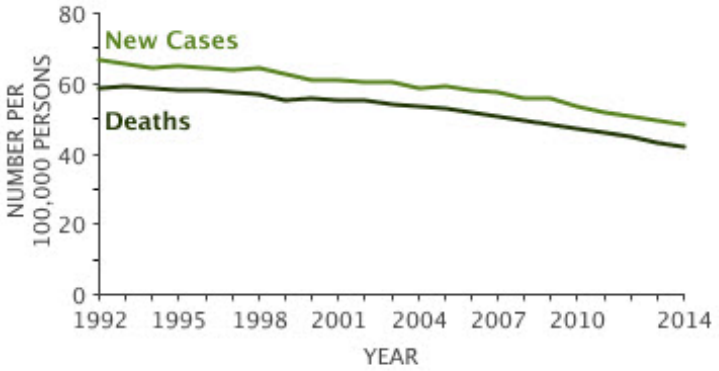
Optimizing EBUS-TBNA in an Era of Personalized Medicine

Nancy P. Caraway, M. D.

Lung Cancer Incidence

Estimated New Cases in 2017	222,500
% of All New Cancer Cases	13.2%
Estimated Deaths in 2017	155,870
% of All Cancer Deaths	25.9%

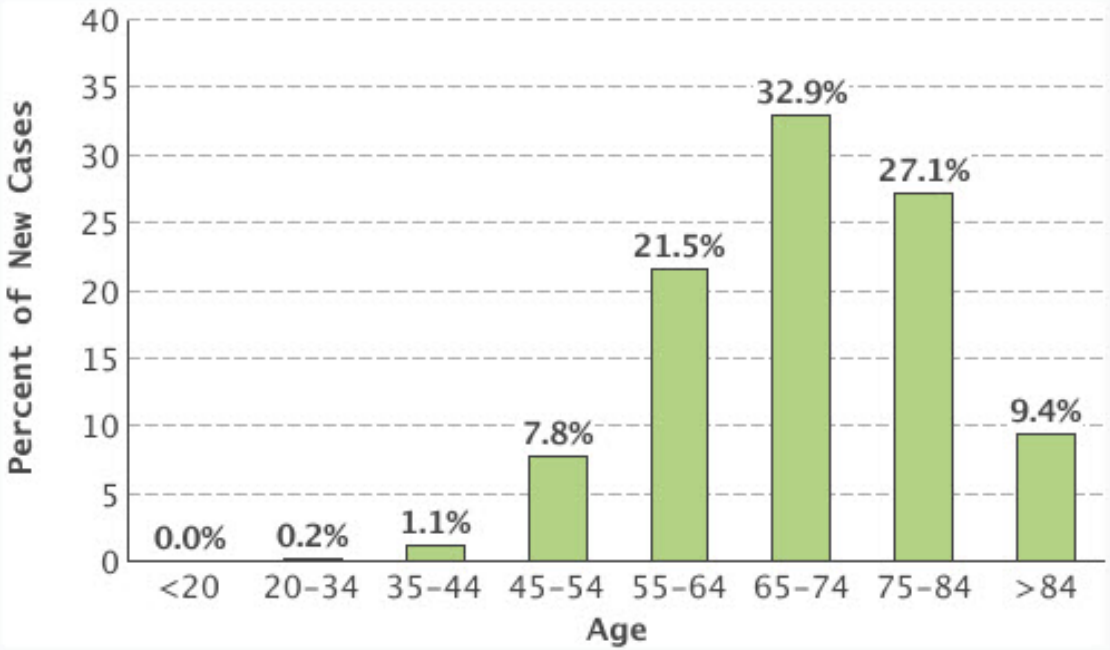


Percent Surviving 5 Years

18.1%

2007–2013

Percent of New Cases by Age Group: Lung and Bronchus Cancer



Lung and bronchus cancer is most frequently diagnosed among people aged 65–74.

Median Age At Diagnosis

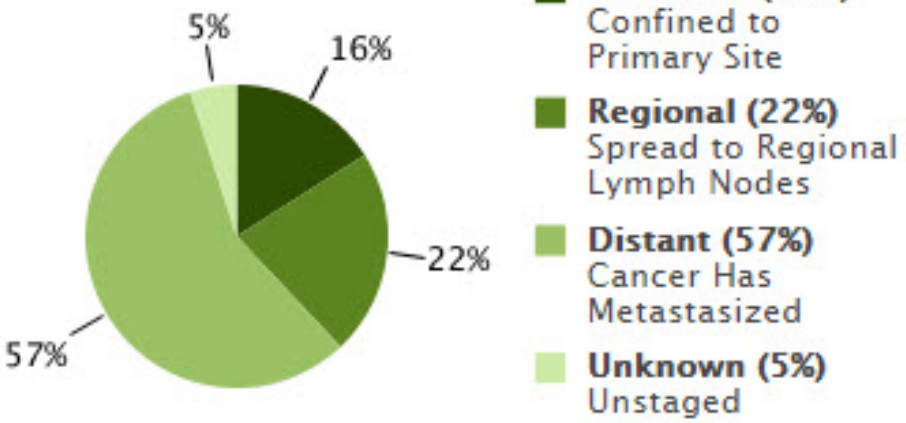
70

SEER 18 2010–2014, All Races, Both Sexes

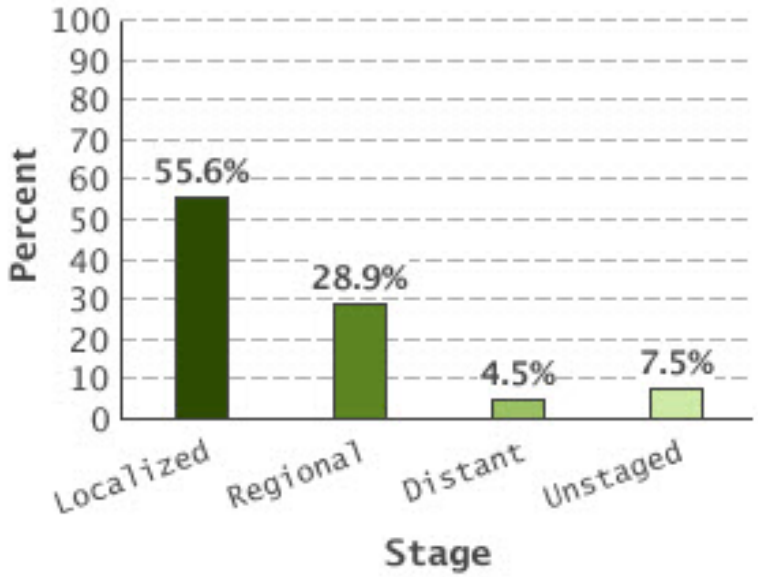
Survival by Stage at DX

Percent of Cases & 5-Year Relative Survival by Stage at Diagnosis: Lung and Bronchus Cancer

Percent of Cases by Stage



5-Year Relative Survival



SEER 18 2007-2013, All Races, Both Sexes by SEER Summary Stage 2000

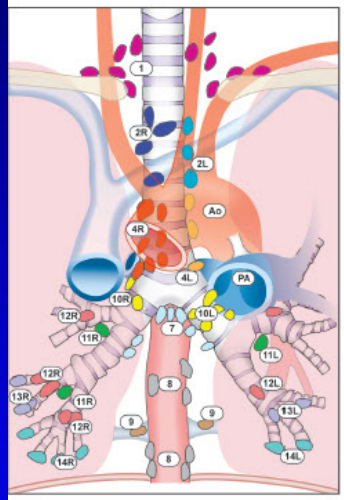
AJCC on Lung Cancer Staging

7th Edition

American Joint Committee on Cancer

Lung Cancer Staging

7th EDITI



Supraclavicular zone

1 Low cervical, supraclavicular, and sternal notch nodes

Superior Mediastinal Nodes

Upper zone

- **2R** Upper Paratracheal (right)
- **2L** Upper Paratracheal (left)
- **3a** Pre-vascular
- **3p** Retrotracheal
- **4R** Lower Paratracheal (right)
- **4L** Lower Paratracheal (left)

Aortic Nodes

AP zone

- **5** Subaortic
- **6** Para-aortic (ascending aorta or phrenic)

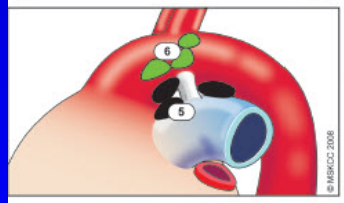
Inferior Mediastinal Nodes

Subcarinal zone

- **7** Subcarinal

Lower zone

- **8** Paraesophageal (below carina)
- **9** Pulmonary ligament



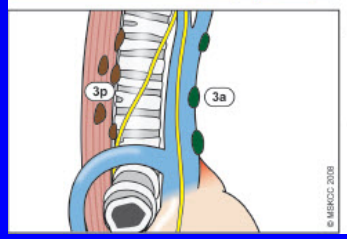
N_1 Nodes

Hilar/Interlobar zone

- **10** Hilar
- **11** Interlobar

Peripheral zone

- **12** Lobar
- **13** Segmental
- **14** Subsegmental



Regional Lymph Nodes (N)

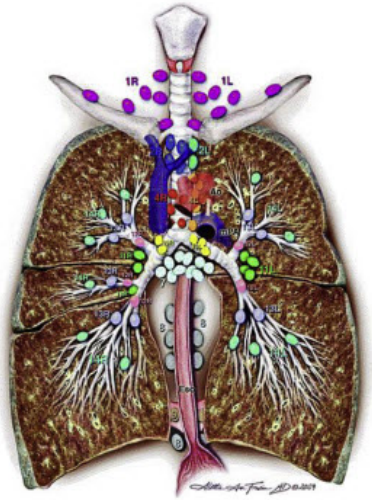
- **NX** Regional lymph nodes cannot be assessed
- **N0** No regional lymph node metastases
- **N1** Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
- **N2** Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
- **N3** Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

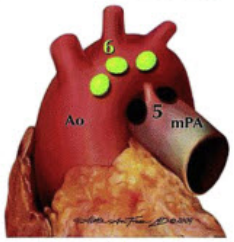
ILLUSTRATION

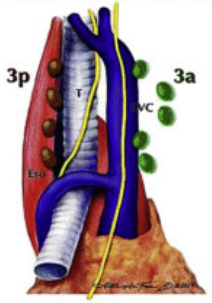
The IASLC lymph node map shown with the proposed amalgamation of lymph into zones.

(© Memorial Sloan-Kettering Cancer Center, 2009.)

8th Edition







Supraclavicular zone

1 Low cervical, supraclavicular, and sternal notch nodes

SUPERIOR MEDIASTINAL NODES

Upper zone

- 2R Upper Paratracheal (right)
- 2L Upper Paratracheal (left)
- 3a Prevascular
- 3p Retrotracheal
- 4R Lower Paratracheal (right)
- 4L Lower Paratracheal (left)

AORTIC NODES

AP zone

- 5 Subaortic
- 6 Para-aortic (ascending aorta or phrenic)

INFERIOR MEDIASTINAL NODES

Subcarinal zone

- 7 Subcarinal

Lower zone

- 8 Paraesophageal (below carina)
- 9 Pulmonary ligament

N1 NODES

Hilar/Interlobar zone

- 10 Hilar
- 11 Interlobar

Peripheral zone

- 12 Lobar
- 13 Segmental
- 14 Subsegmental

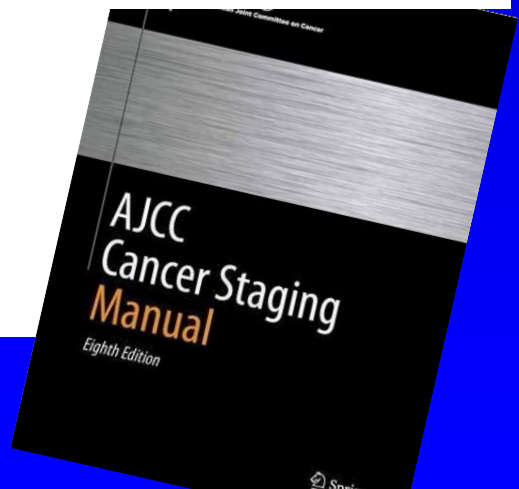
N Subclassification & Distant Mets

N (Regional Lymph Nodes)	
N0	No regional node metastasis
N1	Metastasis in ipsilateral pulmonary or hilar nodes
N2	Metastasis in ipsilateral mediastinal/subcarinal nodes
N3	Metastasis in contralateral mediastinal/hilar, or supraclavicular nodes

M (Distant Metastasis)		
M0	No distant metastasis	
M1a	Malignant pleural/pericardial effusion[c] or pleural /pericardial nodules	M1a *Pl Dissem*
	or separate tumor nodule(s) in a contralateral lobe;	M1a *Contr Nod*
M1b	Single extrathoracic metastasis	M1b *Single*
M1c	Multiple extrathoracic metastases (1 or >1 organ)	M1c *Multi*

TABLE 4] N Subclassification

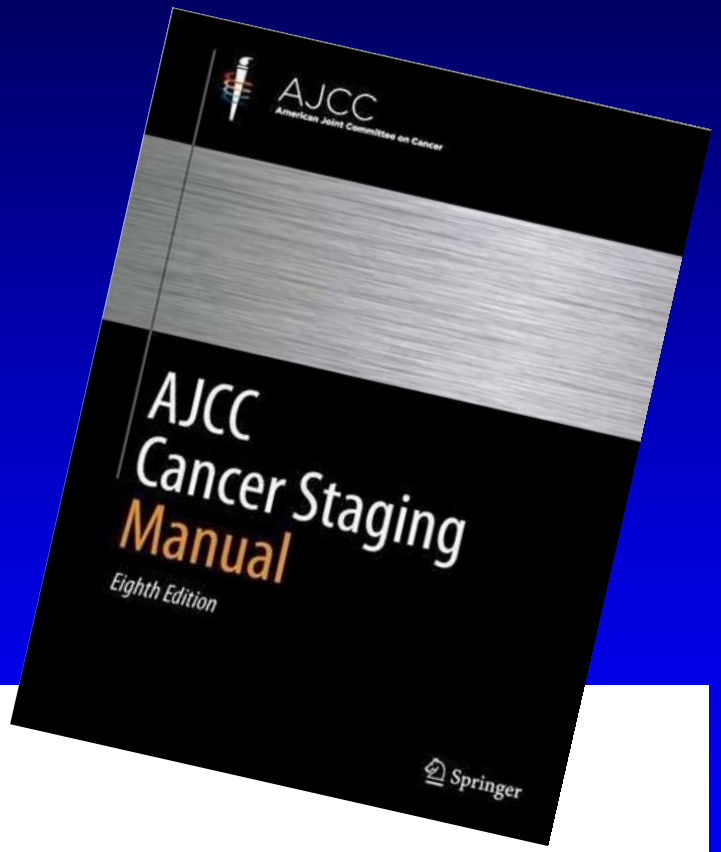
Category	Subclass	Description
Nx		Regional lymph nodes cannot be assessed
N0		No regional lymph node involvement
N1	N1a	Single-station N1 involvement
	N1b	Multiple-station N1 involvement
N2	N2a1	Single-station N2 without N1 involvement (skip)
	N2a2	Single-station N2 with N1 involvement
	N2b	Multiple-station N2 involvement
N3		N3 lymph node involvement



Lung Cancer Grouping & Survival

TABLE 5] Lung Cancer Stage Grouping (Eighth Edition)

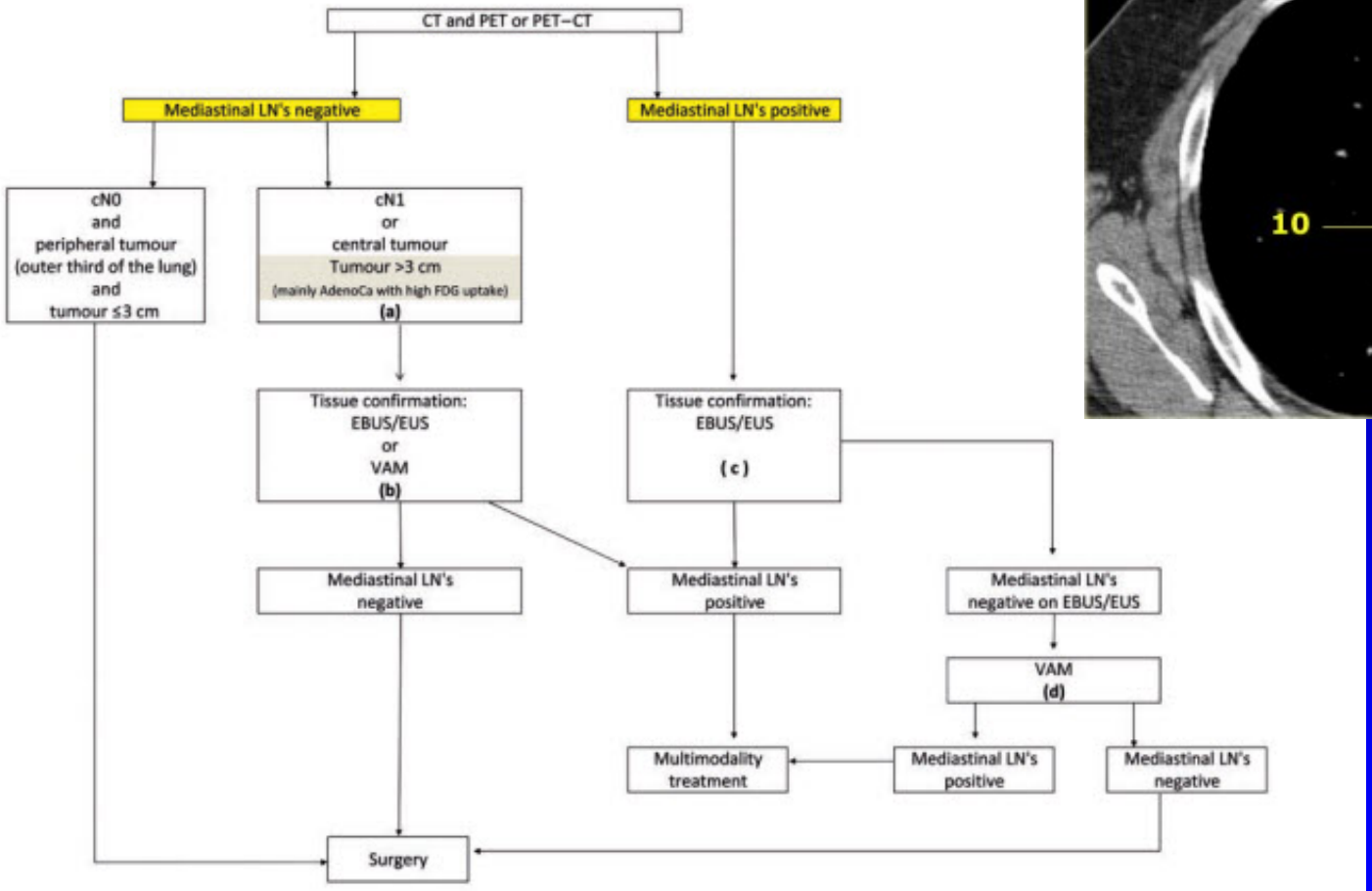
T/M	Label	N0	N1	N2	N3
T1	T1a ≤1	IA1	IIB	IIIA	IIIB
	T1b >1-2	IA2	IIB	IIIA	IIIB
	T1c >2-3	IA3	IIB	IIIA	IIIB
T2	T2a Cent, Visc Pl	IB	IIB	IIIA	IIIB
	T2a >3-4	IB	IIB	IIIA	IIIB
	T2b >4-5	IIA	IIB	IIIA	IIIB
T3	T3 >5-7	IIB	IIIA	IIIB	IIIC
	T3 Inv	IIB	IIIA	IIIB	IIIC
	T3 Sanel	IIB	IIIA	IIIB	IIIC
T4	T4 >7	IIIA	IIIA	IIIB	IIIC
	T4 Inv	IIIA	IIIA	IIIB	IIIC
	T4 Ipsi Nod	IIIA	IIIA	IIIB	IIIC
M1	M1a Contr Nod	IVA	IVA	IVA	IVA
	M1a Pl Dissem	IVA	IVA	IVA	IVA
	M1b Single	IVA	IVA	IVA	IVA
	M1c Multi	IVB	IVB	IVB	IVB

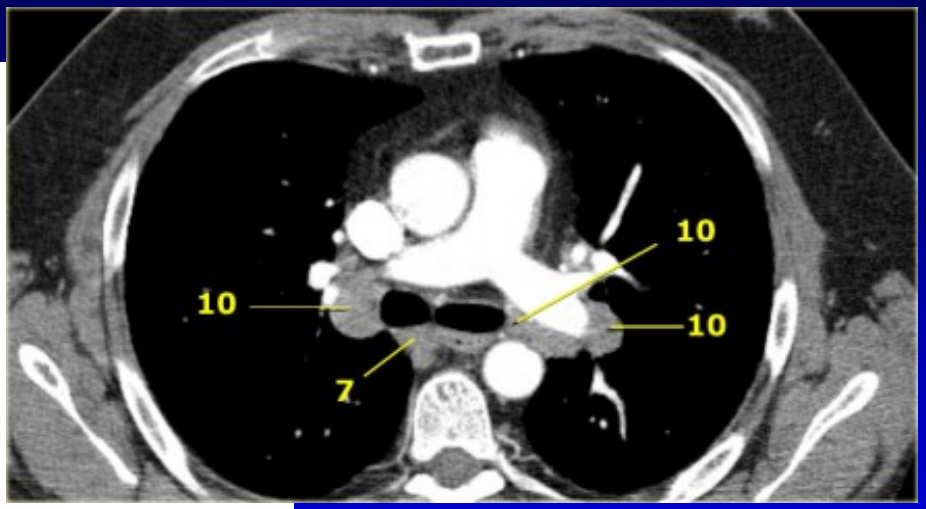


5-Year Survival (%)

Type	IA1	IA2	IA3	IB	IIA	IIB	IIIA	IIIB	IIIC	IVA	IVB
Clinical	92	83	77	68	60	53	36	26	13	10	0
Pathologic	90	85	80	73	65	56	41	24	12	-	-

CT & PET or PET-CT in Staging Lung Cancer



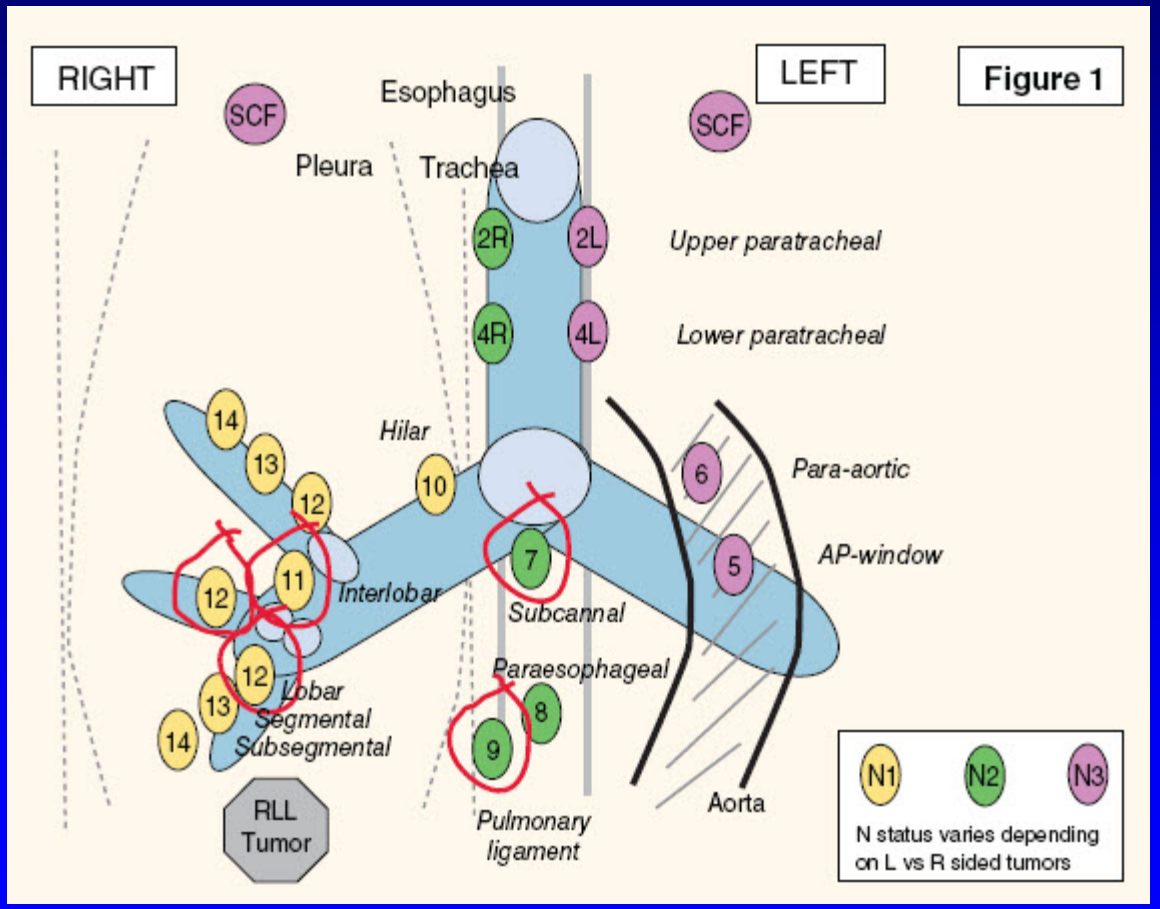


http://www.radiologyassistant.nl/en/p4646f1278c26f/mediastinum-lymph-node-map.html

Annals of Oncology 28 (Supplement 4);iv1-iv21,2017

EBUS-TBNA & EUS

- **EBUS-TBNA: LN sample**
  - 2R and 2L
  - 4R and 4L
  - 7
  - 10R and 10L
  - 11R and 11L
  - Sometimes 12
- **EUS: LN sample**
  - 8
  - 9

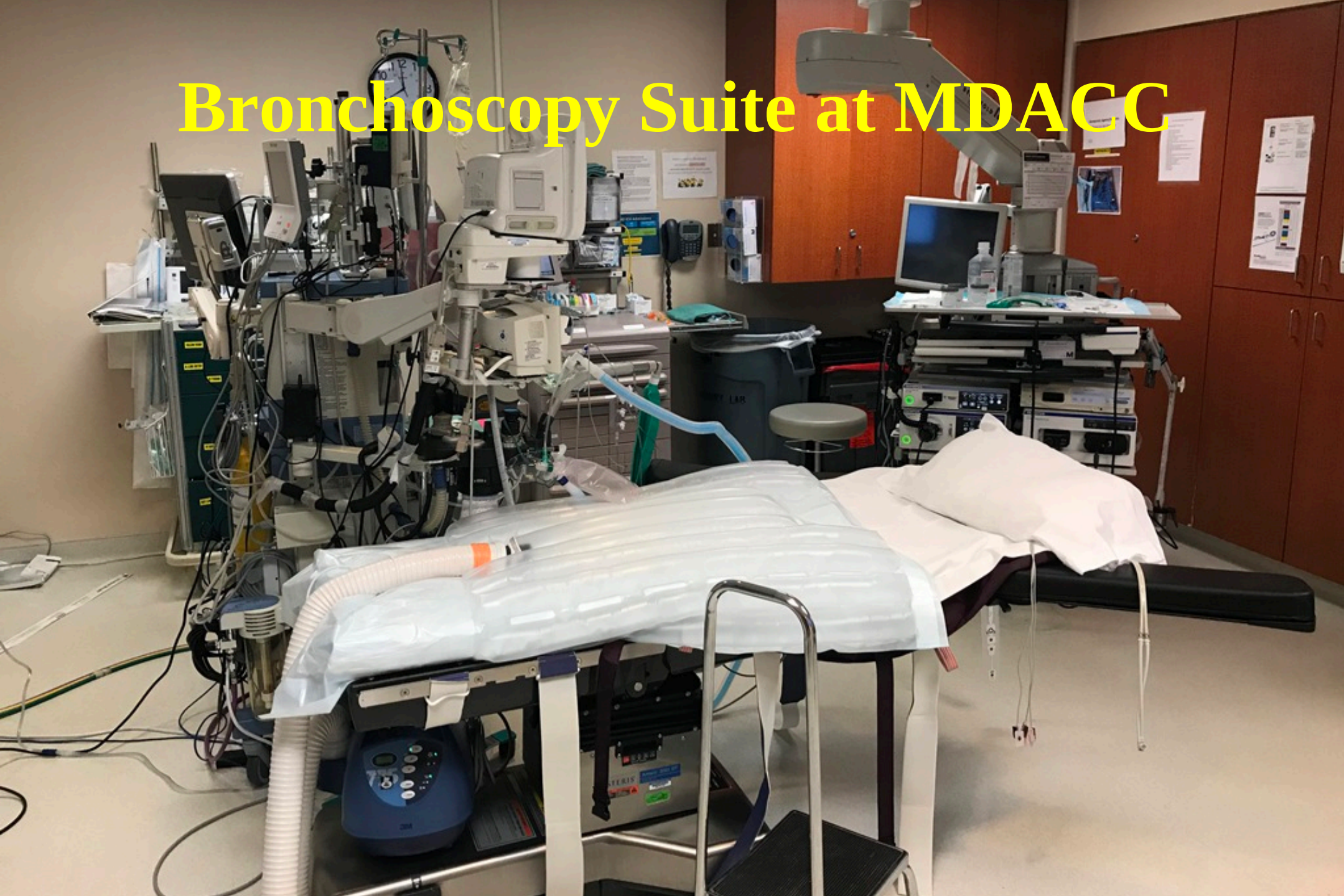


Indications for EBUS-TBNA

- Initial staging & restaging of lung cancer
- Evaluating mediastinal adenopathy with no prior malignancy
- Evaluating mediastinal adenopathy with non-lung primary
- Evaluating mediastinal adenopathy with known non-lung carcinoma and now new lung mass
- Obtain material for mutational analysis

The EBUS-TBNA ROSE Advantage

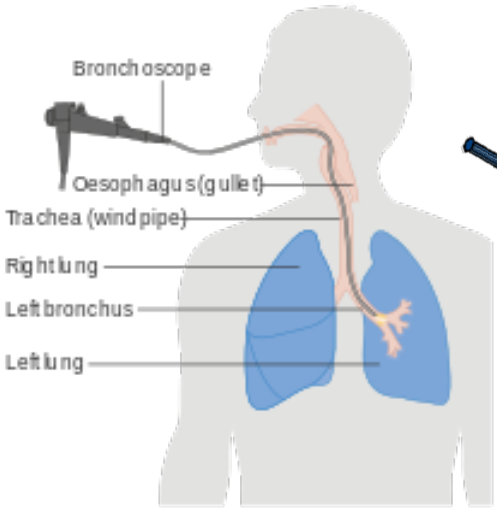
- Real time evaluation of procured material
- Improves diagnostic yield
- Sampling stopped when adequate material obtained
- Specimen triaged for ancillary studies
- Communication between the pulmonologist and pathology faculty & staff


Bronchoscopy Suite at MDACC

Goals for Tissue Procurement

- Adequate sampling of lymph node
- Benign vs. Malignant
- Metastatic lung vs. non-lung carcinoma vs. other
- Histologic subtype
- Molecular testing

EBUS TBNA with ROSE for Dx & Mutational Analysis



ROSE--Diff Quik & Pap-stained smears

Cytology Preparations

IPOX or Special stains

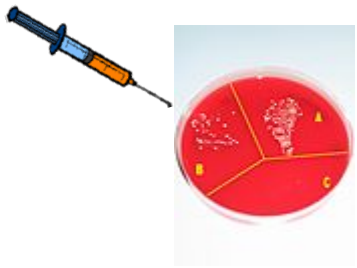
Diagnosis

Mutational Analysis

Cytospin or Cell block

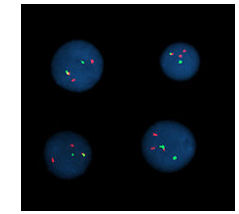
RPMI

FLOW-- suspected lymphoma

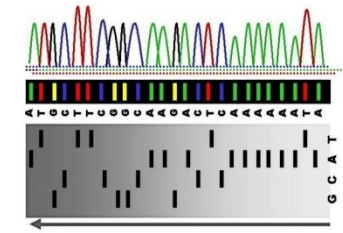


Microbiology--cultures

FISH



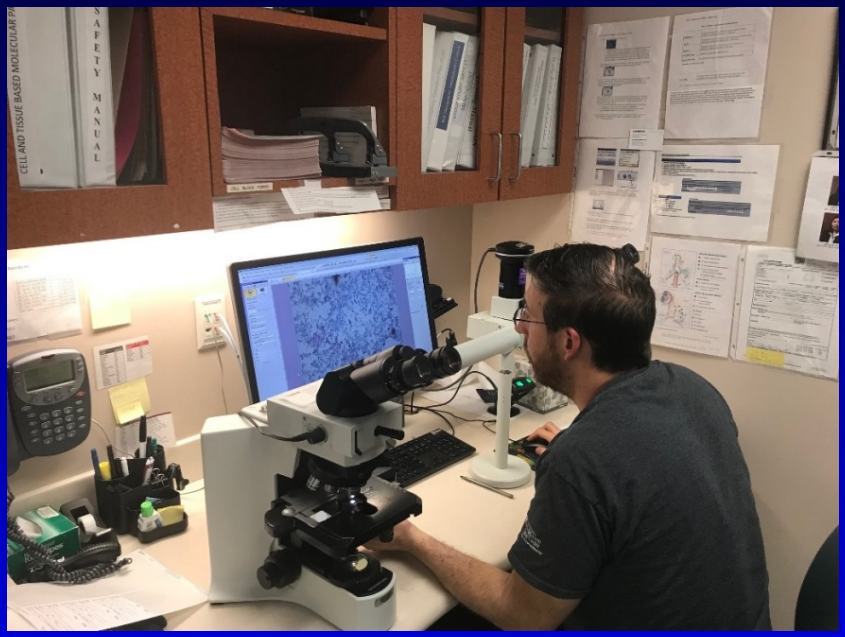
MDL



IPOX

ROSE for EBUS-TBNA

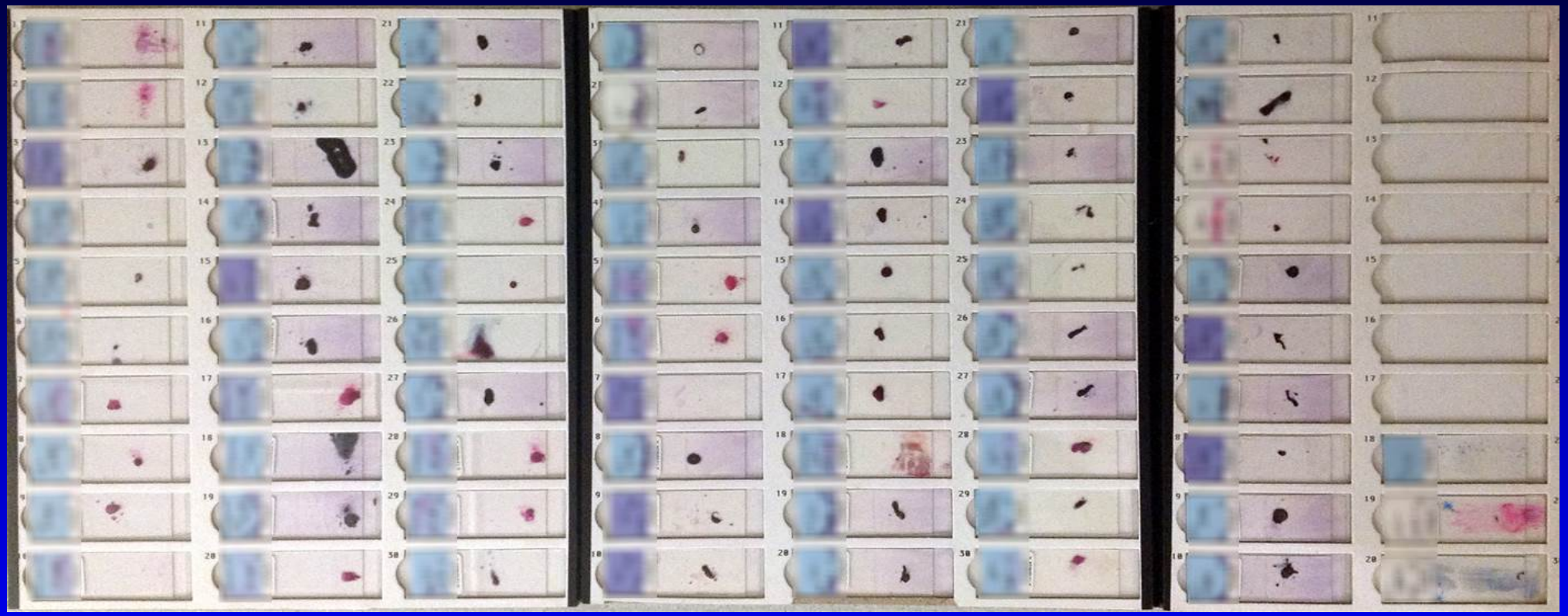
- Bronchoscopy suite contains a lab prep area & microscope
- ~3 min from main cyto lab
- Cytotech performs ROSE
  - Adequate sampling of LN
  - Malignant ➡ ancillary studies
- Telepathology capability; not used on every case



Performance ROSE

- Senior Cytotechnologists
- Cytopathologists
  - Direct viewing of slides
  - Telepathology
- Trainees (fellows and residents)

What EBUS Can Look Like Without ROSE

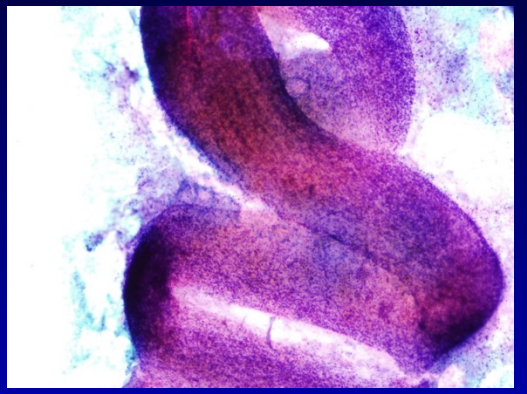


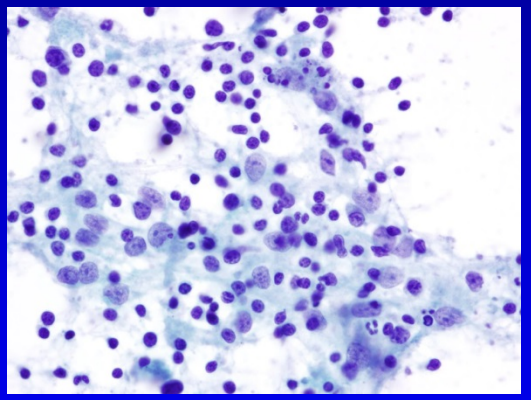
EBUS, station 7, FNA

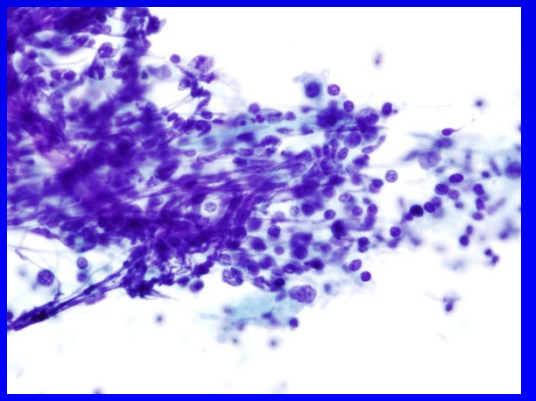
Received 72 direct smears and 1 cell block prepared from 6 needle passes. Passes #1-5 non-diagnostic. Pass #6 has some lymphocytes and two slides with few cells of carcinoma.

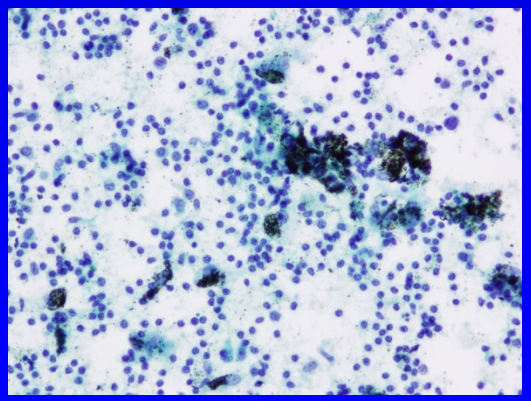
Specimen Adequacy

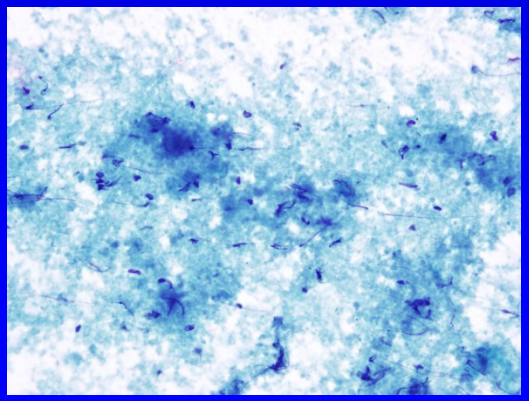
- Number of lymphocytes/hpf
- Germinal center cells
- Anthracotic pigment-laden macrophages
- Extensive necrosis











Positive Cases on ROSE for Non-Small Cell Lung Carcinoma

- 10 smears (5 DQ & 5 Pap)
- Extra Pass for CB
- 1st & last H&E slide
- 10 USS up front
- Not uncommon need more USS for MDL

ROSE Reimbursement

- **Utilization Review and reimbursement of cytology services in EBUS-TBNA**

Table 4 Cost calculation.

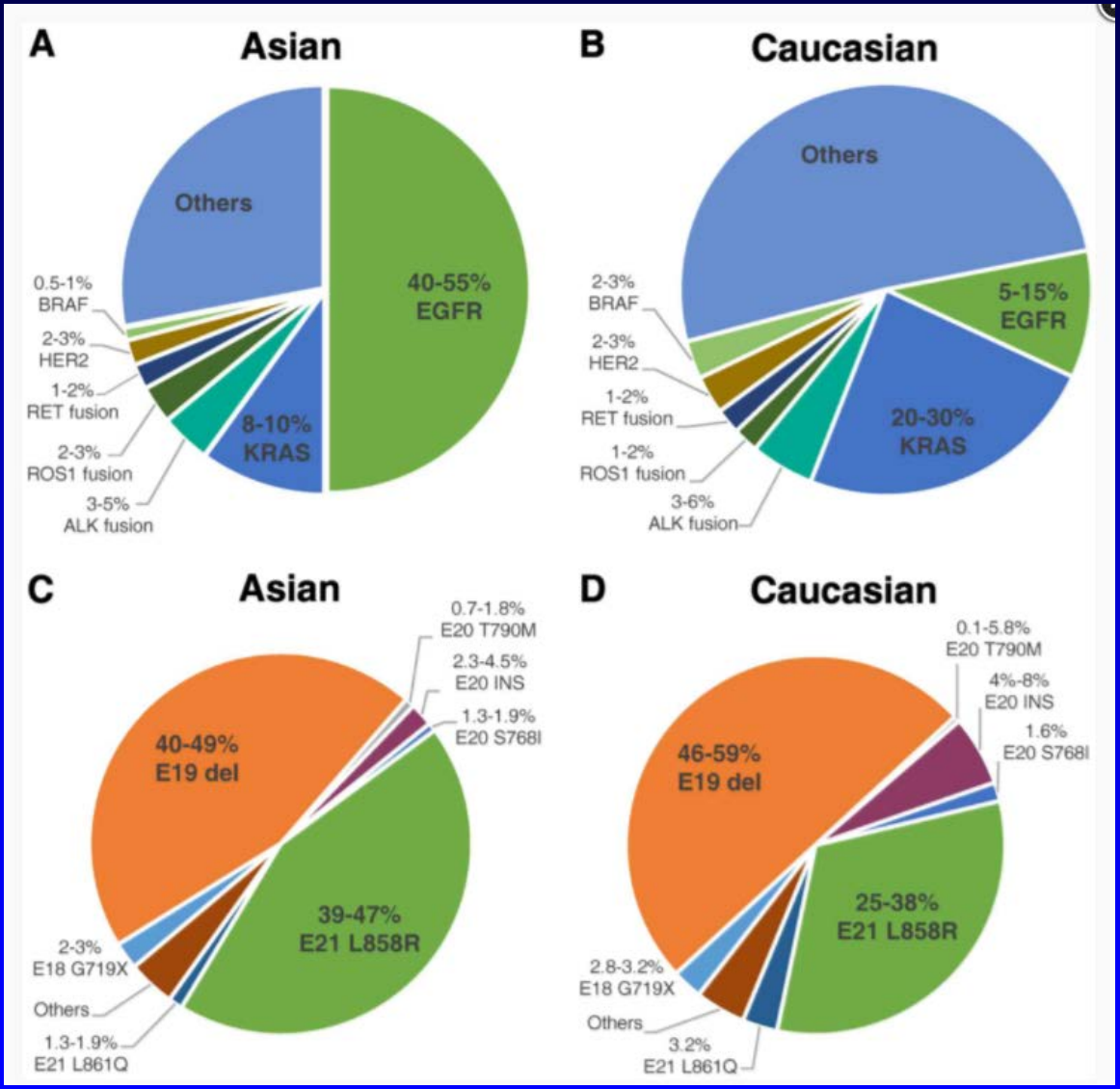
Financial considerations	Technical component	Professional component	Global component	Net gain /loss (range)
Reimbursement	$46.73	$113.97	$160.70	
Cost (using telecytology)	$109.00	$55.71	$164.71	-$4.01
Cost (non-telecytology)	$109.00	$101.45	$210.45	-$49.75
Cost (overall average)	$109.00	$85.60	$194.60	-$33.90

Miller R, et al. JASC. 2016;5:139-144.

Triaging Specimens for Non-Small Cell Lung Carcinoma

- **Diagnosis**
  - **Adenocarcinoma vs. Squamous cell carcinoma Limited immunopanel (TTF-1 and p40); save material for mutational analysis (MDL)**
  - **1-2% of NSCLC cannot be classified as adenocarcinoma or squamous carcinoma; MDL may be helpful**

Mutation Frequencies in Asian and Caucasian Populations



Oncology Reports.2017:37;1347-1358

Targeted Therapy

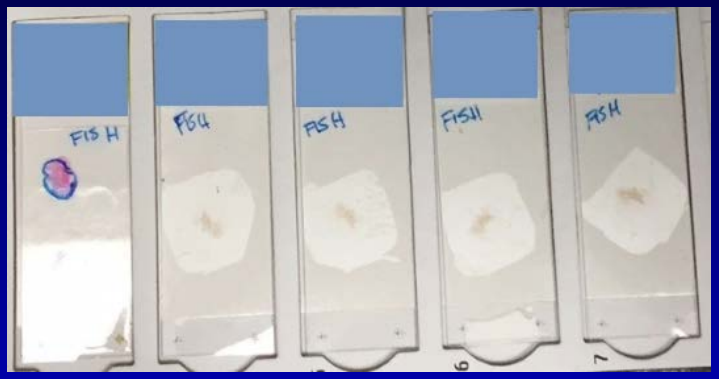
DRUG	TARGET	INDICATION
Erbtinib (Tarceva) Afatinib (Gilotrif) Gefitnib (Iressa)	EGFR	NSCLC (EGFR exon 19 del or 21 substitution)
Osimertinib (Tagrisso)	EGFR T790M	NSCLC with T790M mutation
Necitumumab (Portazza)	EGFR	Squamous cell ca
Ceritnib (Zykadia) Alectinib (Alecensa) Brigatinib (Alunbrig)	ALK	NSCLC with ALK fusion
Crizotinib (Xalkori)	ALK, ROSI	NSCLC with ALK fusion or ROSI gene alteration
Debrafenib (Tafinlar)	BRAF	NSCLC with BRAF V600E mutation
Tranmetinib (Mekinist)	MEK	NSCLC with BRAF V600E mutation
Pembrolizumab (Keytruda)	PD-L1	NSCLC

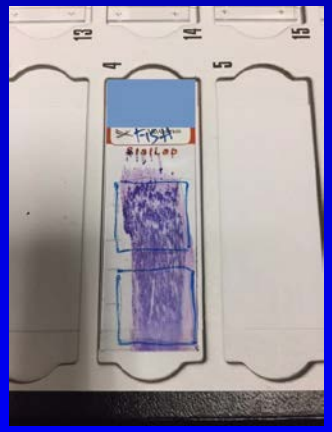
PD-L1

- **An immuno-peroxidase stain for PD-L1, 22C3 AB, performed on tumor cells within the cell block preparation, yielded the following result: membranous staining in ____% of cells (___+ staining intensity).**

Triaging FNA for FISH Analysis

- Most frequent FISH request
  - ALK, RET, MET, ROSI
- Need monolayer smear with tumor cells in the center of the slide
- Slide preparations for cytogenetics
  - 4 USS & 1 H&E (tumor circled)
  - or DQ smears scored and marked (1-2 probes can be applied per slide)





EX: Mutational Analysis Request at MDACC for Lung Adenocarcinoma

- FISH Tests
  - ROS1
  - RET
  - CMET
  - ALK
- Immuno Tests
  - PD-L1
  - MSI
- MDL Tests
  - BRAF V600E
  - EGFR Mutation
  - KRAS Mutation
  - RET Mutation
  - EML4/ALK FUSIONS
  - ROS1 FUSIONs

What Tissue is Suitable for MDL?

2017 Statement
Recommendation: Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing.
Expert consensus opinion: Laboratories should use, or have available at an external reference laboratory, clinical lung cancer biomarker molecular testing assays that are able to detect molecular alterations in specimens with as little as 20% cancer cells.
Strong recommendation: Laboratories should not use total EGFR expression by IHC testing to select patients for EGFR-targeted TKI therapy.

(Arch Pathol Lab Med. doi: 10.5858/arpa.2017-0388-CP)

Table 1 Tumor fraction and DNA yield from concurrently acquired fine needle aspiration and core needle biopsy samples

	FNA smears (n = *24*)	*CNB* (n = *24*)	P-*value*
Tumor fraction			
Mean	54%	39%	
Median	60%	30%	$P = 0.003$
Range	25–90%	20–70%	
DNA yield			
Mean	6.6 ng/μl	17.5 ng/μl	
Median	3.6 ng/μl	12.9 ng/μl	$P = 0.01$
Range	0.36–21 ng/μl	0.27–55 ng/μl	

Abbreviations: CNB, core needle biopsy; FNA, fine needle aspiration.

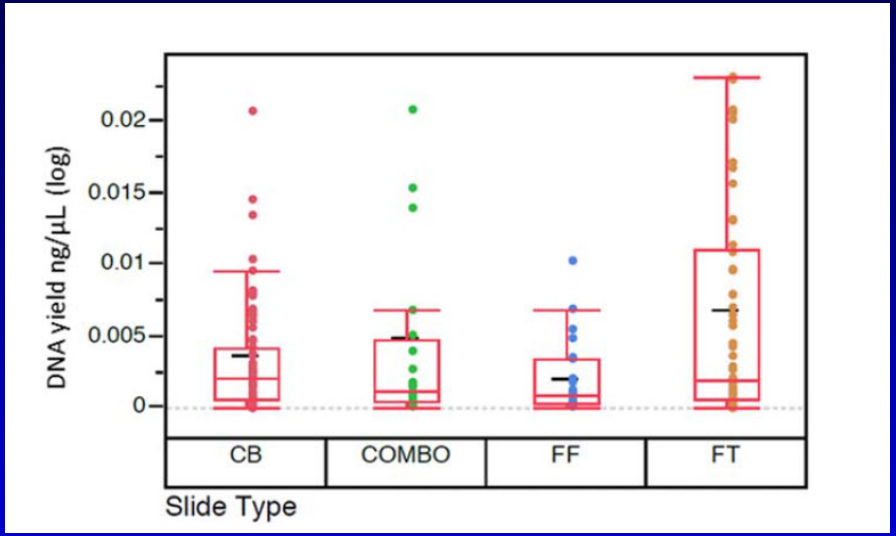
Roy et al.

Optimal Cytology Preparations for Mutational Analysis

- Validation studies needed for laboratory
- Types of preparations
  - Smears vs. liquid-base preparations
  - Papanicolaou vs. Diff-Quik smears
  - Role of cell block preparations
  - Combining different preparations
  - Other techniques (cell transfer/lift)
  - ProCore Bx??? (Acta Cytologica 2106;60:254-259)

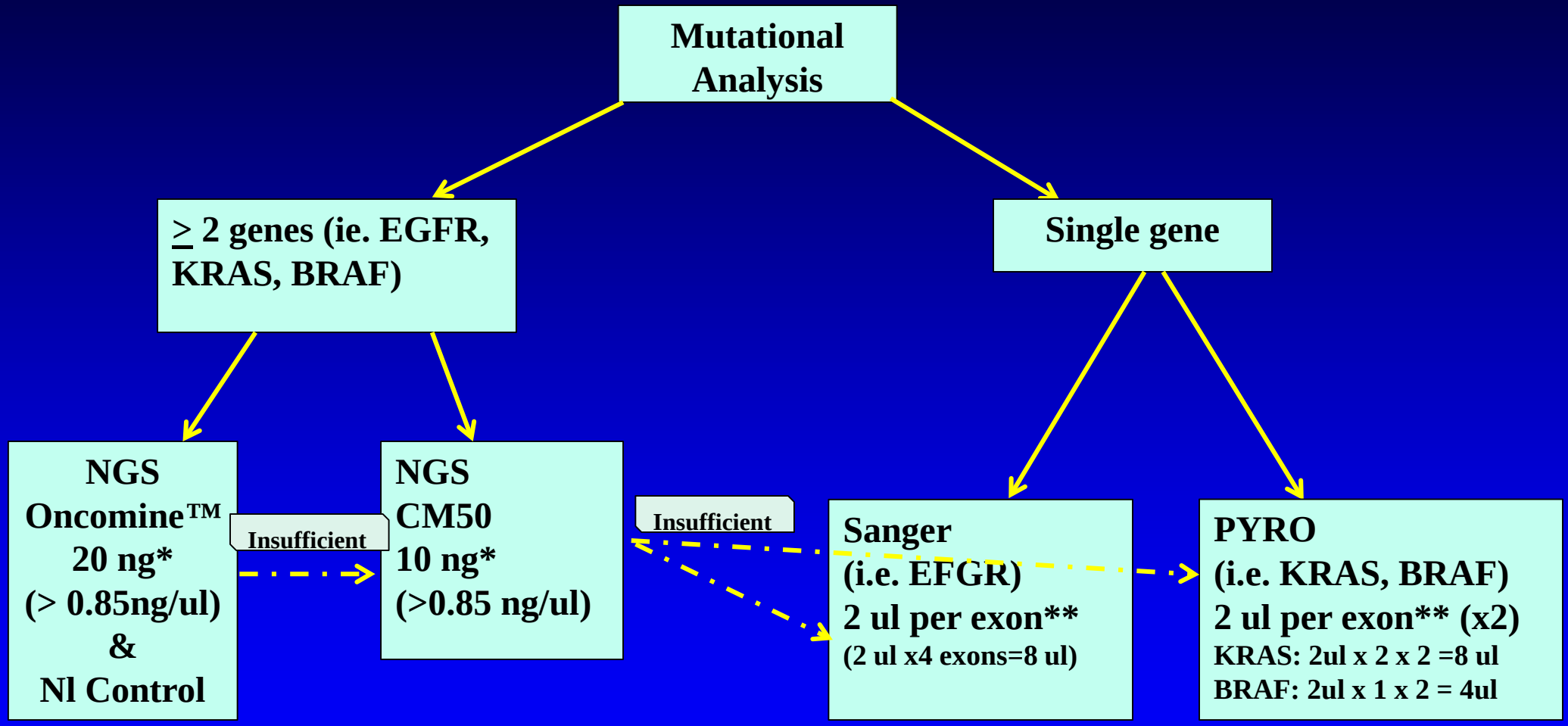
Cytology Preparations vs. DNA Yield

- Frosted tip slides had highest DNA yield over cell blocks, frosted slides and combination
- No experience with liquid base specimens



Roy et al

Mutation Testing Algorithm (MDL)



*Recommended

**Recommended (1000ng/ul) Almost never have that

Courtesy of Dr. Sinchita Roy-Chowdhuri

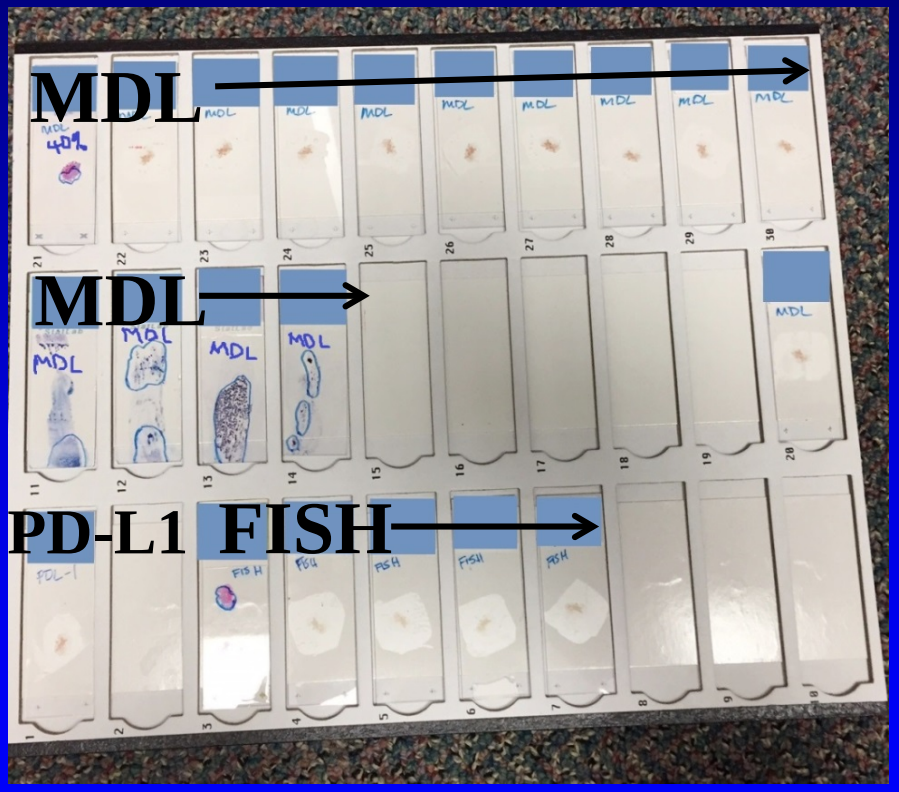
How Much Material Do I Send for MDL???

"As much as You can!!!"

- 1 cell ~ 6-7 pg of DNA
- For every 1 ng of DNA need ~143-166 intact cells
- NGS (Ion Torrent PGM) needs 10 ng, so rounding up ~ 2000 intact tumor cells
- Oncomine needs 20 ng, so 2X (~4000)
- Future ????

Triaging FNA for Mutational Analysis

- MDL
  - 10 USS & 1 H&E (tumor circled; % of tumor) plus 2 DQ & 2 PAP smears (tumor circled, estimate % of tumor present) submitted
- FISH
  - ALK, RET, MET, ROSI
  - 4 USS & 1 H&E (tumor circled) submitted to cytogenetics
- Immunostains
  - PD-L1
  - MSI (4 slides +)

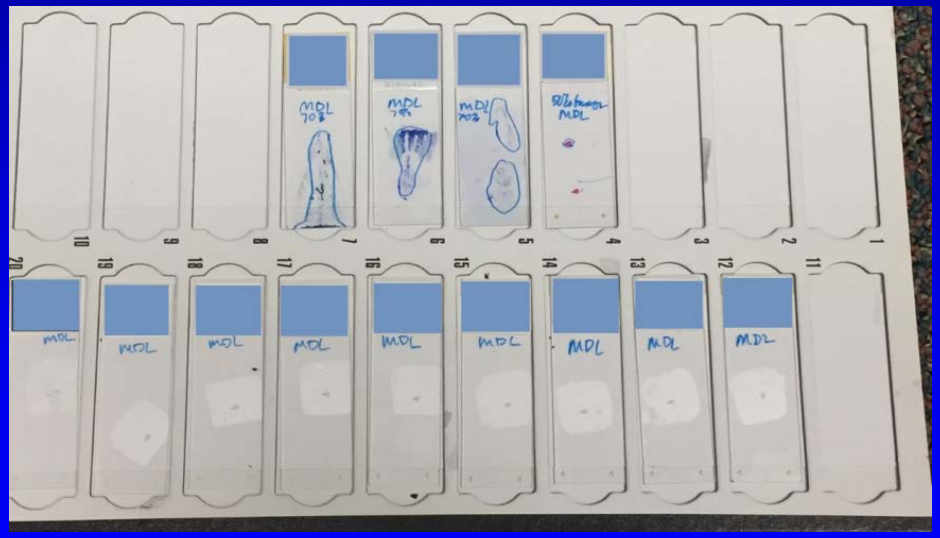


Triaging FNA for Mutational Analysis

- MDL requested on limited material
- Circle tumor; give tumor %
- Write a note to document slides sent
- Request slide to be returned with remaining tumor
- Designated areas circled scrapped (DQ & Pap-stained combined)

Mutational Analysis with CM50

- Smears and cell block from the same case can be combined for MDL



Solid Tumor Genomics Assay v1

Clinical test requisition for mutation studies on the following genes was received: *BRAF, EGFR, KRAS*

A next generation sequencing (NGS)-based analysis for the detection of somatic mutations in the coding sequence of 128 genes and selected copy number variations (amplifications) in 49 genes (overlap: 134 genes total) was performed on the DNA extracted from the sample in our CLIA-certified molecular diagnostics laboratory. Interpretative findings are reported in the gene summary table(s) below followed by specific details of detected amplification and/or mutations.

Interpretation Key:

Circled/Bold:	Mutation or amplification detected
Underlined:	Mutation testing requested (ordered gene)
Asterisk:	Additional confirmation studies in progress

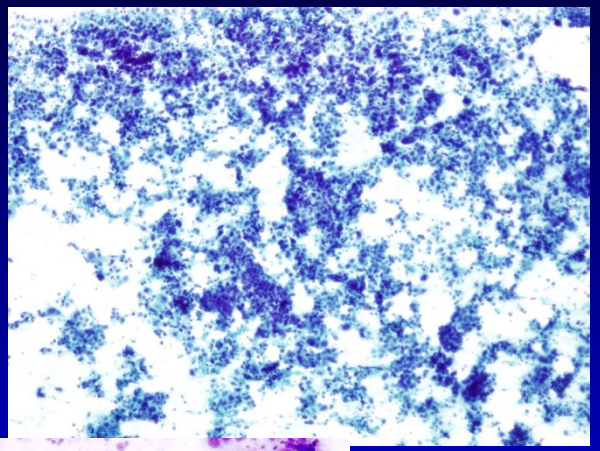
GENE SUMMARY:

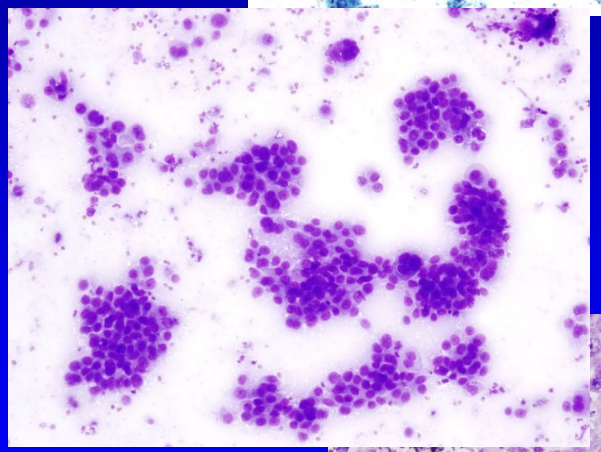
Molecular Diagnostics

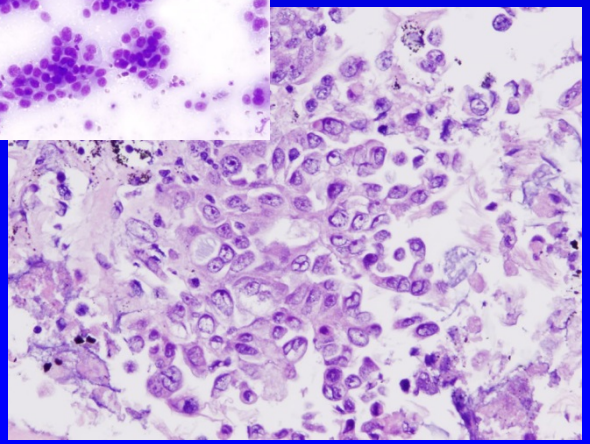
ABL1	*CSF1R*	*FGFR2*	*IDH1*	*MLH1*	*PTPN11*	***TP53***
AKT1	*CTNNB1*	*FGFR3*	*IDH2*	*MPL*	*RB1*	*VHL*
ALK	*EGFR*	*FLT3*	*JAK2*	*NOTCH1*	*RET*	
APC	*ERBB2*	*GNA11*	*JAK3*	*NPM1*	*SMAD4*	
ATM	*ERBB4*	*GNAQ*	*KDR*	*NRAS*	*SMARCB1*	
BRAF	*EZH2*	*GNAS*	*KIT*	*PDGFRA*	*SMO*	
CDH1	*FBXW7*	*HNF1A*	*KRAS*	*PIK3CA*	*SRC*	
CDKN2A	*FGFR1*	*HRAS*	*MET*	*PTEN*	*STK11*	

Mutational Analysis with Oncomine

- **Request: BRAF, EGFR, KRAS**
- **Submit Tumor & Normal control**







Mutational Analysis Performed on FNA specimen with Oncomine

Solid Tumor Genomics Assay v1

Clinical test requisition for mutation studies on the following genes was received: *BRAF, EGFR, KRAS*

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Interpretation Key:

Circled/Bold:	Mutation or amplification detected
Underlined:	Mutation testing requested (ordered gene)
Asterisk:	Additional confirmation studies in progress

GENE SUMMARY:

Molecular Diagnostics

ABL1	CCND1	EZH2	IGF1R	MPL	PIK3R1	SRC
ACVRL1	CCNE1	FBXW7	IL6	MSH2	PNP	STAT3
AKT1	CD274	FGFR1	JAK1	MTOR	PPARG	STK11
ALK	CD44	FGFR2	JAK2	MYC	**PPP2R1A**	TERT
APC	CDH1	FGFR3	JAK3	MYCL1	PTCH1	TET2
APEX1	CDK4	FGFR4	KDR	MYCN	PTEN	TIAF1
AR	CDK6	FLT3	KIT	MYD88	PTPN11	**TP53**
ARAF	CDKN2A	FOXL2	KNSTRN	MYO18A	RAC1	TSC1
ATM	CHEK2	GAS6	**KRAS**	NF1	RAF1	TSC2
ATP11B	CSF1R	GATA2	MAGOH	NF2	RB1	U2AF1
BAP1	CSNK2A1	GATA3	MAP2K1	NFE2L2	RET	VHL
BCL2L1	CTNNB1	GNA11	MAP2K2	NKX2-1	RHEB	WT1
BCL9	DCUN1D1	GNAQ	MAPK1	NKX2-8	RHOA	XPO1
BIRC2	**DDR2**	GNAS	MAX	NOTCH1	RPS6KB1	ZNF217
BIRC3	DNMT3A	HNF1A	MCL1	NPM1	SF3B1	
BRAF	EGFR	HRAS	MDM2	NRAS	SMAD4	
BRCA1	ERBB2	IDH1	MDM4	PAX5	SMARCB1	
BRCA2	ERBB3	IDH2	MED12	PDCD1LG2	SMO	
BTK	ERBB4	IFITM1	MET	PDGFRA	SOX2	
CBL	ESR1	IFITM3	MLH1	PIK3CA	SPOP	

Summary

- **EBUS-TBNA aids in staging patients with lung cancer**
- **EBUS-TBNA with ROSE can help ensure adequate material for diagnosis and molecular testing**
- **Triaging and optimizing material prior to mutational analysis is an important step**
- **Mutational analysis can be performed on cytology material**


Thank You