MATCHED TUMOR NORMAL SEQUENCING: THE ROLE FOR GENETIC COUNSELORS

By Taylor Warner
Genetic Counselor
University of Iowa Hospitals and Clinics

October 13, 2016
Heartland Genetics Service Collaborative - Annual Conference and Ancillary Meetings
OBJECTIVES

• Briefly describe the evolution of cancer genetic testing.
• Define matched tumor normal sequencing.
• Discuss the role of the genetic counselor in matched tumor normal sequencing.
HISTORY OF CANCER GENETICS

• Prior to personalized therapy, cancer treatment was “trial and error”

• Start of personalized therapy → prognostic markers/growth signal inhibitors (factors that predict disease outcome and contribute to the growth and spread of cancer cells; gene-drug interactions)
  • EGFR, KRAS, BRAF, KIT, Her2, etc.

• Addition of panels to tumor profiling → no longer looking at specific alleles but at the whole gene
  • More genes = more targets for therapy → potentially better patient outcome
TUMOR PROFILING UTILIZING NGS

• Quickly becoming an integral part of clinical care in oncology

• Utilizes next generation sequencing (NGS) technologies to test for a targeted panel of known cancer-causing genes.

• Identifies acquired (somatic) alterations in the tumor which lead to the malignant phenotype.

• Somatic alterations are targets for personalized therapies and treatments
  • Safer, more effective, and better patient health outcome than traditional treatment
TUMOR GENOTYPING

• Tumor genotyping will identify **BOTH** somatic alterations and germline changes.

• Testing the tumor only cannot always easily distinguish between somatic and germline changes.
CASE EXAMPLE #1

- 62 y/o female
- Breast cancer dx. 44 and leiomyosarcoma dx. 62
- Tumor Profiling

- Referred to Genetics
- MyRisk Panel negative
  - TP53 R175C VUS
TP53 Family

LEGEND
- Breast cancer
- Leiomyosarcoma
- Skin Cancer
- Hodgkin's lymphoma
- Non-Small Cell Lung Cancer
- Lung cancer
CASE EXAMPLE #2

• 33 y/o male
• Colon cancer and polyposis dx. at 31 y/o
• Met clinical criteria of FAP
  • $\rightarrow$ full gene sequencing *APC*
    • Y1075* and E1317Q
• Tumor Profiling one year later...

![Diagram with text: 33 yrs
Familial adenomatous polyposis
APC Y1075*
APC E1317Q]
APC Family
Case Examples

• Both received tumor profiling and germline sequencing
  • Timing different
  • Different stages of in their cancer history
    → essentially matched tumor normal sequencing

• Genetic counseling involvement only during germline sequencing
MATCHED TUMOR-NORMAL SEQUENCING

• Matched tumor-normal sequencing
  • Comparing tumor and matched normal sequencing pairs
  • Allows for correct identification of somatic and germline alterations

• Not widely utilized
  • Due to cost and turn-around-time

• Why is this important?
  • Allows for informed and appropriate treatment decisions
  • Identifies personal and familial clinical implications
    • Could be missed without performing matched tumor-normal sequencing
WHAT HAS BEEN SEEN SO FAR...

• One study indicated that 31% of alterations identified in tumor samples may be incorrectly classified. This leads to pathogenic germline changes being missed or germline variants mistakenly classified as actionable somatic changes. (Jones at al. 2015)

• Another study suggested that 16% of tumor alterations were germline.
  • Almost every participant had more than one germline variant of uncertain significance.
  • (Schrader et al. 2015)

• Indicates a high incidence of tumor alterations are germline rather than somatic alterations.
AN ADDITIONAL MATCHED TUMOR NORMAL STUDY

• My graduate school thesis project (through UAMS)

• Conducted at Sanford Health in Sioux Falls, SD
GEMMA TRIAL

• Genetic Exploration of the Molecular Basis of Malignancy in Adults
  • Oncogenetic clinical trial
  • Individuals with cancer whose disease has progressed following their first line of treatment
    • Purpose – determine a better course of cancer treatment

• All participants received tumor profiling
  • 106 enrolled (as of September 2015)

• Trial already in place – patients already consented to germline testing in original GEMMA protocol

• The purpose of GEMMA was not to identify germline variants but it is a question that comes up
## TUMOR PROFILING

<table>
<thead>
<tr>
<th>PATIENT RESULTS</th>
<th>TUMOR TYPE: STOMACH NEUROENDOCRINE CARCINOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 genomic alterations</td>
<td>Genomic Alterations Identified</td>
</tr>
<tr>
<td>0 therapies associated with potential clinical benefit</td>
<td>CCNE1 amplification</td>
</tr>
<tr>
<td>0 therapies associated with lack of response</td>
<td>TP53 R110_L111insR</td>
</tr>
<tr>
<td>2 clinical trials</td>
<td>KDM6A loss exons 18-29</td>
</tr>
<tr>
<td></td>
<td>RB1 loss exons 21-27</td>
</tr>
</tbody>
</table>

### APPENDIX

**VARIANTS OF UNKNOWN SIGNIFICANCE**

Note: One or more variants of unknown significance (VUS) were detected in this patient’s tumor. These variants have not yet been adequately characterized in the scientific literature. We choose to include them here in the event that they become clinically meaningful in the future.

<table>
<thead>
<tr>
<th>AKT2</th>
<th>ALK</th>
<th>MAP2K1</th>
<th>NTRK3</th>
</tr>
</thead>
<tbody>
<tr>
<td>R208K</td>
<td>R1231Q</td>
<td>P15A</td>
<td>S716Y</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PTCH1</th>
<th>ZNF703</th>
</tr>
</thead>
<tbody>
<tr>
<td>R13G</td>
<td>H402_D403&gt;PTHLG</td>
</tr>
<tr>
<td></td>
<td>GSSCSTCSAHD</td>
</tr>
</tbody>
</table>
FREQUENCY OF ALTERATIONS

• Actionable Variants
  • 146 actionable variants in 57 genes
  • Average number of actionable variants = 4.29 actionable variants per individual
  • Range = 1-12 actionable variants

• VUSs
  • 419 VUSs in 199 genes
  • Average number of VUSs = 12.32 VUSs per individual
  • Range = 3-93 VUSs
Germline Sequencing

- Performed at Sanford Health
- Next Generation Sequencing
- TruSightOne focused exome kit from Illumina
  - 4200 genes
  - Ran on HiSeq 2500
- GenomePilot software (Appistry) was utilized to run BWA-MEM and GATK to generate the variant call files (VCF)
- VCF files loaded into the Codified software
  - Only looked at TP53 and the 314 other genes that were on the Foundation One reports
FREQUENCY OF GERMLINE ALTERATIONS

- **None** of the actionable variants identified in the tumor were found in germline.

- **VUSs**
  - 84 VUSs in 57 genes
  - Average number of VUSs = 2.47 VUSs per individual
  - Range = 0-5 VUSs

- **31 of 34 (91.18%)** individuals had at least 1 germline VUS
TUMOR REPORTED vs GERMLINE REPORTED - MUTATIONS and VUS

- **Germline and Tumor VUS**: 20.05%
- **Tumor Only VUS**: 14.87%
- **Overall Germline and Tumor**: 85.13%
- **Overall Tumor Only**: 14.87%

- **% Germline Actionable Variants**: 0%
- **100% Tumor Actionable Variants**
- **% Germline Variants of Uncertain Significance**: 79.95%
- **20.05% Germline and Tumor VUS**
- **% Total Germline Variants**: 85.13%
- **14.87% Overall Germline and Tumor**
- **% Germline Variants of Uncertain Significance**: 0%
- **100% Tumor Variants**
MATCHED TUMOR NORMAL SEQUENCING
SUMMARY

• Comparing tumor and matched normal sequencing pairs

• Allows for correct identification of somatic and germline alterations

• Allows for informed and appropriate treatment decisions

• Identifies personal and familial clinical implications

• Previous studies indicate a high proportion of alterations identified on the tumor profile are germline rather than somatic
GENETIC COUNSELING IN MY STUDY

• 4 individuals were referred for genetic counseling
  • 1 passed prior to genetic counseling
  • 3 received genetic counseling and germline testing
    • 1 prior to tumor profiling
    • 2 after tumor profiling
GENETIC COUNSELING EXAMPLE

• Male

• Lung adenocarcinoma – dx. 67 y/o

• Family history
  • Brother – lung cancer dx 59 y/o
  • Sister – breast cancer dx 50s
  • Sister – breast cancer dx 58 y/o
  • Maternal uncle – lung cancer
  • Maternal uncle – esophagus and colon cancer
  • Paternal grandmother – uterine cancer dx 33 y/o
  • Paternal uncle – bone cancer/sinus cancer dx 70s
**GENETIC COUNSELING EXAMPLE**

- **Tumor Profiling:**

<table>
<thead>
<tr>
<th>PATIENT RESULTS</th>
<th>TUMOR TYPE: LUNG ADENOCARCINOMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>14 genomic alterations</td>
<td>Genomic Alterations Identified†</td>
</tr>
<tr>
<td>4 therapies associated with potential clinical benefit</td>
<td>$BRCA1\text{G401}\text{C}$</td>
</tr>
<tr>
<td>0 therapies associated with lack of response</td>
<td>$CDK4$ amplification – equivocal*</td>
</tr>
<tr>
<td>21 clinical trials</td>
<td>$PTEN\text{L57S}$</td>
</tr>
<tr>
<td></td>
<td>$INPP4B$ splice site 2135+2_2135+2delT</td>
</tr>
<tr>
<td></td>
<td>$MYC$ amplification</td>
</tr>
<tr>
<td></td>
<td>$TP53\text{I254F}$</td>
</tr>
<tr>
<td></td>
<td>$ARID1A\text{S610fs*9}$</td>
</tr>
<tr>
<td></td>
<td>$DAXX\text{M389fs*1}$</td>
</tr>
<tr>
<td></td>
<td>$FAT1\text{K316*}$</td>
</tr>
<tr>
<td></td>
<td>$LRP1B$ splice site 10531+1G&gt;C, W1962*</td>
</tr>
<tr>
<td></td>
<td>$MAGI2\text{Y893*}$</td>
</tr>
<tr>
<td></td>
<td>$MSH2$ splice site 2210+1G&gt;T</td>
</tr>
<tr>
<td></td>
<td>$SPTA1\text{G822*}$</td>
</tr>
</tbody>
</table>

- **MSH2, BRCA1, PTEN, TP53**

- Referred for germline testing after tumor profiling
GENETIC COUNSELING EXAMPLE

• Germline Testing:
  • BRCA1, BRCA2, CHEK2, EPCAM, MLH1, MSH2, MSH6, PMS2, MUTYH, PTEN, TP53
  • 2 VUSs identified
    • MLH1 c.-28A>G
    • MLH1 duplication exon 16-19
  • Neither were identified in the tumor profile
WHAT ROLE DO GENETIC COUNSELORS PLAY IN MATCHED TUMOR NORMAL SEQUENCING?
• Assess family history regardless of cancer diagnosis

• Follow-up on tumor profiling reports – both actionable variants and variants of uncertain significance

• Facilitate germline sequencing (if appropriate)

• Identify other family members who would benefit from genetic counseling and/or genetic testing

• Provide risk assessment and communication

• Patient education and assure patient understanding
INTEGRATING GENETIC COUNSELING
• Integration into multidisciplinary oncology teams

• Involvement in tumor boards and case conferences

• Involvement in the patient’s initial or follow-up oncology visits

• Genetic counselors can help coordinate care
  • Help clinicians determine appropriate follow-up
  • Coordinate familial testing and care
  • Bring together/connect patients and their families that have seen multiple providers (oncology, genetics, etc.)
TAKE HOME POINTS

• Matched tumor-normal sequencing is effective in detecting somatic versus germline variants.

• Establishing tumor-normal profiling as clinical practice will assist in the care and treatment of cancer patients, as well as assess future health risks.

• There should be greater involvement of genetic counselors in these types of cases.
  • Genetic counselors play an important role in risk assessment, patient counseling and education, and case management.
QUESTIONS

REFERENCES


REFERENCES


ACKNOWLEDGEMENTS AND THANK YOUS

- Lori Dean and the Heartland Genetics Service Collaborative
- The Sanford Health genetic counselors and research team
- Quinn Stein
- Megan Landsverk
- Megan Bell
- Larissa Risty
- Lori Williamson Dean
- Steven Powell
- Lora Black
- Chun-Hung Chan
- Jason Flanagan
- Megan Cornwell

http://careerconfidential.com/category/thank-you-notes/