CCTC Clinical Chemistry Trainee Council | AACC Better Health Through Laboratory Medicine

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: Introduction to Cancer Genetics
PRESENTER: Deepika Sirohi, MD

Slide 1:

Hello, my name is **Deepika Sirohi**. I am a **Molecular Genetic Pathology Fellow at University of California, San Francisco**. Welcome to this Pearl of Laboratory Medicine on "**Introduction to Cancer Genetics**."

Slide 2:

Molecular genetics is increasingly being applied to solid tumors for establishing definitive diagnosis; providing a tool to inform clinical management decisions by serving as prognostic and predictive markers as well as guide treatment decisions to initiate or monitor therapy.

Slide 3:

Some of the more common tumor types that are received for molecular testing are listed here and we will discuss the testing strategies and guidelines for some of these in this talk. Many sarcomas have specific molecular alterations that can be targeted to confirm diagnosis, however these are too vast and for the sake of brevity are not being covered in this module.

Slide 4:

One of the most frequently tested tumors are lung adenocarcinomas that can have specific molecular alterations that can be used to inform treatment strategies. *EGFR* and *KRAS* mutations are the most frequent alterations in lung adenocarcinomas. Mutations in *EGFR* are known to occur in East Asian women with no history of smoking and commonly have a bronchoalveolar histologic pattern. *KRAS* on the other hand,

occurs more often in non-Asians and smokers in poorly differentiated adenocarcinomas with mucinous and bronchoalveolar features. While Tyrosine kinase inhibitors (TKIs) are used to target lung carcinomas with *EGFR* mutations, presence of *KRAS* mutations is a negative predictor of response to TKIs. Other alterations of therapeutic significance are *EML-ALK4* and *ROS1* fusions that are amenable to Crizotinib therapy and *BRAF* mutations, which confer resistance to EGFR inhibition but respond to MEK inhibitors.

Slide 5:

EGFR mutations are seen in up to 25-30% of non- small cell lung carcinomas. Clinically significant mutations occur in the tyrosine kinase domains and are the most reliable predictors of response to TKIs. Mutations such as exon 19 deletion and L858R in exon 21 predict sensitivity to TKIs, whereas insertions in exon 20 suggest resistance. A missense mutation T790M is known to confer secondary resistance in patients being treated with TKIs.

Slide 6:

Codon 12 and 13 are mutation hotspots in the *KRAS* gene that are mutually exclusive of *EGFR* mutations. They are predictive of reduced sensitivity to Cisplatin and better response to Paclitaxel and Pemetrexed. *ALK* rearrangement in lung carcinomas usually involves fusion with *EML4*, but can involve other partner genes. The rearrangement can be tested by FISH break apart testing. A small proportion of lung carcinomas show *BRAF* mutations and *ROS1* gene rearrangements.

Slide 7:

The American Society of Clinical Oncology recommends *EGFR* as the first line test in limited specimens. For biopsy specimens where adenocarcinomas cannot be excluded, *EGFR* and *ALK* testing is still recommended. On the contrary, in resected specimens where no adenocarcinoma component is identifiable, *EGFR* and *ALK* testing is not recommended.

Slide 8:

In the context of colorectal carcinomas (CRC), oncogenesis follows one of the 3 pathways: Familial adenomatosis polyposis (FAP) pathway associated with chromosomal instability and causes downstream activation of APC/WNT signaling; the

serrated pathway with aberrant CpG methylation, a subset of which can have epigenetic silencing of the *MLH1* gene and consequent microsatellite instability; and the hereditary microsatellite instability (MSI) or Lynch syndrome caused by germline mutations in one of the DNA mismatch repair genes.

Slide 9:

Testing for mismatch repair (or MMR) gene alterations is best achieved by an algorithmic approach that starts with immunohistochemical testing for the 4 mismatch repair proteins. The absence of one or more of the MMR proteins by immunohistochemistry (IHC) suggests a MSI carcinoma that could be either sporadic or inherited. These can be differentiated by testing for *MLH1* promoter hypermethylation seen in most cases and *BRAF* mutations that are present in 3/4th of these. If these are positive, this supports a sporadic microsatellite instability and if these are negative, germline testing for alterations in the MMR gene is indicated. Lynch syndrome is caused by germline mutations in one of the four mismatch repair genes which are present in a third of the cases with microsatellite instability

Slide 10:

The clinical significance for MSI testing is substantiated by the better prognosis of MSI-H carcinomas and their resistance to 5-fluorouracil, cisplatin and alkylating agents. Instead they show better response to irinotecan based therapies with early data suggestive of better response to anti- PD-1 /PD-L1 immune checkpoint inhibitor therapy.

Slide 11:

Single gene testing is important in colorectal cancers to guide treatment decisions. Even though *EGFR* mutations are very rare in colorectal cancers, they can be sensitive to EGFR-directed therapy. Therapeutic decisions can be guided by testing for *KRAS* mutations that when present are indicative of resistance to anti- EGFR antibodies. Common *KRAS* mutations differ among smokers and never smokers. Other less frequently encountered genetic alterations occur in *BRAF*, *PIK3CA*, *NRAS*, *MEK1* and *AKT* genes.

Slide 12:

In line with this, the recent ASCO/CAP recommendation for CRCs are to test for *KRAS* codons 12, 13, 61, and 146 and *NRAS* mutations which together are also called an extended RAS panel. Though mutations in *BRAF* (i.e. V600E) and *PIK3CA* have been shown in some studies to correlate with poor outcomes and decreased response to EGFR antibodies, there is insufficient evidence to recommend testing for these genes under current standard of care guidelines.

Slide 13:

Mutations in melanomas show a somewhat site specific pattern with *BRAF* mutations being more frequent in melanomas of intermittently sun damaged skin, *NRAS* in nodular melanomas of sun-damaged skin, *KIT* in acral and mucosal melanomas and *GNAQ*/*GNA11* in uveal melanomas. *BRAF* V600E is the most frequent alteration seen in melanomas and can be targeted with a specific antibody, Vemurafenib, that has changed the management and outcomes of many advanced melanomas.

Slide 14:

Another tumor type in which molecular testing is frequently requested is glial neoplasms of the brain. The 2016 WHO Classification of Tumors of the Central Nervous System now requires both an IDH gene family mutation and a 1p/19q-co-deletion to establish the definitive diagnosis of grade II and grade III oligodendroglioma These two alterations confer a better prognosis as well as predict response to chemotherapy and radiation therapy. A 1p/19q co-deletion pattern has been shown to have a high association with oligodendroglial morphology and is thought to be an early event in tumor development, although they can also be seen in up to 5-10% of Glioblastoma multiforme with unclear significance.

Slide 15:

EGFR amplifications or mutations are seen in up to half of high grade gliomas and the amplification is essentially pathognomonic of glioblastoma multiforme (or GBM). The amplification is more often seen in primary GBMs and in older patients and is not predictive of response to EGFR therapy. Somatic mutations of *IDH1* or *IDH2* can be seen in up to 75% of grade II and III gliomas (including oligodendrogliomas) and can co-occur with *TP53* alterations or 1p/19q deletions. Mutations in *IDH1* and *IDH2* are highly specific for gliomas (although they may be seen in other tumor types including acute

myeloid leukemia, cartilaginous tumors, and thyroid cancer) and are predictive of a favorable prognosis.

Slide 16:

HER2 or *ERBB2* is amplified in approximately 15 to 20% of primary breast cancers and targetable with specific therapy with trastuzumab or lapatinib. Per ASCO/CAP recommendations *HER2* testing should be done in all invasive primary breast cancers and metastatic tumors if specimen is available by IHC and/or FISH. ASCO/CAP guidelines also include specific recommendations regarding tissue fixation and scoring to ensure accuracy of testing and utility as a predictive marker. Additionally, there are germline changes in certain genes that can predispose to breast carcinoma. Identification of these in familial cancers can help guide the health care of family members, including and take preventative measures, if required.

Slide 17:

Another testing modality for predicting prognosis and guiding therapy is gene expression profiling using an array of biomarkers. Oncotype DX uses 21 genes and is recommended for women with early stage (I-IIIa) hormone-receptor-positive, HER2-negative, invasive breast cancer. The recurrence risk is stratified into 3 categories of low, intermediate, and high risk. MammaPrint, in contrast, uses a 70-gene array and is FDA approved for node-negative, treatment naive breast cancer patients with stage I or II disease. Patients are stratified into low or high recurrence risk categories. Other expression profiling tests for breast carcinomas include PAM50 and Theros.

Slide 18:

Gastrointestinal stromal tumors (or GIST) are the most common soft tissue tumors of the GI tract that show *KIT* mutations in up to 95% of spindled lesions that makes them amenable to treatment with tyrosine kinase inhibitors such as imatinib. Exon 11 mutations are the most frequent and have been shown to predict worse outcomes. Exon 9 mutations in *KIT* are more often seen in GISTs of the small intestine and colon. 5% of GISTs have mutations in *PDGFRA* and these tumors typically have epithelioid morphology. Certain mutations in *KIT* and *PDGFRA* confer resistance to imatinib. Some of these mutations, such as those in exon 9, can be managed with a higher dose or alternative TKIs.

Slide 19:

Mutations in thyroid cancers are commonly either point mutations or translocations. The *BRAF* V600E mutation is seen in more than half of papillary thyroid carcinomas (or PTC) and is especially frequent in the classic and tall cell variants. *BRAF* mutation is an early event and is associated with worse outcomes. Translocations involving *RET/PTC* occur in PTC, commonly in radiation induced cancers while *RAS* mutations are seen in papillary or follicular carcinomas and *PAX8/PPAR*γ rearrangements typically are identified in follicular carcinoma in younger individuals. *RET* mutations on the other hand are seen in Medullary Thyroid carcinomas.

Slide 20: References

1. Hunt J.L. Molecular testing in solid tumors: an overview. Arch Pathol Lab Med 2008;132:164-167
2. Travis W.D, Brambilla E, Riely G.J. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol 2013;31:992-1001
3. Sepulveda AR, Hamilton SR, Allegra CJ et al. Molecular biomarkers for the evaluation of colorectal cancer.Guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. J Mol Diagn 2017;19(2):187-225
4. Igbokwe A, Lopez-Terrada D. H. Molecular testing of solid tumors. Arch Pathol Lab Med 2011;135:67–82)
5. Perry A, Fuller CE, Banerjee R, et al. Ancillary FISH analysis for 1p and 19q status: preliminary observations in 287 gliomas and oligodendroglioma mimics. Front Biosci. 2003 Jan 1;8:a1-9.
6. Banin Hirata BK, Oda JM, Losi Guembarovski R et al. Molecular markers for breast cancer: prediction on tumor behavior. Dis Markers. 2014:513158. Epub 2014 Jan 28.
7. Rossi G, Graziano P, Leone A et al. The role of molecular analyses in the diagnosis and treatment of non-small-cell lung carcinomas. Semin Diagn Pathol. 2013;30(4):298-312.
8. Grossmann AH, Grossmann KF, Wallander ML. Molecular testing in malignant melanoma. Diagn Cytopathol. 2012;40(6):503-10.
9. Nikiforov YE. Molecular analysis of thyroid tumors. Mod Pathol 2011 24, S34–S43

Slide 21: Disclosures

I have no disclosures or conflicts of interest.

Slide 22: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Introduction to Cancer Genetics**."

AACC

Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

Pearl Title: Introduction to Cancer Genetics

Name of Presenter: Deepika Sirohi, MD

Affiliation: University of California, San Francisco

DOI:

CCTC Clinical Chemistry Trainee Council

Application of Molecular testing

- Diagnosis
- Prognostic: risk markers
- Therapeutic
  - Selecting therapeutic targets
  - Monitoring treatment response

Solid tumors with clinically significant molecular biomarkers

- Lung
- Colorectal carcinoma
- Melanoma
- Brain Tumors
- Breast
- GIST
- Thyroid
- Sarcomas

Lung carcinoma

Gene	Prevalence	Histology	Clinical characteristics	Available targeted therapy
EGFR	10-35%	Adenocarcinoma with bronchoalveolar features	East Asian, women, never smokers	TKIs- Geftinib, erlotinib
KRAS	25-30%	Adenocarcinomas, poorly differentiated, mucinous, bronchiolalveolar	Smokers Non-Asians > Asians	Negative predictor of response to EGFR TKIs
EML-ALK4 fusions	5%	Solid/ acinar, signet ring adenocarcinoma	Non/ light smokers Younger age	Response to Crizotinib
BRAF	2-3%	Adenocarcinoma, micropapillary, papillary	Current/former smokers M=F	Resistance to EGFR inhibition. Benefit from MEK inhibitors
ROS1 fusions			Never smokers, Asian, younger	Response to Crizotinib

EGFR

- Mutations seen in 25-30% of NSCLC
- EGFR TK domain mutations: Most reliable predictor of response to TKIs

Sensitive to TKIs	Resistant to TKIs
Exon 19 deletion (codons 746-750)	Insertions in exon 20
L858R (exon 21)	T790M (secondary resistance)
G719 (exon 18)	S768I, L747S, D761Y and T854A
L861 (exon 21)	

Other mutations

- *KRAS*
  - Mutually exclusive of *EGFR*
  - G12C:
    - More common in smokers
    - Reduced sensitivity to Cisplatin
    - Better response to Paclitaxel and Pemetrexed
  - G12D
    - Never smokers
- *EML4-ALK*
  - FISH Dual color break apart probe
  - Other partner genes: *KIF5B*, *KLC-1*, *TFG*
- *BRAF*
  - V600E (exon 15)- most common mutation
  - G469A (exon 11)- 2nd most common
- *ROS1* fusions
  - Fusion partners- *SLC34A2*, *CD74*, *TPM3*, *SD4*, *EZR*, *LRIG3*

ASCO recommendations/ Algorithm

- *EGFR* should get priority over other testing
- On limited specimens *EGFR* and *ALK* testing can be performed when an adenocarcinoma cannot be excluded
- In resected specimens, *EGFR* and *ALK* testing is not recommended if there is no adenocarcinoma

Colorectal Carcinoma (CRC)

- 3 distinct pathways
  - FAP (suppressor) (chromosomal instability pathway)
    - APC/β -catenin/Wnt signaling pathway
  - Serrated pathway (aberrant CpG island methylation-CIMP)
    - Microsatellite stable (MSS)
    - Microsatellite instability high (MSI-H): epigenetic silencing (hypermethylation) of *MLH1*
  - Hereditary MSI (Lynch syndrome)
    - Germline mutation in *MSH2*, *MLH1*, *MSH6*, or *PMS2*

Microsatellite Stability

- Mismatch repair (MMR)
  - Sporadic
    - MSI- Most show CpG methylation of MLH1 promoter
    - 3/4 of these will have *BRAF* mutation
  - Germline
    - < 1/3 of MSI/ MMR deficient- germline mutation of 1 of the 4 MMR genes: Lynch syndrome- AD

MSI- Clinical Significance

- MSI-H
  - Better prognosis
  - Resistant to 5-FU, alkylating agents, and platinum compounds
  - More sensitive to irinotecan
  - Early data: better response to anti- PD-1 /PD-L1 immune checkpoint inhibitor therapy

Colorectal Cancer: Mutation profile

- *EGFR* mutation is very rare in CRC
- EGFR monoclonal Ab resistance
  - *KRAS* mutations
  - *BRAF* V600E
- *KRAS* ~ 40%
  - Never smokers- G12D, G>A transitions (G13D, G12S)
  - Smokers- G12C, G>T transversion (G13C , G12V)
- *BRAF* V600E ~4%
- *PIK3CA* ~ 3%
- *NRAS* ~ 2%
- *MEK1*, *AKT* ~ 0.5%

CRC testing recommendations

- *KRAS* and *NRAS* codons
  - 12 and 13 (exon 2)
  - 59 and 61 (exon 3)
  - 117 and 146 (exon 4)
- *BRAF*
  - V600E: poor outcomes, decreased response to EGFR-targeted therapy
- *PIK3CA*- poor response rate and progression free survival
  - E542, E545, H1047 (exons 9, 20)
- *EGFR*- no role for testing
- CAP guidelines: *KRAS* codons 12/13/61/146 and *NRAS*

Melanoma

- *BRAF*~ 40—60%
  - Intermittently sun damaged skin
  - V600E (70-85%), V600K (5-30%)
  - Vemurafenib- active against BRAF
- *NRAS* (15-20%)
  - Nodular melanomas, sun damaged skin
  - Mutually exclusive of *BRAF* alterations
  - Codon 61- most common
- *KIT*
  - Acral and mucosal melanoma
- *GNAQ*/*GNA11*: mutually exclusive
  - Uveal melanoms (83%)
  - Blue nevus like melanomas

Brain: Oligodendroglioma

- Oligodendroglioma
  - 1p/19q co-deletion
    - Chemosensitive and radiation sensitive
    - Favorable prognosis
  - *IDH* mutations
  - WHO Classification- *IDH* mutations and 1p/19q co-deletion required for grade II and III oligodendrogliomas
  - No *EGFR* amplification

Brain

- *EGFR*
  - Amplified/ mutated in 40-45% of high grade gliomas
  - Constitutive TK activation
  - Does not predict response to EGFR therapy.
- *IDH1*/*IDH2* (exon 4)
  - ~75% of grade II and III gliomas
  - Diagnostic
    - Tumor vs reactive gliosis
    - Pilocytic astrocytoma vs. diffuse astrocytoma or oligodendroglioma
  - Favorable prognosis
  - *IDH1* R132H- most common

Breast Cancer

- *ERBB2* (HER2) amplification- ~20%
  - Higher grade, metastatic tumors
- Response to trastuzumab and lapatinib
- Testing: IHC and/ or FISH
- Familial breast cancer
  - *BRCA1*, *BRCA2*
  - *TP53* - Li-Fraumeni syndrome
  - *STK11* - Peutz-Jeghers syndrome
  - *PTEN* - Cowden syndrome
  - *CHEK2*
  - *ATM*
  - *PALB2*
  - *BRIP1*

Gene expression profiles

- Oncotype DX
  - Predicts a recurrence score which can be used to guide treatment decisions
  - 21 genes (16 cancer related, 5 reference), PCR based
  - Women recently diagnosed with invasive breast cancer (stage I-IIIa), ER+, HER2-
  - Recurrence score: low (<18)/intermediate (18-30)/high risk (>31)
- MammaPrint
  - Predicts likelihood to benefit from adjuvant chemotherapy
  - 70 genes
  - FDA approved for node-negative, treatment naive breast cancer patients with stage I or II disease
  - Classification into low or high risk
- PAM50
  - 50 genes to identify the intrinsic subtype of breast cancer
- Theros (Biotheranostics Breast Cancer Index)

GIST

- *KIT*- 95%, spindled
  - Exon 11 (gastric)
    - p.W557del and/or K558del - worse prognosis
  - Exon 9 (small intestine and colon)
    - Relatively resistant to imatinib (higher dose)
- *PDGFRA*- 5%, epithelioid
  - Gastric, extra-GI
  - D842V- resistant to imatinib
- Imatinib resistance-
  - Primary- *KIT* WT, exon 9, *PDGFRA* D842V
  - *KIT* exon 13 K642E, *KIT* exon 17 Y823D

Thyroid

- *BRAF* V600E
  - PTC - 35-70%
  - Tall cell variant, classic
- *RET/PTC* rearrangement
  - Sporadic PTC - 10-20%
  - Radiation exposure, younger age
  - Lymph node metastases
- *RAS*
  - Papillary (follicular variant) and Follicular
- *PAX8*/peroxisome proliferator-activated receptor (*PPARγ*) rearrangements
  - Follicular
  - Younger, smaller size, solid/nested growth
- *RET* mutations
  - Medullary thyroid carcinomas

References

1. Hunt J.L. Molecular testing in solid tumors: an overview. Arch Pathol Lab Med 2008;132:164-167
2. Travis W.D, Brambilla E, Riely G.J. New pathologic classification of lung cancer: relevance for clinical practice and clinical trials. J Clin Oncol 2013;31:992-1001
3. Sepulveda AR, Hamilton SR, Allegra CJ et al. Molecular biomarkers for the evaluation of colorectal cancer.Guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. J Mol Diagn 2017;19(2):187-225
4. Igbokwe A, Lopez-Terrada D. H. Molecular testing of solid tumors. Arch Pathol Lab Med 2011;135:67–82)
5. Perry A, Fuller CE, Banerjee R, et al. Ancillary FISH analysis for 1p and 19q status: preliminary observations in 287 gliomas and oligodendroglioma mimics. Front Biosci. 2003 Jan 1;8:a1-9.
6. Banin Hirata BK, Oda JM, Losi Guembarovski R et al. Molecular markers for breast cancer: prediction on tumor behavior. Dis Markers. 2014:513158. Epub 2014 Jan 28.
7. Rossi G, Graziano P, Leone A et al. The role of molecular analyses in the diagnosis and treatment of non-small-cell lung carcinomas. Semin Diagn Pathol. 2013;30(4):298-312.
8. Grossmann AH, Grossmann KF, Wallander ML. Molecular testing in malignant melanoma. Diagn Cytopathol. 2012;40(6):503-10.
9. Nikiforov YE. Molecular analysis of thyroid tumors. Mod Pathol 2011 24, S34–S43

Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**:
- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

*****This slide will be completed by CCJ Staff based on disclosure form completed electronically in the submission site.*****

AACC Better health through laboratory medicine.

Thank you for participating in this
Clinical Chemistry Trainee Council
Pearl of Laboratory Medicine.

Find our upcoming Pearls and other
Trainee Council information at
www.traineecouncil.org

Download the free *Clinical Chemistry* app
on iTunes today for additional content!

Follow us:

CCTC Clinical Chemistry Trainee Council