Genomic testing has resulted in a paradigm shift in the diagnosis and treatment of lung cancer.

Demand for greater amounts of tumor tissue

"The needle aspiration lung biopsy is dead"

--Tumor board oncologist
November 2013

Radiologists and oncologists both need to understand:

- The role of mutation testing in the treatment and prognosis of lung cancer
- How and when biopsy specimens can and should be procured for testing
- The tissue requirements of specific genomic testing vendors

49 year old female smoker with seizure

Imaging workup reveals a lung mass, brain metastases

Coaxial needle biopsy was performed
(2 FNAs and 3 cores)
**Cytological evaluation:**

**Adenocarcinoma**

- Smears (Papanicolaou stain)
- Cell Block (H&E stain)

**Immunohistochemistry panel:**

- Subtype of lung cancer
- Tissue of origin for metastases

Thyroid transcription factor 1 (TTF-1) positive c/w lung adenocarcinoma

**Core specimens confirmed adenocarcinoma**

- AdenoCa
- Squamous
- Large cell

Cores are not significantly better than FNA for diagnosis of malignant lung lesions


**Clinical Stage IV NSCLC was diagnosed and our patient was treated with platinum based chemotherapy but progressed**

**How do tumor cells differ from normal cells?**

- Platinum based drugs work by cross-linking DNA preventing repair/replication in 2nd half of cell cycle
- Dose is limited by high toxicity to normal tissues
- Modest survival benefits

*Modified from T. SANDAL, The Oncologist 2002;7:73-81*
Mutations that cause tumor behaviors primarily act in the first half of the cell cycle.

These mutations offer unique targets for therapy not found in normal cells, with low toxicity to normal tissues.

Tumor mutation basics:
- "Wild-type": Normal, non-mutated gene
- Germline mutations (all cells in body)
- Somatic mutations (tumor only)
- Most tumors have multiple mutations, with one dominant "driver" mutation
- Carcinogen induced cancers have more mutations
- Tumors can be heterogeneous with different mutational clones

Various types of mutations can be seen in NSCLC:

- Oncogene: EGFR MT
  - Epidermal Growth Factor Receptor
- Oncogene: KRAS MT
- Oncogene: ALK rearrangement
- Oncogene: p53

Li, Gandara et al: JCO 2013

"Druggable" mutations
Drugs in development
**EGFR mutations**  
(Epidermal Growth Factor Receptor)  
- Common in many cancers  
- Most common actionable mutation in NSCLC  
- Most common in females, non-smokers, Asians, but must test

These cell surface receptors have an intracellular component and are activated by binding with ligands such as Epidermal Growth Factor (EGF)

This stimulates their intrinsic intracellular tyrosine kinase activity resulting in autophosphorylation and downstream activation of growth signaling cascades

**Tyrosine kinase activating** mutations cause EGFR to always be switched “on” and the tumor cell requires this constant growth signaling to survive

**Potential therapeutic agents:**
- Monoclonal antibodies (cetuximab, panitumumab)
- Tyrosine kinase inhibitors (TKI) (erlotinib, gefitinib, afatinib)
- Downstream kinase inhibitors

**The EGFR is a validated target for NSCLC therapy**
- TK-activating mutations of EGFR occur in 10-15% of NSCLC tumors  
- In EGFR-mutated tumors, EGFR TKIs are highly effective with >70% response rate and prolonged survival  
- In EGFR wild-type tumors, EGFR TKIs are far less effective

**Phase III IPASS Trial:**

Efficacy of TKIs vs. platinum based chemotherapy based on **EGFR Mutation Status**

- **EGFR mutation positive**
- **EGFR mutation negative**

Response after 2 months of TKI

- **Gefitinib (Tarceva)**

TKIs produce few serious side effects but rarely can cause severe interstitial lung disease

- Often from new or pre-existing T790M mutation
- Genomic retesting after treatment failure
- Irreversible TKIs are being developed

**ALK rearrangements:** *(Anaplastic Lymphoma Kinase)*

- Another “druggable” target but only 4% of NSCLC
- Most common rearrangement seen in NSCLC is a fusion: EML4-ALK
- Adenocarcinomas, non-smokers or light smokers
- Will not respond to EGFR specific TKIs
- 60% respond to crizotinib, an ALK specific kinase inhibitor

**ALK Fusion Genes in Lung Cancer**

- Crizotinib

**Response after 2 months of TKI**

- Biopsy EGFR MT+
- After gefitinib
- Relapse, rebiopsy

**ALK Fusion Genes in Lung Cancer**
**PROFILE 1001**
(Crizotinib first-in-human phase I study)
Waterfall plots of best percent change in target lesions from baseline

**Drug resistance develops**

<table>
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<tr>
<th>8 cycles Crizotinib</th>
<th>10 cycles Crizotinib</th>
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**KRAS mutation**
(Kirsten rat sarcoma viral oncogene)
- Present in 1/3 of human malignancies
- A “loss of function” mutation
- Leads to constant activation of downstream signaling pathways independent of EGFR
- KRAS mutations are associated with poor prognosis regardless of treatment
- Smokers, Caucasians

**Ongoing drug development of specific kinase inhibitors active downstream to KRAS**

**Our patient’s genomic tumor testing revealed:**
- EGFR mutation negative
- ALK fusion negative
- KRAS mutation positive (smoker)

No FDA approved drug yet
Which patients may benefit from biopsy and genomic testing?

- No surgical specimen forthcoming
- Second primary vs. metastasis staging issue
- Re-testing after treatment failure to look for:
  - New driver mutation
  - Drug resistance mutation
  - New dominant tumor clone
- All advanced adenocarcinomas should be tested, prioritizing EGFR and ALK (College of American Pathologists)

DNA can be extracted from a variety of biopsy specimens

- Paraffin embedded cell block or cores
- Fresh frozen tissue
- Slides from FNA/cell block
- Macrodissection of larger samples or laser microdissection from slides to isolate pure tumor tissue

Tissue requirements for genomic testing

- High percentage of cells nucleated
- At least 20% of cells are tumor tissue (otherwise false negatives)
- Larger amounts of tissue more likely to yield accurate results
- Amount of tissue required dependent on vendor’s method of testing
- Instructions on vendor website

Methods for genomic testing vary widely among vendors

- Serial testing by a variety of methods for a limited panel of likely and/or actionable mutations (Lower cost)
- Next generation sequencing: Simultaneously extract all known oncogenes from mechanically fragmented tumor DNA using probes, PCR, and gene sequencing with computer analysis (Higher cost)

Representative vendor instructions: 3-5 cores requested

Next generation sequencing (NGS) for 182 cancer related genomic alterations

Tissue requirement: 1 cubic mm
Why test for hundreds of mutations when so few are actionable?

- In all tumor types, many carry unknown mutations
- Further goals of research and drug development
- Unexpected druggable mutations may be found

Lung biopsy for mutation testing

- Start with FNA to confirm presence of viable tumor before taking core biopsies
- Avoid areas of necrosis
- Biopsy different parts of a solid mass
- Larger amounts of tissue increase the chances of positive results
- Confine the throw of the needle to the mass; avoid including normal lung

Safety concerns for core biopsy: Hemorrhage and air embolism

- Larger lesions tamponade bleeding
- Avoid including adjacent normal lung in core specimen
- Examine thin section images of a contrast CT before planning biopsy approach and avoid large vessels in and adjacent to the tumor

Core biopsy of smaller lesions may hemorrhage into adjacent lung parenchyma
A preponderance of recent air embolism cases in the literature and the legal sphere involved core biopsies

Summary

- Test all advanced/progressing adenocarcinomas for EGFR and ALK mutations
- Only small minority of patients with NSCLC will have a druggable target
- Biologics will have better response rates and lower toxicity than standard chemotherapy, but resistance will develop
- Vendor specific requirements for tissue testing vary; determine prior to biopsy