Clinical Utility for Oncology Cases

Presenters:
Pranil Chandra, DO: Hematologic Malignancies
Rajyasree Emmadi, MD: Solid Tumor Oncology

Moderated by: Loren Joseph, MD

Outline

• Background and introduction to genomic profiling in oncology
  • Brief review of high-throughput technologies

• Clinical utility and guiding appropriate testing utilization

• Examples of clinical applications across hematologic malignancies with select case studies
Reasons For Growth of Precision Oncology

• Explosion of literature
  – Identification of genomic aberrations that have clinical significance
    • Numerous genomic research efforts such as Cancer Genome Atlas Project (TCGA) and others
• Marked increase in therapeutic drug development
  – Greater than 500 targeted/cancer therapies in development over the next few years
• Marked decrease in costs to identify genomic aberrations
  – Exponential decrease in sequencing costs
    • Rapid adoption of massively parallel (NGS) and other high throughput technologies (i.e. microarray)

Opportunity – Actionable Information

Pre-Genomic Molecular Technologies

• PCR-based
  – Sanger Sequencing
  – Pyrosequencing
  – Others
• Limitations
  – Often small amount of tissue which limits the number of genes tested for.
  – Longer TAT when assessing for alterations in multiple genes in an algorithmic and sequential fashion.
    • JAK2➔MPL➔CALR
  – Multiplex testing may become expensive when sequentially assessing for alteration in multiple genes and/or targets.
  – Allelic sensitivity

High-throughput Genomic Testing Methods

• Basic principles
  – Disruptive technologies that allow for high throughput DNA/RNA analysis
    • Assess for multiple (up to hundreds) of genomic aberrations at once
  – Next generation sequencing
    • Also known as massively parallel sequencing
    • Various platforms and chemistries commercially available
  – Cytogenomic microarray
    • SNP and array based platforms commercially available
High-throughput Genomic Testing Methods

• Advantages
  – NGS is more sensitive and accurate than Sanger Sequencing
    • Each section of DNA/RNA is sequenced multiple times.
      – Allows for greater depth of coverage (i.e. 500-1000X in oncology samples)
    • Can pick up mutations at low percentage compared with Sanger sequencing.
      – Advantageous in situations of molecular/tumor heterogeneity
  – SNP-based microarray can identify alterations that are not detected by traditional methods
    – Copy neutral-based loss of heterozygosity

High-throughput Genomic Testing Methods

• Advantages
  – Cost-efficient utilization of limited tissue samples to yield actionable information
    • Needs less DNA
    • Can test more than one sample at a time
    • Faster TAT compared to sequential testing
  – Information on multiple genes at once
  – Can process multiple patient samples at once
  – Usually more cost effective when assessing for alterations in multiple genes
High-throughput Genomic Testing Methods

• Disadvantages
  – Expensive instrumentation and validation costs
  – Increased technical, scientific, and medical expertise required
  – Increased risk of contamination
    • Requires robust and standardized pre-analytic and analytic processes
  – Significant pressure from a regulatory and reimbursement perspective

Multiplex Testing Will Become Standard of Care in Certain Clinical Contexts

• Paradigm of one gene, one companion diagnostic assay, and one therapy is unsustainable.
• Not enough tissue or time.
  – More cost efficient utilization.
  – Presence of multiple mutations which may affect clinical decision making.
• Cost of multiplex testing utilizing high-throughput technologies is dropping rapidly.
• Consolidation of molecular testing into multiplex panels utilizing higher-throughput and more efficient technologies.
What is Clinical Utility?

• Payer centric definition in the era of value-based care
  – Improve patient outcomes
  – Decrease health care costs

Expanded Definition of Clinical Utility

• Provide valuable information to the pathologist, treating oncologist, patient/family member, or other clinician—patient centric

<table>
<thead>
<tr>
<th>Diagnostic and predictive</th>
<th>Various hematologic malignancies</th>
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<tr>
<td></td>
<td>MDS, MPN, and MDS/MPN, end the “diagnostic odyssey”</td>
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<td>Minimal residual disease monitoring</td>
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<td>Imminent relapse</td>
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<tr>
<th>Prognostic</th>
<th>Alter clinical management of patient</th>
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<td>Heightened vigilance</td>
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<td>Institute therapeutic decisions earlier</td>
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<tr>
<th>Therapeutic</th>
<th>On or “off label” use of FDA approved targeted therapy</th>
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<td>Investigational agent in a clinical trial setting</td>
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<td>Greater than 500 targeted therapeutics in clinical development over next 5 years</td>
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Clinical Utility and Utilization Management Based on the Clinical Context

**Single Analyte Tests**
- Targeted NGS and/or Multiplex Panel(s)
  - Performed on Tissue and/or Peripheral Blood

**Broader Genomics Panels**
- Performed on Tissue and/or Peripheral Blood

Diagnosis +/- metastasis → Relapse/refractory → Advanced

Diagnosis
- Prognosis
- Chemotherapy
- Targeted Therapy
- Clinical Trials
- Other Management Decisions

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Clinical Utility and Utilization Management Based on the Morphologic Context

- BIC +/- Image Analysis
- Flow cytometry
- Cytogenetics and Cytogenomics
- In-situ hybridization
- Polymerase chain reaction (PCR)
- Gene Expression Profiling
- Sequencing
  - Sanger, Pyro, and NGS

Images courtesy of Pranit Chandra, D.O. PathGroup
Integration of Clinical, Morphologic, Genomic, and other Data

Clinical Utility and Evidence Generation

Guideline recommendations by agencies and professional societies
Randomized prospective clinical trials
Meta analysis
Retrospective clinical trials

Case studies
Case reports
Clinical Utility in Hematologic Malignancies

• Diagnosis
  – Myeloid stem cell disorders
    • Identify aberrations in genes to establish clonality required for a diagnosis of MDS, MPN, or MDS/MPN
      – JAK2, MPL, CALR, CSF3R, KRAS, NRAS, SETBP1, others
      – > 15 genes referenced in NCCN guidelines that provide diagnostic, prognostic, and/or therapeutic information across myeloid stem cell disorders including MDS

• Risk prediction
  – BCR/ABL monitoring in CML
  – Clearance of mutations in AML
Clinical Utility Cont.

- Prognosis
  - Acute Myeloid Leukemia
    - *FLT3, NPM1, CEBBPA, KIT, PHF6, RUNX1, TP53, U2AF1, ASXL1, and TET2* associated with prognosis
  - Other myeloid stem cell disorders
    - Mutation in *ASXL1, TP53, TET2, DNMT3A, SRSF2* and others generally associated with aggressive clinical course
    - More than 1 mutation by technologies such as NGS generally associated with aggressive clinical course


Better Prognostic Risk Stratification in AML

- Multiplex testing has allowed for refined prognostic risk stratification
- Mutations with **good** prognosis
  - *IDH1 or IDH2 and NPM1* with wild type *FLT3 ITD* status
- Mutations with **poor** prognosis
  - *TET2, ASXL1, PHF6* or *MLL-PTD* with mutant *FLT3 ITD* status

Refined Distribution of Prognostic Categories in AML

- Mutational profiling increases overall precision in AML prognosis
  - More precise identification of patients with favorable and unfavorable risk

Numerous Emerging Publications

- Genomic Classification and Prognosis in Acute Myeloid Leukemia
  - By various authors from New England Journal of Medicine
Clinical Utility Cont.

• Prognosis
  – Plasma cell myeloma
    • Multiplex FISH panel standard of care, per NCCN guidelines
  – Chronic Lymphocytic Leukemia
    • IgVH somatic hypermutation
    • Multiplex FISH panel per NCCN guidelines
    • TP53, SF3B1, BIRC3, NOTCH1, and ATM mutation associated with aggressive clinical course


Clinical Utility Cont.

• Therapy
  – Chronic myelogenous leukemia
  – MDS with 5q-
  – Other myeloproliferative neoplasms
  – Advanced, aggressive, and/or refractory tumors
    • Investigational use of targeted therapies in a clinical trial setting
      – Retrospective data demonstrate clinical benefit
      – Ongoing prospective clinical trials
    • Targeted therapies are in various stages of clinical development
      – PI3K, MEK, AKT, mTOR, FLT3, TRK, NOTCH, KIT, IDH, Dot1L, FGFR, JAK2, and many other inhibitors
Case Study: 76-Year-Old Man with Anemia

• Clinical findings
  – Macrocytic anemia
  – Normal white blood cell count
  – Normal platelet count

• Morphologic findings
  – Tri-lineage dysplasia with ring sideroblasts
    • No increase in blasts
  – Highly suspicious for myeloid stem cell disorder
    • RCMD and ring sideroblasts
  – Atypical ill-defined lymphoid aggregates
Case Example: Summary of Ancillary Findings

- Flow cytometry
  - Immunophenotypic abnormalities of myeloid lineage
  - Monoclonal B-cell population
- Conventional and molecular (FISH-based) cytogenetics
  - Normal conventional cytogenetic karyotype
  - FISH was negative for the commonly seen aberrations in myeloid stem cell disorders
- Next generation sequencing detected 3 somatic mutations
  - CBL
  - SF3B1
  - MYD88

Clinical Significance of Genomic Findings

- Diagnostic
  - Presence of neoplastic clone established which supported the morphologic impression in establishing a diagnosis of MDS
  - Concurrent lymphoplasmacytic lymphoma
    - Patient had IgM monoclonal gammopathy
- Prognostic
  - Literature suggests a less than favorable prognosis for MDS and LPL
- Therapeutic
  - Targeted therapies may be available in an investigational context, which may be utilized upon disease refractoriness
    - FLT3, MEK, and/or PI3K inhibitors

Case Study: 71 year old man with leukocytosis

- CBC findings
  - White blood cell count
    - **43,200** with marked absolute neutrophilia
  - Red blood cell count/Hgb/Hct
    - 5.36 million, 13.8, 47.4
  - Platelet count
    - 257,000
Peripheral smear morphology

Bone marrow
Summary of other ancillary findings

- **Flow cytometry**
  - 1.3% abnormal myeloid blast population
  - Evidence of left shifted granulocytic maturation

- **Conventional cytogenetic studies**
  - Within normal limits

- **FISH myeloid panel studies**
  - Within normal limits
    - Negative for BCR/ABL or other aberrations commonly seen in MPN

- **PCR-based studies**
  - JAK2 V617F negative
  - CALR negative

- **Next generation sequencing**
  - CSF3R T618I mutation
  - SRSF2 P95H mutation

Clinical Significance of Genomic Findings

- **Diagnostic**
  - Chronic neutrophilic leukemia

- **Prognostic**
  - Literature suggests aggressive clinical course

- **Therapeutic**
  - Targeted therapies may be available in an investigational context, which may be utilized upon disease refractoriness
    - JAK/STAT or SRC/LYN inhibitors

Key Take Aways

- Multiplex and high-throughput testing technologies offer many advantages over traditional molecular testing methods
- Such testing has the potential to improve outcomes and/or decrease health care spend by providing valuable information
  - Diagnosis
  - Prognosis
  - Therapy
- Definitions of clinical utility for molecular and genomic testing in hematologic malignancies need to be accordingly expanded
- The role of the laboratorian (pathologist, molecular pathologist/geneticist, and other molecular professionals) will continue to evolve
  - Larger and more integral part in the delivery of health care to guide both utilization and interpretation

Clinical Utility in Solid Tumor Molecular Testing

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Associate Professor of Clinical Pathology
University of Illinois at Chicago

October 5, 2016
Disclaimers/COI

1. The mention of some assays is not intended as an endorsement of some assays over others, but is merely a consequence of the limitation of time.

2. Common stock ownership in Cepheid, Inc

October 5, 2016

Solid Tumor Oncology

**Learning objectives:**

1. Appreciate the breadth of clinical utility in solid tumor molecular testing

2. Efficiencies and economies of molecular oncology testing
Solid Tumor Oncology

- Diagnostic
- Prognostic
- Predictive

Solid Tumor Oncology

- Thyroid lesions
- Colon lesions
- Brain tumors
- Cystic neoplasms of the pancreas
- Soft tissue tumors
- Melanoma
Solid Tumor Oncology

Indeterminate thyroid FNA

- Gene expression profiling, microRNA profiling and/or mutational profiling can help yield diagnostic information.
- Certain mutations associated with higher likelihood of malignancy.
  - BRAF V600E mutation and RET fusion are very specific for malignancy.
  - RAS mutation and PAX-8/PPAR gamma fusion associated with increased likelihood of lesion being malignant.

Tumor type | Abnormality
--- | ---
Medullary thyroid CA | RET translocation
Papillary thyroid CA | BRAF, RET-PTC fusion
Follicular thyroid CA | RAS, PAX8, PPARγ1
Colon -FAP | APC truncation, deletion, missense
Colon- HNPCC/sporadic | dMMR genes/BRAF V600E
Juvenile polyposis | SMAD4/DPC4 deletions
Cowden | PTEN
Li Fraumeni | TP53
Peutz-Jeghers | LKB1/STK11
Solid Tumor Oncology

Diagnostic

- **Brain tumors**
  - Loss of 1p and 19q in 60-80% of oligodendrogliomas
  - EGFR amplification in high grade astrocytomas (GBM)
  - IDH1 mutations in up to 75% of grade II-III diffuse gliomas

- **Cystic neoplasms of the pancreas** - these are a heterogenous group of tumors
  - 25% being mucinous, harboring a risk of malignancy ranging from 10-15% (KRAS mutation, p53 overexpression, loss of DPC4)
  - 50% of IPMNs found to harbor GNAS activating mutations
  - Sporadic & familial serous cystic neoplasms: VHL alterations, overexpression of VEGF, GLUT-1, HIF1-a, and CAIX
### Solid Tumor Oncology

#### Diagnostic

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewing family of tumors</td>
<td>t(11;22)(q24;q12) - EWS-Fli1</td>
</tr>
<tr>
<td>Desmoplastic small round cell tumor</td>
<td>t(11;22)(p13;q12) - EWS-WT1</td>
</tr>
<tr>
<td>Alveolar rhabdomyosarcoma</td>
<td>t(2;13)(q35;q14) - Pax3-FKHR</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>t(X;18)(p11.23;q11) - SYT-SSX1, 4</td>
</tr>
<tr>
<td>Liposarcoma (myxoid and round cell)</td>
<td>t(12;16)(q13;p11) - TLS (FUS)-CHOP</td>
</tr>
<tr>
<td>Atypical lipomatous tumor, well differentiated liposarcoma</td>
<td>12q rings and giant markers - HMGIC, CDK4, and MDM2 amplification</td>
</tr>
<tr>
<td>Extraskeletal myxoid chondrosarcoma</td>
<td>t(9;22)(q22;q12) - EWS-CHN (TEC)</td>
</tr>
</tbody>
</table>

Melanocytic lesions: diagnostically challenging
- **myPath Melanoma test:**
  - performed on RNA extracted from FFPE tissue
  - qRT-PCR of 14 signature and 9 housekeeping genes
  - generate a myPath Melanoma score
Solid Tumor Oncology

Prognostic

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Abnormality/Assay/Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic Breast CA</td>
<td>p53 missense, Oncotype Dx/Mammaprint</td>
</tr>
<tr>
<td>Familial Breast CA</td>
<td>BRCA1, BRCA2</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>p16INK4A loss; KRAS, p53 mutation; EGFR, MET amplification</td>
</tr>
<tr>
<td>Colon carcinoma: Good</td>
<td>MSI-H, CIMP;</td>
</tr>
<tr>
<td>Prognosis</td>
<td></td>
</tr>
<tr>
<td>Colon carcinoma: Poor</td>
<td>CIN, p53, BRAF and KRAS mutations</td>
</tr>
<tr>
<td>Prognosis</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal Stromal</td>
<td>KIT exon 11 deletion- poor prognosis but good response to TKIs</td>
</tr>
<tr>
<td>Tumor (GIST)</td>
<td></td>
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</tbody>
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Solid Tumor Oncology

Prognostic: Sporadic Breast CA

- Oncotype Dx: ASCO, NCCN, St. Galen
- Mammaprint: St. Galen
- Prosigna PAM-50: ASCO, NCCN, St. Galen
- Breast Cancer Index: ASCO, St. Galen
- Endopredict: ASCO, St. Galen
Solid Tumor Oncology

Prognostic: Colon CA

- Good prognosis: MSI-H
  - MSI status is consistently associated with survival of CRC: a recent meta-analysis showed MSI-high CRC to be associated with a 40% better overall survival rate than MSS CRC
- Poor prognosis: CIN, p53, BRAF and KRAS mutations
- Oncotype Dx: multi-gene test for predicting recurrence risk in patients with stage II and stage III A/B colon cancer

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Prognostic: Prostate adenoCA

- Oncotype Dx:
  - The majority of patients diagnosed with low-risk prostate cancer undergo surgery or radiation Rx despite only a 3% chance of mortality.
  - Genomic Health’s bx-based, multi-gene test has been clinically validated to predict aggressive cancer at the time of diagnosis, helping to stratify patients into immediate Rx vs active surveillance groups.
Solid Tumor Oncology

Predictive: Lung adenoCA

- Positive predictor of response to Rx:
  - EGFR TK domain mutations (exon 19 del, L858R; exon 18 G719X; exon 21 L858R)
  - EML4-ALK fusions

- Negative predictor of response to Rx:
  - KRAS mutations
  - MET amplification
  - Resistance mutations: EGFR - insertions in exon 20; S768I, L747S, D761Y, T854A and T790M (secondary)

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Predictive: Colon CA

- KRAS and NRAS mutations predict lack of response to monoclonal EGFR therapy
- Poor response of MSI-H to 5FU based chemoRx
- Emerging evidence that BRAF mutations may respond better to a therapeutic strategy utilizing a combined approach
  - (i.e. EGFR, PIK3CA, and/or MEK therapy)
- Limited studies show PIK3CA mutation associated with improved survival with aspirin use
Solid Tumor Oncology

Predictive: Colon CA

- Recommended mutations for routine genotyping (>1% prevalence):
  - KRAS: codons G12, G13 (could be first step in algorithm approach)
  - KRAS: minor codons Q61, A146
  - NRAS: codon Q61
  - BRAF: codon V600
  - PIK3CA: codons E542, E545, H1047

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Predictive: Colon CA

- Additional markers:
  - Additional negative predictors: PTEN mutation/loss, AKT1 E17K mutation, possibly HER2 amplification
  - Positive predictor: Expression of EGFR ligands epiregulin (EREG) and amphiregulin (AREG) by RT-PCR
### Solid Tumor Oncology

#### Colon CA

- Economic value of screening for KRAS mutations as predictors of response to EGFR inhibitors*:

<table>
<thead>
<tr>
<th></th>
<th>KRAS screening</th>
<th>No screening</th>
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<tr>
<td>Cetuximab (KRAS neg) n=60**</td>
<td>$600,000</td>
<td>$600,000</td>
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<tr>
<td>Cetuximab (KRAS pos) n=40</td>
<td>$0</td>
<td>$400,000</td>
</tr>
<tr>
<td>KRAS testing analysis cost***</td>
<td>$80,000</td>
<td>$0</td>
</tr>
<tr>
<td>Total cost of care</td>
<td>$680,000</td>
<td>$1,000,000</td>
</tr>
<tr>
<td>Cost savings</td>
<td>$320,000</td>
<td>$0</td>
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*For 100 Metastatic CRC patients being considered for Cetuximab therapy  
**Based on a hypothetical cost of $10,000/month for Cetuximab  
***Based on a cost of $800 for KRAS testing

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### Solid Tumor Oncology

#### Predictive: Cutaneous Melanoma

- **BRAF V600 mutations**
  - found in 35-66% of cutaneous melanomas – Vemurafenib potent inhibitor but ineffective in wt BRAF tumors (OS 6m/RR/PFS= 84%/48%/5.3m vs 64%/5%/1.6m for dacarbazine)
  - Acquired resistance develops in <1 year with single agent, therefore dual inhibition of MAPK pathway recommended

- **NRAS exon 2 and exon 1 mutations**

- **KIT mutations**
Doctors would often diagnose illnesses by examining urine and were taught that this would demonstrate the body was imbalanced, according to the theory of the four humours. This medieval approach to illness, originally developed by ancient Greek physicians Hippocrates and Galen, stated that illness was caused when one of four bodily liquids (or humours), namely blood, phlegm, black bile and yellow bile, fell out of balance.

Physicians used many different techniques to analyze the imbalance of the four humours in the body. Uroscopy was most widely used for diagnosing illness. Physicians would collect patients urine in a flask called “matula”. 
Solid Tumor Oncology

An evolving science

- We are still in the early stages of figuring out the secrets of the abnormalities of the human genome
- It behooves us to be more permissive in discovery testing, but more stringent in inference once we have sufficient data
- Stringency in discovery testing would leave large holes in oncology knowledge and set back implementation of Precision Medicine

Gefitinib, the first EGFR-TKI evaluated in a phase III trial was initially said to demonstrate response in only 10% of the cohort and no survival benefit was conferred. Lack of significant clinical benefit led to withdrawal of gefitinib from the US market.

- But patients were not prospectively stratified according to mutation status. Follow-up analysis identified activating EGFR mutations in 8 of 9 responders and no mutations were seen in 7 patients who did not respond to this Rx.
- Gefitinib returned in July 2015 with restricted availability
- FDA approved it as first-line Rx for metastatic lung NSCLC with exon 19 del or exon 21 (L858R) mutations based on IFUM trial.
An evolving science: Another consideration

- Perhaps we should move from a ‘tumor-centric’ classification to a ‘gene-’ or ‘pathway-’ or ‘omics-’ centric classification?
  - Guinney et al.* – colon CA reclassification into CMS1, CMS2, CMS2-3, CMS4 and Mixed groups
  - Hoadley KA et al.** – multiplatform analysis reveals molecular classification within and across tissues of origin

** Cell, 2014. 158(4):929-944

Solid Tumor Oncology

An evolving science: Another consideration

- TCGA and BROAD institute data covering 4,742 tumor-normal paired samples determined a set of 254 significantly mutated genes
- About 127 mutations had a prevalence of 1% or higher, suggesting neither WGS nor WES will be routinely necessary

Slide courtesy Dr. L. Joseph
Solid Tumor Oncology

An evolving science

“To boldly go where no one has gone before”

“If you eliminate the impossible, whatever remains, however improbable, must be the truth.” - Spock quoted from The Sign of the Four by Sir Arthur Conan Doyle

Image courtesy: http://www.phrases.org.uk/images/enterprise.jpg