NGS in Cancer Pathology
After the Microscope: From Nucleic Acid to Interpretation

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Disclosures

Not a paid consultant to Agilent, nor a share holder
Outline

1. Introduction to Cancer Genomics
2. NGS Oncology Assay Basics
   a. Instrument Footprint
   b. Nucleic Acid: DNA, RNA, Both?
   c. Library Prep: Hybrid Capture vs Amplicon
   d. Sequencing: Depth of Coverage and Data Quality
   e. Bioinformatics: Compute, Storage, Workflow
   f. Data Interpretation: “Knowledge is power”
   g. Reporting
3. Questions
Cancer Genomics
Hallmarks of Cancer: The Next Generation

Douglas Hanahan\textsuperscript{1,2,*} and Robert A. Weinberg\textsuperscript{3,*}

- EGFR inhibitors
- Cyclin-dependent kinase inhibitors
- Sustaining proliferative signaling
- Evading growth suppressors
- Immune activating anti-CTLA4 mAb
- Avoiding immune destruction
- Enabling replicative immortality
- Telomerase Inhibitors
- Inducing angiogenesis
- Activating invasion & metastasis
- Tumor-promoting inflammation
- Selective anti-inflammatory drugs
- Inhibitors of VEGF signaling
- Inhibitors of HGF/c-Met
- Deregulating cellular energetics
- Resisting cell death
- Genome instability & mutation
- Resisting apoptosis

\textsuperscript{5} Cell 144, March 4, 2011
TCGA PanCancer Data

Cirello et al., Nature Genetics 45, 1113-1120 (2013)
Survival Comparisons

ALK indicates anaplastic lymphoma kinase gene; EGFR(s), epidermal growth factor receptor gene (sensitizing); EGFR(o), epidermal growth factor receptor gene (other); KRAS, Kirsten rat sarcoma; NA, not applicable.

A, Median survival (95% CI): oncogenic driver + no targeted therapy, 2.38 (1.81-2.93); oncogenic driver + targeted therapy, 3.49 (3.02-4.33); no oncogenic driver, 2.08 (1.84-2.46). B, Survival by oncogenic driver detected for patients with the 5 most frequent oncogenic drivers and targeted treatment. Median survival (95% CI): EGFR(s), 3.78 (2.77-NA); EGFR(o), 2.70 (1.42-NA); ALK, NA (2.80-NA); KRAS, 4.85 (1.30-NA); doubletons (oncogenic drivers in 2 genes), 2.69 (1.94-NA). Vertical tick marks are censoring events.

Figure Legend:
Acquired Resistance to Lorlatinib and Resensitization to Crizotinib in NSCLC

Mutational heterogeneity in cancer and the search for new cancer-associated genes


Figure 1: Somatic mutation frequencies observed in exomes from 3,083 tumour-normal pairs
**Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma**

Alexandra Snyder, M.D., Vladimir Makarov, M.D., Taha Merghoub, Ph.D., Jianda Yuan, M.D., Ph.D., Jesse M. Zaretsky, B.S., Alexis Desrichard, Ph.D., Logan A. Walsh, Ph.D., Michael A. Postow, M.D., Phillip Wong, Ph.D., Teresa S. Ho, B.S., Travis J. Hollmann, M.D., Ph.D., Cameron Bruggeman, M.A., Kasthuri Kannan, Ph.D., Yanyun Li, M.D., Ph.D., Ceyhan Elipenahli, B.S., Callian Liu, M.D., Christopher T. Harbison, Ph.D., Lisi Wang, M.D., Antoni Ribas, M.D., Ph.D., Jedd D. Wolchok, M.D., Ph.D., and Timothy A. Chan, M.D., Ph.D.

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**Nivolumab in Previously Untreated Melanoma without BRAF Mutation**

Caroline Robert, M.D., Ph.D., Georgina V. Long, M.D., Ph.D., Benjamin Brady, M.D., Caroline Dutriaux, M.D., Michele Maino, M.D., Laurent Motzer, M.D., Jessica C. Hassel, M.D., Piotr Rutkowski, M.D., Ph.D., Catriona McKenzi, M.D., Ph.D., Ewa Kalinka-Warzocha, M.D., Ph.D., Kerry J. Savage, M.D., Michae M. Hembeg, M.D., Ph.D., Celeste Leblé, M.D., Ph.D., Julie Charles, M.D., Ph.D., Catalin Mihalciuc, M.D., Vanna Chiarion-Sileni, M.D., Cornelia Mauch, M.D., Ph.D., Francesco Cognetti, M.D., Ana Arranze, M.D., Ph.D., Henrik Schmidt, M.D., D.M.Sc., Dirk Schadenhof, M.D., Helen Gogas, M.D., Lotta Ludgren-Erliksson, M.D., Christine Horak, Ph.D., Brian Sharkey, Ph.D., Ian M. Waxman, M.D., Victoria Atkinson, M.D., and Paolo A. Ascierto, M.D.

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**Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer**

Naiyer A. Rizvi, 1,3,5*, Matthew D. Hellmann, 1,3,5* Alexandra Snyder, 1,3,5* Pia Kvistborg, 4,5 Vladimir Makarov, 2,9 Jonathan J. Havel, 8 William Lee, 8 Jianda Yuan, 8 Phillip Wong, 8 Teresa S. Ho, 6 Martin L. Miller, 7 Natasha Rekhtman, 8 Andi L. Moreira, 8 Fawzia Ibrahim, 4 Cameron Bruggeman, 4 Bilel Gasmil, 10 Roberta Zappasodi, 9 Yuka Maeda, 10 Chris Sander, 11 Edward B. Garon, 11 Taha Mergnhouf, 1,10 Jedd D. Wolchok, 1,3,10 Ton N. Schumacher, 11 Timothy A. Chan 1,3,5,*
Oncology Testing Paradigm

**Initial Diagnosis**
- **Comprehensive Testing (Discovery)**
  - Hybrid Capture RNA-Seq (Differential Gene Expression, Coding Fusions/Mutations)
  - Hybrid Capture DNA-Seq (Copy Number, Mutations, Fusions)

**Minimal Residual Disease**
- **Targeted Testing (Response to Treatment)**
  - Ultra Deep DNA Sequencing
  - Neoantigen
  - ctDNA/exome (Digital PCR, Lower complexity Hybrid Capture or Amplicon NGS Panels)

**Disease Progression**
- **Comprehensive Testing (Discovery)**
  - RNA-Seq (Differential Gene Expression, Coding Fusions/Mutations)
  - Hybrid Capture DNA-Seq (Copy Number, Mutations, Fusions)
Data Management
NGS Oncology Assay Basics

Instrument Footprint
Nucleic Acid: DNA, RNA, Both?
Library Prep: Hybrid Capture vs Amplicon
Sequencing: Depth of Coverage and Data Quality
Bioinformatics: Compute, Storage, Workflow
Data Interpretation: “Knowledge is power”
Reporting
- **Laboratory Management System (LIMS)**
- **Nucleic Acid QC**
  - Spectrophotometer
  - Pico Green Fluorometer
  - DIN/RIN Measurement
  - Quantitative PCR
- **Library Prep Automation / Liquid Handling**
- **Sequencer**
- **Compute and Storage (Cloud vs Local)**
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Reporting
Development and Validation of a Scalable Next-Generation Sequencing System for Assessing Relevant Somatic Variants in Solid Tumors

http://dx.doi.org/10.1016/j.neo.2015.03.004
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Reporting
DNA and RNA Libraries

Hybrid Capture

Whole Transcriptome

Fusions

Amplicon (PCR)
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Reporting
Uniform Coverage

0-213

0-221

0-208

0-208
Mutation Detection


Total count: 212
A : 0
C : 66 (31%, 40+, 26-)
G : 0
T : 146 (69%, 95+, 51-)
N : 0
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Bioinformatics Conundrum?

Expensive

MUSINGS

The $1,000 genome, the $100,000 analysis?

Elaine R Mardis*

Complicated
FASTQ – Data QC – Align (.BAM/.BAI) – Call Variants (.VCF) – Annotate (.MAF/.TSV)

https://www.coursera.org/learn/galaxy-project
Open pipelines for integrated tumor genome profiles reveal differences between pancreatic cancer tumors and cell lines

Jeremy Goecks¹, Bassel F. El-Rayes², Shishir K. Maithel², H. Jean Khoury³, James Taylor⁴ & Michael R. Rossi⁵

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Jeremy Goecks
GEORGE WASHINGTON UNIVERSITY
NGS Oncology Assay Basics

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- Reporting
The integrity of the output is dependent on the integrity of the input.

George Fuechsel
C>T or G>A

Lawrence et al., Nature 499, 214-218 (11 July 2013)
Is a normal control required to call somatic or germline mutations?

No

Yes
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Reporting
# Lung Cancer Biomarkers Guidelines

## Lung Biomarker Test Report

<table>
<thead>
<tr>
<th>Specimen adequacy:</th>
<th>Adequate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated tumor cellularity:</td>
<td>50%</td>
</tr>
</tbody>
</table>

### Results

- **EGFR mutational analysis:** Mutation identified (exon 21 Leu858Arg)
- **ALK rearrangement:** Not detected
- **KRAS mutational analysis:** No mutations detected (wild-type KRAS allele)

### Methods

- **EGFR**
  - Exons assessed: 18, 19, 20, and 21
  - Test method: PCR, allele-specific hybridization
- **ALK**
  - Test method: Fluorescence in situ hybridization
- **KRAS**
  - Codons assessed: 12, 13, 61, and 146
  - Test method: PCR, allele-specific hybridization
MSK Levels of Evidence

Level 1: FDA-recognized biomarker predictive of response to an FDA-approved drug in this indication

Level 2A: Standard of care biomarker predictive of response to an FDA-approved drug in this indication

Level 2B: Standard of care biomarker predictive of response to an FDA-approved drug in another indication but not standard of care for this indication

Level 3A: Compelling clinical evidence supports the biomarker as being predictive of response to a drug in this indication but neither biomarker and drug are standard of care

Level 3B: Compelling clinical evidence supports the biomarker as being predictive of response to a drug in another indication but neither biomarker and drug are standard of care

Level 4: Compelling biological evidence supports the biomarker as being predictive of response to a drug but neither biomarker and drug are standard of care

Level R1: Standard of care biomarker predictive of resistance to an FDA-approved drug in this indication

Standard Therapeutic Implications
Includes biomarkers that are recommended as standard of care by the NCCN or other expert panels but not necessarily FDA-recognized for a particular indication

Investigational Therapeutic Implications
Possibly directed to clinical trials

Hypothetical Therapeutic Implications
Based on preclinical, non-clinical data

Standard Therapeutic Implications
Questions?