Emerging NGS Applications at the Intersection Germline and Somatic Cancer Genetics

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ARUP Conference Park City
August 17, 2018
Disclosure Information

Nothing to disclose
Learning Objectives

1. Understand when and how testing for inherited mutations in *BRCA1*, *BRCA2*, and other homologous recombination DNA repair genes is used to guide cancer treatment.

2. Describe the clinical scenario and utility of tumor sequencing of mismatch DNA repair genes as part of a Lynch syndrome workup.

3. List at least two types of tumor findings that increase the probability that a germline variant in a cancer predisposition gene is pathogenic.
Interplay

Tumor

Targeted Therapy

Cancer Syndrome Screening

Variant Interpretation

Germline
Outline

• DNA repair gene mutations and cancer treatment
  – Background
  – Testing Approaches

• Tumor sequencing in a Lynch workup

• How tumors can help with variant classification

• Case vignette
# DNA Repair Genes Guide Cancer Treatment

<table>
<thead>
<tr>
<th>DNA Repair Pathway</th>
<th>Example Genes</th>
<th>Germline Syndrome</th>
<th>Treatment Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homologous Recombination Repair (HR)</td>
<td>BRCA1, BRCA2</td>
<td>Hereditary breast/ovarian/prostate</td>
<td>PARPi, platinum</td>
</tr>
<tr>
<td>Mismatch Repair (MMR)</td>
<td>MSH2, MLH1</td>
<td>Lynch</td>
<td>PD1/PDL-1 inhibitors</td>
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</table>

PARPi= poly(ADP) ribose polymerase inhibitor  
PD1/PDL-1= programmed cell death 1/ligand 1
FDA Approves PARPi for Ovarian and Breast

• Three PARPi approved, 2014 (ovary), 2018 (breast)

• Two drugs based on $BRCA1/2$ mutation status

• Germline + somatic $BRCA1/2$ testing now standard

• Other cancers close behind: prostate, pancreatic
Extraordinary PARP Inhibitor Responses in DNA Repair-Mutated Prostate Cancer

Response to PARPi | Lack of Response
---|---
| 14/16 (88%) with bi-allelic DNA repair defects responded | 2/33 (6%) without bi-allelic DNA repair defects responded

Germline DNA Repair Mutations Are Common in Metastatic Prostate Cancer

12% (82/692) with deleterious germline mutations in 16 DNA repair genes

59% (36/61) with avail. tumors had second allele loss-of-function mutation

Pritchard et al. NEJM 2016
FDA Approves PD-1 Inhibitor for Any MMR-Deficient Cancer

FDA grants accelerated approval to pembrolizumab for first tissue/site agnostic indication
MSI by NGS (mSINGS)

Exome

Large Panel NGS (e.g. UW-OncoPlex)

Targeted PCR (MSI-plus)

Salipante et al. 2015 *Clin. Chem.* and Hempelmann et al. 2015 *JMD.*
“Long Tail” of MSI Cancers Now Being Tested for MMR

Hause et al. (2016) *Nat. Med.* PMID:27694933

MMR= mismatch DNA repair
MSI by NGS Outperforms Traditional Methods in New Cancer Types

Adapted from Hempelmann et al. (2018) JITC. PMID:29665853
<table>
<thead>
<tr>
<th>DNA Repair Pathway</th>
<th>Functional</th>
<th>Mutation-Based</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMR</td>
<td>IHC, MSI</td>
<td>NGS Panel (e.g. ColoSeq), Total Mutation Burden</td>
</tr>
<tr>
<td>HR</td>
<td>LOH Burden</td>
<td>NGS panel (e.g. BROCA), HRD Mutation Signature</td>
</tr>
</tbody>
</table>

MMR= mismatch repair, HR= homologous recombination, HRD= homologous recombination deficiency
IHC= immunohistochemistry, MSI= microsatellite instability, LOH= loss of heterozygosity
Implementation of Cancer NGS Testing: 
Not One-Size Fits All
Approach at UW/BBI/SCCA

- Upfront **germline and tumor paired sequencing**

- DNA repair-focused NGS panels – exons **AND** introns
  – e.g. BROCA-tumor
Practice of Genomic Medicine: Patient-Tailored Expert Review
SCCA Prostate Cancer Genetic Care Clinic (launched 2016, Heather Cheng)

**Two-Part Consultation**

1. Medical Oncology
   - Discuss genetics (somatic and germline) for treatment planning
   - Discuss trial/research options

2. Genetic Counselor
   - Risk assessment, pre- and post-test counseling, as relevant
   - Education and guidance on discussing with family
Outline

• DNA repair gene mutations and cancer treatment

• Tumor sequencing in a Lynch workup
  – After germline testing
  – As first-line screening

• How tumors can help with variant classification

• Case vignette
Universal Lynch Syndrome Screening: Colorectal Cancer

Tumor MSI/IHC POS

\[ \text{BRAF V600E/MLH1 methylation NEG} \]

Germline testing NEG

Lynch excluded??

MSI Flavors in CRC

- Microsatellite Stable ~84%
- 3% Lynch ??!!
- 2-3% Double Somatic
- <1% Unexplained MSI
- ~10% MLH1 Methylated

MSI= microsatellite instability, CRC= colorectal cancer
After Germline Testing is Unexpectedly Negative: Sometimes Called “Lynch Like”

- Lynch syndrome in ~3% of colorectal cancer

- “Lynch-like” also in ~2-3% of colorectal cancer
  - Positive tumor screening and no germline mutation
  - Increasingly clinically important with universal screening
Double Somatic MMR Mutations Common

- Explain most “Lynch-like” cases (up to 75%)
- About as common as Lynch syndrome
- Positive screening results explained by somatic mutation
- Patients unlikely to have Lynch syndrome (“undiagnosed”)

MMR = mismatch repair genes
Recommendation to consider somatic MMR testing in some scenarios when germline testing is negative was added to the 2015 NCCN guidelines

MMR= mismatch repair genes
Tumor NGS Can Explain MMR Deficiency After Germline is Unexpectedly Negative

- Double Somatic Mutation 75%
- Missed Lynch 7%
- Unexplained 10%
- MLH1 Methyl 5%
- False Positive IHC 3%

Jacobson et al. (2018), manuscript in preparation
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Tumor Sequencing as First-Line Lynch Screening Can Simplify Testing

**TRADITIONAL SCREENING**

- **MSI Testing**
  - MSI-High
  - MSI-Low or MSS
- **IHC Testing**
  - Absent MSH2 & MSH6, or MSH6 or PMS2
  - Absent MLH1 & PMS2
- **BRAF and/or MLH1 Methylation Testing**
  - BRAF and/or MLH1 methylation negative
  - BRAF and/or MLH1 methylation positive
- **Germline Positive**
  - Refer to Genetics Germline NGS panel test offered
- **Germline Negative**
  - KRAS
  - NRAS
  - Actionable Tumor Findings
  - Tailored Therapy
- **Cascade testing offered to family**

**TUMOR NGS SCREENING**

- **One Test: MSI, MMR mutation status + BRAF, KRAS, NRAS**
  - **POS**
    - Germline
  - **NEG**
    - DONE

- **Unexplained dMMR**
  - Double somatic mutations
  - Tailored Therapy
  - Cascade testing offered to family
  - Actionable Tumor Findings
  - Tailored Therapy

DONE
Study Design

• 419 OSU prospective cases with known MMR IHC, MSI, BRAF, MLH1-methylation status by conventional assays

• Tumor-only NGS at UW, blinded expert review

• MMR genes, MSI by NGS, BRAF, KRAS, NRAS
Tumor NGS as First-Line Lynch Screening Performs Better Than Traditional Screening

<table>
<thead>
<tr>
<th></th>
<th>Tumor NGS</th>
<th>MSI + BRAF</th>
<th>IHC + BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>100% (94-100)</td>
<td>91% (81-97)</td>
<td>90% (79-96)</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>95% (93-97)</td>
<td>95% (92-97)</td>
<td>95% (92-97)</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>40% (30-51)</td>
<td>34% (25-45)</td>
<td>33% (24-44)</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>&gt;99% (99-100)</td>
<td>&gt;99% (98-100)</td>
<td>&gt;99% (98-100)</td>
</tr>
<tr>
<td><strong>Lynch Cases Missed</strong></td>
<td>0 missed</td>
<td>5 missed</td>
<td>6 missed</td>
</tr>
</tbody>
</table>

PPV = positive predictive value; NPV = negative predictive value (95% confidence intervals)

Hampel et al. *JAMA Oncology* 2018 PMID: 29887214
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• Tumor sequencing in a Lynch workup

• How tumors can help with variant classification
  – In patients with germline VUS
  – Incorporating somatic data into classification

• Case vignette
Tumor Data Is Being Used To Clarify Germline Variants

• Increasing tumor testing to clarify “Lynch-like” cases

• Same tests used in patients with germline MMR VUS

VUS= variant of uncertain significance
MMR= mismatch repair genes
“Lynch-like”= patients with positive Lynch screening tests, but negative germline testing
When Tumor Testing *Might* Help

“High” germline VUS, close to likely pathogenic

OR

“Low” germline VUS, close to likely benign

VUS= variant of uncertain significance
Clues From Tumor: Germline VUS Pathogenic

Loss of Heterozygosity (LOH) + No Somatic Explanation

OR

Single Somatic Mutation Consistent With 2nd Hit
Clues From Tumor: Germline VUS Benign

Double somatic mutation

AND

Absence of LOH
Pitfalls

• Missed germline or somatic mutation

• Double somatic mutations are *in cis*

• Multiple clones
Tumor NGS Enables Variant Re-Classification

- 40 patients with germline MMR VUS

- 5 re-classified based in part on tumor sequencing
  - 4 to likely pathogenic
  - 1 to likely benign

MMR= mismatch repair gene
VUS= variant of uncertain significance
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Incorporating Somatic Data into Classification: Lynch is the Perfect Model

- Highly specific tumor phenotype (e.g. MSH2 loss)

- Somatic mutations are *de novo*

- Analogous to *de novo* germline mutation in a patient with a matching phenotype - strong criteria for pathogenicity
Somatic Mutations That Explain IHC

- Somatic missense mutations often explain IHC
- Same mutations may be seen as germline VUS
- Can we use the tumor information to reclassify?
- Yes!
Tumor NGS for **Variant Classification**: Passenger or Driver?

**Normalized Variant Allele Fraction**

Shirts et al. (2018), *Am. J. Hum Genet.* PMID:29887214
Likelihood Modeling

- Prior probability based on InSiGHT

- Likelihood ratio (LR) from normalized variant allele fraction

- Posterior probability >95% = Likely Pathogenic
Results

- 61 somatic missense MMR mutations that fit IHC
- 20 reported as germline
  - 10/20 classified as pathogenic/likely pathogenic
  - 10/20 classified as VUS
- 4 of 10 VUS reclassified as pathogenic/likely pathogenic
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Case

• 45 year old woman with colorectal cancer that loss of MSH2 and MSH6 protein by IHC

• Father had colon cancer at age 55

• Colon cancer predisposition panel testing identifies a rare deep intronic variant in *MSH2*
Case

- Nucleotide position is completely conserved

- Computer prediction strongly suggests the creation of a cryptic splice site

- Variant is reported as VUS with offer of RNA functional studies to follow up
Case

• Same *MSH2* deep intronic variant is observed *twice* as a somatic mutation in colorectal cancer that had loss of MSH2 and MSH6

• Functional RNA splicing studies confirm introduction of a cryptic exon and frameshift in ~50% of transcripts

Thanks to Silvia Casadei
Lynch syndrome caused by an inherited pathogenic deep intronic mutation in *MSH2* that results in the introduction of a cryptic exon and a premature frameshift.
Summary

• **Germline and tumor** NGS testing of DNA repair genes is increasingly **needed to guide cancer treatment**

• **Tumor NGS** testing increasingly **used in a Lynch workup**

• **Tumor NGS testing assists with DNA repair gene variant classification**
Thank You!

- **UW/SCCA/FHCRG/BBI Clinical**
  - Genetics and Solid Tumors Lab
  - NGS Analytics Lab
  - GPS Team
  - Brian Shirts
  - Angie Jacobson
  - Andrew McFaddin
  - Eric Konnick
  - Steve Salipante
  - Noah Hoffman
  - Tina Lockwood
  - Robin Bennett
  - Liz Swisher
  - Sheena Todhunter
  - Brice Colbert
  - Ed Gow
  - Mallory Beightol
  - Jennifer Hempelmann
  - Moon Chung
  - Bob Livingston
  - Pete Nelson
  - Heather Cheng
  - Bruce Montgomery
  - Nola Klemfuss

- **UW Research**
  - Mary-Claire King
  - Tom Walsh
  - Silvia Casadei
  - Maribel Harrell
  - Jessica Mandell
  - Jay Shendure

- **The Ohio State**
  - Heather Hampel
  - Sisi Haraldsdottir
  - Albert de la Chapelle
  - Rachel Pearlman

- **Funding**
  - DOD
  - NIH/SPORE
  - PCF
  - SU2C
  - IPCR