Case Study: Next-Generation Sequencing Implementation for Precision Oncology Testing

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Learning Objectives

• Describe the decision making process in deciding whether to implement next-generation sequencing in a clinical pathology lab setting
• Identify the variety of testing strategies and chemistries available
• Review example case studies where NGS is uniquely suited to provide novel clinical insights
Background about our lab

• Serves a community hospital system (now 50+ hospitals)
• Also serving a cancer research institute (Earle A Chiles Research Institute)
Background

Our molecular genomics laboratory:

• Began NGS testing in 2015
• Housed within the larger clinical testing laboratory
• Affiliated with our Pathology Department
Lab services have evolved over time

- RNA-seq
- Whole-genome sequencing
- Single-cell RNA sequencing
- Real-time PCR-based tests
- 50-gene hybrid DNA and RNA solid tumor panel
- 363-gene DNA/RNA solid tumor panel
- 50-gene heme malignancy panel
- 170-gene DNA/RNA panel
- Sanger-based tests
- CITE-Seq
- TCR-Seq
- ATAC-Seq
- Whole-exome sequencing
- microbiome
Distribution of tumors tested: 363-gene panel

Breast carcinoma  
Cholangiocarcinoma  
Duodenal adenocarcinoma  
Ependymoma  
Gastric carcinoma  
Glioma  
Lung adenocarcinoma  
Melanoma  
Ovarian serous carcinoma  
Prostate carcinoma  
Salivary gland carcinoma  
Thymoma  
Urothelial carcinoma

Cancer, other  
Colorectal carcinoma  
Endometrial carcinoma  
Esophageal carcinoma  
Glioblastoma  
Head and neck squamous cell carcinoma (HNSCC)  
Lung squamous cell carcinoma  
Meningioma  
Pancreatic carcinoma  
Renal cell carcinoma  
Testicular cancer  
Thyroid carcinoma  
Uterine carcinoma
Why NGS testing for somatic cancer?
Top therapeutic mutation targets in lung cancer.

Lin and Shaw, Trends Cancer 2016
The growing field of immuno-oncology is intrinsically linked to genomics

- The success of Tumor Mutational Burden (TMB; # of mutations per megabase) as a biomarker for I-O therapy.
- These successes have also required an expansion in the percent of the genome we test.

Carbone et al NEJM 2017
The good news: sequencing has never been more affordable
Why bring NGS in-house versus external testing providers

- Complete flexibility over the content (gene list, chemistry, methodology, reporting).
- Access to complete datasets for research and reanalysis (fastqs, bams etc.).
- NGS is an integral part of research biomedicine.
Which sequencing platform should I choose?
Considerations when choosing NGS platform(s)

1. What is your expected patient test volume?
2. Percentage of the genome that your test(s) will interrogate (e.g. number of Mb per sample)?
3. How fast can you deliver results?

Solid answers to these questions can help to narrow down the platform of choice.
Ok, you’ve generated data. So now what?

Bioinformatics!

It looks like you're trying to do bioinformatics in Excel.

Download R
Lots of options here as well

• End-to-end vendor pipelines
• Build your own pipelines
• Local storage and compute vs cloud
The final piece: Interpretation and Reporting

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**PATIENT INFORMATION**

- **Patient Name:** Example Patient Two
- **Date of Birth:** 01/01/1901
- **Gender:** M
- **MRN:** 200000000
- **Referring Physician:** Dr. Prov Portland
- **Referring Pathologist:** Dr. Prov Portland
- **Accession Number:** 17-000-00000
- **Specimen Type:** FFPE
- **Tissue Type:** Omentum
- **Surgical Path, Case Number:** PS-17-000000, A1
- **Indication:** Melanoma metastatic
- **Date Collected:** 01/01/2018
- **Date Ordered:** 01/01/2018

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**TRISEQ CLINICAL SEQUENCING PANEL - FINAL REPORT**

**Report Summary**

- 1 Genomic Findings
- 4 FDA approved in patient's tumor type
- 0 FDA approved in other tumor types
- 5 Clinical Trials

**Tissue Pathology**

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**REPORT DETAILS**

<table>
<thead>
<tr>
<th>Genomic Variant</th>
<th>Interpretation Summary</th>
<th>FDA Approved therapies in patients tumor type</th>
<th>FDA Approved therapies in other tumor types</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF</strong> c.1799T&gt;A; p.V600E</td>
<td><strong>YES</strong></td>
<td><strong>YES</strong></td>
<td></td>
<td>See clinical trials sections</td>
</tr>
<tr>
<td><strong>BRAF-V600E</strong> is an activating mutation. BRAF encodes the signaling protein Braf, which is downstream of Ras and activates the MAPK pathway (21447722). Activating mutations in BRAF may predict sensitivity to Raf or MEK inhibitors, some of which have been FDA-approved for certain indications (16273091, 20818644, 22723336, 23020132, 22735584, 23436872, 22663011, 27283866).</td>
<td></td>
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</tbody>
</table>

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*Note: The content above is a simulated representation of a clinical report. Actual clinical reports are significantly more detailed and complex.*
Summary:

- It has never been easier to bring NGS testing on-line in your lab/institute.
- New targeted therapy and immune-oncology developments will further increase the value of these results for oncology patients.
Case study: A prototypical NGS application
Case study - 50 y.o. female
• Presented to clinic with a range of symptoms
  ▪ Facial numbness
  ▪ Partial hearing loss
  ▪ Persistent cough
• Brain MRI, chest CT were performed
• Diagnosis of primary lung cancer with brain metastasis
• Median survival for this diagnosis historically has been 5-6 months (Ali et al. Curr. Oncol. 2013).
Case study (continued):

- Lung biopsy was performed.
- Tissue preserved in formalin, embedded in paraffin wax (FFPE).
- Sections cut and affixed to microscope slides for review by pathologist.
- Genomic sequencing was ordered.
- DNA and RNA were extracted from tissue sample and sequenced.
Case study (continued):

Sequencing result:

- **EGFR** c.2573T>G: p.L858R
EGFR – epidermal growth factor receptor

Growth factors EGF, TGF-α, etc.

EGFR phosphorylation

Cell membrane

Cytoplasm

JAK SRC
PI3K
PTEN
Ras Raf MEK
PLCγ DAG
MKP1
ERK
PKC

Survival
Proliferation
Oncogenesis

Angiogenesis
Tumorigenesis
Inhibition of apoptosis

Gene expression
Cell-cycle progression

Transformation
Differentiation
Apoptosis
Case study (continued):

• Patient was put on a therapy targeting EGFR L858R.
• Erlotinib is a tyrosine kinase inhibitor (TKI).
• Tumors exhibited rapid reduction in size.
• **Patient still alive ~2 years later.**

Park *et al.* Biochem. Journal 2012
Survival in patients with EGFR-activating mutations (Phase III Data)

Figure 2: Progression-free survival in both treatment groups
PFS = progression-free survival. HR = hazard ratio.

Zhou et al. Lancet Oncol. 2011
Back to our case study:

• At ~2 year mark, new scans revealed that patient tumors now progressing again.
• Sequencing of new biopsy sample reveals the presence of EGFR T790M mutation.
• T790M is a common acquired resistance mechanism for TKI therapies.
• What to do now? Immunotherapy?
Case study: The atypical case
Case study 2 – 38 y.o. female

• Stage IIIA triple negative metastatic breast cancer.

• Due to family history and age of diagnosis, patient was referred to genetic counseling.

• Identification of pathogenic germline PALB2 4-bp frameshift deletion.

• Carboplatin added to treatment plan; tumor exhibited resistance to carbo.

• Tumor and germline whole exome sequencing performed.
PALB2 forms complex with BRCA1/2 in DNA repair.
Confirmation of 4-bp $PALB2$ frameshift deletion in both germline and carbo-resistant tumor.

Formal HGVS indication:

$PALB2$

c.172_175delTTGT:p.Gln60fs
Identification of novel 5’ 8-bp deletion in tumor only.

- Deletion restores *PALB2* reading frame in the tumor.

Formal HGVS indication:
*PALB2* c.172_175delTTGT:p.Gln60fs

*PALB2* c.[146_153del; c.172_175del]:
p.Lys49_Cys57delinsSerArgArgThrArg

Germline
Restoring mutations have been identified as mechanism for resistance in other \textit{BRCA} complex genes.

\textbf{Secondary \textit{BRCA1} Mutations in \textit{BRCA1}-Mutated Ovarian Carcinomas with Platinum Resistance}

Elizabeth M. Swisher,\textsuperscript{1,2} Wataru Sakai,\textsuperscript{3,4} Beth Y. Karlan,\textsuperscript{5} Kaitlyn Wurz,\textsuperscript{1,2} Nicole Urban,\textsuperscript{1} and Toshiyasu Taniguchi\textsuperscript{1,4}

Departments of Obstetrics and Gynecology and Medicine, University of Washington, Seattle, Washington; and Research Institute, Los Angeles, California

\textbf{Abstract}

Although ovarian carcinomas with mutated \textit{BRCA1} or \textit{BRCA2} are sensitive to platinum compounds, such carcinomas eventually develop platinum resistance. Previously, we showed that acquired resistance to cisplatin in \textit{BRCA2}-mutant tumors can be mediated by secondary intragenic mutations.
The original pre-carbo core biopsy was obtained and exome sequencing was performed.

- Secondary $PALB2$ reversion mutation is only detected in the post-carboplatin sample.

- $PALB2$ frame restoration likely occurred as resistance mechanism to carbo.
The frameshift and reversion are present in the RNA-seq data as well.

RNA-seq data also confirm LOH in original *PALB2* frameshift.
Follow-up for *PALB2* restoration case:

- Patient unlikely to benefit from PARP inhibitor therapy
- Patient considering immunotherapy trials
Case study: Immunotherapy considerations
Metastatic melanoma patient – 71 y.o. male

- Patient with history of metastatic melanoma (primary lesion not known).
- Prior lesions:
  - 15 years ago: right upper back lesion
  - 8 years ago: new back lesion distal site
- Current lesion: adrenal resection
Metastatic melanoma case - initial 50-gene targeted hotspot panel sequencing results

RESULT SUMMARY

Clinically Significant:
- BRAF c.1798_1799delinsAA: p.V600K
- IDH1 c.394C>T: p.R132C
- CTNNB1 c.53_54del: p.R18fs

- BRAF inhibitor therapy an option
- Patient also considering immunotherapy trials
- Larger sequencing panel was utilized.
Sequencing with 363-gene panel and whole exome

- 43 mutations found in the NGS panel, 8 of known clinical significance.
- Tumor mutational burden analysis on exome is clear TMB-high (>30 mut/Mb).

### RESULT SUMMARY

**Clinically Significant:**

**Unknown Clinical Significance:**
Extensive sequencing panel revealed frameshift mutation in the \textit{B2M} gene (Beta-2-Microglobulin)

- B2M a requirement for MHC class I antigen presentation
B2M frameshift also detected in RNA
Loss of B2M a recently discovered immunotherapy evasion mechanism in melanoma.

Loss of Functional Beta₂-Microglobulin in Metastatic Melanomas From Five Patients Receiving Immunotherapy

Nicholas P. Restifo, Francesco M. Marincola, Yutaka Kawakami, Jeff Taubenberger, John R. Yannelli, and Steven A. Rosenberg

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Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma


Patient unlikely to benefit from immunotherapy
Summary:

- Extended sequencing panels can have a significant impact on treatment decisions
- Routine WES, WGS, RNA-seq likely not far off in clinical practice
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