Clinical Next Generation Sequencing: Just Another Lab Test

The presentation will start momentarily!
Clinical Next Generation Sequencing: Just Another Lab Test

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- Professor of Pathology, Professor of Obstetrics and Gynecology, and Director of the Molecular Genetic Pathology Fellowship
- He has led development of Genomics and Pathology Services at WUSTL
- He is involved in next generation sequencing clinical test design and in the evaluation of different platforms for clinically oriented next-generation sequencing
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Disclosure

Vendor-Client relationships with:

- Illumina
- Agilent
- Life Technologies
- Affymetrix
What I’m not going to do…

• Compare and contrast the various platforms for Next Generation Sequencing (NGS)

• Compare and contrast the various chemistries

• Discuss the different approaches to library preparation, analysis of RNA or epigenetic changes, and so on

Instead focus on what we’ve learned at GPS@WUSTL regarding the clinical application of NGS, in a CAP/CLIA environment, as a component of direct patient care, that is reimbursed.
… regarding the clinical application of NGS, in a CAP/CLIA environment, as a component of direct patient care…”

- NGS is a technique for DNA sequence analysis
- Because it is by definition massively parallel, it provides the opportunity to evaluate panels of genes (in addition to single genes or small sets of genes)
- Other methods can also be used to evaluate panels of genes, but NGS provides more flexibility to detect a broader range of mutation types in a larger target region, which we’ve leveraged for a panel of cancer genes
- Clinical application creates novel regulatory issues
- Can get reimbursed to do the testing
Over 150 faculty and staff support GPS function
- Acquisition of pharmaceutical industry talent
- Board-certified geneticists and physicians
- Board certified anatomic & molecular pathologists
- Computational biologists, bench scientists
- Software engineers, informaticians
- Biostatisticians, IT administrators

Pathology Services
- 53,000+ Surgical Pathology Cases/yr
- 1,800+ Consults/yr
- Over 700,000 well annotated research specimens

Major Investments in capital, space, & software development
- ~15,000 sq ft dedicated labs; majority CAP/CLIA
- Clinical Genomicist Workstation
## General differences between NGS for clinical versus basic science applications

<table>
<thead>
<tr>
<th></th>
<th>Clinical</th>
<th>Basic Science</th>
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<tbody>
<tr>
<td><strong>Goals</strong></td>
<td>aid diagnosis, provide prognostic information, direct therapy in a clinically relevant time frame</td>
<td>discovery</td>
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<tr>
<td><strong>Operational Measures</strong></td>
<td>traditional clinical laboratory metrics (sensitivity, specificity, PPV, NPV, etc.)</td>
<td>quality metrics vary based on scientific question</td>
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<tr>
<td><strong>Regulation</strong></td>
<td>CAP/CLIA, HIPPA, IRB</td>
<td>IRB</td>
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<tr>
<td><strong>Reimbursement</strong></td>
<td>insurance companies, institutional payors, research grants</td>
<td>research grants</td>
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General implications of these differences

Clinical NGS
- Need high coverage (about 1000x) for low FP rate, high PPV
- Panels of genes
- Must detect full spectrum of mutations
- Must be CAP/CLIA licensed to get reimbursed

Discovery NGS
- Low coverage (in the 20-30x range, or lower) is acceptable
- Larger number of genes
- Generally limited to a subset of mutations dictated by research question
- Accreditation not an issue
Operational issues

• For constitutional disorders, mutations are homozygous (100% allele frequency) or heterozygous (50% allele frequency)

• Somatic tumors contain mutations that may be homozygous or heterozygous; contain different clonal subpopulations; and contain tumor cells that are diluted by stromal cells, endothelial cells, and inflammatory cells

• For most tumor types, only FFPE tissue is available for analysis

• The bioinformatic pipeline must detect the full range of relevant mutations, including SNVs, CNVs, indels, and translocations
WUCaMP28

- Oncology panel, not constitutional disease panel
- 28 genes, all with an established roll in patient care for Dx, Px, or Tx
- For each gene, target region includes all exons, about 200 bp of upstream and downstream intron, and about 3 kb of promoter region; entire target region is about 500 kb
- Target 1000X depth of coverage
- Currently reimbursed
WUCaMP28 Cancer Set

**Solid tumors**
- PDGRFA
- BRAF, PTEN
- CTNNB1, KRAS
- NRAS, PIK3CA
- TP53, MAP2K2
- MAPK1, MET
- RET, EGFR

**Hematologic cancers**
- ALK
- KIT
- WT1
- CSF1R
- IDH1
- IDH2
- JAK2
- PTPN11
- CEBPA
- DNMT3A
- FLT3, MLL
- NPM1, RUNX1
- CHIC2
The bulk of a tumor is **not** comprised of tumor cells.

If 50% of the tumor is stroma, then the relative allele frequency of heterozygous somatic mutations will be 25%.
Must be optimized for Formalin Fixed Paraffin Embedded (FFPE) tissue

- Most surgical pathology specimens (greater than 95%) exist as FFPE tissue only
- Robust clinical assays must work from FFPE tissue
- All GPS cases undergo surg path review prior to testing
There is significant coverage variation by gene, but little variation in coverage by gene between fresh and FFPE tissue.

References:
M Kerick et al. *BMC Medical Genomics* 2011;4:68
Sensitivity of SNV detection varies by software tool

**GATK**

**SamTools**

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Tuning the bioinformatics can increase sensitivity and PPV for SNVs

Detection of 10% Allele Frequency versus Coverage (GATK)

- About 1000x coverage results in a sensitivity of about 90% for 10% allele frequency mutations.
- The positive predictive value remains >99% even with lower coverage.
Detection of indels also varies by software tool: FLT3 ITD detection is an example

- Tested a set of 24 cases with known FLT3 ITDs by WUCaMP28
- Pindel correctly identified ITDs in 23/24 cases
- Identified all ITDs (24/24) by de-novo assembly methods (detected allele frequencies of less than 4%)
- No false positive results

<table>
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<tr>
<th>PCR/GC Size</th>
<th>PINDEL</th>
<th>ASSEMBLY</th>
<th>SAMTOOLS</th>
<th>GAATK</th>
<th>DINDEL</th>
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red = not detected  
green = detected

Reference: EJ Duncavage et al. submitted
Detection of larger scale structural variation is also dependent on the bioinformatic pipeline

- **Structural Variation**
  - Translocations
  - Large insertions or deletions
  - Inversions

- **Account for up to 5-10% of genomic variation in malignancy**

- **Difficult to detect in NGS data due to short read lengths**
All of which requires a novel bioinformatic pipeline

research databases are not CAP/CLIA compliant

“in the cloud” is not HIPPA compliant
• The economics of reimbursement dictate that:

• CGW use a rules-based approach to template the report using the clinical history (employing natural language functionality), the panel ordered, and known genotype-phenotype correlations (influenced by results at other genes in the panel)

• A report is issued only on those genes that were ordered (also for liability reasons)

• Provides the option for reinterpretation at a later date
Reimbursement

• Were we to use traditional “code stacking” the CMS allowable for the WUCaMP28 would be $180,028.77

• We therefore use CPT code 81407, which is currently a CMS “non-valued code”

• Reimbursement varies between institutional payors, Medicare, private insurance carriers, and research organizations
Reimbursement, continued

• We initially performed all the payor contact (precertification and predetermination) of ordered tests to establish “best practices”

• Thus far, precertification is not required for about 55% of patients, requires a phone call for about 33%, and requires additional information for about 12%

• Thus far, testing has been approved for 88% of patients and denied for 5% (decision pending for 7%).
Although clinical next generation sequencing may be just another lab test…

A man who carries a cat by the tail learns something he can learn in no other way.

- Mark Twain
Acknowledgements

• Karen Seibert, PhD
• Shashi Kulkarni, PhD
• Rich Head, MS
• Andy Bredemeyer, PhD
• Rakesh Ragarajan, MD, PhD
• Robi Mitra, PhD

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• David Spencer, MD, PhD
• Kris Rickhoff
• Andy Drury
• Skip Virgin, MD, PhD
• Jeff Milbrandt, MD, PhD
Molecular Diagnosis of Acute Myeloid Leukemia: present and future challenges  
Thursday, May 31, 12:00-1:00 pm Central  
- David Czuchlewski, MD, FCAP and Mohammad Vasef, MD, FCAP

While personalized molecular approaches to solid tumors continue to evolve, genetic information has long been critical in the diagnosis and classification of acute myeloid leukemia (AML). In this webinar, we will review the development of current diagnostic tools that further refine the distinct subtypes of AML, with a practical focus on questions likely to occur in the setting of a general pathology practice, including: How can molecular data be used to guide patient care? Which patients need molecular testing? Which genes should be evaluated, and by what methods? In addition, we will present an update to the 2008 WHO classification, including new information on established targets and novel gene mutations likely to impact clinical practice in the near future. Finally, we discuss the revolutionary changes coming to molecular diagnosis of AML in the dawning era of next-generation sequencing.
Don’t Forget to Check Out Past Webinars!

- Past Webinars Available Now Online at www.cap.org/institute
  - Molecular Testing Selection Guidelines for Selecting Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors
  - Clinical Use of Whole Genome and Whole Exome Sequencing Today
  - Validating Whole Slide Imaging Systems for Diagnostic Use in Pathology
  - The Why, What and How of Identifying Patients at Risk
  - Molecular Diagnosis for Lung Cancer Patients
  - Whole Genome Analysis as a Universal Diagnostic: A Pathologist’s Perspective
  - Next-Generation Sequencing for the Clinical Laboratory

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## CAP Learning – Molecular Oncology CME

<table>
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<tr>
<th>Course</th>
<th>Learning Objectives</th>
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| **Molecular Pathology: An Introduction to DNA Technology and Diagnostic Applications (SAM eligible)**<br>CME/SAM – 2.0 | -Identify potential application of molecular pathology  
-Describe the chemical structure and properties of DNA and RNA  
-Explain the different types of genetic variations  
-Identify diagnostic techniques in molecular pathology|
| **Archives Applied: KRAS (SAM eligible)**<br>CME/SAM – 1.0            | -Identify whether anti-EGFR therapy is an appropriate treatment method for a patient case  
-Describe advantages and limitations of specific KRAS mutation testing methods  
-Identify the appropriate elements to include in the report for a patient case  
-Describe the current role of KRAS mutation testing for management of patients with metastatic colorectal cancer |
| **Archives Applied: Molecular Test Validation (SAM eligible)**<br>CME/SAM = 1.0 | -Identify the appropriate:  
-Test parameters for an analytic quantitative or qualitative test  
-Clinical performance characteristics for test validation  
-Performance characteristics for a quantitative or qualitative test  
-Elements to include in test validation documentation  
-Identify pre-validation considerations for a proposed molecular pathology test |
| **Archives Applied: Molecular Diagnostics of Soft Tissue Tumors (SAM eligible)**<br>CME/SAM = 1.0 | -Recognize which genetic alterations seen in soft tissue tumors are amenable to molecular diagnostics using routine clinical genetic approaches  
-Describe characteristics of chromosomal translocations in soft tissue sarcomas  
-Identify the advantages and limitations of conventional cytogenetic analysis for soft tissue tumors  
-Identify approaches for assessing inactivation of a tumor suppressor gene, for example the SMARCB (INI1) in soft tissue tumors  
-Identify the advantages and limitations of molecular cytogenetic analysis for soft tissue tumors |
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| **Molecular Testing for AML Cases**        | - Recognize molecular oncology knowledge and skills required of pathologists that can mitigate problems and enhance patient care with respect to specimen handling  
- Realize the effects that appropriate specimen handling and communication throughout all stages of diagnosis have in enhancing patient care  
- Reflect on your own knowledge and skills in specimen handling and patient care, and identify what can help you and your practice be more effective in these areas of molecular oncology |
| CME – .5                                   |                                                                                                                                                                                                                      |
| **BRAF Mutation Testing in Melanoma**      | - Follow quality assurance policies and procedures to ensure adequate sample collection and proper handling techniques for molecular oncology tests  
- Use appropriate result reporting principles for incorporating molecular test results into surgical pathology reports |
| CME – .5                                   |                                                                                                                                                                                                                      |
| **Molecular Testing for Lymphoma Cases**   | - Recognize molecular oncology knowledge and skills required of pathologists that can mitigate problems and enhance patient care with respect to specimen handling  
- Realize the effects that appropriate specimen handling and communication throughout all stages of diagnosis have in enhancing patient care  
- Reflect on your own knowledge and skills in specimen handling and patient care, and identify what can help you and your practice be more effective in these areas of molecular oncology |
| CME - .5                                   |                                                                                                                                                                                                                      |
| **Molecular Testing for EGFR and KRAS Mutations** | - Recognize the indications for EGFR and KRAS molecular testing as they pertain to non-small cell lung cancer  
- Interpret molecular diagnostic test results and correlate them with the diagnosis pertaining to non-small cell lung cancer |
| CME - .5                                   |                                                                                                                                                                                                                      |
| **BRAF Mutations in Thyroid Cases**        | - Recognize the importance of BRAF mutation testing for preoperative diagnosis of thyroid cancer.  
- Recognize the important role that interpreting molecular testing results has in patient management.  
- Recognize how selecting patients with cytologically indeterminate thyroid nodules for molecular testing can enhance the accuracy of cytologic diagnosis. |
| CME - .5                                   |                                                                                                                                                                                                                      |
## Course Learning Objectives

| Course | Molecular Diagnosis of Ewing Sarcoma  
| CME - .5 | - Review sample requirements and handling for RT-PCR, FISH, and cytogenetic analysis as they pertain to evaluating mesenchymal neoplasms  
- Describe the advantages and limitations of genetic approaches commonly used in the classification of mesenchymal neoplasms to include conventional karyotyping, FISH, and RT-PCR  

| Course | BPFT Testing Self Study  
| CME /SAM – 2.5 | - Explain the ASCO-CAP ER/PR Testing Guidelines and their implications for lab procedures, test results and patient care.  
- Explain the ASCO-CAP HER2 Testing Guidelines and their implications for lab procedures, test results and patient care.  
- Determine if the assay and tissue sample are appropriately matched per the ASCO/CAP Guidelines.  
- Explain the biology of fixation interactions with assay performance.  
- Explain the potential use of molecular analysis in patient care decisions.  
- Mitigate problems and enhance patient care with respect to specimen handling  

| Course | HER2 FISH Test Interpretation Accuracy  
| CME/SAM – 1.5 | - Accurately interpret HER2 FISH tests.  
- Correct for HER2 FISH interpretative errors.  
- Recognize the relationship between HER2 FISH test results and patient treatment.  

| Course | BPFT Reporting  
| CME/SAM – 1.5 | - Apply the ASCO-CAP ER/PR and HER2 Guideline criteria to all reports in a standardized manner.  
- Create consistent, standardized and integrated reports.  
- Remediate inconsistent data and provide a resolution in an integrated report.  
- Create patient friendly reports.  
- Use formatting techniques to create clear and understandable reports.  

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## CAP Learning – Molecular Oncology CME

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| **ER IHC Test Interpretation Accuracy**  <br>CME/SAM – 2.0 | - Plan and perform a proper ER IHC test validation.  
- Accurately perform and interpret ER IHC tests, including the proper evaluation of appropriate controls and test tissues.  
- Evaluate and integrate ER staining patterns with clinical and morphologic findings.  
- Identify the relationship and impact of ER IHC test results on patient treatment. |
| **HER2 IHC Test Interpretation Accuracy**  <br>CME/SAM – 2.0 | - Plan and perform a proper HER2 IHC test validation in accordance with ASCO-CAP guidelines for HER2 testing.  
- Accurately perform and interpret HER2 IHC tests, including the proper evaluation of appropriate controls and test tissues.  
- Evaluate and integrate HER2 staining patterns with clinical and morphologic findings to help improve concordance with HER2 FISH results.  
- Identify the relationship and impact of HER2 IHC test results on patient treatment. |
| **Archives Applied: Acute Erythroid Leukemia (SAM Eligible)**  <br>CME/SAM – 1.0 | - Identify the current criteria for the diagnosis of AEL.  
- Identify the clinical features of AEL.  
- Identify typical morphologic and cytochemical findings of AEL.  
- Identify Immunophenotypic findings of AEL.  
- Distinguish the features that indicate a diagnosis of AEL versus other types of AML. |
| **Archives Applied: Low-Grade Papillary Urothelial Carcinoma (SAM Eligible)**  <br>CME/SAM – 1.0 | - List the 2004 WHO/ISUP criteria for low-grade papillary urothelial carcinoma (LG-UrCa).  
- Identify the clinical outcome of patients with a confirmed LG-UrCa diagnosis.  
- Recognize indicators of risk for recurrence, grade progression, or stage progression of LG-UrCa.  
- Distinguish the features that indicate a diagnosis of LG-UrCa versus high-grade papillary urothelial carcinoma (HG-UrCa) for a noninvasive papillary urothelial neoplasm.  
- Summarize the outcome of patients with LG-UrCa following the 2004 WHO/ISUP consensus grading classification. |
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The CAP Learning Portal includes new tools to support the learning needs of pathologists such as:

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- Competency Model for Pathologists
- Personal Progress Check (member only tool)
- My Learning Plan (member only tool)
- Help Center

Benefits
- Increase effectiveness to plan and manage learning
- Increase efficiency to target learning needs and identify premium learning solutions
- Increase satisfaction with learning solutions that meet specific learner needs
- Increase capability to maintain professional certifications
To learn more…

For more details and to register for/access Molecular Oncology educational offerings:

1. Log in to the cap.org website
2. Click on Launch Portal
3. Click on the Learning Options tab
4. Type Molecular Oncology in the Search box

A list of available learning options displays
THANK YOU!

Thank you for attending our webinar “Clinical Next Generation Sequencing: Just Another Lab Test” by John D. Pfeifer, MD, PhD, FCAP.

For comments about this webinar or suggestions for upcoming webinars, please contact Jill Kaufman, PhD, Director of Personalized Health Care at jkaufma@cap.org

NOTE: There is no CME/CE credit available for today’s free webinar.