Challenges of Tissue Acquisition & Biomarker Testing in The Community and Academic Settings

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Auris J and J	X	X
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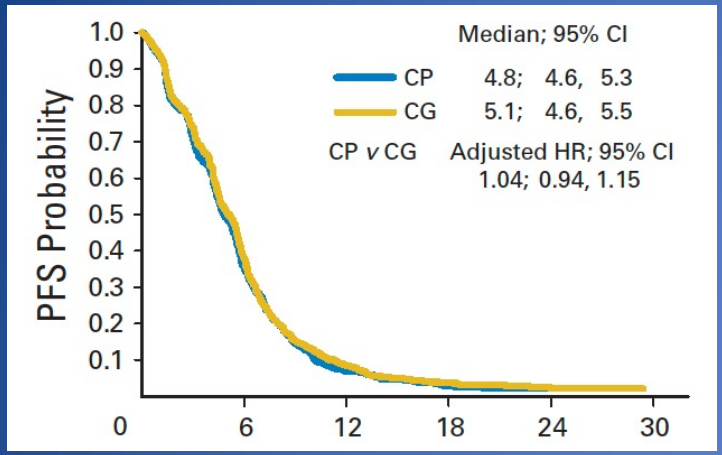
Amgen	X	
ACS	X	

Outline

- The Rationale for Biomarker Testing
- The Challenges in getting and testing specimens
- Is EBUS good enough?
- Is liquid biopsy good enough?
- Potential solutions

Biomarker testing in NSCLC

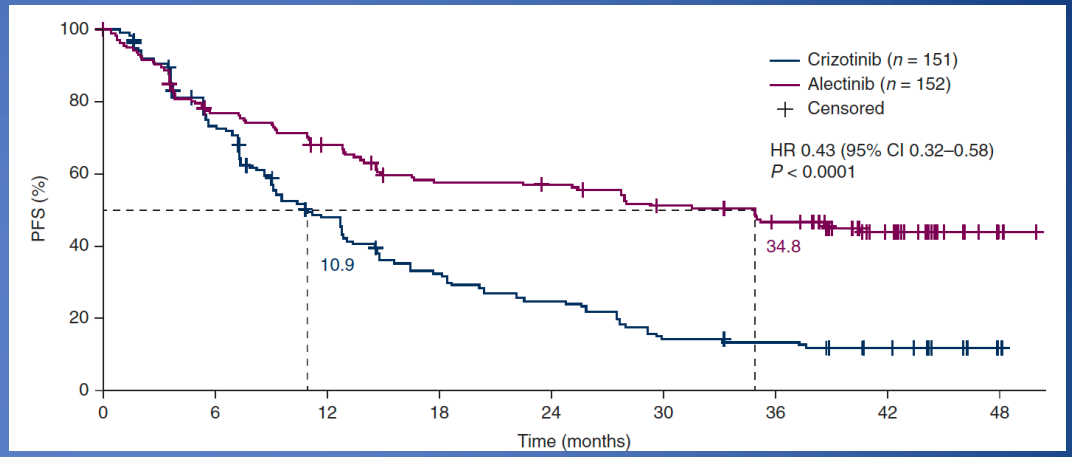
Scagliotti et al. J Clin Oncol. 2008 Jul 20;26(21):3543-51



Cisplatin/pemetrexed vs Cisplatin/gemcitabine; Stage IIIB/IV NSCLC

Median PFS ~5 months with either

Mok et al. Ann Oncol. 2020 Aug;31(8):1056-1064

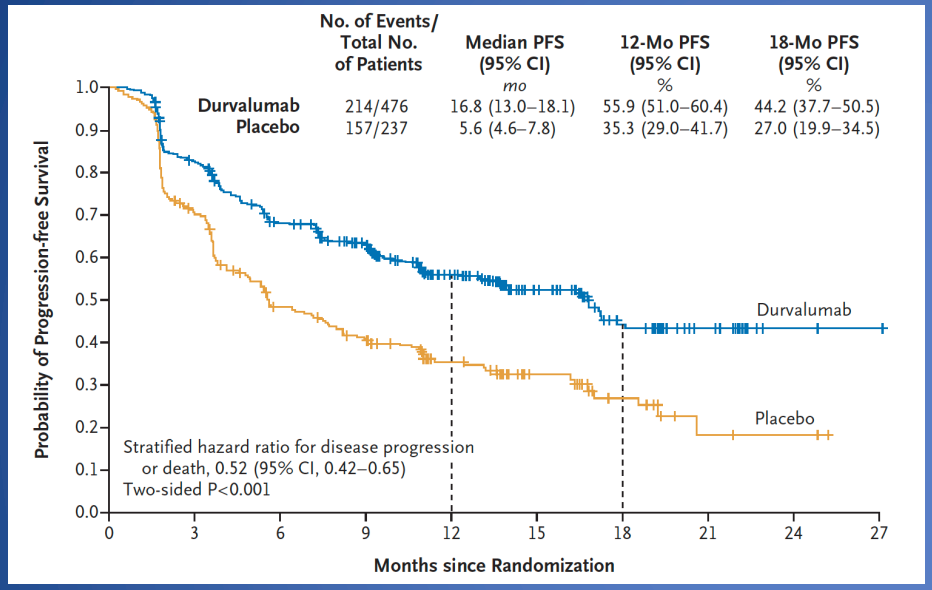


Alectinib vs Crizotinib Stage III/IV ALK (+) NSCLC

Median PFS 34.8 months with Alectinib

Biomarker testing in NSCLC

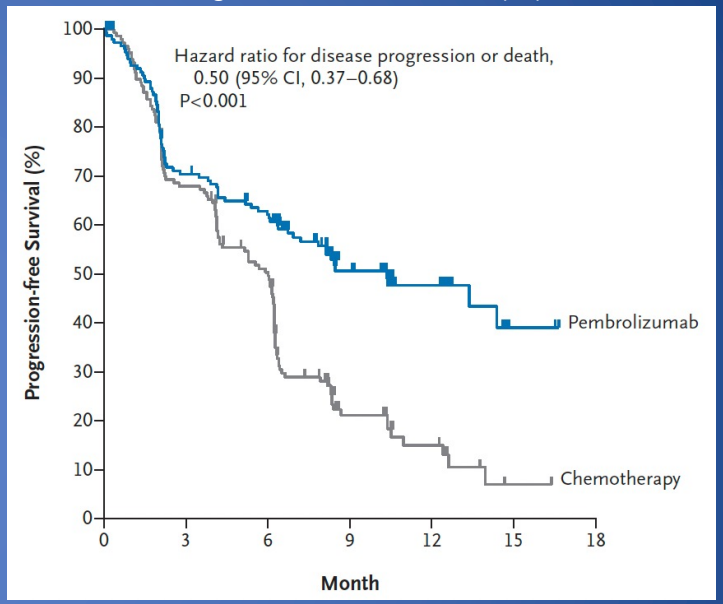
PACIFIC TRIAL. Antonia et al. N Engl J Med. 2017 Nov 16;377(20):1919-1929



Chemo + (Immunotherapy vs placebo)
Stage III NSCLC

Median PFS 16 vs 5 months

Reck et al. N Engl J Med. 2016 Nov 10;375(19):1823-1833



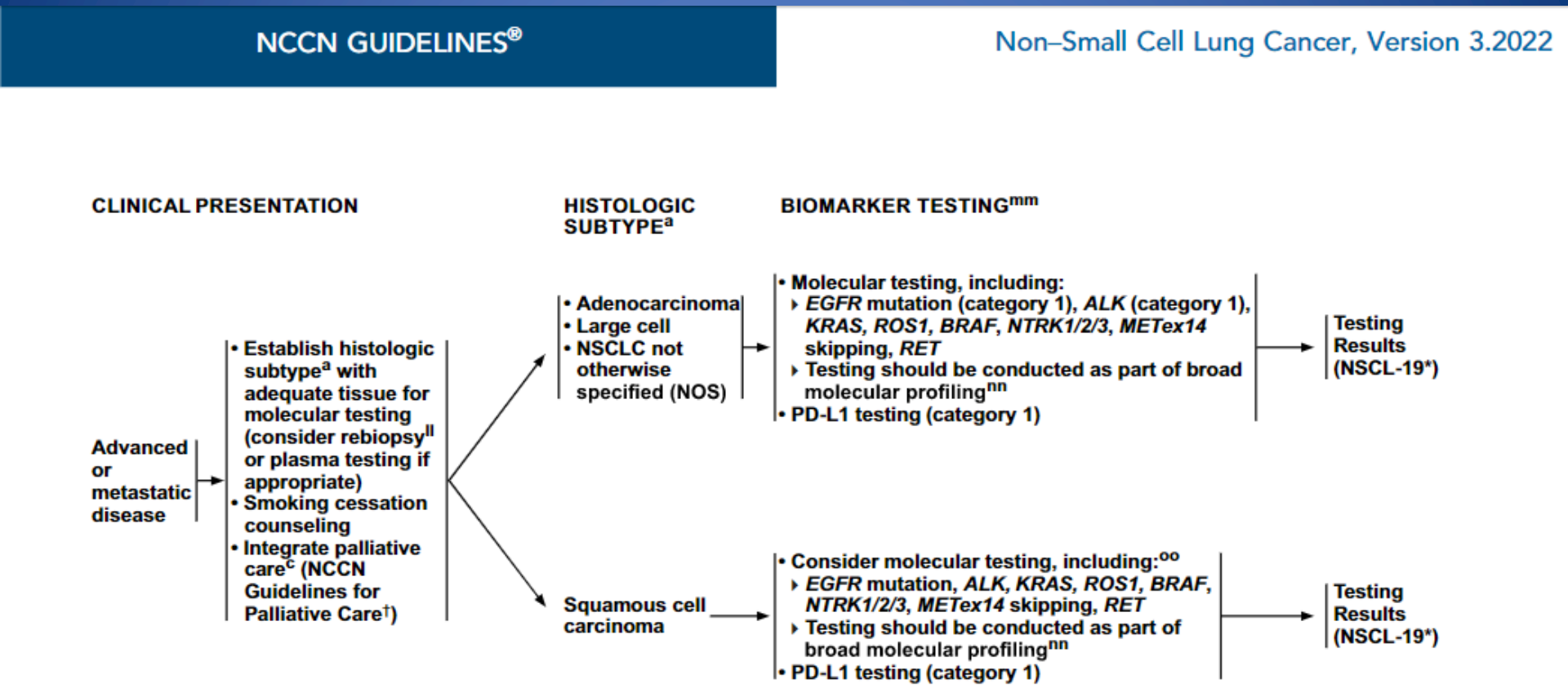
Immunotherapy vs Chemotherapy
Stage IV NSCLC with PD-L1 > 50%

Median PFS 10.3 vs 6.7 months

Immune and Targeted Therapies in Early-Stage Disease

- ADAURA – Adjuvant osimertinib versus placebo in **EGFR(+) *resected* lung cancer**.
  - At 2 years, **disease-free survival 90% vs 44%**
- IMpower010 - adjuvant atezolizumab vs supportive care in *resected* lung cancer
  - Patients with PD-L1 ≥ 1%, **HR 0.66** (95% CI 0.50–0.88; p = 0.0039) **for death or progression**
  - Did allow EGFR and ALK to enroll
- Checkmate 816 - Neoadjuvant immunotherapy plus platinum-doublet chemotherapy versus chemotherapy alone in *resectable* lung cancer.
  - Median event-free survival was 31.6 months vs 20.8 months
  - **EGFR and ALK mutations were excluded**

NCCN Guidelines for Testing



The List of Targeted Agents Continues to Grow

TARGETED THERAPY OR IMMUNOTHERAPY FOR ADVANCED OR METASTATIC DISEASE[a,b]

EGFR Exon 19* Deletion or *L858R
- First-line therapy
  - Afatinib[1]
  - Erlotinib[2]
  - Dacomitinib[3]
  - Gefitinib[4,5]
  - Osimertinib[6]
  - Erlotinib + ramucirumab[7]
  - Erlotinib + bevacizumab[c] (nonsquamous)[8]
- Subsequent therapy
  - Osimertinib[9]

EGFR S768I, L861Q,* and/or *G719X
First-line therapy
- Afatinib[1,10]
- Erlotinib[2]
- Dacomitinib[3]
- Gefitinib[4,5]
- Osimertinib[6,11]
- Subsequent therapy
  - Osimertinib[9]

***EGFR Exon 20* Insertion Mutation Positive**
- Subsequent therapy
  - Amivantamab-vmjw[12]
  - Mobocertinib[13]

***KRAS G12C* Mutation Positive**
- Subsequent therapy
  - Sotorasib[14]

***ALK* Rearrangement Positive**
- First-line therapy
  - Alectinib[15,16]
  - Brigatinib[17]
  - Ceritinib[18]
  - Crizotinib[15,19]
  - Lorlatinib[20]
- Subsequent therapy
  - Alectinib[21,22]
  - Brigatinib[23]
  - Ceritinib[24]
  - Lorlatinib[25]

***ROS1* Rearrangement Positive**
- First-line therapy
  - Ceritinib[24]
  - Crizotinib[27]
  - Entrectinib[28]
- Subsequent therapy
  - Lorlatinib[29]
  - Entrectinib[28]

***BRAF* V600E Mutation Positive**
- First-line therapy
  - Dabrafenib/trametinib[30,31]
  - Dabrafenib[30]
  - Vemurafenib
- Subsequent therapy
  - Dabrafenib/trametinib[31,32]

***NTRK1/2/3* Gene Fusion Positive**
- First-line/Subsequent therapy
  - Larotrectinib[33]
  - Entrectinib[34]

***MET* Exon 14 Skipping Mutation**
- First-line therapy/Subsequent therapy
  - Capmatinib[35]
  - Crizotinib[36]
  - Tepotinib[37]

***RET* Rearrangement Positive**
- First-line therapy/Subsequent therapy
  - Selpercatinib[38]
  - Pralsetinib[39]
  - Cabozantinib[40,41]

PD-L1 ≥1%
- First-line therapy[d]
  - Pembrolizumab[42-44]
  - (Carboplatin or cisplatin)/pemetrexed/ pembrolizumab (nonsquamous)[45,46]
  - Carboplatin/paclitaxel/bevacizumab[c]/ atezolizumab (nonsquamous)[47]
  - Carboplatin/(paclitaxel or albumin-bound paclitaxel)/pembrolizumab (squamous)[48]
  - Carboplatin/albumin-bound paclitaxel/ atezolizumab (nonsquamous)[48]
  - Nivolumab/ipilimumab[49]
  - Nivolumab/ipilimumab/pemetrexed/ (carboplatin or cisplatin) (nonsquamous)[50]
  - Nivolumab/ipilimumab/paclitaxel/carboplatin (squamous)[50]

PD-L1 ≥50% (in addition to above)
- First-line therapy[d]
  - Atezolizumab[51]
  - Cemiplimab-rwlc[52]

Next Generation Sequencing (NGS)

- Technology of high-throughput
- Massively parallel DNA sequencing
- Thousands of variants (somatic or germline) from hundreds of genes can be identified in a single test.
- **Paradigm change:** single drug with a single companion diagnostic test measuring variants in a single gene → multiple drugs being developed and used in the clinic using a single NGS test as the companion diagnostic test.

Blumethal et al. JAMA Oncol. 2016;2(1):13-14

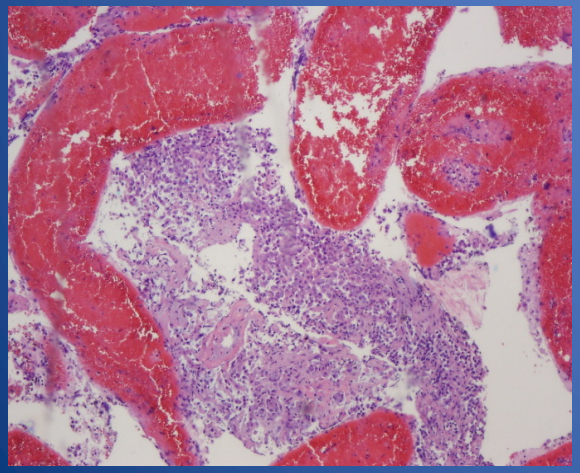
Advantages of NGS

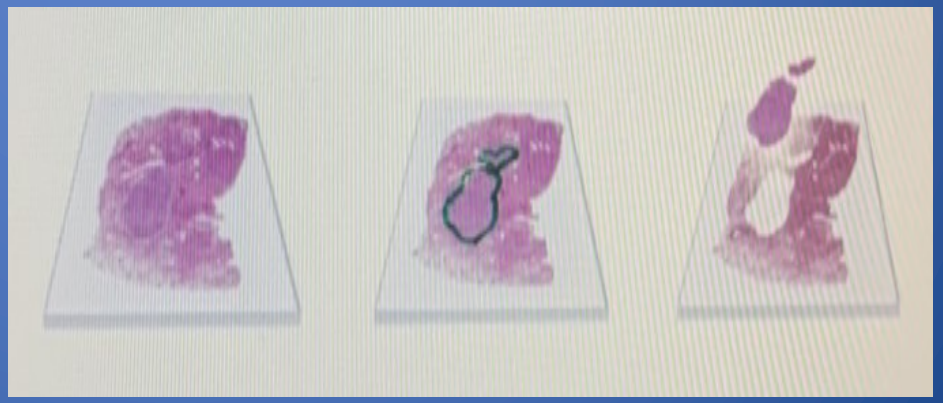
- Single test identifies thousands of somatic or germline mutations from hundreds of genes
- Preferred over direct sequencing as it is more sensitive in specimens with low tumor cellularity.
- May be more cost-effective than single gene testing modalities
- Driver mutations detected in 50-60% of NSCLC patients, of which 50% can be treated with a targeted agent.

Drilon et al. Clin Cancer Res 2015;21: 3631-9
Pennel et al. J Clin Oncol 2018;36 abstr9031
Lim et al. Oncotarget 2016;7:24172-8
Kaderbai et al. Oncotarget 2016; 724860-70

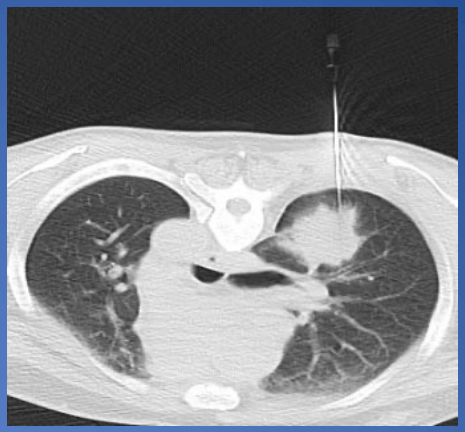
DNA Requirements

- Depends on the NGS platform
- Large panel (> 200 genes) requires up to 50ng of DNA
- A 50 gene panel may require 1-10ng of DNA (>1000 cells) while 1200 gene panels may require 25-100ng of DNA (>200,000 cells)

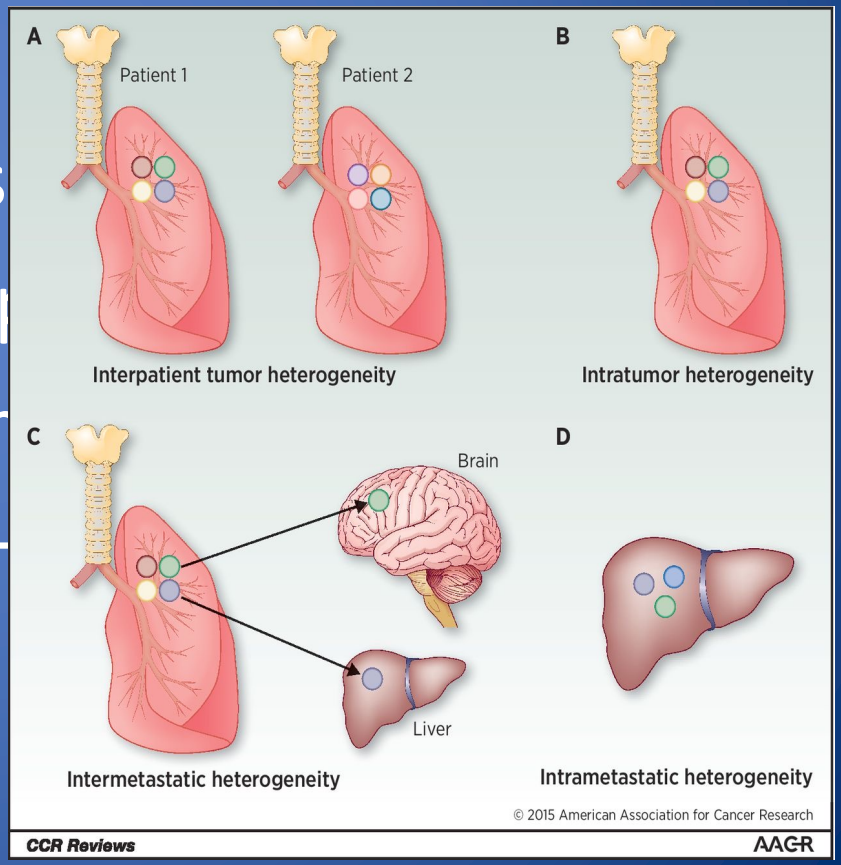




Challenges of tissue-based genomic profiling



- Invasive
- Need for repeated biops
- Non representative samp
- Variable cellularity amor
- Competing needs, i.e., IH
- Tumor heterogeneity



Sholl et all .J Thorac Oncol. 2015;10(5):768-777.
Gerlinger et al.N Engl J Med.2012;366(10):883-892.
Rijavec et al. Cancers. 2020 Jan;12(1):17.
Jamal-Hanjani et al. Clinical cancer research. 2015 Mar 15;21(6):1258-66.

Challenges cont.

- 1/3 of patients with advanced NSCLC may die within the first 2 months after initial diagnosis
- Poor ECOG status of many patients
- Need for significantly shorter turnaround times
- Up to 80% of patients with NSCLC having advanced disease will only have tissue from small biopsies or cytology samples, limiting the potential to perform additional tests.
  - Up to 31% of patients do not have accessible tissue
  - Up to 20% of biopsies are inadequate for molecular testing due to insufficient tissue amounts

Globus, *et al.* J Clin Oncol, 37 (2019) Abst 9103.
Sholl, *et al.* Arch Pathol Lab Med, 140 (2016), pp. 825-829
Chouaid, *et al.* Lung Cancer, 86 (2014), pp. 170-173

EBUS for genetic testing

- Initially evaluated for single gene testing
  - Pooled analysis of 28 studies (2,497 patients) reported sufficient sample for EGFR in 94.48%
  - Analysis of 12 studies (607 patients) reported sufficient sample for ALK in 95%
  - Smaller studies for ROS-1 showed sufficient sample in 83%

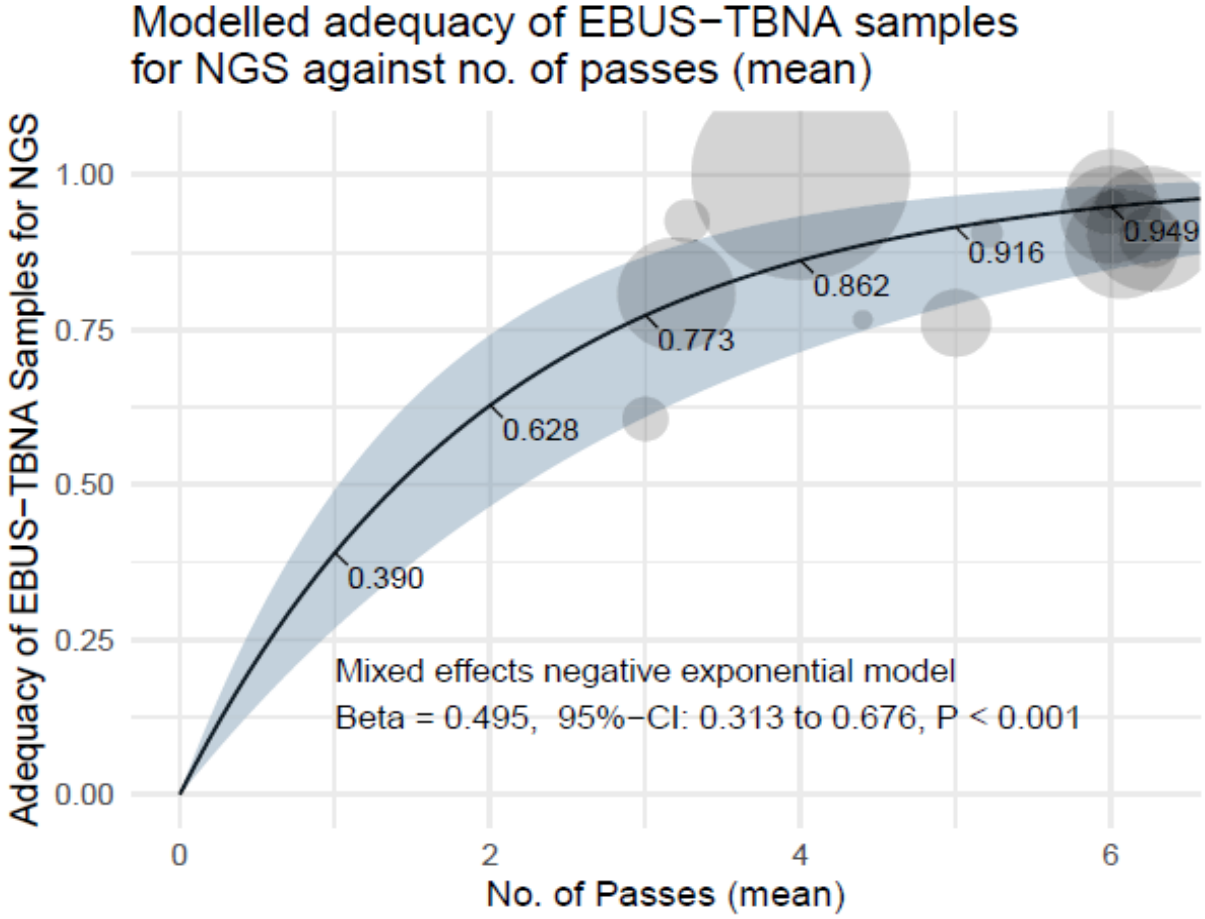
Labarca et al. Ann Am Thorac Soc 2018;15:1205-1216
Fernandez-Bussy et al. Arch Bronchopneumol 2017; 53: 172-174

EBUS for NGS?

- Twenty-one studies 1,175 patients
- The pooled proportion of adequate EBUS-TBNA samples for NGS (yield) was 86.5% (95%-CI: 80.9% to 91.4%).
- Pooled mean weight of DNA extracted from EBUS-TBNA samples was 868.7 ng (95%-CI: 446.3 ng to 1291.1 ng).
- Considerable heterogeneity among studies
- Meta-regression with a mixed-effects negative exponential model showed an increased proportion of adequate EBUS-TBNA samples for NGS as mean number of passes increases ($\beta = 0.495$,95%-CI 0.313 to 0.676, $P < 0.001$).
- **Modeled yield rates were 77.3%, 86.2%, 91.6% and 94.9% at mean passes of 3, 4, 5 & 6 respectively.**

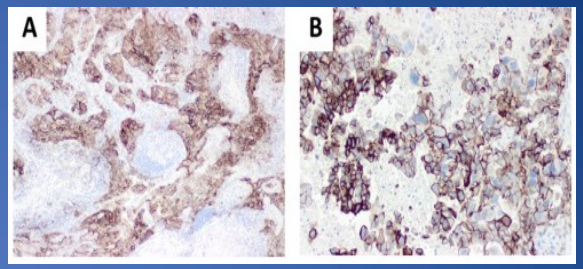
Zhao et al. Lung Cancer 166 (2022) 17–26

TAKE HOME POINT
6 passes for adequacy



Zhao et al. Lung Cancer 166 (2022) 17–26

PD-L1 testing on the EBUS-FNA cytology specimens of non-small cell lung cancer



- Consecutive patients with NSCLC undergoing EBUS
- Cell blocks used for PD-L1 testing
- 265 EBUS-FNA specimens
- **230 (86.8%) were adequate for PD-L1 testing.**
- Of 34 NSCLC patients with both histology and EBUS-FNA cytology specimens tested for PD-L1 - concordance of 91.3%.
- The PD-L1 results from 16 paired specimens from the same anatomic site had 100% agreement.
- The rates of PD-L1 TPS≥50% were significantly higher in the metastatic tumors in the lymph nodes than in the lung primary lesions.

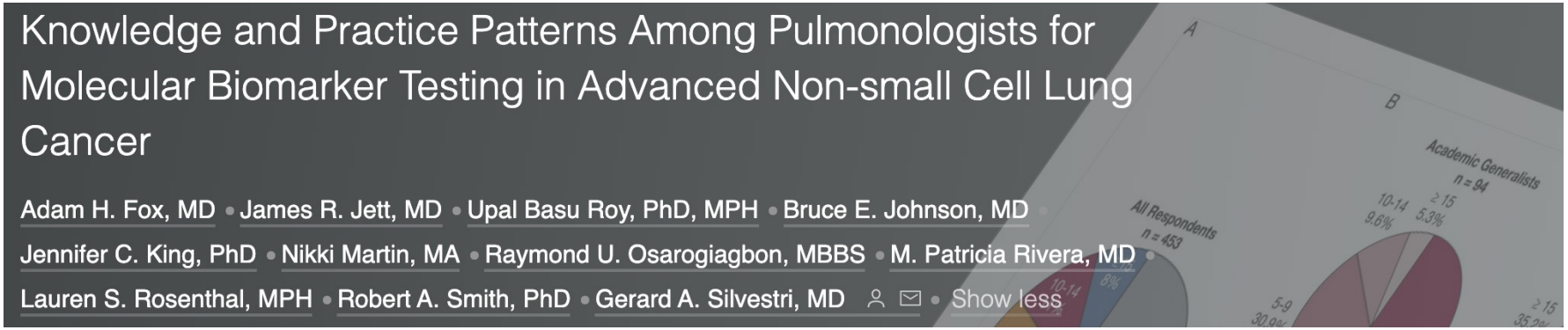
Wang et al. Lung Cancer 2019, 136:1-5.

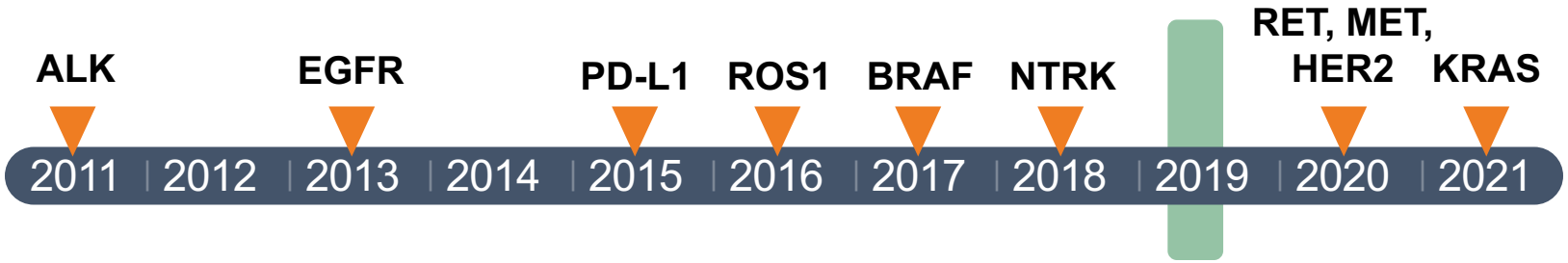
Suitability of Bronchoscopic Biopsy Tissue Samples for Next-Generation Sequencing (Other types of Bronchoscopy)

- Success rate of DNA sequencing 84.1% and RNA 92.7%.
- Success rate of DNA (RNA) sequencing was 57.1% (71.4%) for **small EBUS-GS** (n = 14), 93.4% (96.9%) for **large EBUS-GS** (n = 32), 62.5% (100%) for **EBB** (n = 8), and 100% (100%) for **EBUS-TBNA** (n = 15).
- Tissue surface area of **≥ 1 mm2 (tumor content > 30%)** was adequate for samples to be tested with NGS (all devices).
- Mean tumor content ratios were 32.2 ± 19.6% in the small EBUS-GS subgroup, 31.7 ± 17.4% in the large EBUS-GS subgroup, 46.4 ± 28.1% in the EBB subgroup, and 26.2 ± 17.3% in the EBUS-TBNA subgroup

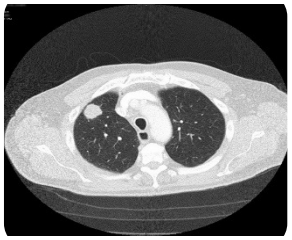
Murakami et al. Diagnostics 2021, 11, 391

PULMONOLOGISTS ROLE IN BIOMARKER TESTING



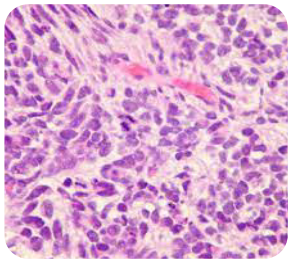


Common Diagnostic Pathway in Advanced Lung Cancer



Advanced lung cancer is suspected

Biopsy Performed



Advanced lung cancer is confirmed

Biomarker testing is ordered and/or sent

Biomarker results available for treatment decisions

? How often do pulmonologists encounter advanced lung cancer in their practice?

? How often are pulmonologists ordering biomarker testing?

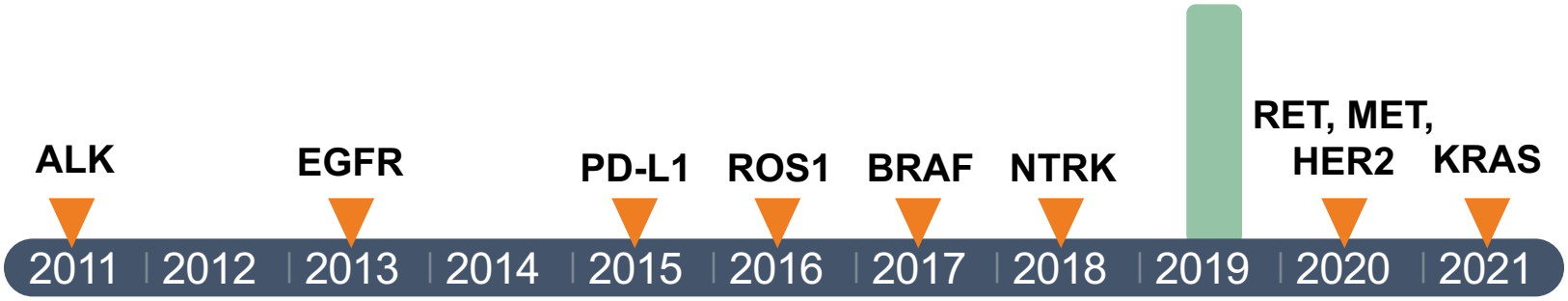
? What assays and testing strategies do they use?

? Do pulmonologists perform or have access to technology such as EBUS and ROSE?

? What do pulmonologists know about individual biomarkers and therapies?

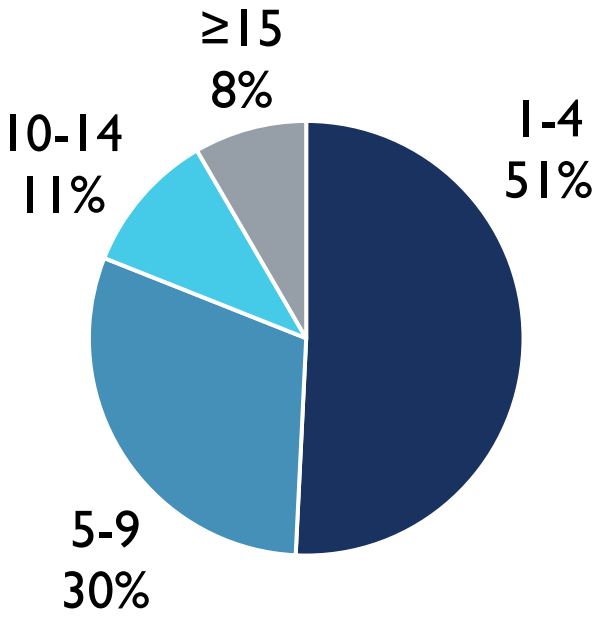
PULMONOLOGISTS ROLE IN BIOMARKER TESTING

- Cross-sectional survey of over 450 pulmonologists in the CHEST database
- Key question domains:
  - Practices for diagnosing advanced lung cancer
  - Collaboration between sub-specialties
  - Knowledge of individual biomarkers

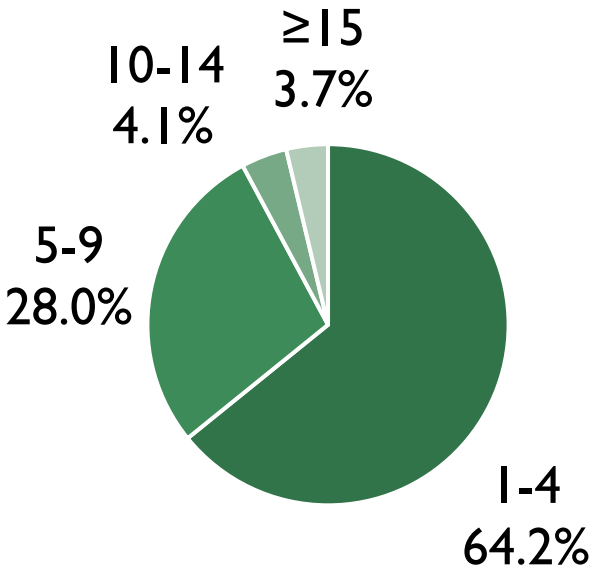


Number of New Patients with Lung Cancer Per Month

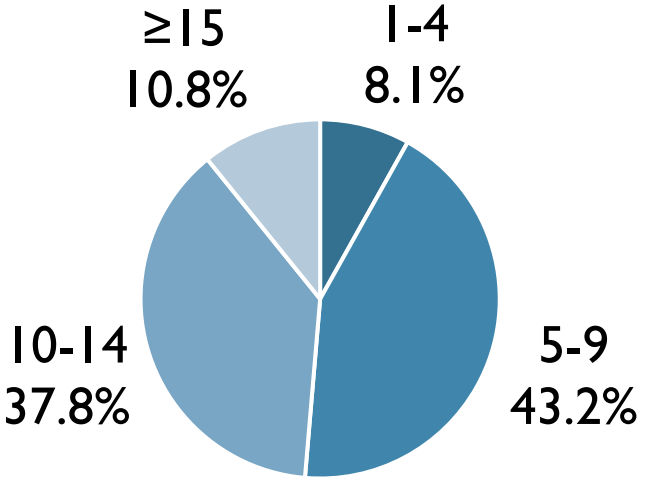
All Respondents
n = 453



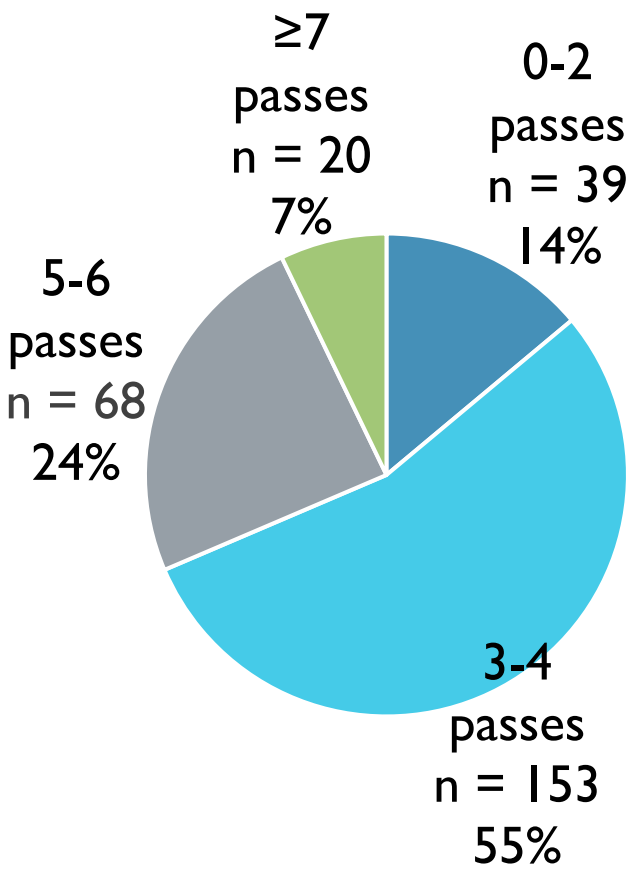
Community Generalists
n = 268



Community Interventionalist
n = 37



Number of Needle Passes During EBUS to Collect Tissue for Biomarker Testing



- Responsible for ordering:
  - Oncologists (37%)
  - Pathologists (31%)
  - Pulmonologists (23%)
  - Tumor board (7%)
- 48% reported an institutional policy to guide biomarker testing
- Location:
  - In-house (20%)
  - Outside testing (44%)
  - Combination (31%)

A Solution?

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

FRONTIERS IN MEDICINE

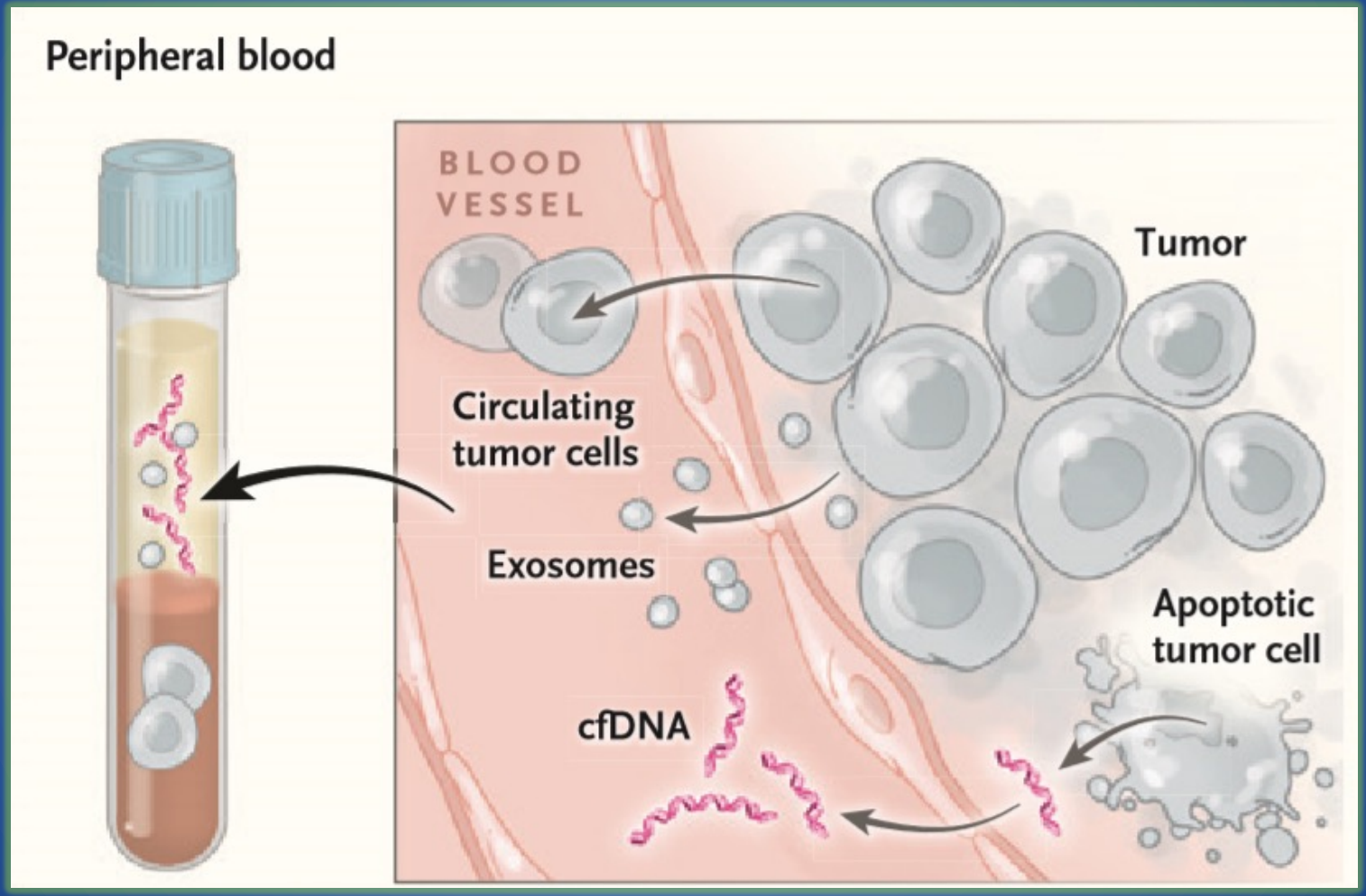
Application of Cell-free DNA Analysis to Cancer Treatment

Ryan B. Corcoran, M.D., Ph.D., and Bruce A. Chabner, M.D.

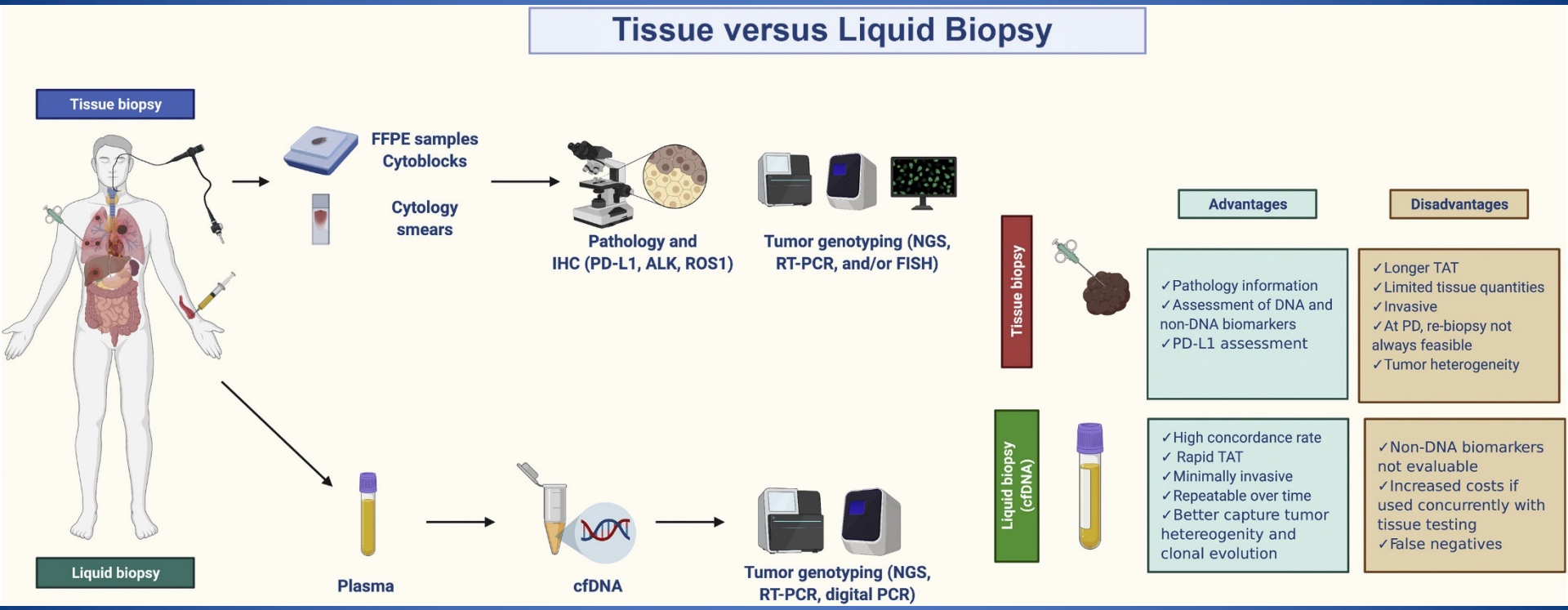
TUMOR BIOPSIES REPRESENT THE STANDARD FOR CANCER DIAGNOSIS and the primary method for molecular testing to guide the selection of precision therapies. Liquid biopsies, particularly those involving cell-free DNA (cfDNA) from plasma, are rapidly emerging as an important and minimally invasive adjunct to standard tumor biopsies and, in some cases, even a potential alternative approach. Liquid biopsy is becoming a valuable tool for molecular testing, for new insights into tumor heterogeneity, and for cancer detection and monitoring. Here, we review the current and potential clinical applications of cfDNA analysis in patients with cancer (see video).

N ENGL J MED 379;18 NEJM.ORG NOVEMBER 1, 2018

What's in the "Liquid"?



N ENGL J MED 379;18 NEJM.ORG NOVEMBER 1, 2018



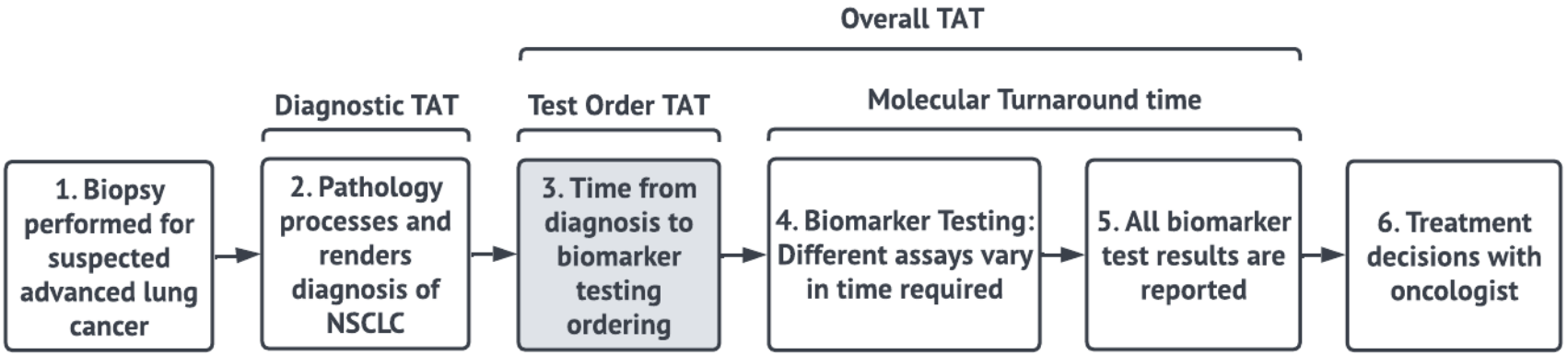
Rolfo et al. JTO 2021, 16(10); 1647-1662

What effect does this have on care?

- Surveyed 170 oncologists.
- For non-squamous NSCLC, 97% of respondents reported ordering tests for *EGFR*, *ALK*, *ROS1*, and *BRAF*.
- Testing for *MET*, *RET*, and *NTRK* > higher among academic and thoracic versus community providers
- Most considered 1 (46%) or 2 weeks (52%) acceptable turnaround time,
- 37% usually waited three or more weeks.
- Respondents who waited ≥3 weeks were more likely to defer treatment until results were reviewed (63%).
- Community and generalists respondents who waited ≥3 weeks were more likely to initiate non-targeted treatment while awaiting results.
- Respondents <5 years out of training more likely to cite their concerns about waiting for results as a reason for not ordering biomarker testing (42%, vs. 19% with ≥6 years of experience).

Millheim 2021 Cancer Medicine

One Solution and Rationale for Reflex Testing



- Systematic and/or automatic evaluation of eligible patients
  - Should reduce disparities in testing based on patient characteristics
  - Should improve timeliness of testing
  - Consolidates the need for knowledge
- Multiple potential means to achieve systematic testing

Barriers to Reflex Testing

- Communication and consensus on:
  - Eligible patients
  - Biomarkers, panel, laboratory
- Integration across practices
- Must keep pace with changes in biomarkers, assays, and guidelines, etc.
- Compliance issues:
  - Limiting unnecessary testing
  - Issues of self-referral, orders from “treating provider”

Mazer. J. Health Care Compliance. 2018

Example of Reflex Testing

- 2016-2018
- Single hospital molecular lab
- Intervention: Pathologist orders institution-approved biomarker panel for any patient newly diagnosed with adenocarcinoma of the lung

Turnaround time (averages):

- 1 year prior: **52.6* days**
- Intervention Year: **26.5 days**
- Year after intervention: **15.6 days**

  (decreased average by 37 days)

- Switched from send-out PCR/FISH to mostly in-house NGS
- Only those for whom biomarker testing was completed (no analysis of any missed cases)
- Lack of implementation evaluation

Anand et al. *Clin Lung Cancer*. 2020

What's happening in our lab

- Leading a multicenter trial of testing tissue (EBUS) and blood simultaneously in 200 consecutive patients with advanced NSCLC

- Performing a pilot trial of reflex testing in rural and underserved hospital with the intention of doing a pragmatic group RCT focused on testing and TAT

- SEER – Medicare linkage to assess who is NOT being treated for lung cancer and not being tested for biomarkers

Summary

- Targeted therapy options are increasing, providing better patient outcomes and is now recommended for nearly all stages of lung cancer
- Requires mutational testing which is being performed at variable rates, Lengthy TAT, and often has tissue that is inadequate for testing.
- EBUS is a first line test in NSCLC for diagnostic and staging purposes.
- EBUS bronchoscopy is a validated method for acquiring tissue for biomarker analysis and PDL-1 testing.
- There is an opportunity to improve awareness of best methods for biomarker testing in EBUS bronchoscopy.

Summary

- Challenges exist when obtaining adequate tissue for biomarker analysis in NSCLC, and the role of “liquid biopsy” will need further investigation and clarification.
- Reflex testing can work, but if not communication between pulmonary, path, oncology, and external testing companies is needed. QNS rates should be kept at the hospital level.