Case Studies in Molecular Medicine

Christine M. Walko, PharmD, BCOP, FCCP
James Saller, MD
DeBartolo Family Personalized Medicine Institute
Moffitt Cancer Center
Clinical Pearls of Genomic Analysis
(as told through real cases)

• Additional expression testing may add additional clinical value
  – ERBB2 and FGFR2

• Hotspot testing is valuable and economical but also has limitations
  – EGFR mutations

• Commercial lab test reports may not provide the most complete data to make a clinical decision
  – NAB2-STAT6 fusions

• Genetics can be valuable when working through cancers of unknown primary (CUP)
  – Refining the subtype in sarcomas
  – Tissue of origin testing

• Collaboration is **ESSENTIAL!**
  – High grade spindle cell neoplasm found in the brain
Value from Additional Expression Testing

- **Case #1**: 74 yo male with trigeminal nerve tumor diagnosed 2013
  - Initially read as squamous cell/spindle cell carcinoma
    - Radiation and cisplatin
  - Bottom lip lesion biopsied and showed **melanoma**
    - Nivolumab x 8 cycles then progression
  - Recent MRI showed PD in the trigeminal nerve tracking along his 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th}, 7\textsuperscript{th} and 8\textsuperscript{th} cranial nerves

**Genomic Alterations Identified\(^\dagger\)**

- **ERBB2** amplification, S310F
- **ARID2** A1547fs*14, E1264*, E1413fs*5
- **CDKN2A** p14INK4a R58* and p14ARF P72L, p16INK4a
- V51fs*83 and p14ARF H66fs*94
- **LRP1B** W2657*, W3570*
- **TERT** promoter -146C>T
- **TOP2A** amplification
- **TP53** G266V, P47*, R158C

**Additional Findings\(^\dagger\)**

- **Tumor Mutation Burden** TMB-High; 135 Muts/ Mb

**Additional Disease-relevant Genes with No Reportable Alterations Identified\(^\dagger\)**

- **BRAF**
- **KIT**
- **NRAS**

PD = progressive disease
Clinical Discussion: Case 1

• ERBB2 directed therapy:
  – ERBB2 amplification (copy number =157)
  – ERBB2 S310F: activating mutation
  – Clinical data supports response to anti-Her2 therapies such as lapatinib, trastuzumab, and pertuzumab
  – MATCH Trial, arm B = afatinib

• But does this translate in clinical ERBB2 protein expression?

HER2/neu ISH (Ventana INFORM HER2) testing ordered to confirm

HER2 amplification (HER2/CEP17 >8:1)
Case #2: 62 yo female with lung biopsy showing adenocarcinoma

- PET showed multiple bilateral lung lesions, update in thoracolumbar spine and pancreas.
- Laminectomy and T12 tumor resection
- Pathology showed mucinous adenocarcinoma consistent with colonic origin
  - Irregular mucinous glands with no squamous differentiation, focal necrosis with rare mitoses < 1/hpf
  - Positive: CK7, CK20, and CDX2
  - Negative: TTF1, napsin, GATA3 BRAF V600E, and ALK
  - Ki-67 variable proliferative index ranging from 1%-10%

Genomic Alterations Identified

- **FGFR2 TACC2-FGFR2 fusion**
- **PTEN G36**

Additional Findings

- Microsatellite status: MSI-Unknown
- Tumor Mutation Burden: TMB-Unknown

Additional Disease-relevant Genes with No Reportable Alterations Identified

- **BRAF**
- **KRAS**
- **NRAS**
Clinical Discussion: Case 2

• Fusion between TACC2 exons 1-10 and FGFR2 exon 18
  – FGFR2 variants truncated at exon 17 have been reported as oncogenic
  – FGFR2 mutations reported in 8% of colorectal cancers
  – High FGFR2 expression correlated with lung or bone mets (c/w case presentation)
  – FGFR2 is targetable w/ a TKI, like pazopanib or more selective FGFR2 inhibitor could be considered for future therapy.

• But does this translate in clinical FGFR2 protein expression?

  Unknown: availability of CLIA assay very limited
Hotspot Testing Limitations

• **Case #3**: 46 yo female never smoker with newly diagnosed stage IV adenocarcinoma of the lung

• Left upper lobectomy pathology showed moderately differentiated adenocarcinoma
  – Molecular analysis (Clarient) for EGFR alterations was negative for
    • Exon 19 deletions
    • Exon 20 insertions
    • G719S/A/C, L858R, L661Q, S788I and T790M.
  – The tumor was also negative for alterations in KRAS, ALK, PIK3CA, RET and BRAF.

• Treatment started with SRS to brain lesion then cisplatin and pemetrexed with radiation to the left chest wall

• Recurrence was again treated with carboplatin and pemetrexed
Clinical Discussion: Case 3

  - RB1 and RAF variants of unknown significance (VUS) at 0.1%

- Guardant Report 7/2016
  - EGFR H835L (19.0%)
  - EGFR L833V (19.4%)
  - TP53 R282W (19.0%)

Complex rare mutations of L833V and H835L in exon 21 of EGFR that are CIS (on the same allele) are predicted to have an effective response with gefitinib.

J Clin Oncol. 2011;29(16):e468-9
EGFR Mutations in GBM

<table>
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<tr>
<th>EGFRvI</th>
<th>N-terminal deletion</th>
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<tbody>
<tr>
<td>EGFRvII</td>
<td>Exons 14–15 deleted</td>
</tr>
<tr>
<td>EGFRvIII</td>
<td>Constitutively active exons 2–7 deleted frequent in GBM</td>
</tr>
<tr>
<td>EGFRvIV</td>
<td>Ligand-binding exons 25–27 deleted</td>
</tr>
<tr>
<td>EGFRvV</td>
<td>Ligand-binding exons 25–28 deleted</td>
</tr>
<tr>
<td>Point mutants</td>
<td>Domain interfaces R84K, A265V/D/T, P545L, G574V</td>
</tr>
</tbody>
</table>

EGFR domain structure:
- Domain I exons 1–4
- Domain II exons 5–7
- Domain III exons 8–12
- Domain IV exons 13–16

Transmembrane domain:
- Tyrosine kinase domain
- Intracellular domain
- C-terminal

Ligand-binding domains:
- pTyr site

Going Beyond the Clinical Report

- **Case #4**: 35 yo male with a retrosigmoid solitary fibrous tumor (SFT)
  - Initial diagnosis in 2013 based on tissue from the omentum, negative margins
    - Moderate nuclear pleomorphism; < 4 mitoses per 10 hpf
    - Strongly + CD34, moderately + CD99, focally + CK
    - Negative PLAP, myogenin, C-Kit, desmin, S100, SMA, HHF35, and synaptophysin
  - Local recurrence of SFT in 2016 based on descending colon mass resection
    - Up to 4-5 mitoses per 10 hpf
    - + CD34 and STAT6
  - Restaging scans 3 months later showed extensive liver involvement
  - Treatment plan is temozolomide and bevacizumab
Clinical Discussion: Case 4

• Foundation One testing ordered on 2016 sample:
  – SFT is characterized nearly ubiquitously by a fusion of NAB2 and STAT6 occurring via inversion of 12q13.
  – Specific exon locations of NAB2-STAT6 dictates high vs low risk.

Additional inquiry to Foundation Medicine: Fusion of NAB2-STAT6 occurs between **exons 1-6 of NAB2 and exons 17-22 of STAT6**
Comparison of the two most frequent categories of NAB2-STAT6 fusion variants

Wild-type (negative regulator of fibrosis)

Wild-type (positive regulator of fibrosis)

(Akaike, Kurisaki-Arakawa et al. 2015)
Comparison of the two most frequent categories of NAB2-STAT6 fusion variants

Wild-type (negative regulator of fibrosis)

Wild-type (positive regulator of fibrosis)

Older patients with benign behavior
NAB2 exon 4-STAT6 exon 2/3
- Fusion protein lacks the CDH4-interacting domain (CID) terminal repressor domain of NAB2, but contains the complete STAT6 protein.
- Increased activity of EGR1 \(\rightarrow\) prominent diffuse fibrosis

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**Older patients with benign behavior**

NAB2 exon 4-STAT6 exon 2/3

- Fusion protein lacks the CDH4-interacting domain (CID) terminal repressor domain of NAB2, but contains the complete STAT6 protein.
- Increased activity of EGR1 → prominent diffuse fibrosis

**Younger patients with aggressive behavior**

NAB2 exon 6-STAT6 exon 16/18

- An almost complete NAB2 protein fused to a considerably truncated portion of STAT6, retaining transcriptional activation domain (TAD), but lacking DNA-binding domain (DBD) and SRC-homology 2 domain (SH2) → abolished inflammatory response.
- CID terminal repressor domain intact → less fibrosis
- Higher mitotic count & cellularity, deep-seated

(Akaike, Kurisaki-Arakawa et al. 2015)
Going Beyond the Clinical Report

• **Case #4:** 35 yo male with a retrosigmoid solitary fibrous tumor (SFT)
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Would this have changed management if we knew it in 2013?
Clarifying Tumor Subtypes

• **Case #5**: 46 yo female initially diagnosed 2014 with a schwannoma
  – Laminectomy 2015
  – Recurrent spinal tumor, biopsy 2016
    • Grade 3 spindle cell tumor concerning for malignant peripheral nerve sheath tumor based on intraneural origin.
  – Right L5 resection and L5-S1 posterior lateral arthrodesis 2016.
  – Treated with radiation
  – Metastatic disease developed 3 months later and started single agent doxorubicin

(Akaike, Kurisaki-Arakawa et al. 2015)
Reversible BAF (mSWI/SNF) Complex Disruption in Human Synovial Sarcoma (SS)

(t(X;18) translocation)

Ch18

SS18 (18q11.2)

ChX

SSX (Xp11.2)

BAF (mSWI/SNF)

H3K27Me3 Repression
Sox2 Inactivation and Quiescence

H3K27Me3 Removal
Sox2 Activation and Proliferation

ssBAF

DeBartolo Family
PERSONALIZED MEDICINE INSTITUTE

Cell 2013 153, 71-85DOI: (10.1016/j.cell.2013.02.036)
Clarifying Tumor Subtypes

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**TUMOR TYPE:** SOFT TISSUE SARCOMA (NOS)

Genomic Alterations Identified:
- **SS18-SSX2 fusion**
- **DDX3X rearrangement exon 10**
- **EP300 EP300-ATRX fusion**

Diagnostic of synovial cell sarcoma, chemotherapy changed to ifosfamide and doxorubicin with good response
Cancer of Unknown Primary

• **Case #6**: 60 yo female with history of breast cancer
  – Dx Winter 2013 with IDC, high nuclear grade, poorly differentiated, Ki67 = 67%, ER 90%, PR 90% and Her2 negative
    • Treated with TC x 4 then 6 months of anastrozole
  – Fall 2014 PD → everolimus and exemestane
  – Early 2015 PD → paclitaxel
Clinical Discussion: Case 6

- 3/2016 Cervix and pelvic mass: poorly differentiated carcinoma
  - Neoplastic cells have transitional and glandular differentiation; oncocytic and hobnail features
  - Positive ER, PR, CK7 and vimentin (focal)
  - Negative CK20, calretinin, WT-1, GCDFP-15, mammaglobin, GATA 3, napsin and CEA
  - IHC not specific for metastatic breast carcinoma and cannot rule out a primary gynecologic site

- This sample was sent for further testing:
  - Tissue of origin
  - Foundation One
## TOO tests

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<th></th>
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<th>CGI</th>
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<td>mRNA</td>
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<td>Coverage</td>
<td>64 miRNAs</td>
<td>2,000 genes</td>
<td>92 genes</td>
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<tr>
<td>Tumor types</td>
<td>42-49 sub-types</td>
<td>15 Major types</td>
<td>50 sub-types</td>
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Example Decision algorithm
Tissue of origin testing

Core premise:
Different tissue types have distinct mRNA profiles

2000-gene profile
Degree of utility is case-specific

<table>
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<tr>
<th>Tissue</th>
<th>Similarity Score</th>
<th>Low 0-5</th>
<th>High 100</th>
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When to Order TOO?

- Unresolved differential diagnosis of 2 or more cancer types
- History of multiple cancers
- IHCs are inconclusive or conflicting
- Clinical presentation and histology differ on possible site of origin
- Atypical distribution of metastases
- Not responding to treatment as expected
Clinical Discussion: Case 6

Foundation One Results

- Based on Foundation report, the alterations seem more common in Gyn malignancies vs. breast
- PIK3CA, PPP21A and TP53 have been reported in cases of uterine serous carcinoma and serous endometrial intraepithelial carcinomas
- MLL3 alterations have been reported in 8% of cervical cancers.

Genomic Alteration Identified†

- PIK3CA E542K
- LRP1B loss exons 8-11
- MLL3 C310S
- PPP2R1A W257C
- RB1 R358*
- TP53 H193fs*54

Additional Disease-relevant Genes with No Reportable Alterations Identified†

- ERBB2
Clinical Discussion: Case 6

Tissue of Origin Results

She was changed back to carboplatin and paclitaxel with decrease in pulmonary metastases and no evidence of disease in the pelvis.
Value of Collaboration

- **Case #7**: 62 yo female with right parietal lesion
  - 2016 craniotomy at OSH with pathology consistent with high grade spindle cell neoplasm
  - Primary sarcoma vs. gliosarcoma?

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<tr>
<td>Genomic Alterations Identified†</td>
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<tr>
<td>CDK4 amplification</td>
</tr>
<tr>
<td>EZH2 D730fs*1</td>
</tr>
<tr>
<td>MDM2 amplification</td>
</tr>
<tr>
<td>FRS2 amplification</td>
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<td>GLI1 amplification</td>
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<td>TP53 K132R</td>
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MCC Clinical Genomic Action Committee

- Bioinformatics
- Leukemia
- PCM Fellow
- Genetic Couns
- Medical Gen.
- Thoracic
- Anat Pathology
- GU
- Pharmacy
- Heme/onc fellow
- Mol Pathology
- Sarcoma
- Breast
- Myeloma
- Leukemia
- Bioinformatics
- PCM Fellow
- MCC Clinical Genomic Action Committee
Clinical Discussion: Case 7

- Focal amplification of 12q1-15
  - CDK4
    - Sarcoma (18-24%)
    - GBM (14%)
  - MDM2
    - Sarcoma (27%)
    - GBM (10%)
  - GLI1
    - Rhabdomyosarcoma (60%)
    - GBM (~7-13%)
    - Soft tissue sarcoma (7%)
  - FRS2
    - Well diff liposarcoma (100%)
    - Dediff liposarcoma (93-100%)
    - Undiff HG pleomorphic sarcoma (32%)
    - HG serous ovarian ca (12.5%)

Final Diagnosis: Gliosarcoma
Conclusions

1. NGS may lend further support to traditional IHC or FISH. (e.g. ERBB2)
2. NGS may interrogate less frequent mutations not included in hotspot testing.
3. Be aware of what your test is able to tell you, and what its limitations are.
4. TOO is an additionally helpful method to determine the site of origin.