MOLECULAR TESTING:
Challenges in Delivery of the Service

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DISCLOSURES

- **Advisory Boards** (compensated): BMS, Lilly/Imclone, AstraZeneca, Celgene, Synta Pharm., Bayer, Genentech/Roche, Ventana, Amgen, Biothera, Clovis.

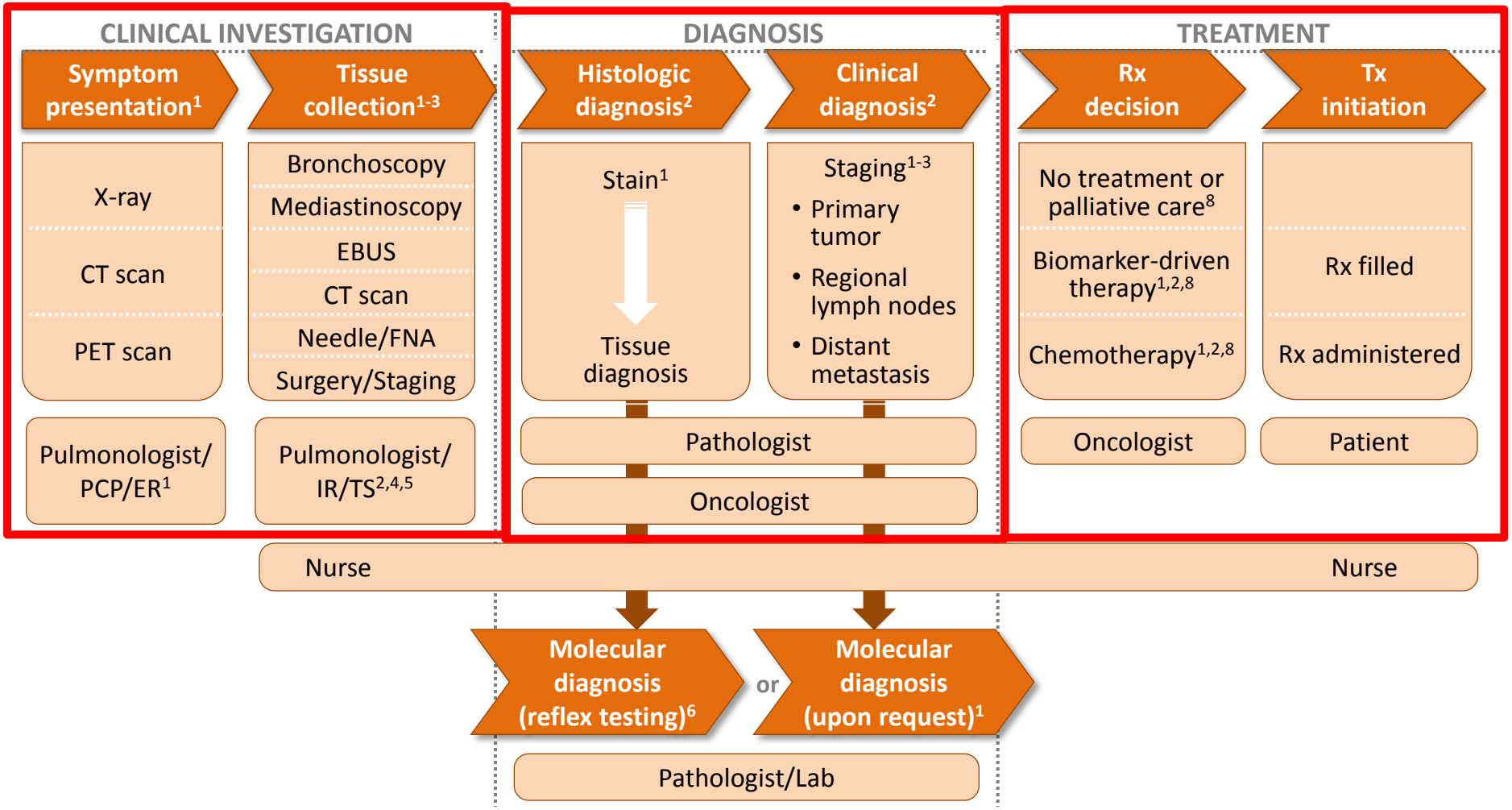
- **Resarch Funding (through Univ. Colorado)**: Celgene, Amgen, Genentech, Lilly/Imclone, Ventana.

WHAT CHALLENGES?

- Building up an multidisciplinary infrastructure
- Understanding of the therapeutic landscape for lung cancer
- Tissue acquisition
- Tissue processing
- Pre-analytic variables
- Assay methodology
- Reporting the results

BUILDING A MULTIDISCIPLINARY INFRASTRUCTURE !

The Patient Journey: NSCLC



Understanding the therapeutic landscape:

- Does histology matter?
- What mutations matter?
- Does resistant mutation matter?
- Should pathologists recommend treatment options?

DOES HISTOLOGY MATTERS?

“New” Therapies in Advanced NSCLC

Agent	Patient Selection
Bevacizumab	Histology (non-squamous)
Pemetrexed	
Nivolumab	Histology (squamous, 2. line)
EGFR TKI (gefitinib, erlotinib,afatinib)	*EGFR* mutation (first line)
Crizotinib/Ceritinib	*ALK* rearrangement

ORIGINAL ARTICLE

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

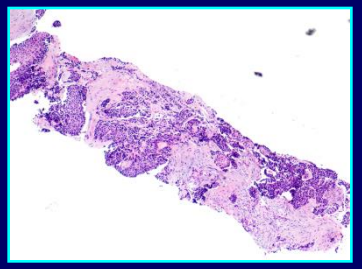
Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Neal I. Lindeman, MD; Philip T. Cagle, MD; Mary Beth Beasley, MD; Dhananjay Arun Chitale, MD; Sanja Dacic, MD, PhD; Giuseppe Giaccone, MD, PhD; Robert Brian Jenkins, MD, PhD; David J. Kwiatkowski, MD, PhD; Juan-Sebastian Saldivar, MD; Jeremy Squire, PhD; Erik Thunnissen, MD, PhD; Marc Ladanyi, MD

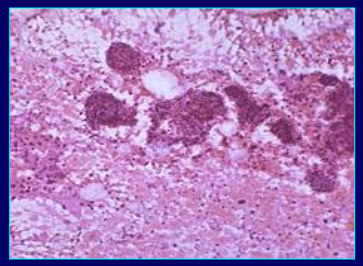
Journal Thoracic Oncol , April 2013

WHOM TO TEST?
HISTOLOGY MATTERS!

Tumor Positive

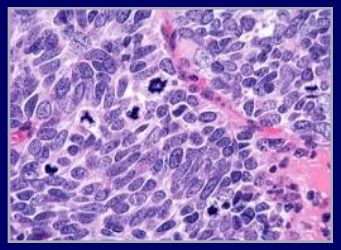


Biopsy



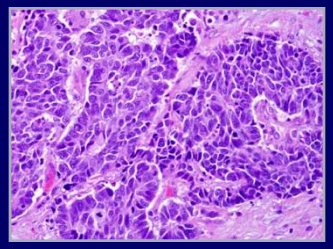
Cytology

SCLC



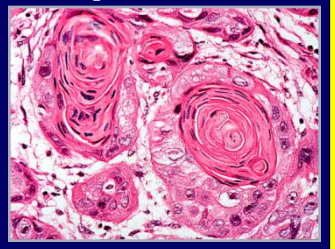
Morphology

LCNEC



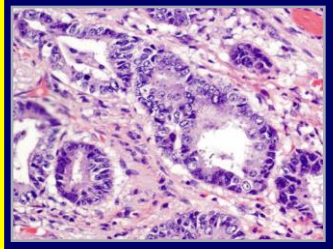
Morphology
IHC NE (+)

Squamous



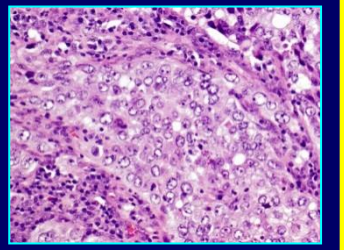
Morphology
IHC p63/p40 (+)

Adenoca



Morphology
IHC TTF1 (+)

NSCLC-NOS



Morphology
IHC (-)

Molecular Testing:
***EGFR* mutation, *ALK* Fusion**

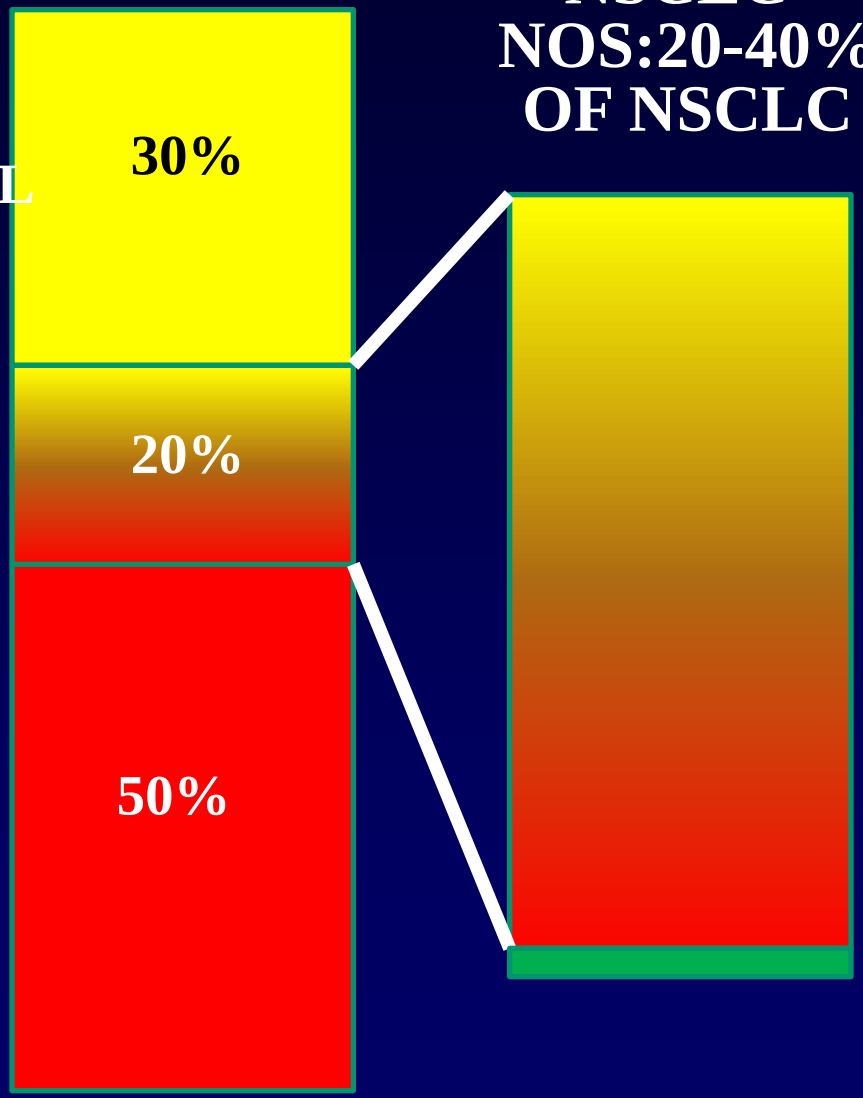
LIGHT MICROSCOPY

FORMER NSCLC-NOS:20-40% OF NSCLC

SQUAMOUS CELL CARCINOMA

NSCLC-NOS

ADENO-CARCINOMA



NSCLC, FAVOR SQUAMOUS CELL CARCINOMA

NSCLC-NOS <5%

NSCLC, FAVOR ADENO-CARCINOMA

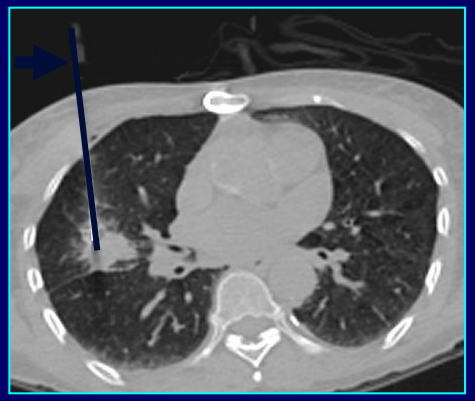
METASTASIS OR OTHER TUMOR

IMMUNOHISTOCHEMICAL MARKERS

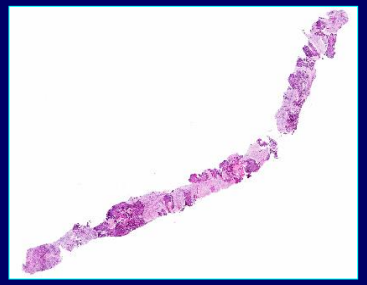
- **ADENOCARCINOMA (ONE MARKER)**
  - **TTF-1 (best), Napsin, PE-10**
- **SQUAMOUS CARCINOMA (ONE MARKER)**
  - **p40 (best), p63, CK5/6, 34βE12**

"THE TISSUE IS STILL THE ISSUE"

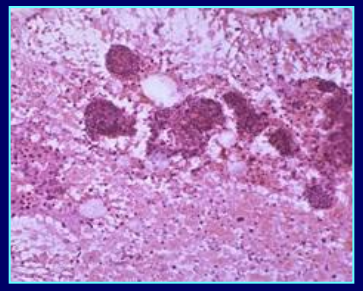
Advanced Tumor

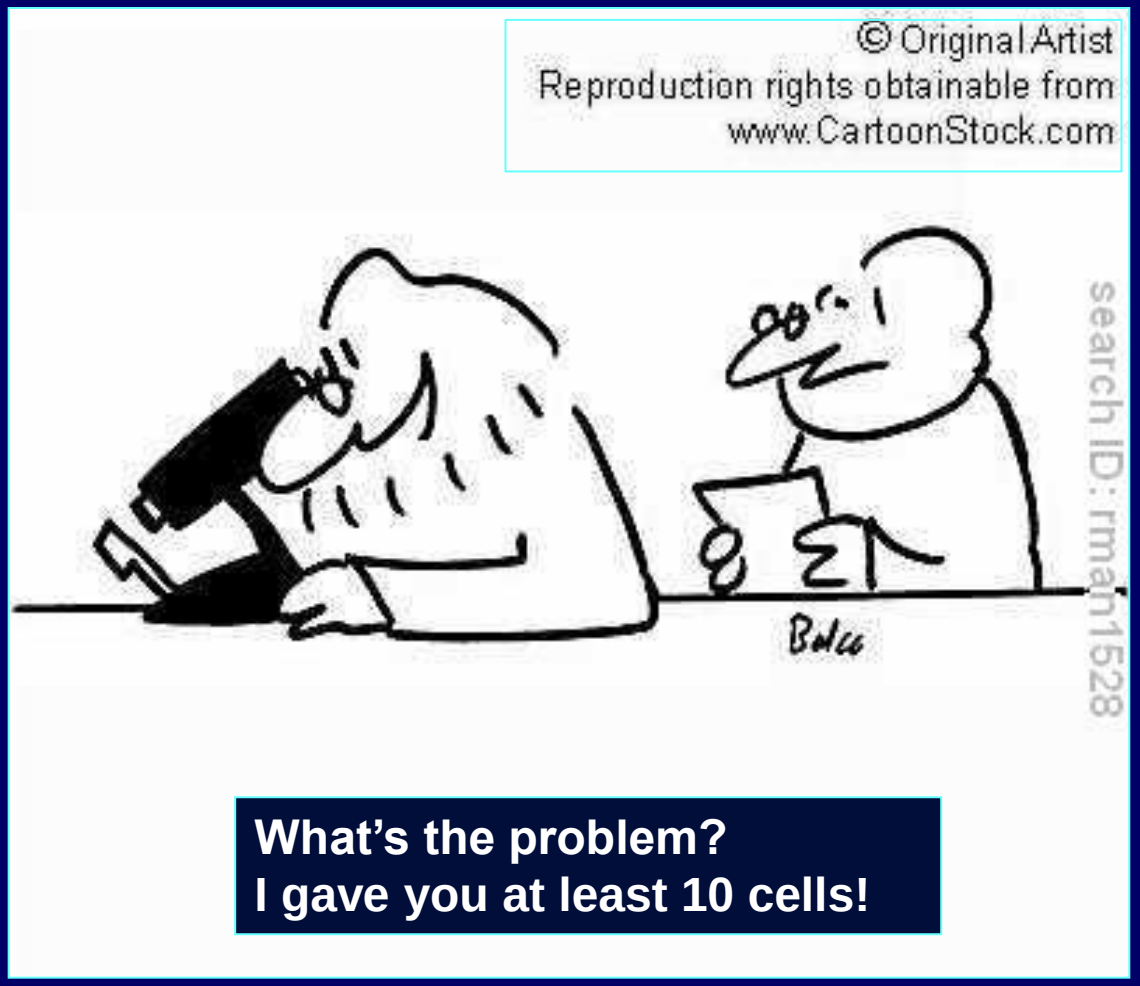


Core Needle Biopsy (CNB)



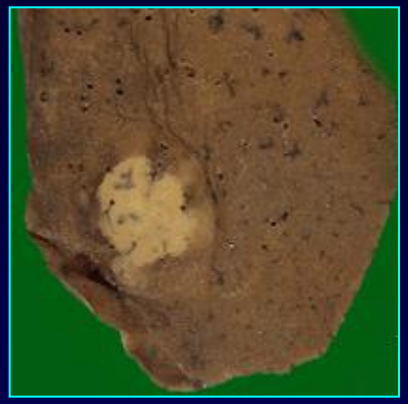
Fine Needle Aspiration (FNA)



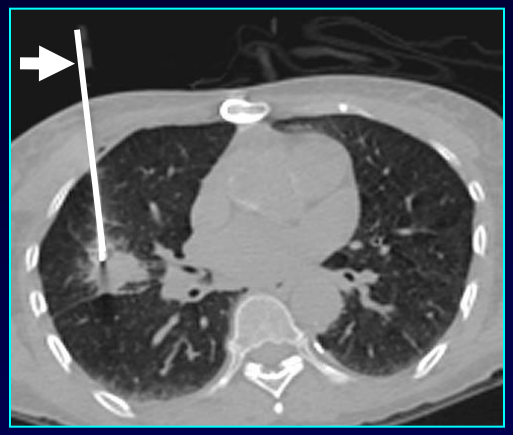


Types of Histology and Cytology Specimens

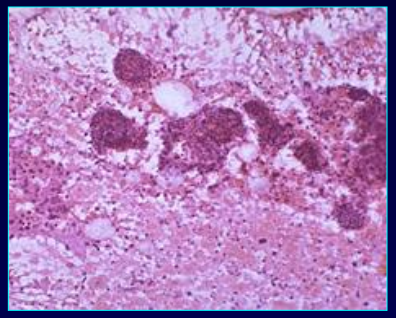
Surgical Resection



Advanced Tumor

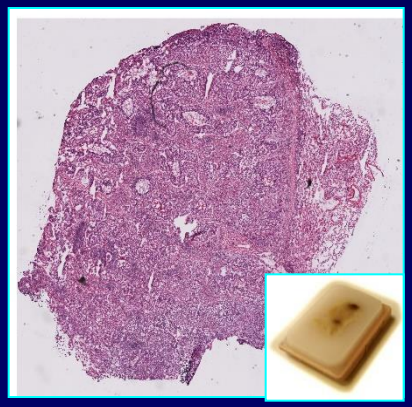


Endobronchial Ultrasound (EBUS) or Pleural Fluid

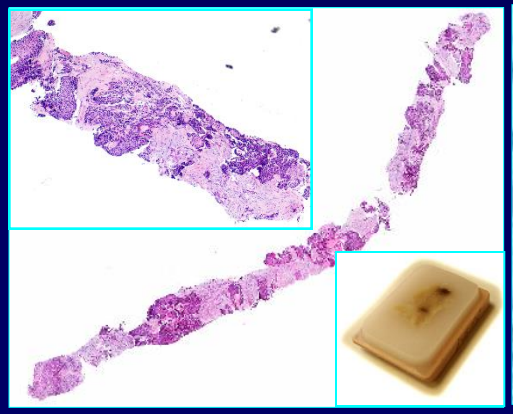


Alcohol-fixed

Histology

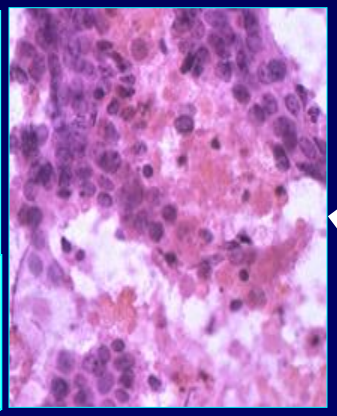


Core Needle Biopsy (CNB)



Formalin-fixed and Paraffin-embedded (FFPE)

Fine Needle Aspiration (FNA)

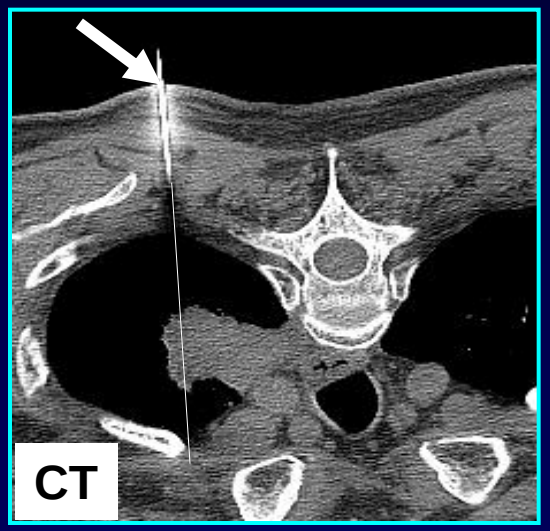


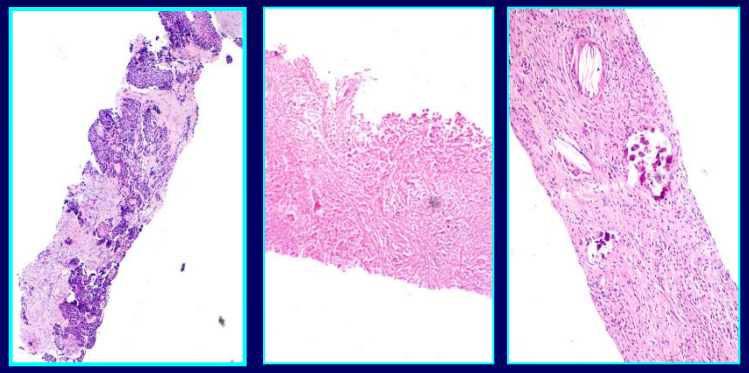
Alcohol-fixed

Alcohol-fixed – Cell Block

Tissue Quality Control for Molecular Testing by Pathologist

Core Needle Biopsy (CNB)

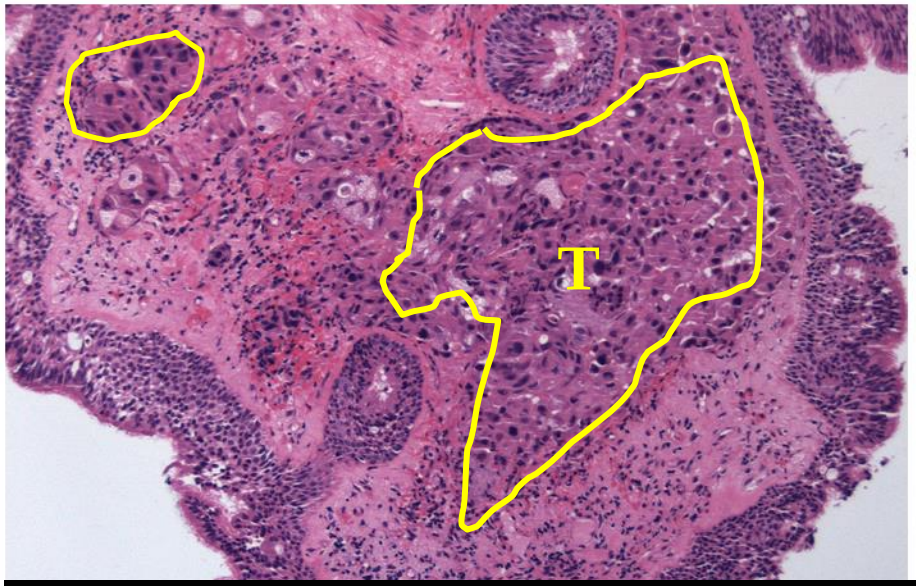


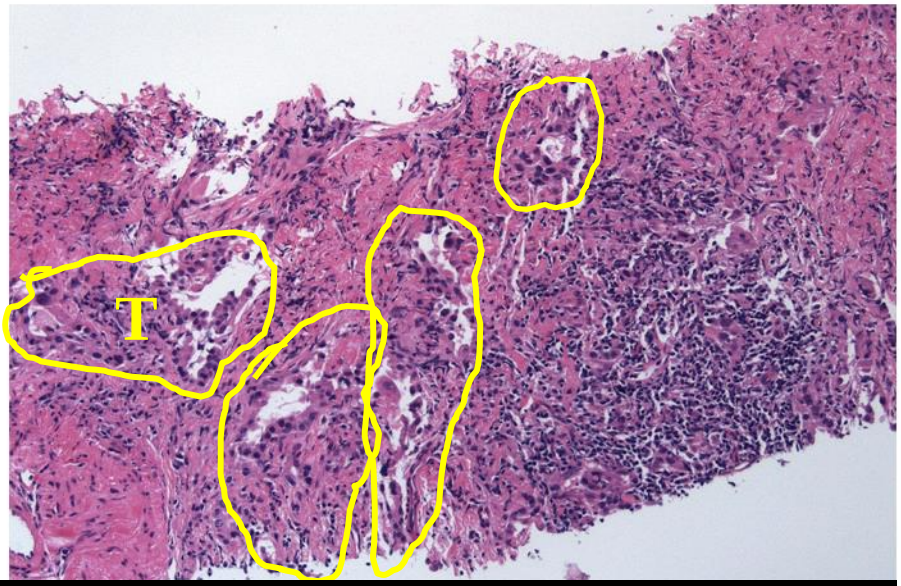


SCC **Necrosis** **Fibrosis**

Adequacy Biopsies for Molecular Profiling (DNA, RNA and Proteins) in NSCLC Refractory Tumors:

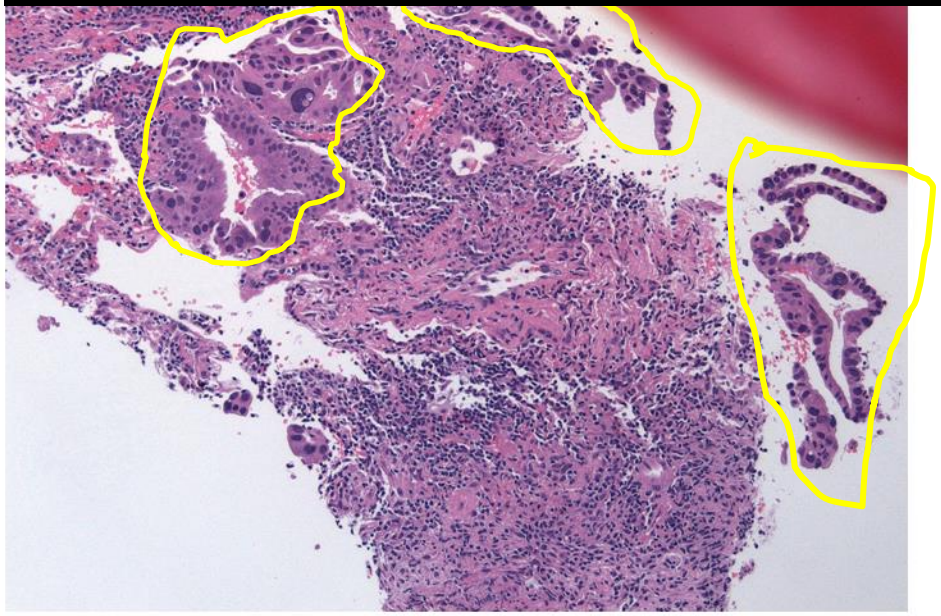
Courtesy: I. Wistuba

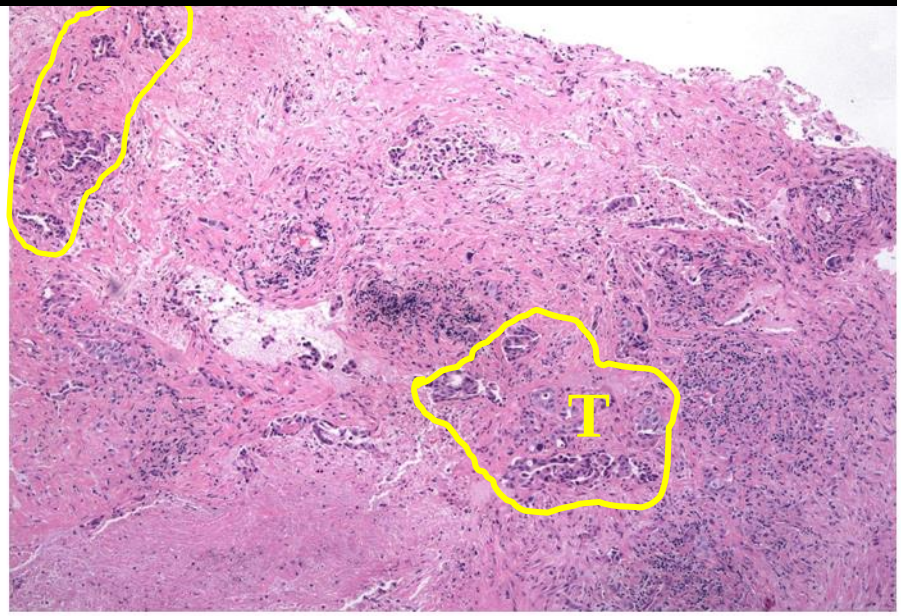




1st Step in Mutation/FISH Testing:

Pathologist review HE slide and mark areas for analysis





IASLC/ATS/ERS Recommendation on Molecular Testing in Lung Cancer

Pathology Consideration for Good Practice

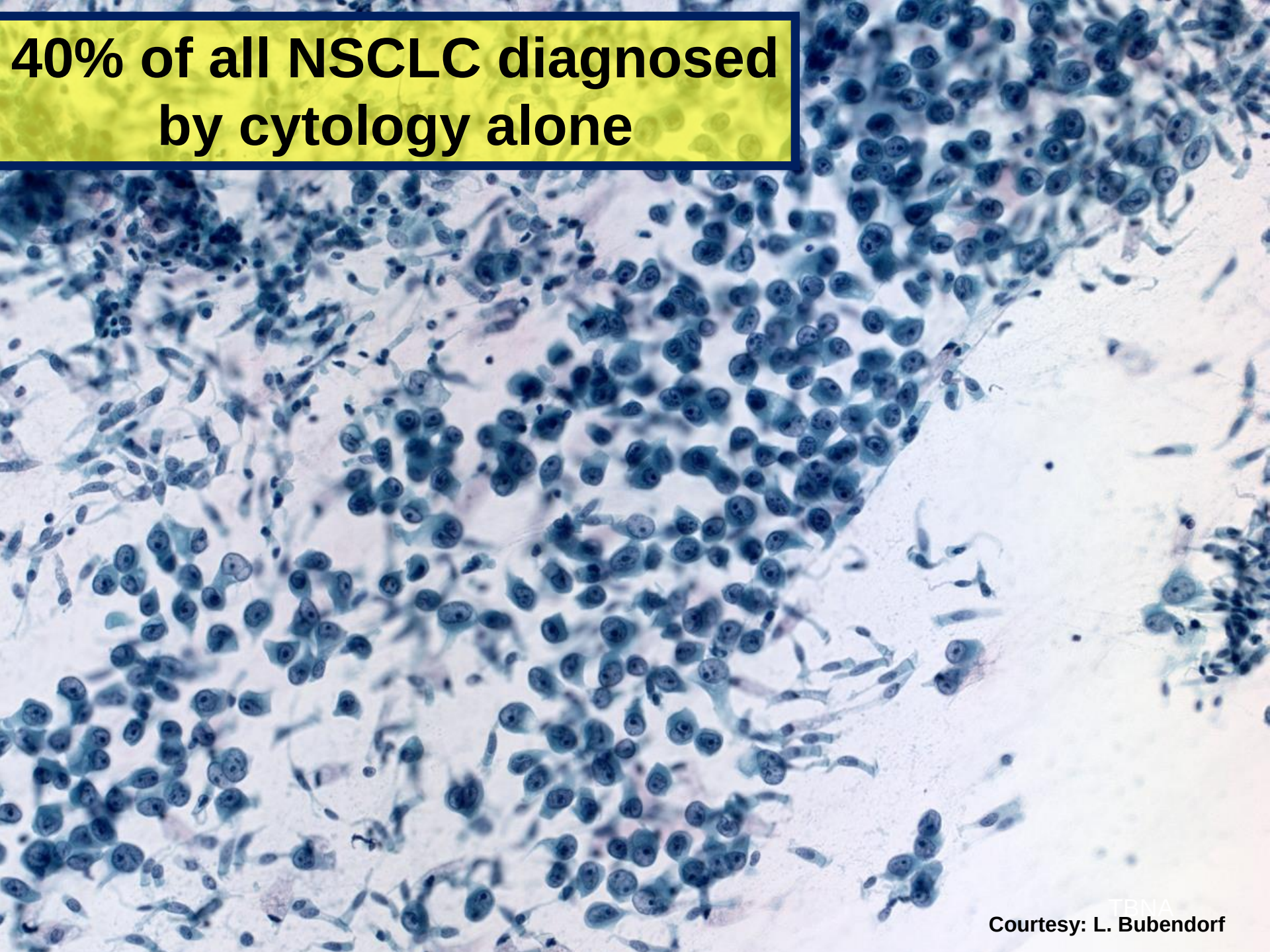
2. Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.
3. To guide therapy for patients with advanced lung adenocarcinoma, each institution should develop a multidisciplinary team that coordinates the optimal approach to obtaining and processing biopsy/cytology specimens to provide expeditious diagnostic and molecular results.
7. Cell blocks should be prepared from cytology samples including pleural fluids.

Bone biopsies can be used if not decalcified, otherwise may give false negative results

IASLC, International Association for the Study of Lung Cancer;
ATS, American Thoracic Society; ERS, European Respiratory Society

Travis WD, et al. *J Thorac Oncol* 2011;6:244–85

40% of all NSCLC diagnosed by cytology alone



Courtesy: L. Bubendorf

CYTOLOGY IS A POWERFUL TOOL FOR CLASSIFYING NSCLC

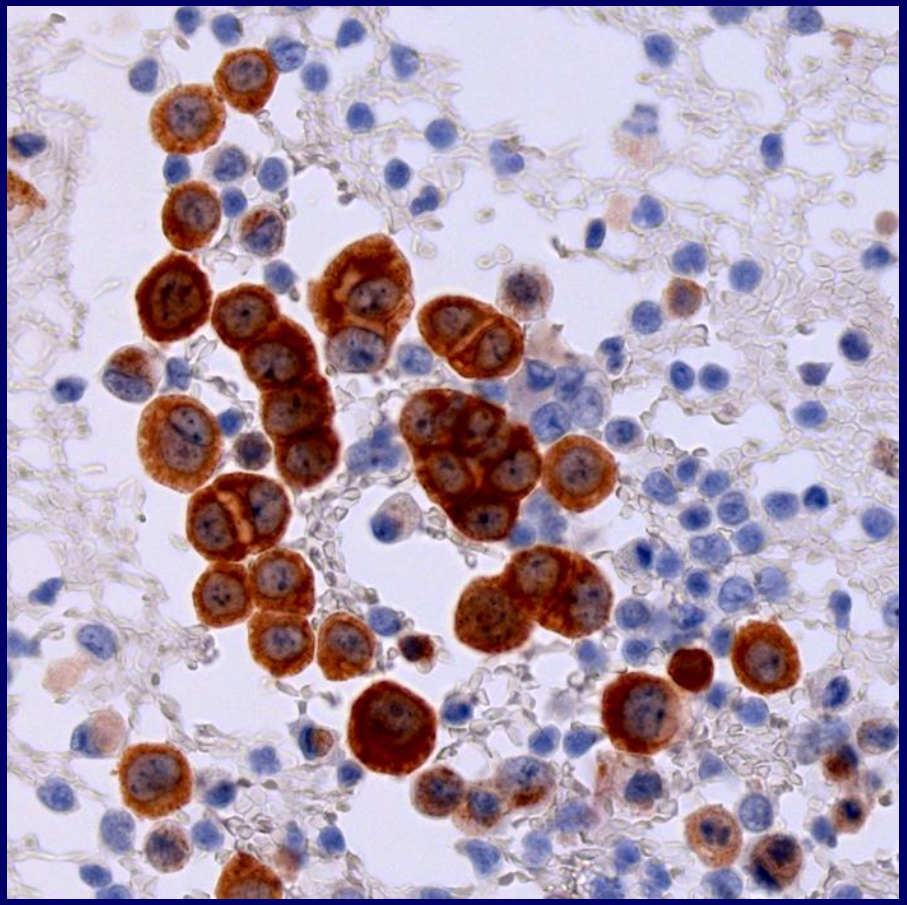
Suitability of Thoracic Cytology for New Therapeutic Paradigms in Non-small Cell Lung Carcinoma

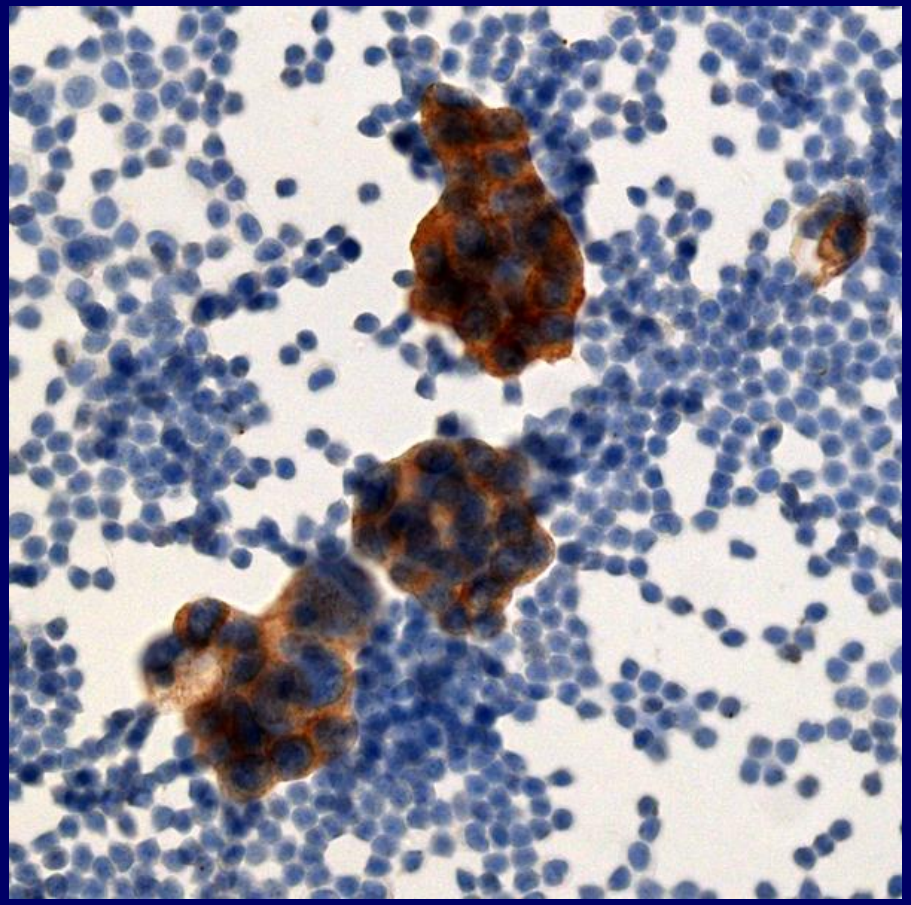
High Accuracy of Tumor Subtyping and Feasibility of EGFR *and* KRAS *Molecular Testing*

Natasha Rekhtman, MD, PhD, Suzanne M. Brandt, MD,* Carlie S. Sigel, MD,* Maria A. Friedlander, MPA, CT (ASCP),* Gregory J. Riely, MD, PhD,† William D. Travis, MD,* Maureen F. Zakowski, MD,* and Andre L. Moreira, MD, PhD**

J Thoracic Oncol 6:451-8, 2011

ALK immunocytochemistry on cytological slides





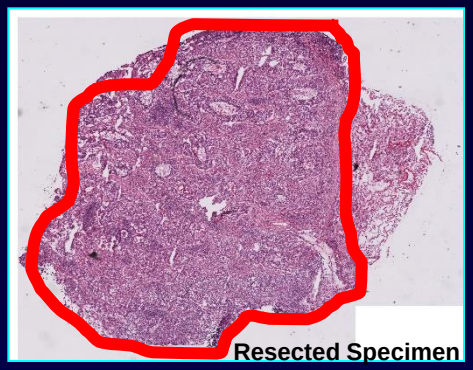
5A4 monoclonal Ab, Novocastra; Leica BondMax

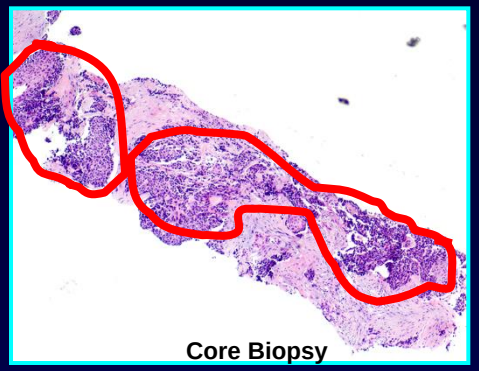
Almost 100% concordance between ALK ICC and FISH

Savic S. et al, *J Thorac Oncol*, in press

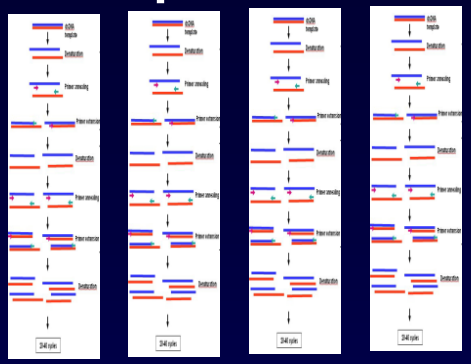
Multiplexed Mutation Assays

Tumor Tissue

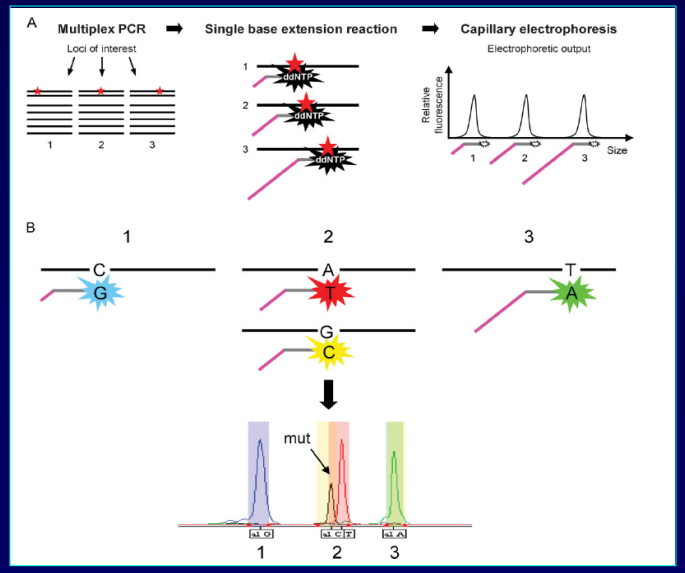




Multiplex PCR

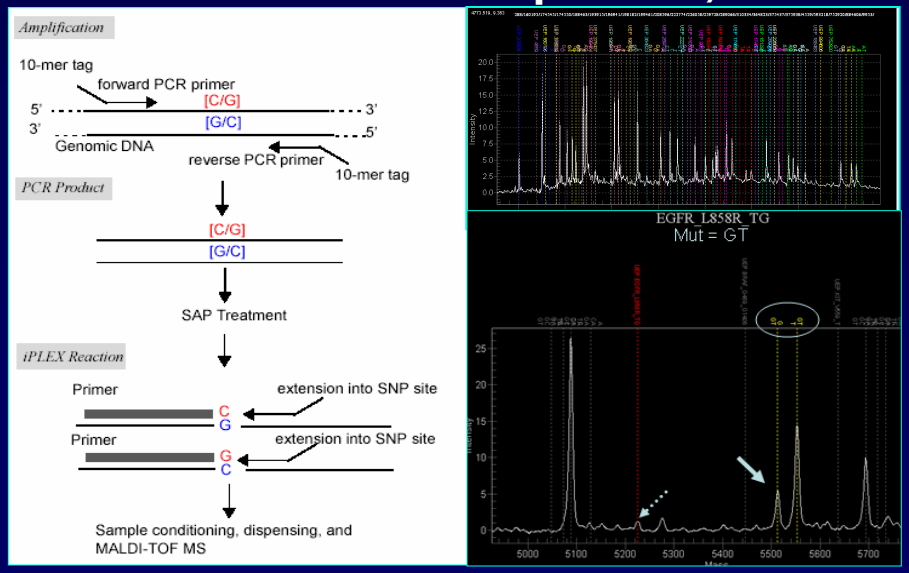


SNaPshot® (Applied Biosystem)



Dias-Santagata, EMBO Mol Med 2:146, 2010

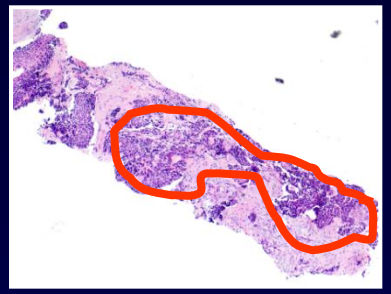
Mass ARRAY SNP - Sequenom, Inc



10% Sensitivity and ~20ng DNA/multiplex reaction

NSCLC Molecular Diagnosis

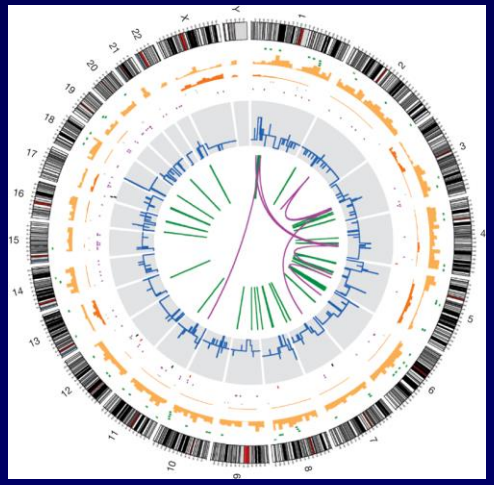
Tumor (CNB)



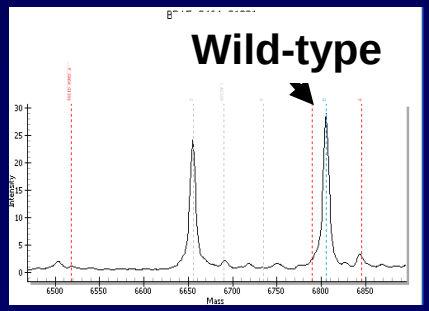
FFPE DNA Extraction

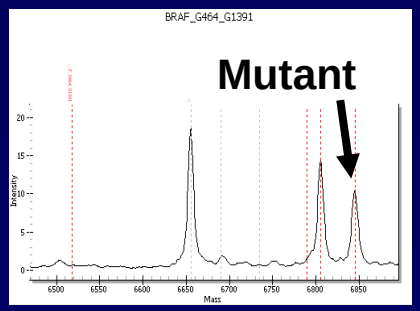
Multiplex PCR
~20ng DNA/multiplex reaction

Next-Generation of Sequencing (NGS): DNA- & RNA-seq



Sequenom™ (*BRAF*: G464-G1391)





~10% Sensitivity

NGS as a Single Platform to Evaluate Multiple Alterations (200-400 Genes) Tumors

- Mutation detection
- DNA copy number detection
- Translocations/gene fusions
- RNA-seq: gene expression, alternative splicing

Next Generation of Sequencing

Current:

Illumina HiSeq 2000

300 – 600 Gigabases
6 – 11 days

Illumina MiSeq

1.5 Gigabases
1 day

Ion Torrent PGM

1 Gigabase
6 hours

Emerging:

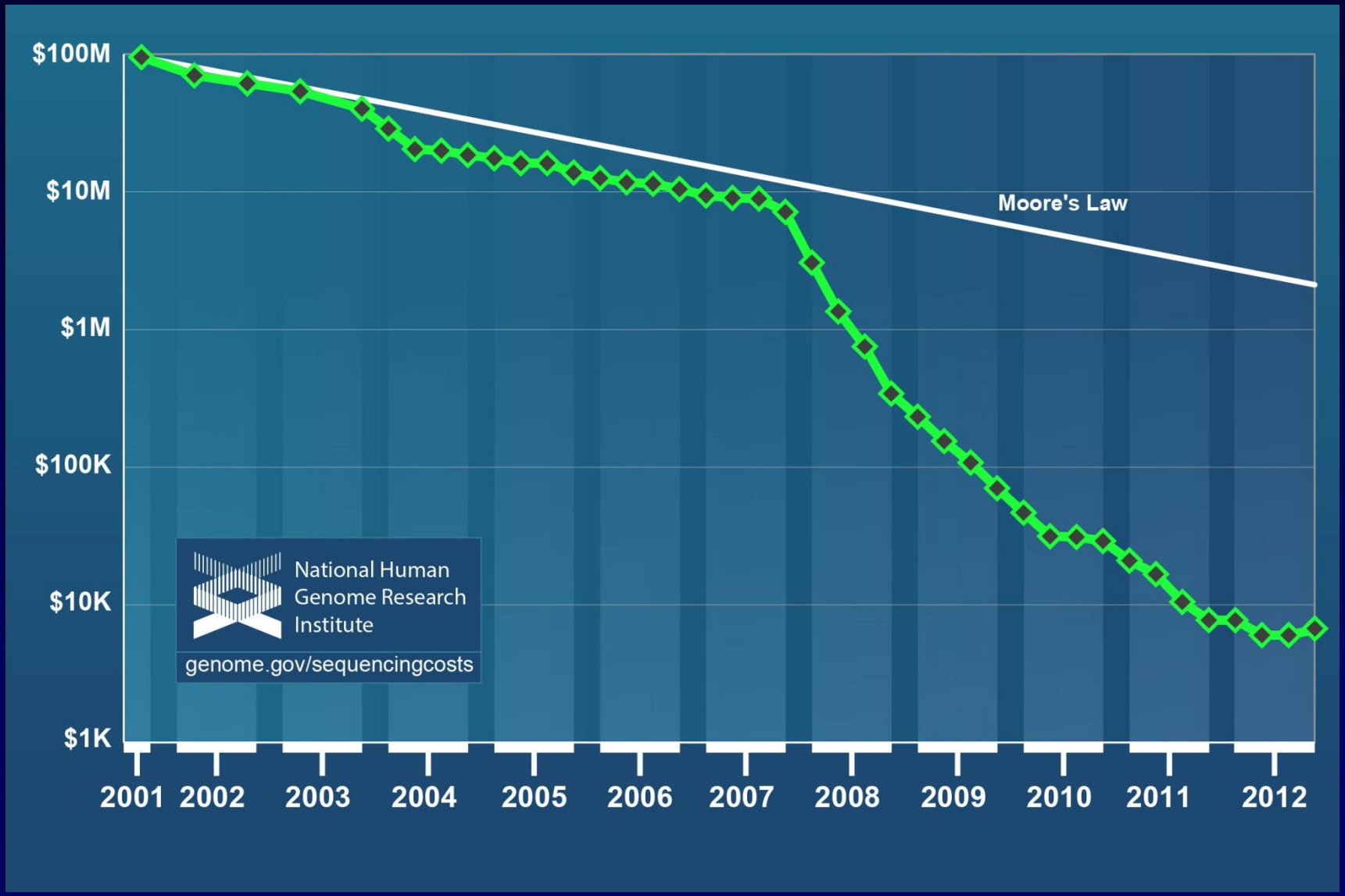
Illumina HiSeq 2500

Ion Torrent Proton

Human Genome in a Day

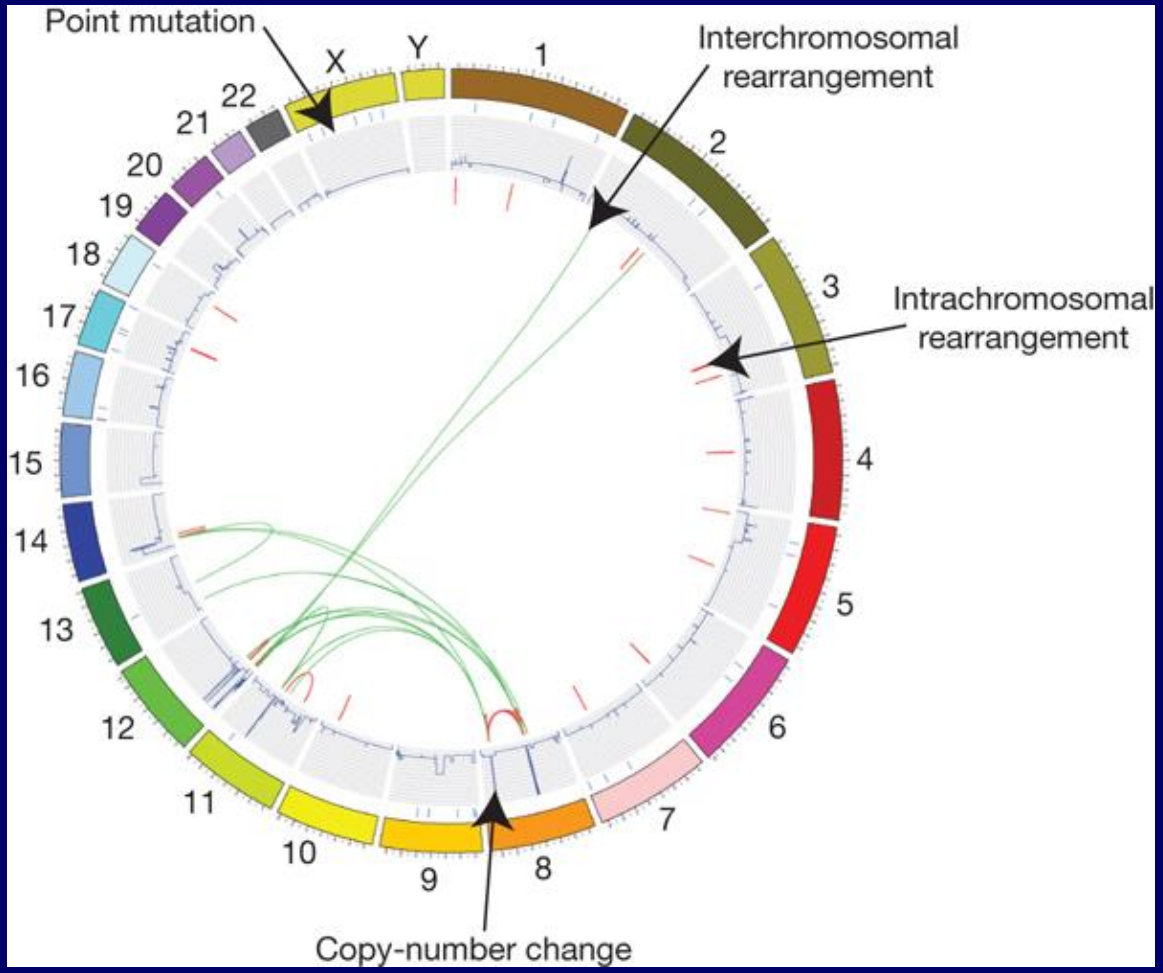
Courtesy of P. Bunn, Colorado (UCCC)

Cost of Genome Sequencing



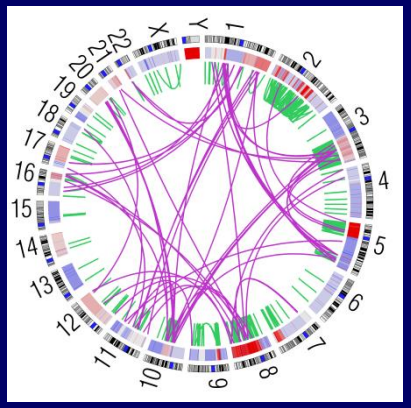
Courtesy of G. Riely

Figurative depiction of the landscape of somatic mutations present in a single cancer genome.

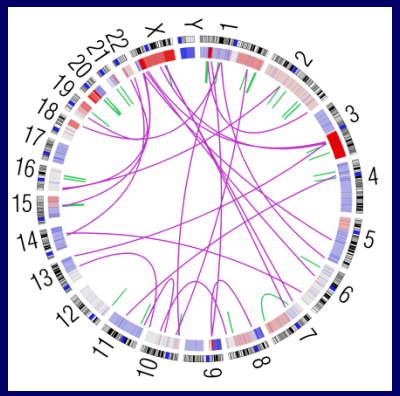


Squamous cell lung cancer: complexity revealed by whole genome sequencing

LUSC-66-2756



LUSC-34-2600



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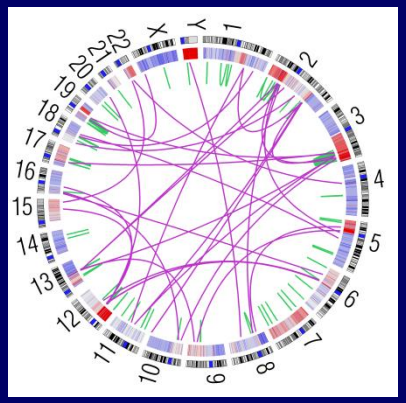

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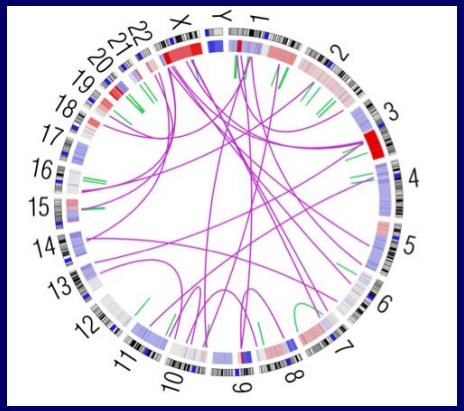

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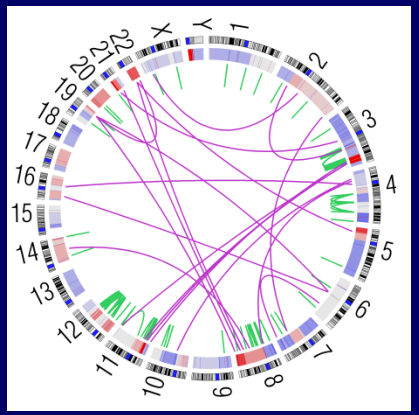

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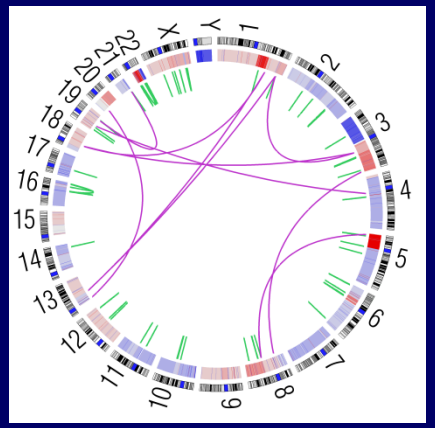
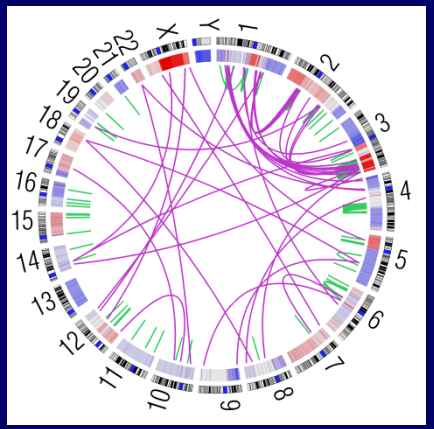
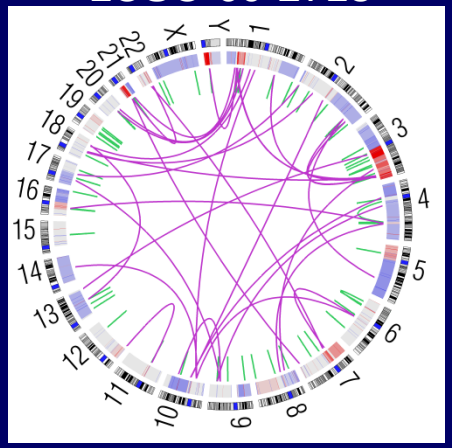


LUSC-60-2711



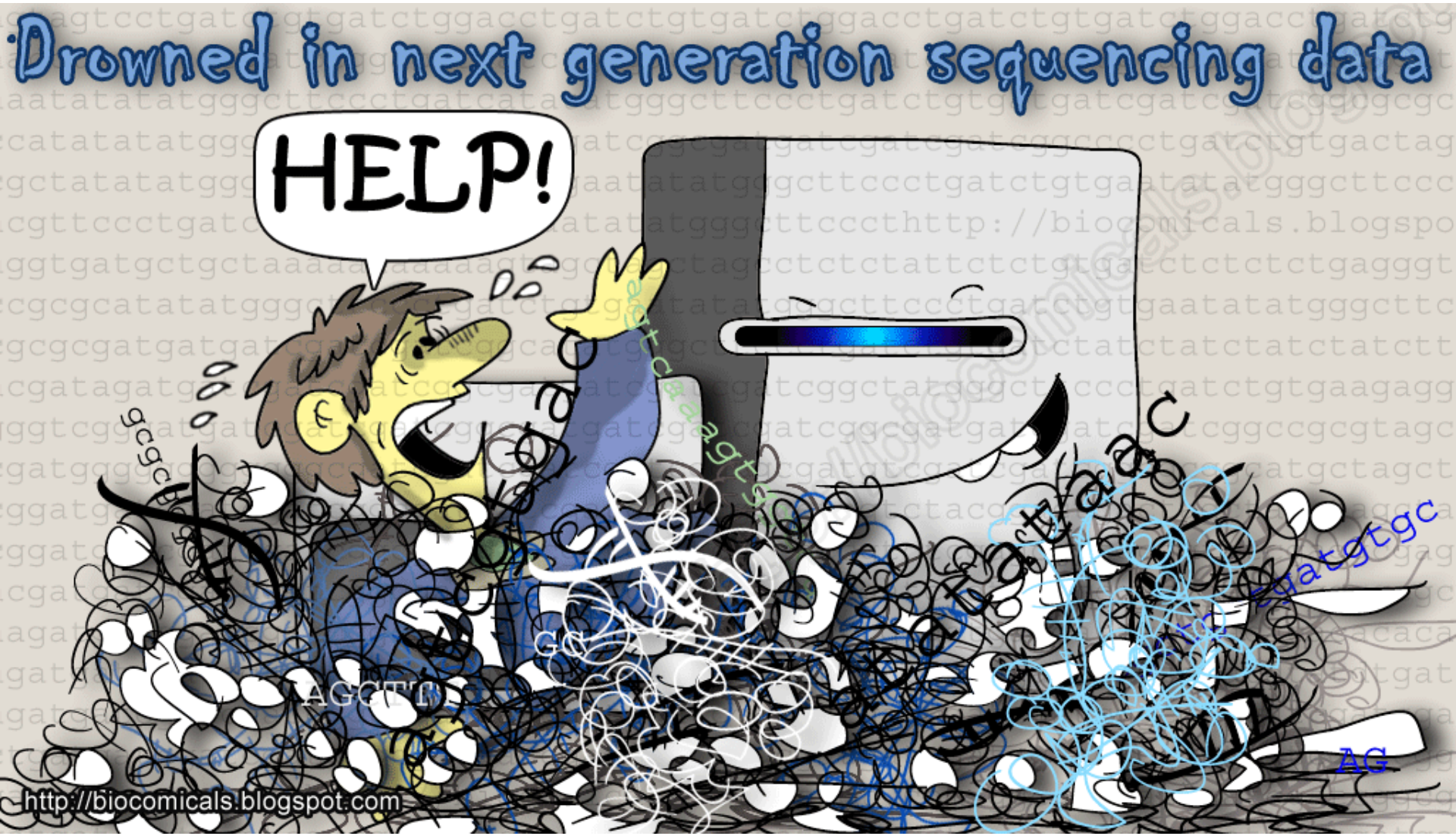
LUSC-60-2713



- **Only NGS allows the multiplexed nature required to obtain the information we need with the specimens that we can obtain**
- **Test development, reporting, and incorporation into clinical practice will require continued development and refinement.**

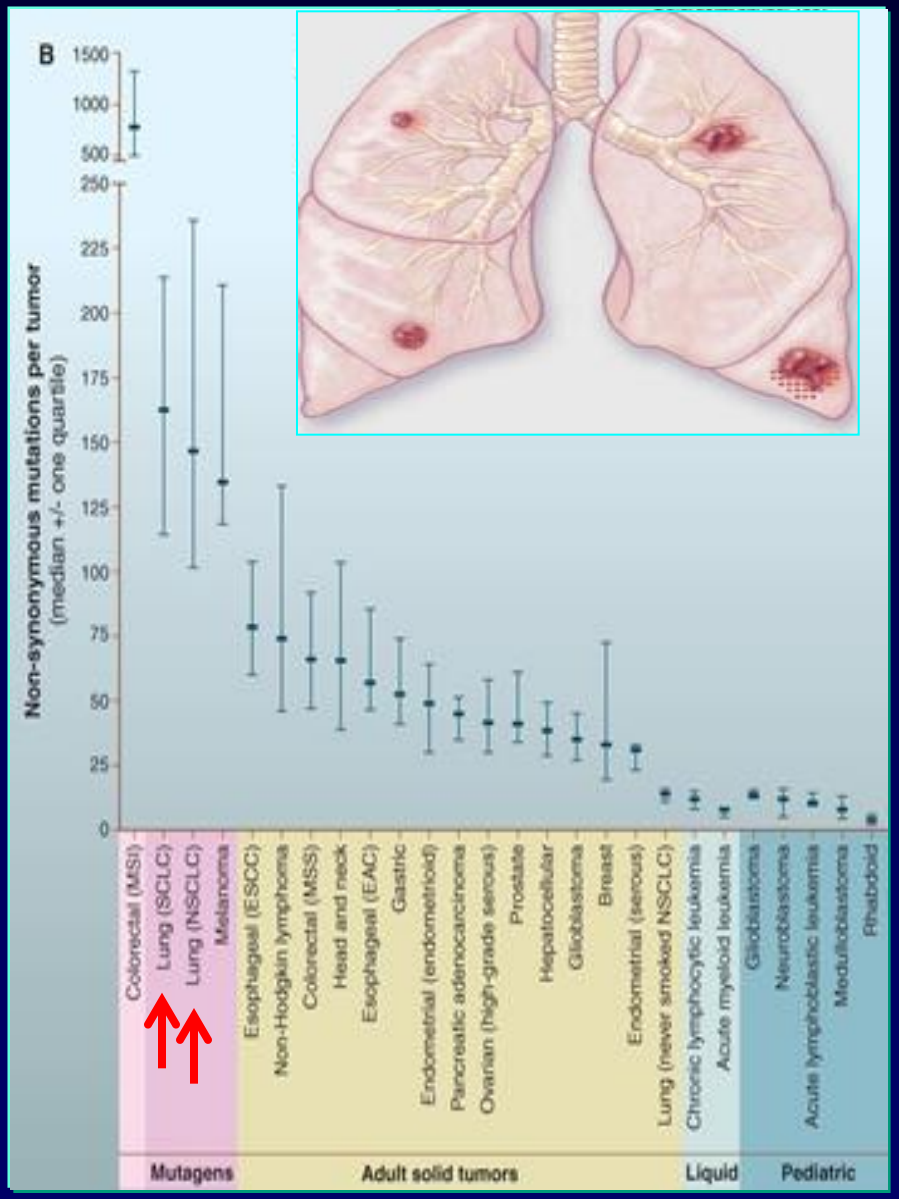
BIOINFORMATIC CHALLENGE !

Modified from G. Riely, Santa Monica 2013


Drowned in next generation sequencing data
HELP!
http://biocomicals.blogspot.com

WHICH MUTATIONS MATTERS?

Lung Cancer Show High Number of Somatic Mutations Detected by Genome-wide Sequencing

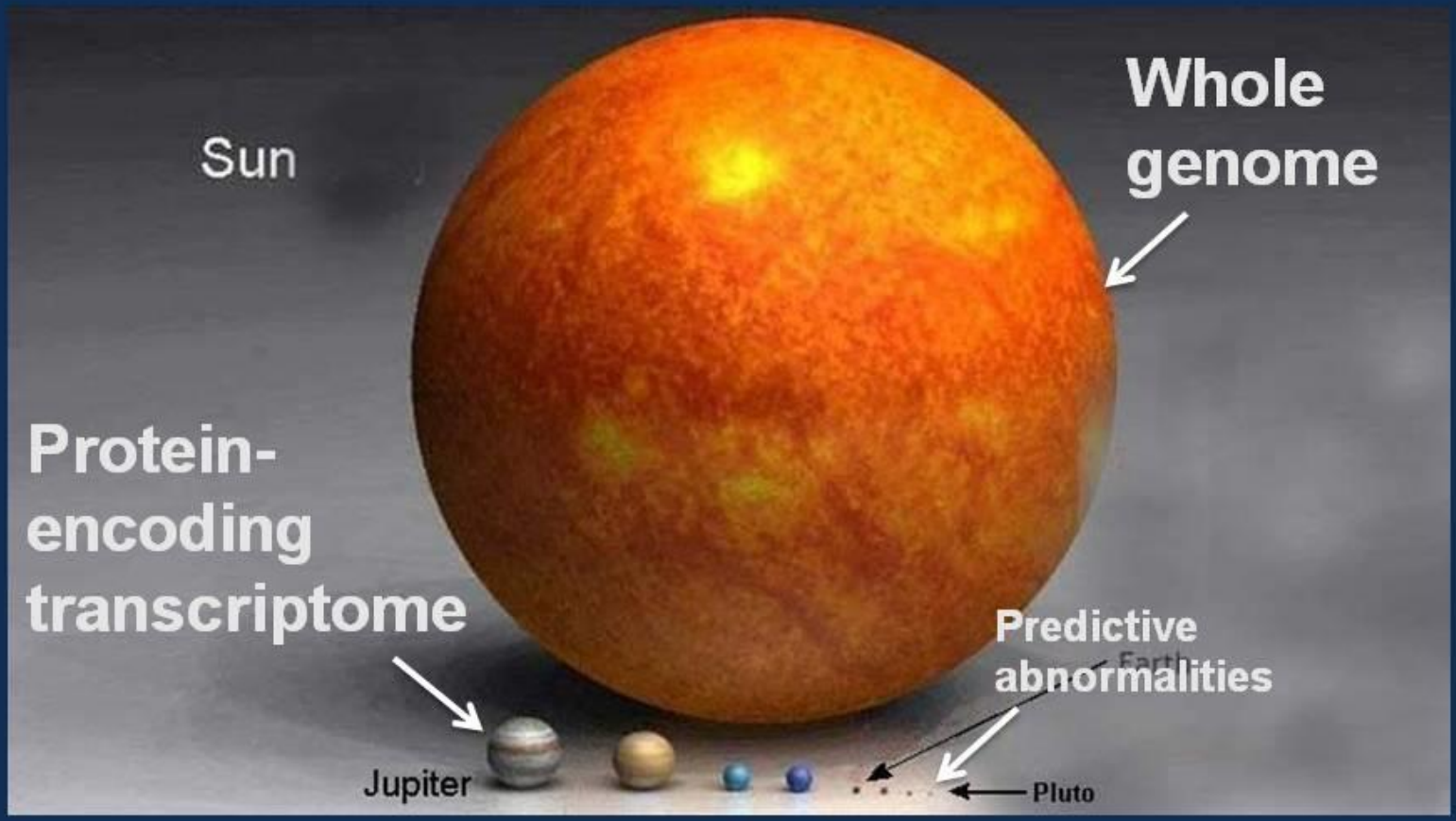


Median number of non-synonymous mutations per tumor:

- Colorectal (MSI) ~700
- SCLC 163
- NSCLC 147
- Melanoma 135
- Esophageal SCC 79
- Colorectal (MSS) 66
- Head and Neck 66
- Gastric 53
- Breast 33
- Glioblastoma 35

B Vogelstein et al. Science 2013;339:1546-1558

Only a fraction of molecular aberrations are clinically relevant

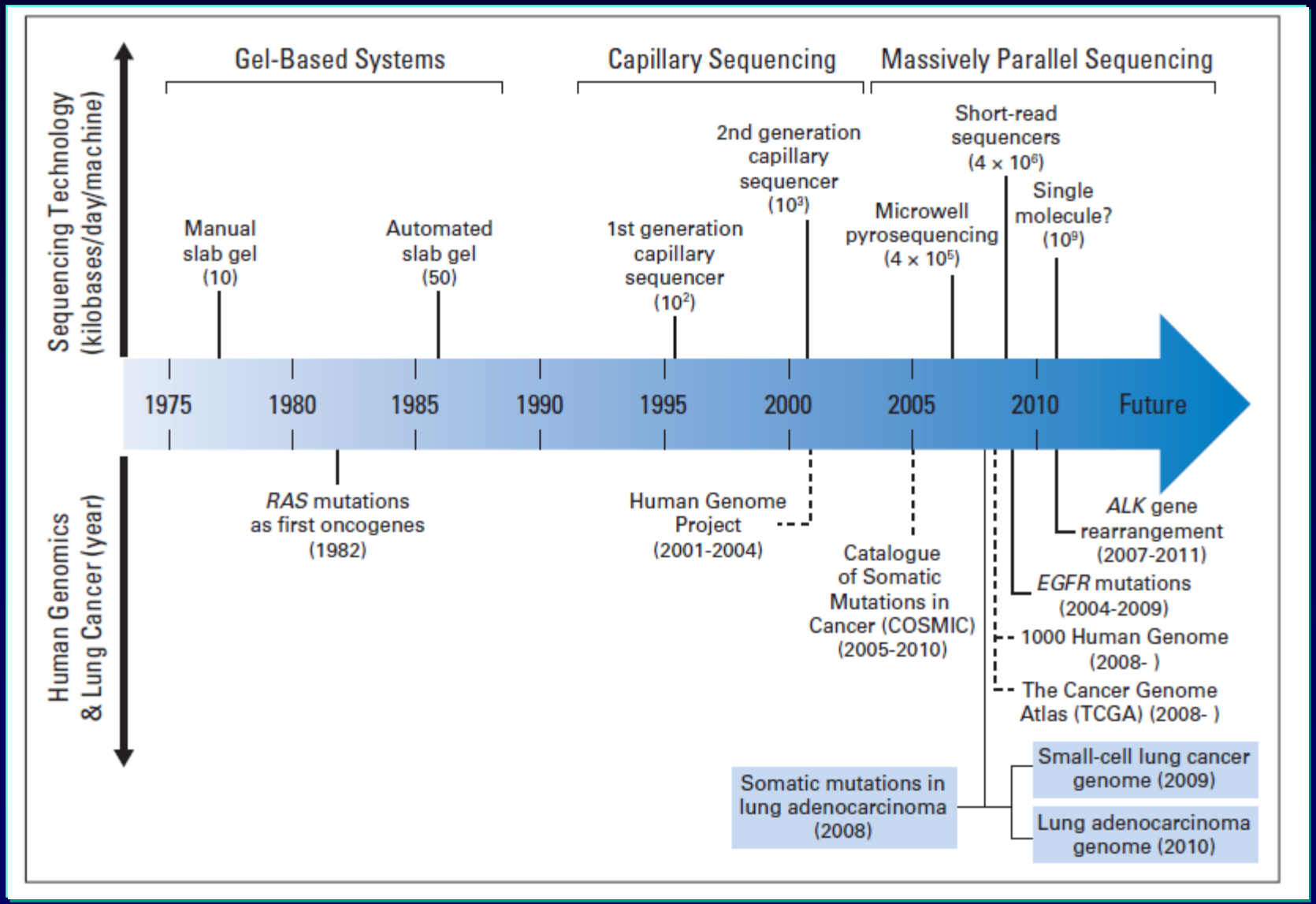


Courtesy of Philip C. Mack, PhD.

Presented by: David E. Gerber, MD

PRESENTED AT: ASCO 50th ANNUAL MEETING SCIENCE & SOCIETY

Advances in Sequencing Methodologies and Human Lung Cancer Genomics



Li et al (D. Gandara), J Clin Oncol 31:1039-1049, 2013

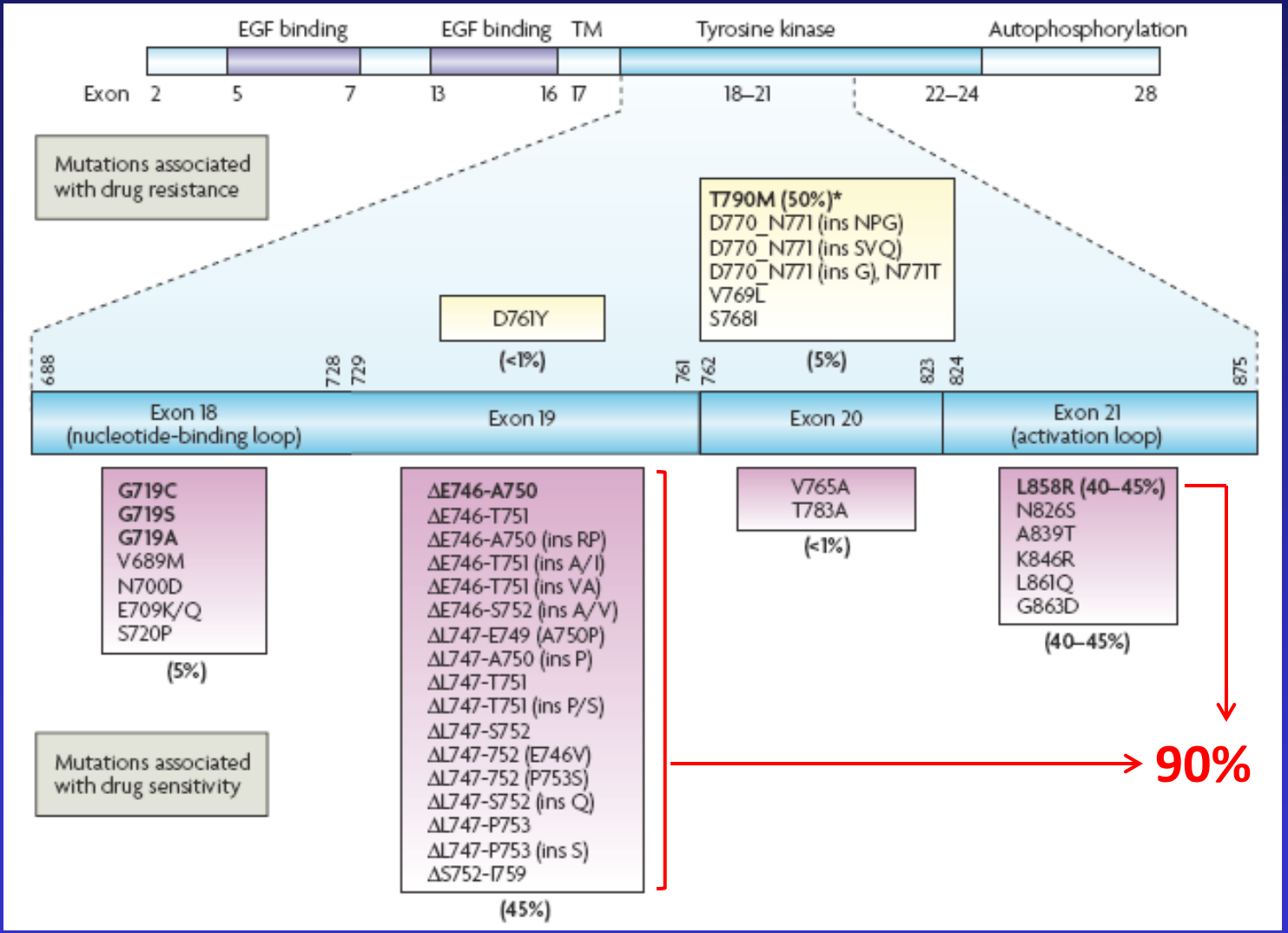
Mutation Tests with Increased Sensitivity

Method	Sensitivity	Mutations Identified
Direct sequencing	25%	Known and new
PCR-SSCP	10%	Known and new
TaqMan PCR	10%	Known only
Loop-hybrid mobility shift assay	7.5%	Known only
Cycleave PCR	5%	Known only
PCR-RLFP (fragment length analysis)	5%	Known only
MassARRAY genotyping	5%	Known only
LNA-PCR clamp	1%	Known only
Scorpion ARMS (DxS)	1%	Known only
dHPLC	1%	Known only
COLD-TaqMan PCR	0.05%	Known only
Parallel (Next Generation) Sequencing	0.01%	Known and Unknown

SSCP, single-strand conformation polymorphism; RLFP, restriction fragment length polymorphism; LNA, locked nucleic acid; ARMS, Amplification Refractory Mutation System; dHPLC, denaturing high performance liquid chromatography

Adapted from Pao W, Ladanyi M. Clin Cancer Res 2007;13:4954–55

EGFR Tyrosine Kinase Domain Mutations

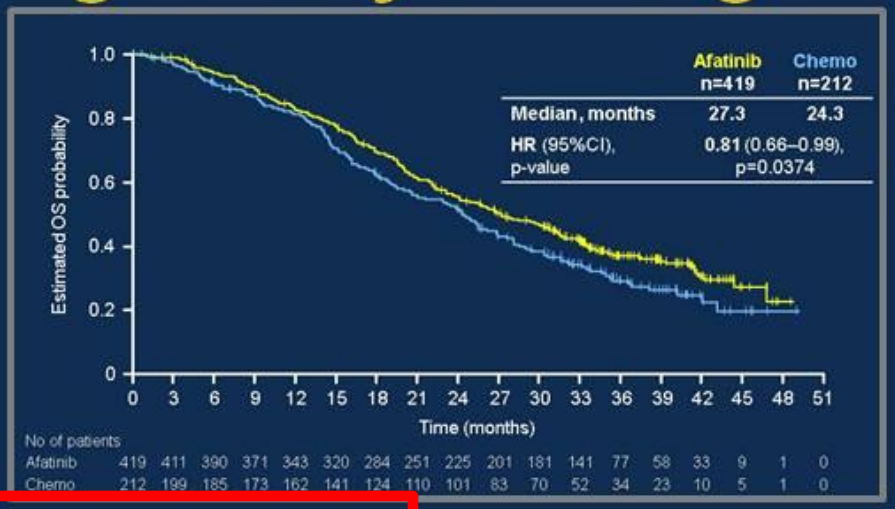


Sharma SV, et al. Nat Rev Cancer 2007;7:169–81

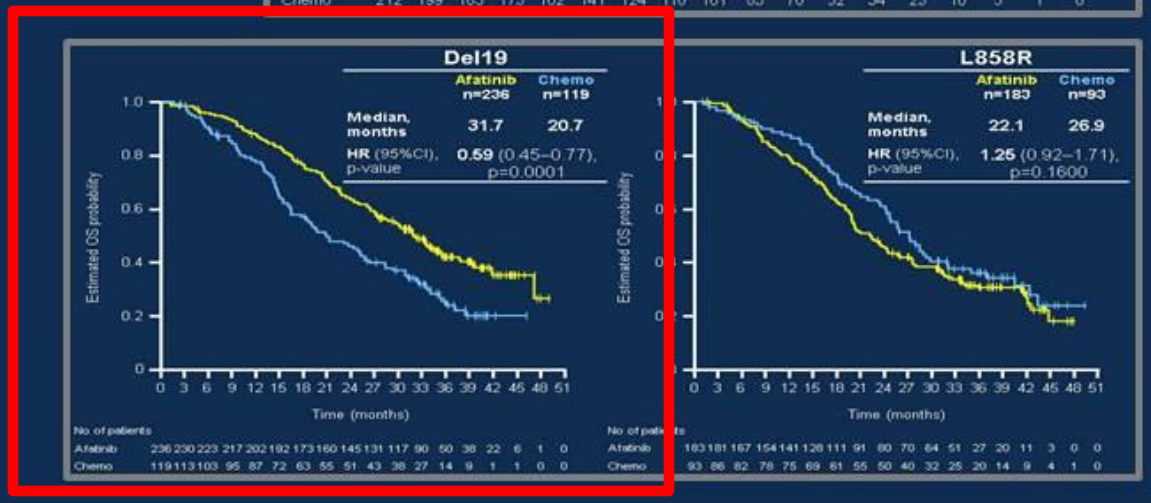
Combined OS Analysis: LUX-Lung 3, LUX-Lung 6: Key Findings

Yang, A#8004

Combined OS analysis, common mutations only



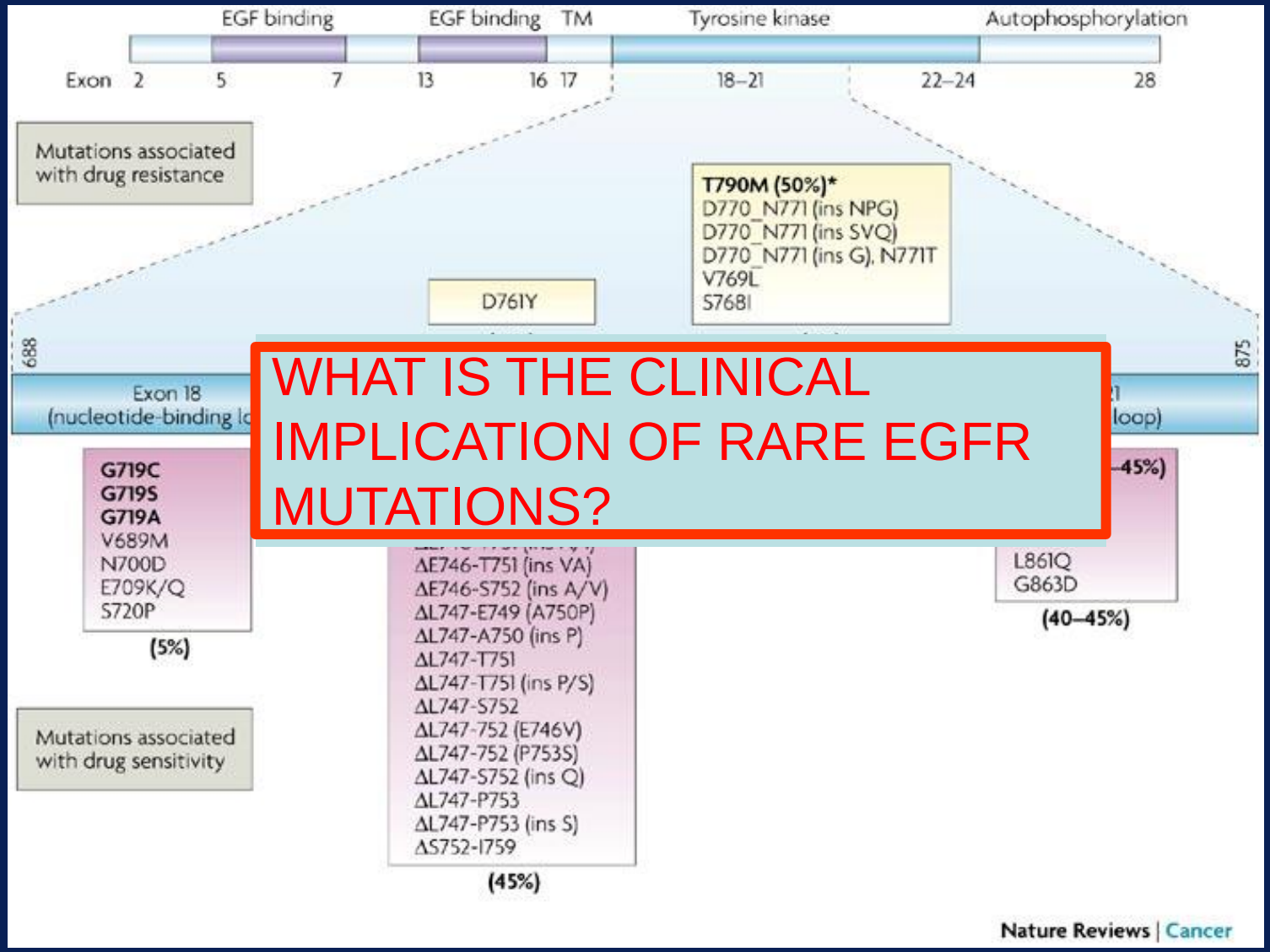
OS by mutation subtype



Presented by: H. Jack West

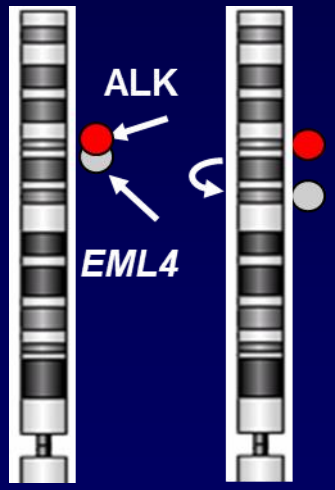
PRESENTED AT: ASCO 50th ANNUAL MEETING SCIENCE & SOCIETY

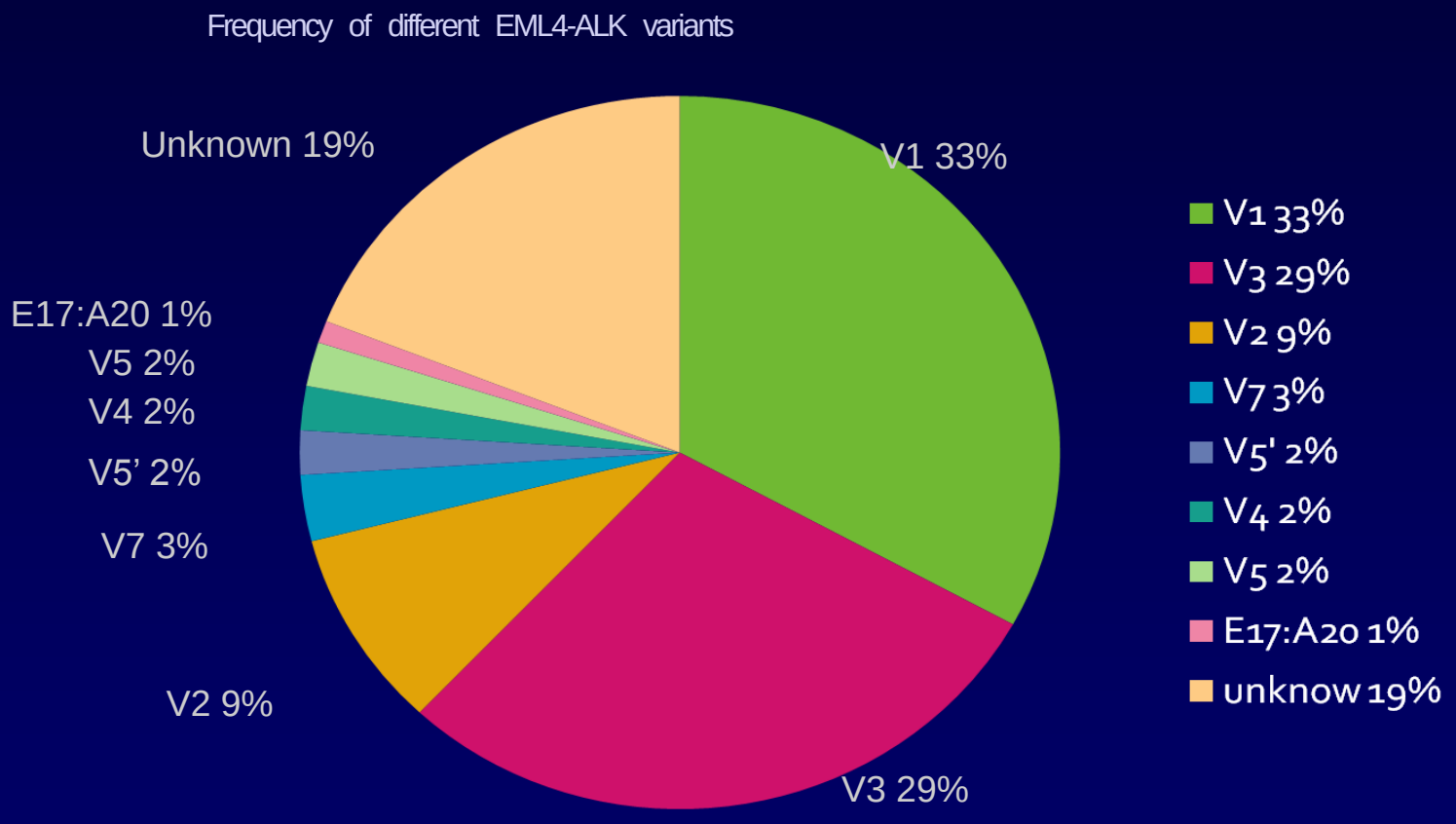
EGFR kinase domain mutations



ALK-DIAGNOSTICS

ALK Fusion Variants in NSCLC

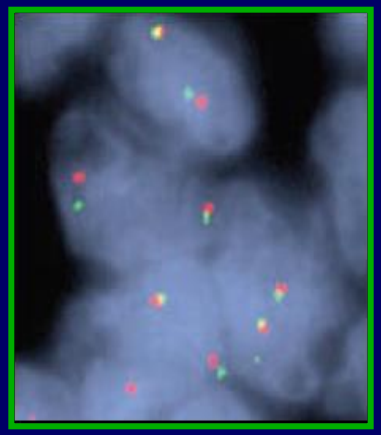




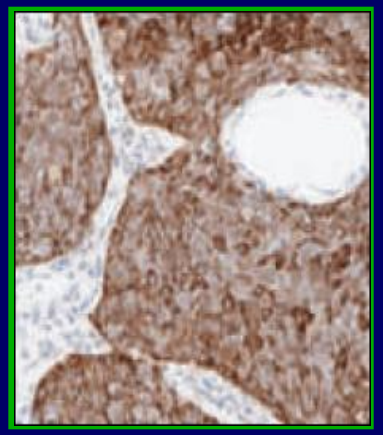
Sasaki T et al. *Eur J Cancer*. 2010.

Proposed methods to detect *ALK*-positive tumors

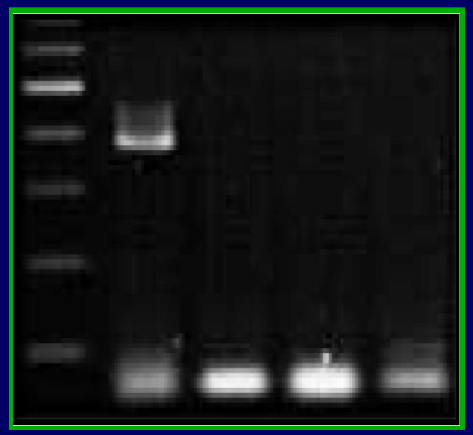
- Four proposed methods of testing
  - Fluorescent in-situ hybridization (FISH)
  - Immunohistochemistry (IHC)
  - Reverse transcriptase polymerase chain reaction (RT-PCR)
  - DNA sequencing



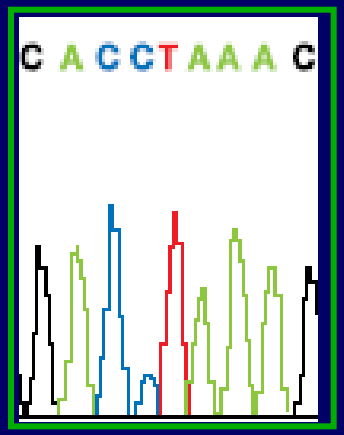
FISH



IHC



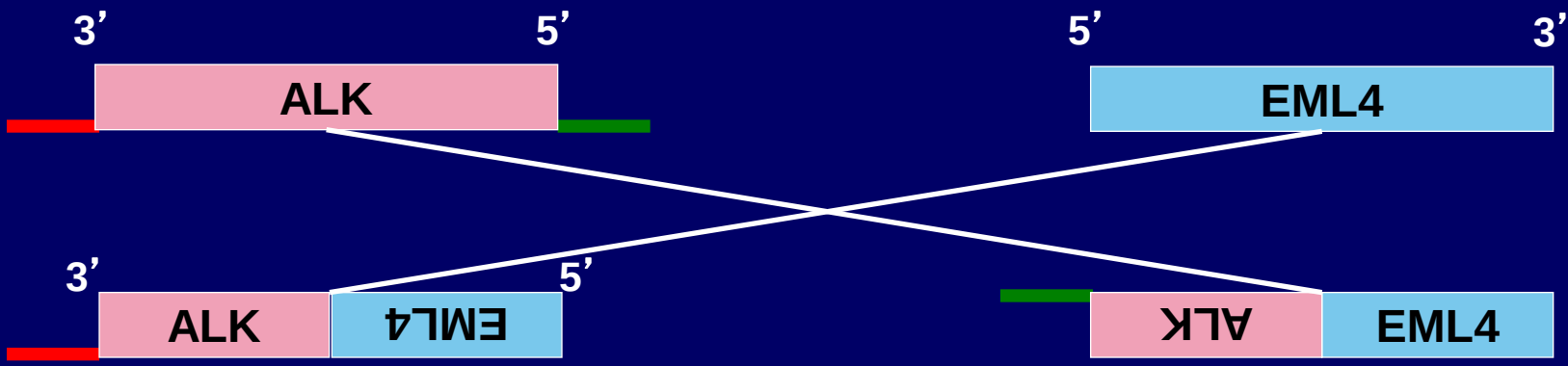
RT-PCR

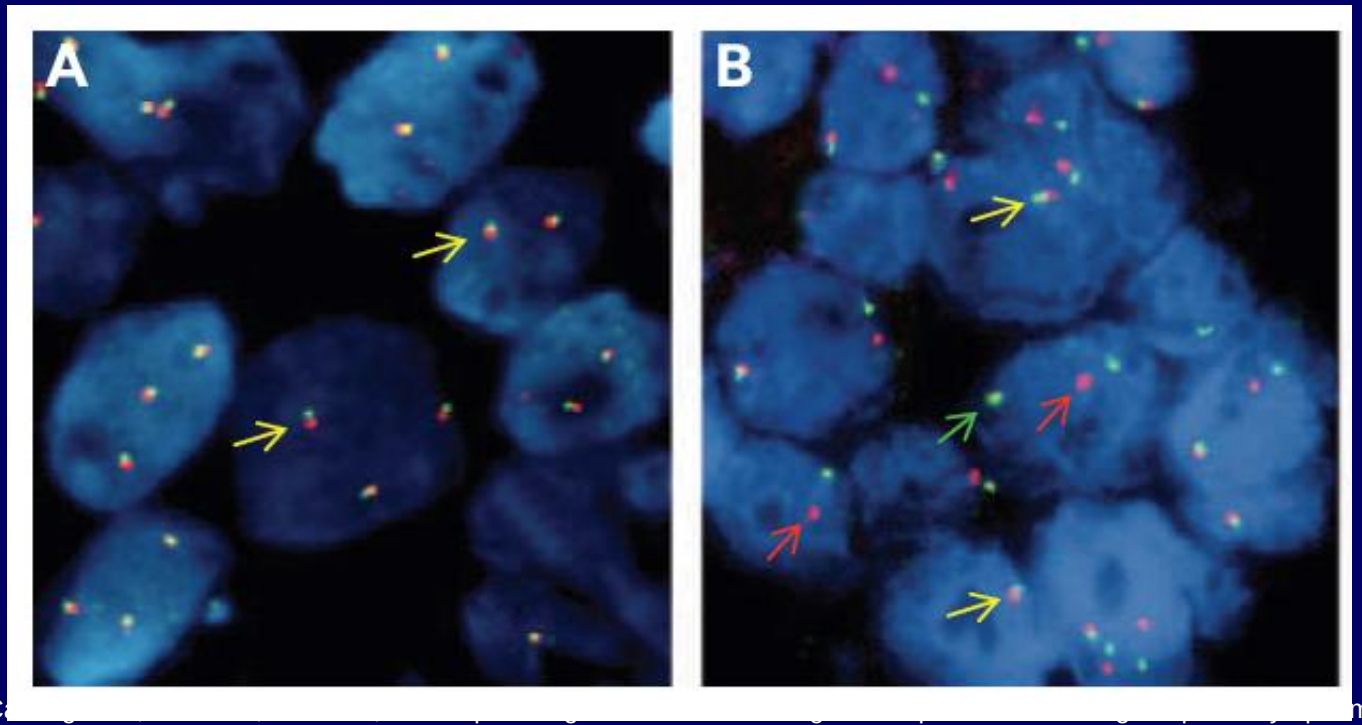


DNA Sequencing

Hirsch F et al. *Clin Cancer Res*. 2010;16:4909–4911

ALK Rearrangement and FISH





Ca… ma kinase (ALK) gene rearrangements potentially suitable for ALK inhibitor treatment. Clin Cancer Res 2010; 16:5581-90.

GOOD AGREEMENT BETWEEN ALK IHC AND FISH; SOMEWHAT DEPENDENT ON ANTIBODY

	Antibody	Total Cases Studied	IHC Negative		IHC Positive		IHC Doubtful (Equivocal)	
			FISH−	FISH+	FISH−	FISH+	FISH−	FISH +
This study	5A4	373	326	0	0	18	29	0
Published studies								
Rodig et al.[20]	ALK1	243	233	2	0	8	—	—
Yi et al.[12]	ALK1	101	69	0	22	10	—	—
Paik et al.[13]	5A4	640	602	0	10	28	—	—
Park et al.[19]	5A4	262	234	0	3	25	—	—
McLeer-Florin et al.[2]	5A4	81	59	0	1	19	0	2
Zhang et al.[21]	SP8	130	110	0	0	15	—	—
Sholl et al.[22]	5A4	176	162	1	0	13	—	—
Minca et al.[10]	D5F3	249	217	0	0	32	—	—
Selinger et al.[23]	ALK1/D5F3	587	581	0	6	7	—	—
Martinez et al.[9]	D5F3	79	73	0	1	5	—	—
Takamochi et al.[24]	5A4	360	347	0	3	10	—	—
Total		2908	2687	3	73	172	0	2

FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

JOURNAL OF THORACIC ONCOLOGY

J Thoracic Oncol, September 2014

ALK IHC+/FISH- TUMORS MIGHT RESPOND TO CRIZOTINIB

D5F3 ALK1

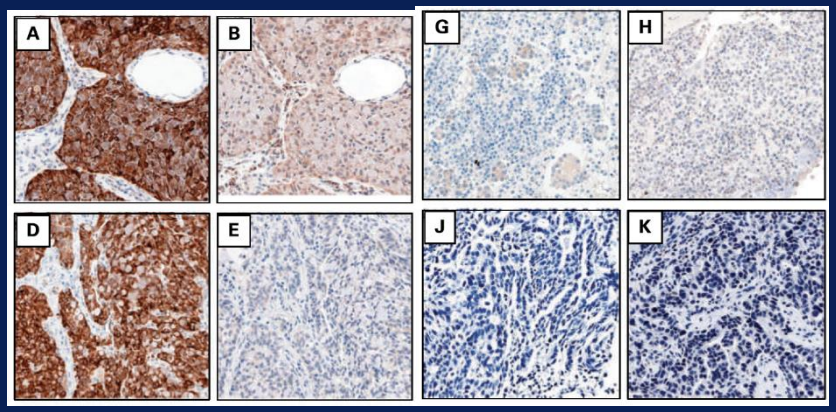


Table 1. Interpretation of IHC staining on lung adenocarcinoma

	D5F3 antibody
Sensitivity (%)*	100
Specificity (%)*	99
Positive predictive value (%)	96
Negative predictive value (%)	100
κ statistic	0.94

*Of the pathologists' IHC interpretation as positive staining in predicting an *ALK*

Mino-Kenudson M, Chirieac LR, Law K, et al.. Cancer Res 2010; 16:156

Next-Generation Sequencing Identifies and Immunohistochemistry Confirms a Novel Crizotinib-Sensitive ALK Rearrangement in a Patient with Metastatic Non–Small-Cell Lung Cancer

J Thorac Oncol 2012;7 (9):e14

Nir Peled, MD, PhD, Gary Palmer, MD,† Fred R. Hirsch, MD, PhD,† Murry W. Wynes, PhD,† Maya Ilouze, PhD,* Marileila Varella-Garcia, PhD,† Lior Soussan-Gutman, PhD,§ Geoff A. Otto, PhD,‡ Philip J. Stephens, PhD,‡ Jeffrey S. Ross, MD,‡ Maureen T. Cronin, PhD,‡ Doron Lipson, PhD,‡ and Vincent A. Miller, MD‡*

A Dramatic Response to Crizotinib in a Non–Small-Cell Lung Cancer Patient with IHC-Positive and FISH-Negative ALK

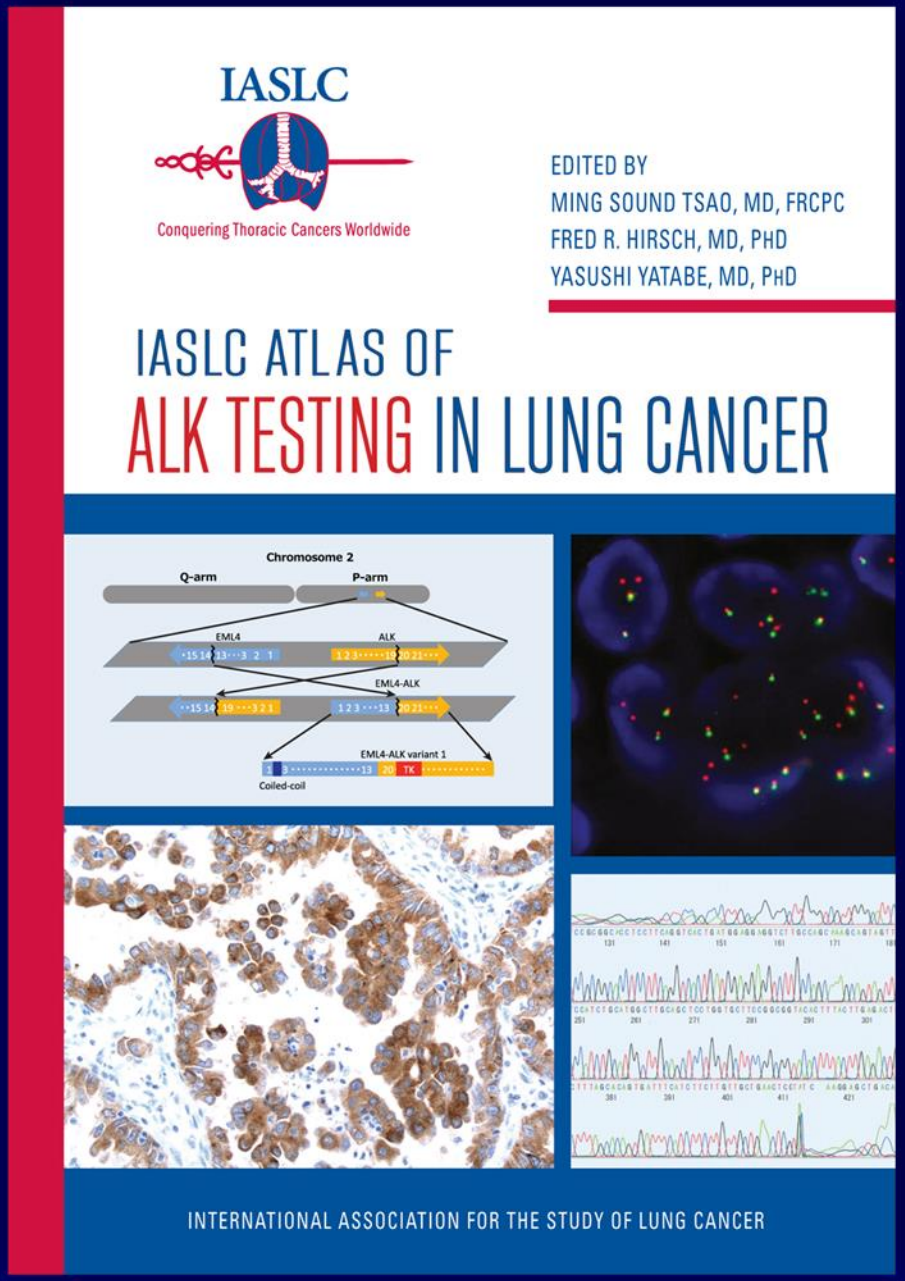
J Thorac Oncol 2012;7 (12):e36

Jong-Mu Sun, MD, PhD, Yoon-La Choi, MD, PhD,† Jae-Kyung Won, MD,‡ Fred R. Hirsch, MD, PhD,§ Jin Seok Ahn, MD, PhD,* Myung-Ju Ahn, MD, PhD,* and Keunchil Park, MD, PhD**

Atypical Negative *ALK* Break-Apart FISH Harboring a Crizotinib-Responsive *ALK* Rearrangement in Non–Small-Cell Lung Cancer

J Thorac Oncol: 2014: Mar 9 (3): e21-23

Shengxiang Ren, MD, PhD, Fred R. Hirsch MD, PhD,† Marileila Varella-Garcia, PhD,‡ Dara L. Aisner, MD, PhD,‡ Theresa Boyle, MD,† Caicun Zhou, MD, PhD,* and D. Ross Camidge, MD, PhD†*



Can be ordered at

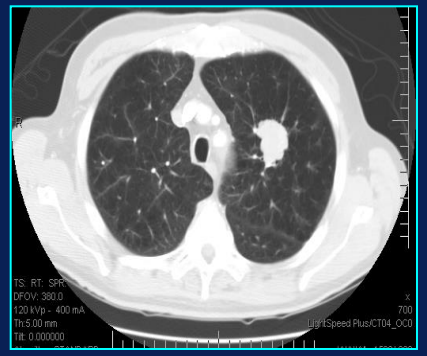
www.iaslc.org

TUMOR HETEROGENEITY?

Personalized Lung Cancer Therapy is Critical in this Heterogeneous Disease

NSCLC

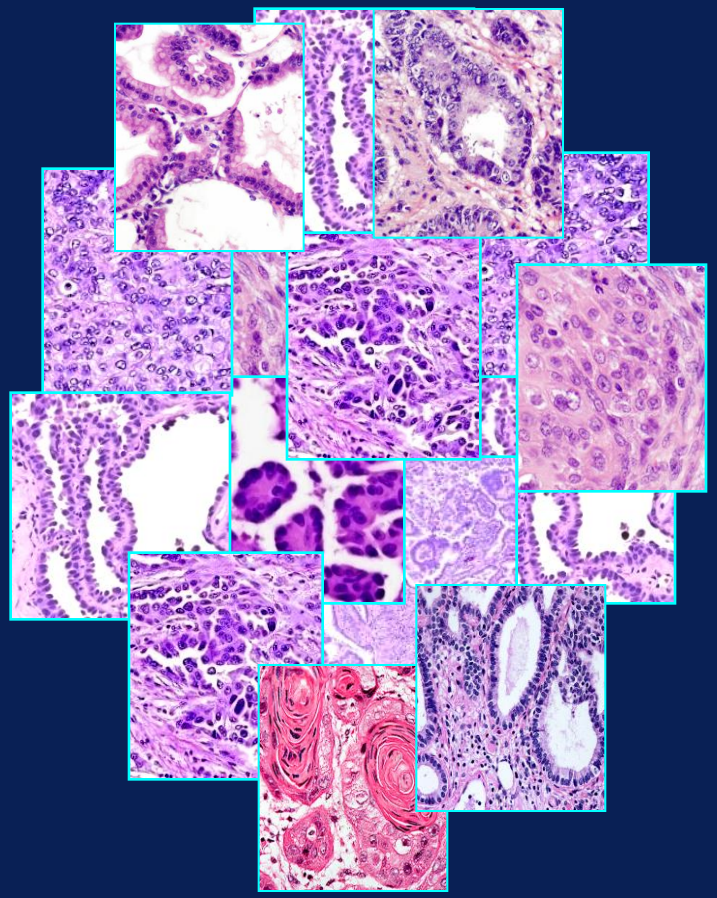
CT



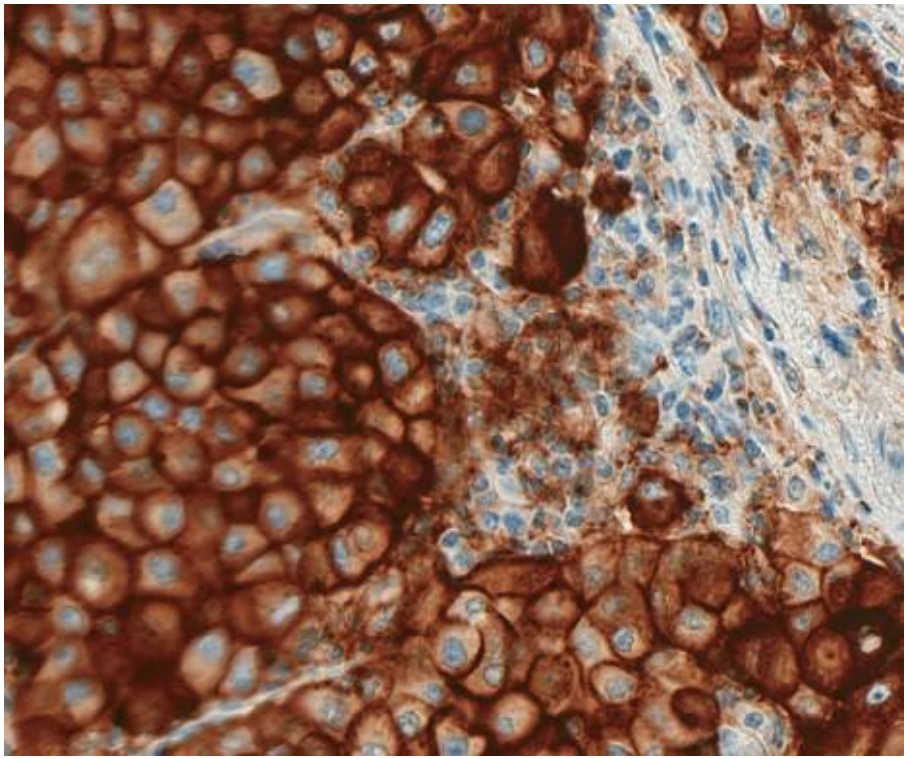
Pathology Specimens

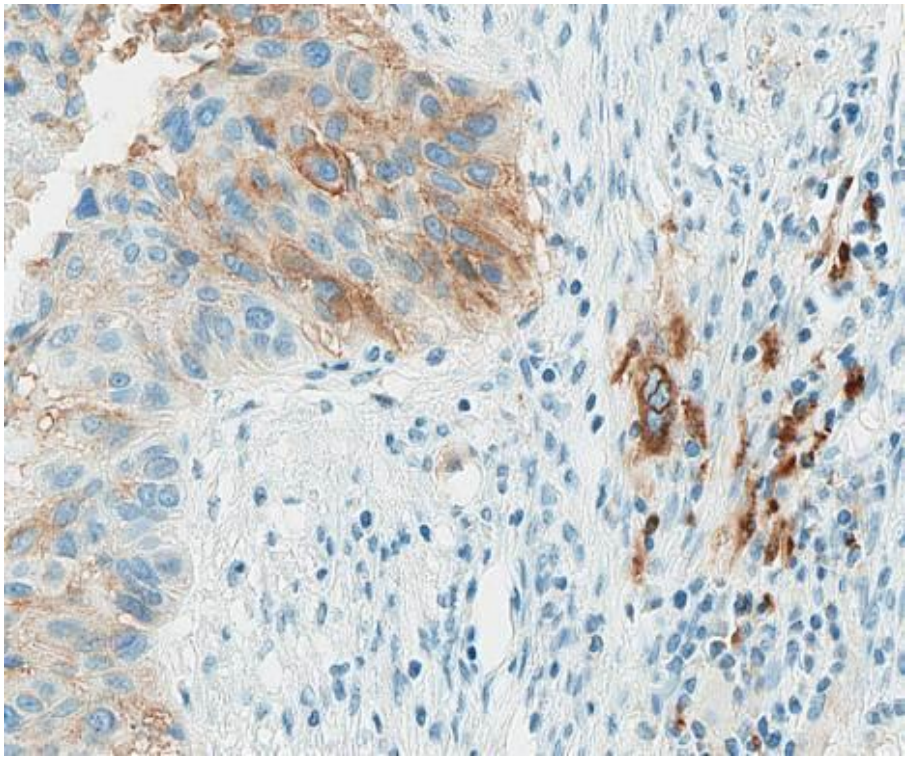
Not All Tumors are the Same

Histopathology



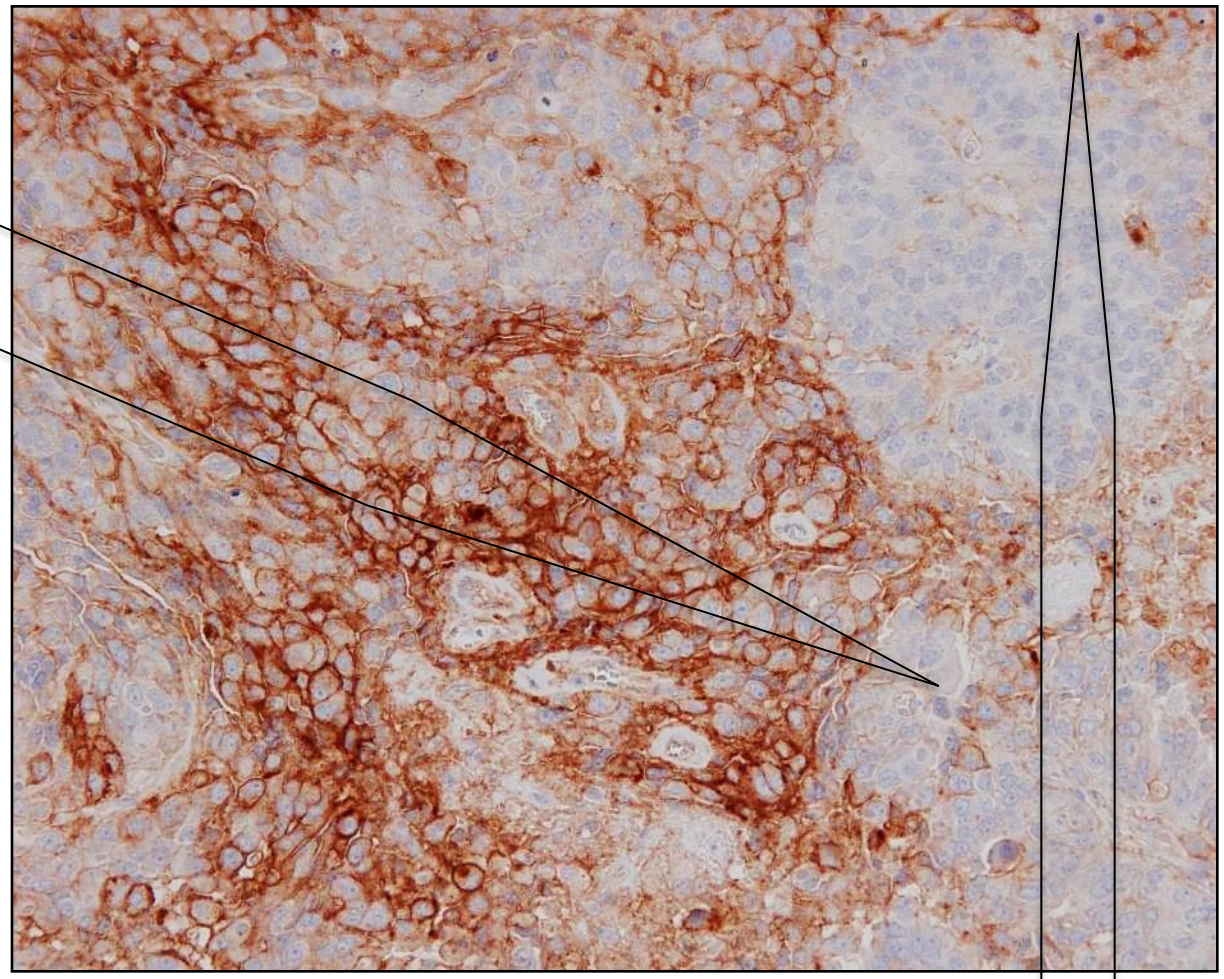
Variable PD-L1 Staining Detected Within Tumor Specimens





AstraZeneca MedImmune

PD-L1 Immunohistochemistry: Expression Heterogeneity and Potential for Sampling Error



Biopsy Core 1

Biopsy Core 2

18g needle = 800 µm

AstraZeneca MedImmune

Temporal heterogeneity of melanoma metastases

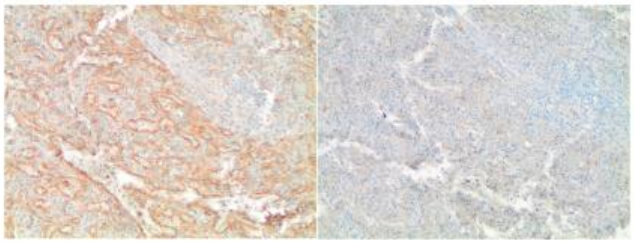
Patient no.	Clinical Resp.	Biopsy site	PD-L1 IHC (%pos. tumor cells)
1	NR	SQ met #1	5-10
		SQ met #2	0
2	NR	Skin primary	20
		LN met	0
3	CR	Skin primary	5
		SQ met	0
		LN met	0
4	NR	Skin primary	5
		LN met #1	0
		LN met #2	5
5	PR	Lung met #1	5
		Lung met #2	50

Topalian, ST et al., NEJM 2012.

Control in tissue TMA

Cell Signaling

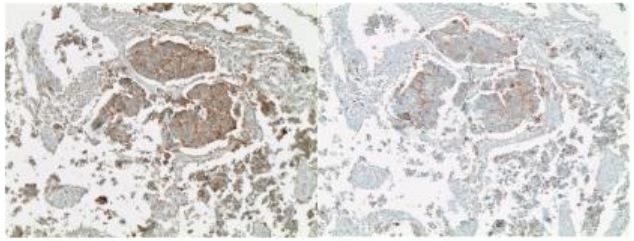
Spring Bioscience



NSCC3 A-7

Cell Signaling

Spring Bioscience



DIFFERENCES IN SPECIFIC AND NON-SPECIFIC STAINING BETWEEN DIIFERENT ANTIBODIES !

Cell Signaling

TIME IS IMPORTANT!

Ideally, Reflex at Diagnosis

Test requested by oncologist

1–7 days

Sample delivery to testing center

1–2 days

Prepare HE & unstained section

Pathologist to QC the HE slides

1–2 days

Lab performance of the assay

7–10 days

Macro-dissection
DNA isolation
Mutation assay

Reporting of results

Total: 10–21 days (average 14 days)

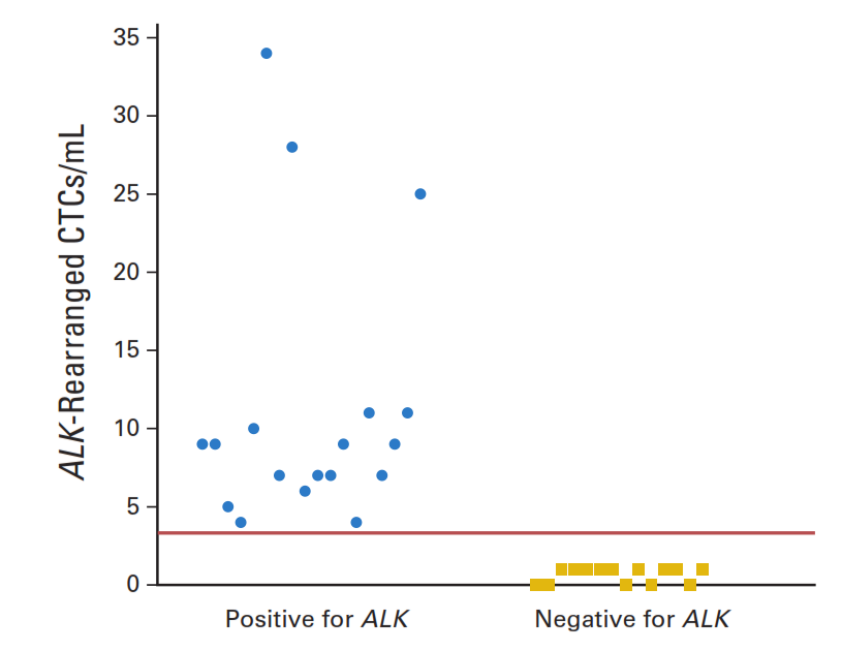
Reflex testing by pathologist at time of diagnosis

Result available at consultation

CIRCULATING TUMOR CELLS OR DNA?

Detection of Circulating Tumor Cells Harboring a Unique *ALK* Rearrangement in *ALK*-Positive Non–Small-Cell Lung Cancer

Emma Pailler, Julien Adam, Amélie Barthélémy, Marianne Oulhen, Nathalie Auger, Alexander Valent, Isabelle Borget, David Planchard, Melissa Taylor, Fabrice André, Jean Charles Soria, Philippe Vielh, Benjamin Besse, and Françoise Farace



ALK+ CTC	ALK status in tumor		Total
	ALK+	ALK-	
<4	0	14	14
≥4	18	0	18
Total	18	14	32

Sensitivity 100%, specificity 100%

NPV 100%, PPV 100%

J Clin Oncol 2013:31:2273-81

ORIGINAL ARTICLE

OPEN

Gefitinib Treatment in *EGFR* Mutated Caucasian NSCLC

Circulating-Free Tumor DNA as a Surrogate for Determination of EGFR Status

Jean-Yves Douillard, MD, PhD, Gyula Ostoros, MD,† Manuel Cobo, MD,‡ Tudor Ciuleanu, MD,§ Rebecca Cole, PhD,‖ Gael McWalter, MSci,‖ Jill Walker, PhD,‖ Simon Dearden, MSc,‖ Alan Webster, MSc,‖ Tsveta Milenkova, MD,‖ and Rose McCormack, PhD‖*

J Thorac Oncol, September 2014

TABLE 1. *EGFR* Mutation Status Summary, Concordance, Sensitivity, Specificity, and Positive- and Negative-Predictive Value for Tumor vs. Plasma 1 Circulating-Free Tumor DNA Samples by *EGFR* Mutation Status (Screened Patients Evaluable for Both Samples, $n = 652$)

	Plasma 1 *EGFR* Mutation Status, *n*		
	Positive	**Negative**	**Total**
Tumor *EGFR* mutation status, *n*[a]			
Positive	69	36	105
Negative	1	546	547
Total	70	582	652

	n	Rate, %	95% Confidence Interval
Concordance	652	94.3	92.3–96.0
Sensitivity	105	65.7	55.8–74.7
Specificity	547	99.8	99.0–100.0
Positive-predictive value	70	98.6	92.3–100.0
Negative-predictive value	582	93.8	91.5–95.6

[a]For the comparison of tumor and plasma data, the tumor DNA mutation status was adjusted for the mutations analyzed in circulating-free tumor DNA from plasma (i.e., for exon 19 deletions, L858R point mutations and T790M point mutations only).

EGFR, epidermal growth factor receptor.

Detection of *EGFR* activating mutations from plasma DNA as a potent predictor of survival outcomes in FASTACT-2

Tony Mok,[1] Yi Long Wu,[2] Jin Soo Lee,[3] Chong-Jen Yu,[4] Virote Sriuranpong,[5] Wen Wei,[6] Julie Tsai,[6] Matt Truman,[7] Barbara Klughammer,[8] and Lin Wu[6]

[1]Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong, China; [2]Guangdong Lung Cancer Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China; [3]National Cancer Center, Goyang, Republic of Korea; [4]National Taiwan University Hospital, Taiwan; [5]The King Chulalongkorn Memorial Hospital & Chulalongkorn University, Thailand; [6]Roche Molecular Systems, Inc. Pleasanton, California, USA; [7]Roche Products Ltd, Dee Why, Australia; [8]F. Hoffmann-La Roche Ltd, Basel, Switzerland

Presented at the 2013 ASCO Annual Meeting. Presented data is the property of the author.

ASCO Annual '13 Meeting

Concordance between tumor and plasma samples

- Total of 224 patients had both tumor and baseline plasma samples with available *EGFR* mutation analysis results (Table 3)
  - Sensitivity: 77% (69/90)
  - Specificity: 96% (129/134)
  - Positive predictive value: 93% (69/74)
  - Negative predictive value: 86% (129/150)
  - Overall concordance: 88% (198/224)

TIME IS IMPORTANT!

Ideally, Reflex at Diagnosis

Test requested by oncologist

1–7 days

Sample delivery to testing center

1–2 days

Prepare HE & unstained section

Pathologist to QC the HE slides

1–2 days

Lab performance of the assay

7–10 days

Macro-dissection
DNA isolation
Mutation assay

Reporting of results

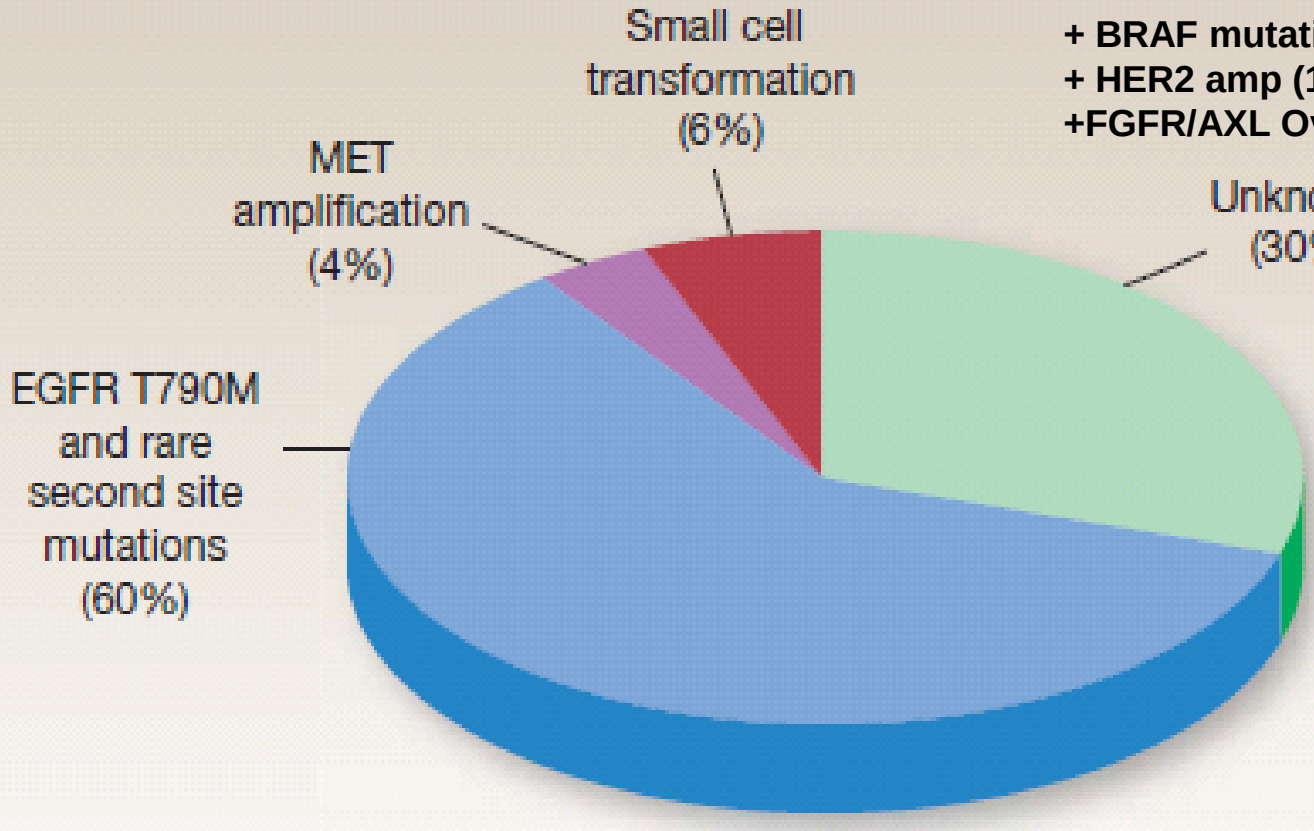
Total: 10–21 days (average 14 days)

Reflex testing by pathologist at time of diagnosis

Result available at consultation

WHAT ABOUT RESISTANT MECHANISMS?

Mechanisms of Acquired Resistance to EGFR TKIs



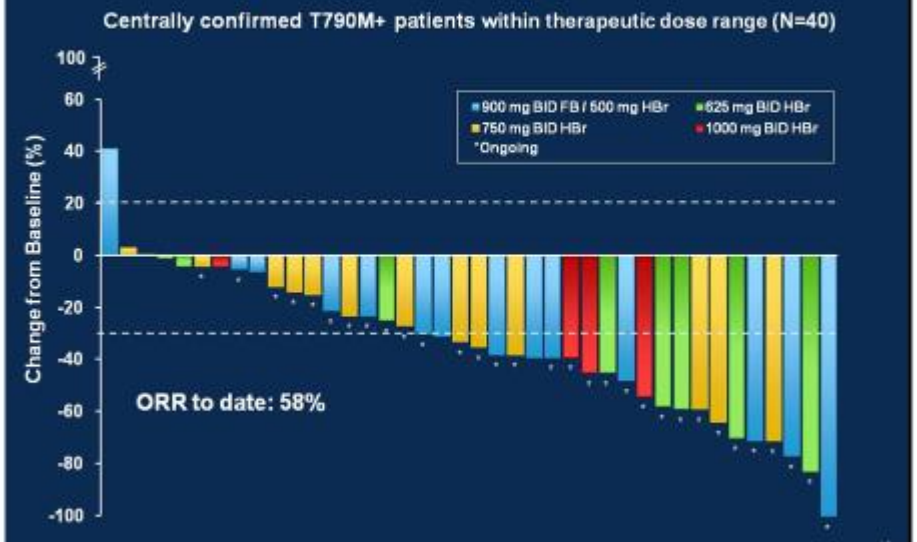
Oxnard et al '11; Ohashi et al '12; Takezawa et al '12

Third Generation EGFR TKIs Targeting T 790 M Mutations

- Irreversible binding of activating and T790M mutations but not wildtype
  - CO-1686 (Clovis)
  - AP26113 (Ariad) – ALK
  - AZD9291 (AZ)

CO 16 86

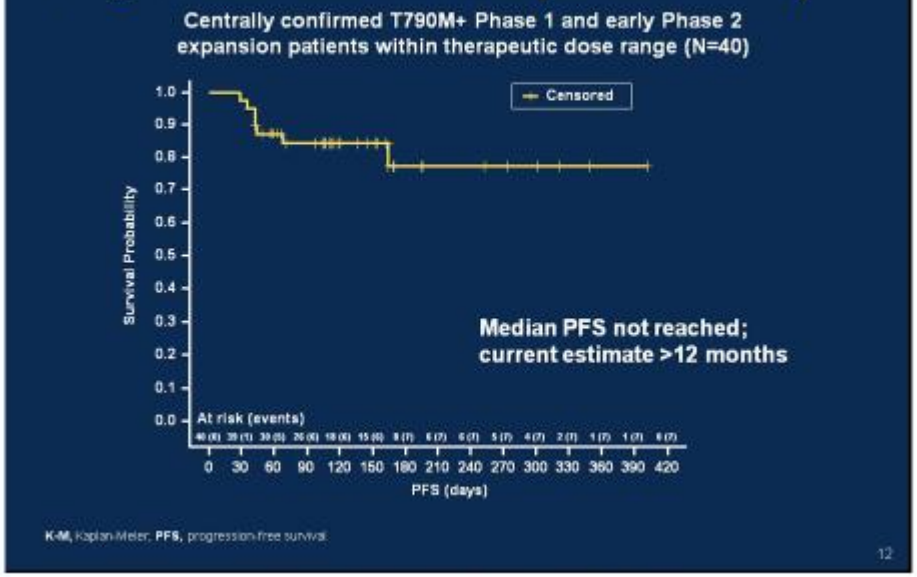
Best response in Phase 1 and early Phase 2 expansion cohort patients



10

Presented By Lecia Sequist at 2014 ASCO Annual Meeting

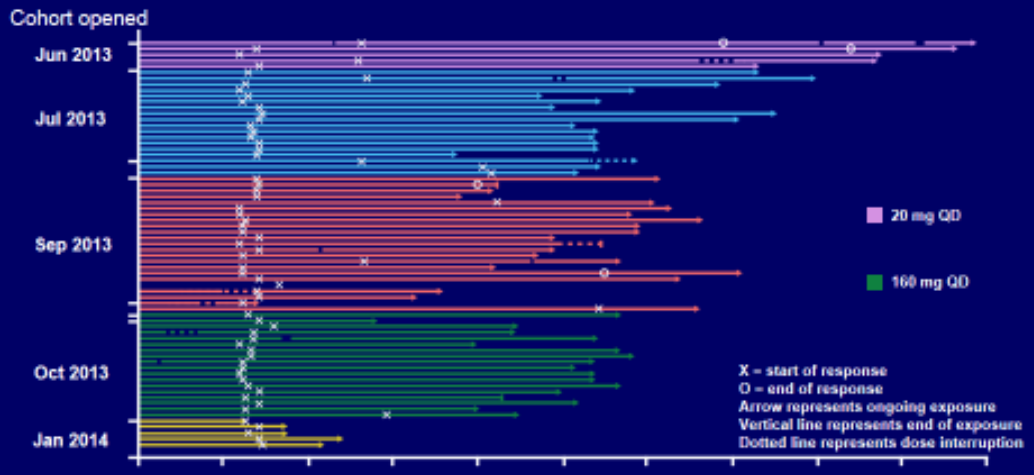
Progression-free survival (K-M estimate)



12

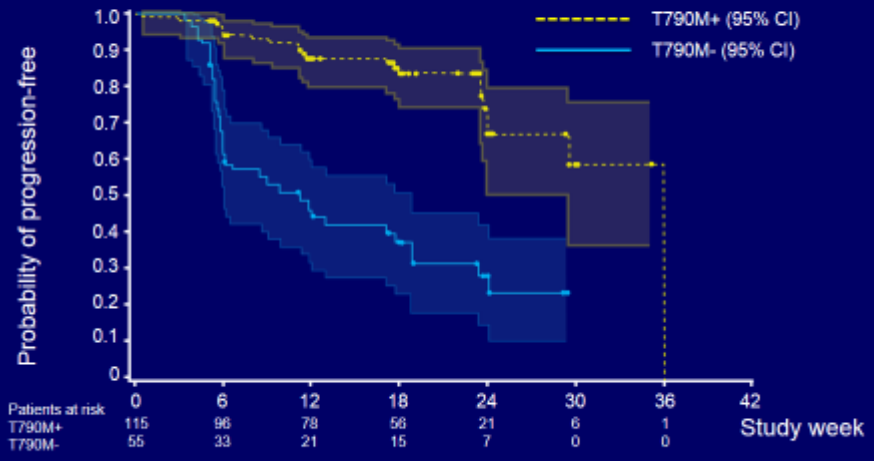
Presented By Lecia Sequist at 2014 ASCO Annual Meeting

AZD 9291 Response Duration in T790M mEGFR



Population: al

AZD9291 PFS in T790M mEGFR post EGFR TKI Rx

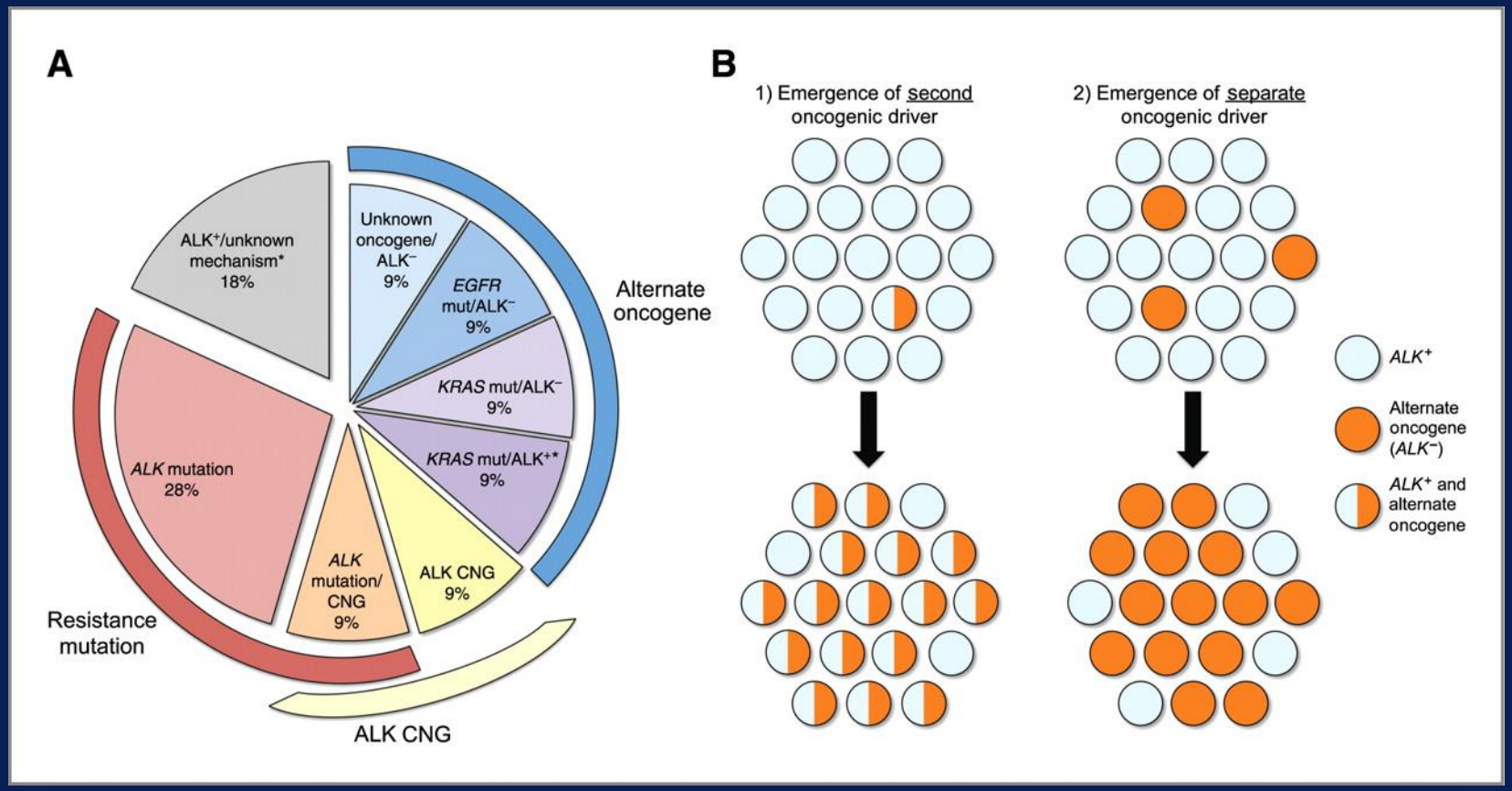


Crizotinib resistance

Mutation	Localization in kinase	Proposed mechanism of resistance
C1156Y	N-terminal of αC-helix	Unknown
L1196M	Gatekeeper	Crizotinib binding
L1152R	N-terminal of αC-helix	Unknown
F1174L	C-terminal of αC-helix	Affinity for ATP
G1202R	Solvent front	Crizotinib binding
S1206Y	Solvent front	Crizotinib binding
1151Tins	N-terminal of αC-helix	Affinity for ATP
G1269A	ATP binding pocket	Affinity for ATP
D1203N	Solvent front	Crizotinib binding

Doebele, CCR 2012

Relative frequencies of crizotinib resistance mechanisms in patients with ALK+ NSCLC and models for potential mechanisms of alternate oncogene acquisition.



Doebele R C et al. Clin Cancer Res 2012;18:1472-1482

REPORTING


Gentris
A CGI COMPANY


PerkinElmer Labs


LabCorp
Laboratory Corporation of America


illumina


GE Healthcare
SeqWright Genomic Services


Baylor Miraca
Genetics Laboratories


Quest
Diagnostics


COVANCE


CARIS
LIFE SCIENCES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GenomOncology
BATTLING CANCER, ONE GENOME AT A TIME


Knight Diagnostic Labs
GeneTrails

FOUNDATIONONE

Patient Name	Report Date	Tumor Type
	22 June 2014	Lung adenocarcinoma

Date of Birth		**Medical Facility**	University of Colorado Hospital	**Specimen Received**	10 June 2014
Sex		**Ordering Physician**	Doebele, Robert	**Specimen Site**	Liver
FMI Case #		**Additional Recipient**	Not Given	**Date of Collection**	29 May 2014
Medical Record #		**Medical Facility ID #**		**Specimen Type**	Block
Specimen ID		**Pathologist**	Dara Aisner		

ABOUT THE TEST:
FoundationOne™ is a next-generation sequencing (NGS) based assay that identifies genomic alterations within hundreds of cancer-related genes.

PATIENT RESULTS

- **4 genomic alterations**
- **4 therapies associated with potential clinical benefit**
- **0 therapies associated with lack of response**
- **6 clinical trials**

TUMOR TYPE: LUNG ADENOCARCINOMA

Genomic Alterations Identified†

EGFR amplification, D770_N771>QVH
MYC amplification
MYST3 amplification

Additional Disease-relevant Genes with No Reportable Alterations Detected
ALK
KRAS

†For a complete list of the genes assayed, please refer to the Appendix
⌘See Appendix for details

THERAPEUTIC IMPLICATIONS

Genomic Alterations Detected	FDA Approved Therapies (in patient's tumor type)	FDA Approved Therapies (in another tumor type)	Potential Clinical Trials
EGFR amplification, D770_N771>QVH	Afatinib	Cetuximab Lapatinib Panitumumab	Yes, see clinical trials section
MYC amplification	None	None	Yes, see clinical trials section
MYST3 amplification	None	None	None

bioT3
Metastatic Cancer Solution

CancerTREATMENT NGS+ Comprehensive IHC & FISH Summary Report

bioTheranostics, Inc.
9640 Towne Centre Dr, Suite 200
San Diego, CA 92121
1 (877) 886-6739

PATIENT

Name:
DOB:
Sex:
Medical Record:

SPECIMEN

Order ID:
Sample ID:
Biopsy: Retroperitoneum Mass Cor
Patient Tumor Type: Unknown
Date Received: 1/14/2015
Date Reported: 1/22/2015
Date of Collection: 1/2/2015

PHYSICIAN

Physician Name: Uchenna O. Njiaju,
Facility: Memorial Medical Oncolog
Address: 525 N Foote Ave., Ste 202
City, State, Zip: Colorado Springs, CO 80909
Phone: (719) 365-9746
Fax: (719) 365-6317

TEST DESCRIPTION: CancerTREATMENT NGS+ uses NGS, IHC, and FISH platforms for comprehensive biomarker analysis. This report summarizes results from IHC and FISH-based biomarker analyses. Please see the Next Generation Sequencing Test Report for NGS biomarkers.

POSITIVE TEST RESULTS (Please see individual test reports for additional information)

Biomarker	Result	FDA Approved Therapies Targeting Molecular Pathway	FDA Approved Indication(s)	Clinical Trials in Solid Tumors
HER2 Amplification (FISH)	*DETECTED*	Kadcyla® (ado-trastuzumab emtansine)	Breast cancer (HER2+)	Yes, see clinical trials section
		Herceptin® (trastuzumab)	Breast cancer (HER2+)	
			Gastric or gastroesophageal junction cancer (HER2+)	
		Tykerb® (lapatinib)	Breast cancer (HER2+)	
		Perjeta® (pertuzumab)	Breast cancer (HER2+)	
PD-L1 Expression (IHC)	*1+, POSITIVE*	None	None	Yes, see clinical trials section
EGFR Expression (IHC)	*1+, POSITIVE*	Erbitux® (cetuximab)	Squamous cell cancer of the head and neck	Yes, see clinical trials section
			Colorectal cancer (KRAS Wild-type Only)	
c-MET Expression (IHC)	*1+, POSITIVE*	None	None	Yes, see clinical trials section

NEGATIVE TEST RESULTS (Please see individual test reports for additional information)

Biomarker	
ALK Rearrangement (FISH)	ROS1 Rearrangement (FISH)
RET Rearrangement (FISH)	c-MET Amplification (FISH)

RESULTS:

MULTIPLEX MUTATION ANALYSIS by Targeted Next-Generation Sequencing
Mutations Identified (SEE INTERPRETATION)

Gene	Predicted Protein Changes	Nucleotide Change
1. *BRAF*	p.V600E	c.1799T>A

No additional significant mutations were identified in *KIT*, *NRAS*, or the remainder of the panel (see Assay Methodology section for panel contents).

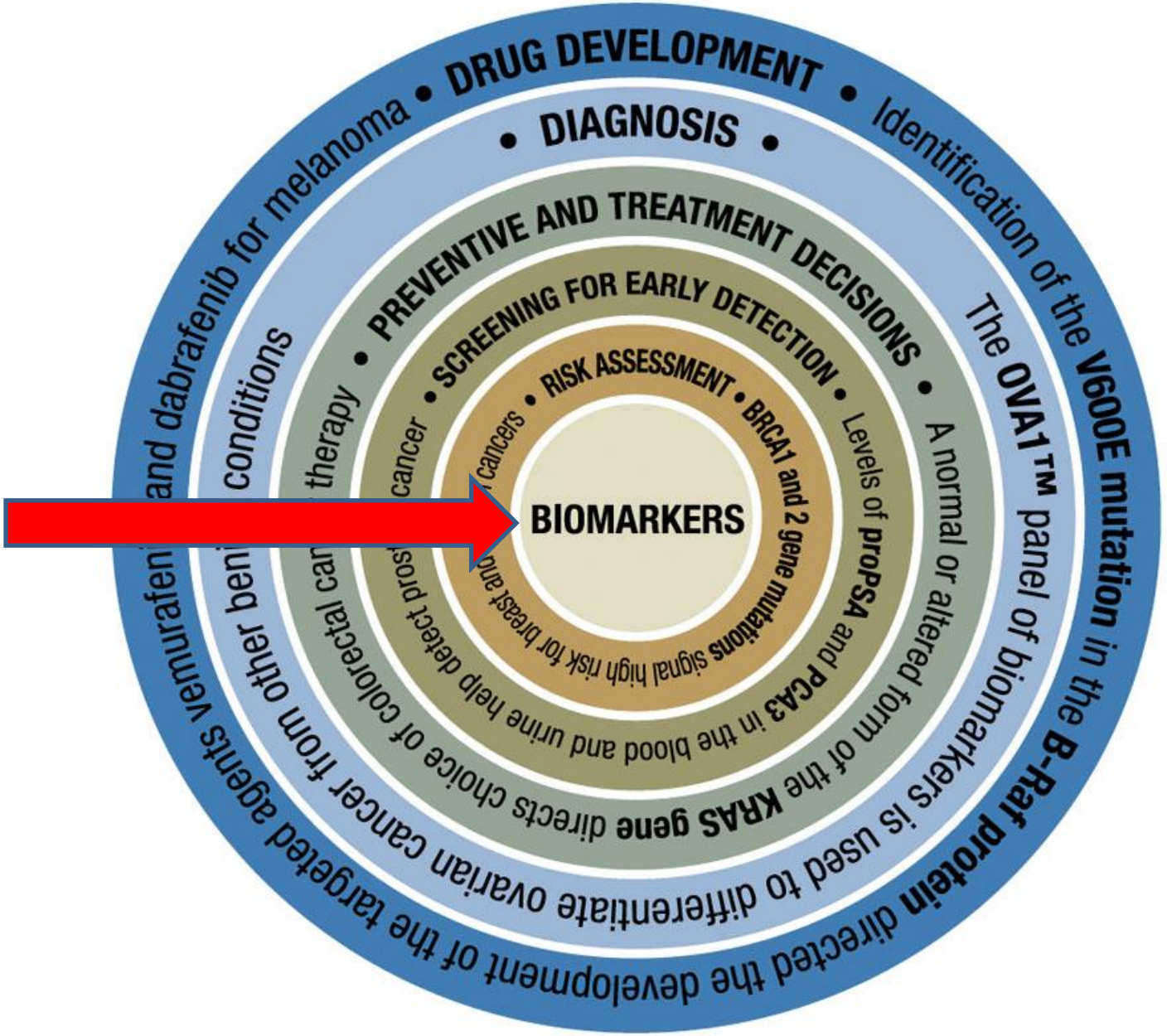
INTERPRETATION
Mutations were identified in this sample of known clinical relevance:
1. There is evidence of a mutation in *BRAF*, resulting in an anticipated single amino acid substitution (p.V600E). This is among the most common mutations reported in malignant melanoma. This finding has been associated with responsiveness to targeted therapy agents. Clinical correlation is recommended. Analysis of *BRAF* is based on reference sequence NM_004333.4

METHODS AND ASSAY LIMITATIONS:

Mutation Analysis
Preanalytical Processing: H&E-stained paraffin sections were examined by a board-certified anatomic pathologist for testing suitability. Tumor cells were isolated by microscope assisted microdissection followed by tumor cell lysis and DNA extraction.

Solid Tumor Sequencing Panel
Sequence analysis of selected coding regions of 26 genes involved with solid tumors (*AKT1, ALK, APC, BRAF, CDH1, CTNNB1, EGFR, ERBB2, FBXW7, FGFR2, FOXL2, GNAQ, GNAS, KIT, KRAS, MAP2K1, MET, MSH6, NRAS, PDGFRA, PIK3CA, PTEN, SMAD4, SRC, STK11, TP53*) was carried out using the TruSight Tumor sequencing panel (Illumina, Inc.). Custom bioinformatic analysis developed at the University of Colorado was applied to map targeted regions, and identify variants and assay artifacts. Specific targeted regions analyzed are available from CMOCO upon request. Variants in intronic regions, or alterations anticipated to produce no amino acid change are not reported.

Assay Limitations:
This assay does not detect all types of mutations. For example, chromosomal translations, gene fusions, and copy number alterations are not detected. Insertions and deletions larger than 40 base pairs may not be detected. The analytic sensitivity for mutations in the genes listed above at 500x minimum coverage of each region is 5% variant allele frequency. Unless otherwise specified, all reported regions met the minimum criteria of 500x coverage. Although microdissection is employed, mutations present at a level below the analytic sensitivity may not be detected by this assay. This assay is not designed for the detection of germline alterations.



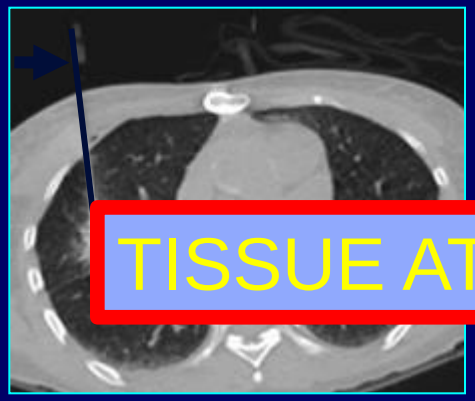
AACR : CANCER REPORT 2013

BEST PRACTICE FOR PATIENTS ON TREATMENT

- ROUTINE TESTING FOR EGFR and ALK AT TIME OF PRIMARY DXG
- Preferentially: Core biopsy
- If FNA: Tissue Block
- In all cases: Quality Control for Viable Tumor Tissue
- Primary Tumor vs Metastases?
- Rebiopsy at time PD? (in clinical study)

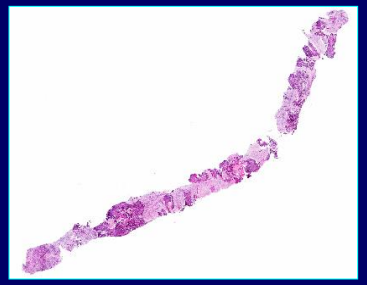
"THE TISSUE IS STILL THE ISSUE"

Advanced Tumor

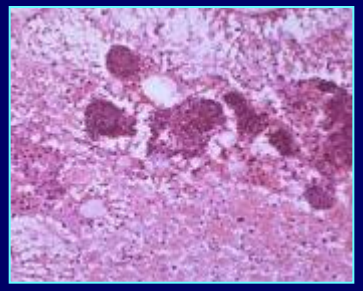


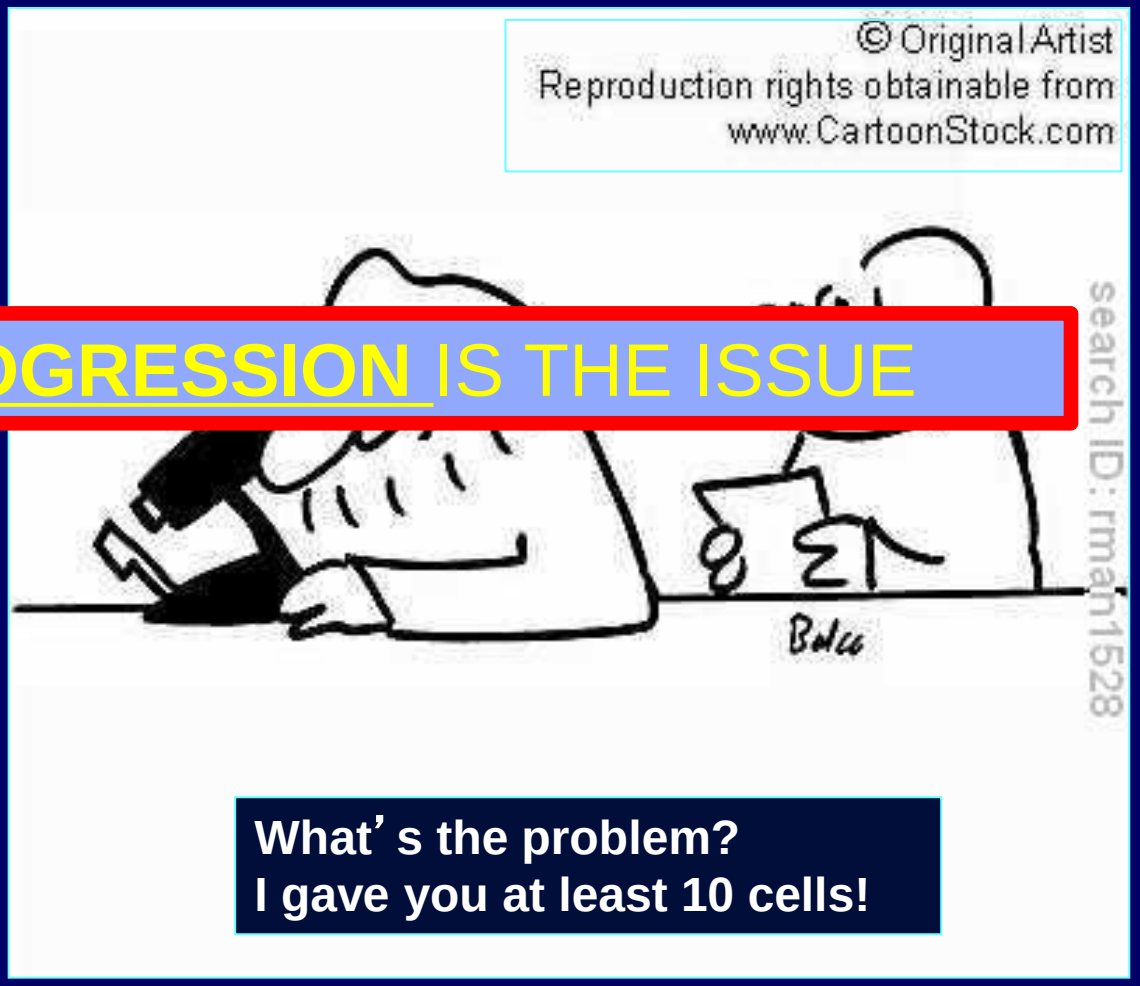
TISSUE AT **PROGRESSION** IS THE ISSUE

Core Needle Biopsy (CNB)



Fine-needle Aspiration (FNA)





16TH WORLD CONFERENCE ON LUNG CANCER

IASLC

INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

WWW.IASLC.ORG

Abstract Submission Open	January 2015
Registration Open	January 2015
Abstract Submission Deadline	April 24, 2015
Abstract Notifications	June 22, 2015
Early Registration Deadline	June 26, 2015
Late Breaking Abstract Submission Deadline	July 10, 2015
Regular Registration Deadline	July 24, 2015

SEPTEMBER 6–10, 2015

→ DENVER, COLORADO, USA

CURE FOR LUNG CANCER