15–30% of all patients with non-small cell lung cancer (NSCLC).
- This alteration is a known activating mutation, though may be less potent than the more common codon 12 and 13 mutations (PMID: 20570890).
- This alteration has not been reported in the COSMIC database for NSCLC. Furthermore, A146 mutations in KRAS were not found in 2 studies comprised 449 cases of NSCLC in which KRAS was sequenced in its entirety (PMID: 18948947, 18632602).
- This alteration has rarely been found in other cancer types. This alteration has only been reported in 15 colorectal cancer cases in the COSMIC database. An additional 68 cases of A146T have been reported in colorectal cancer in the COSMIC database. However, one systematic study of exon 4 mutations in colorectal cancer demonstrated the presence of A146 mutations in 5% of colon cancers (PMID: 20570890).
- Activating mutations in KRAS predict poor survival in patients with NSCLC, though these studies have generally only included codon 12 and 13 mutations.
- Activating mutations in KRAS may predict sensitivity to inhibitors of the RAS/RAF/MEK/ERK pathway. Preclinical studies have shown that MEK inhibitors, in particular, may be effective for KRAS mutant tumors, and these agents are in clinical trials for patients with KRAS mutant cancers.
- Activating KRAS mutations may also predict resistance to anti-EGFR therapies.
A “Critical Path” to Effective Cancer Treatment

- Tumor Dependency
- Rational Therapeutics
- Tumor Response (transient)

Therapeutic Combinations

Cure or Long-term Control

Treatment Resistance
MEK1 mutations and resistance to RAF/MEK inhibition

Eva Goetz, unpublished

Emery et al., PNAS (2009), Wagle et al., JCO (2011)
Genome-scale loss-of-function screens for resistance to RAF inhibition

- 90,000 shRNAs
- Targeting 16,600 genes

Steven Whittaker
**NF1** mutations in patients with intrinsic and acquired resistance to vemurafenib

<table>
<thead>
<tr>
<th>Patient</th>
<th>PFS (months)</th>
<th>Resistance</th>
<th>cDNA</th>
<th>Protein</th>
<th>Candidate splice motif</th>
<th>Splice motif sequence</th>
<th>Site broken?</th>
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<tbody>
<tr>
<td>15</td>
<td>1.5</td>
<td>De novo</td>
<td>c.135C&gt;T</td>
<td>p.N45N</td>
<td>Enhancer</td>
<td>ATCAAT</td>
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<td>45</td>
<td>5</td>
<td>Acquired</td>
<td>c.4023G&gt;A</td>
<td>p.Q1341Q</td>
<td>Splice site</td>
<td>AACCTCCTTCAAGAT</td>
<td>Yes</td>
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<td>46</td>
<td>2.5</td>
<td>De novo</td>
<td>c.7248C&gt;T</td>
<td>p.R2450*</td>
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<td>N/A</td>
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<td>50</td>
<td>2</td>
<td>De novo</td>
<td>c.3018C&gt;T</td>
<td>p.V1008V</td>
<td>Enhancer</td>
<td>ATGGTCC</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Steven Whittaker
Eli Van Allen
Nikhil Wagle
Approaches to Therapeutic Combinations in Melanoma

- B-Raf
- MEK1/2
- ERK1/2
- Oncogenic transcriptional output
- PI3 kinase pathway?
- GPCR pathway?
- GEF/GTPase/PAK signaling?
- Immune checkpoint mechanisms
The Engine of Precision Cancer Medicine

- Patient encounter
- Omic profiling
- Data interpretation
- Management decision
- Clinical response?
- Drug resistance?
- Salvage or new therapy?

Fresh biopsy

The Engine of Precision Cancer Medicine
The Cancer Genomics Vision: Looking Forward

- Completing the mutational atlas for primary tumors
- Expanding the atlas beyond primary tumors
  - Metastases
  - Following relapse to therapy
- Systematic functional annotation
- Systematic clinical implementation
- Worldwide data sharing
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