How to integrate sequencing strategies with other techniques?

Fred R. Hirsch, MD,PhD
Professor of Medicine and Pathology,
Univ. of Colorado Cancer Center, Aurora, CO, USA

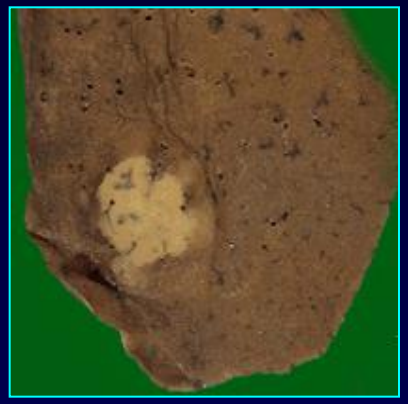
DISCLOSURES

- **Advisory Boards**: Genentech/Roche, BMS, Boehringer-Ingelheim, Novartis, Pfizer, Lilly, Amgen, Celgene, Synta, Biothera, Biodesix

- **Research Fundings**: Genentech, Amgen, Lilly-Imclone, Ventana, Celgene, Morphotek.

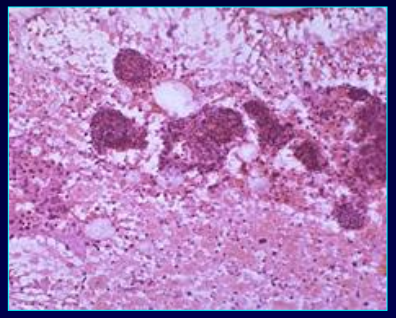
Types of Tumor Specimens In Lung Cancer

Surgical Resection



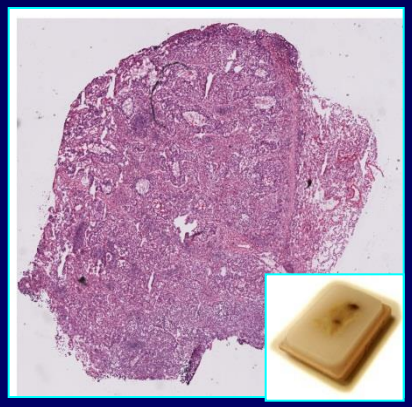
Advanced Tumor

Endobronchial Ultrasound (EBUS) or Pleural Fluid

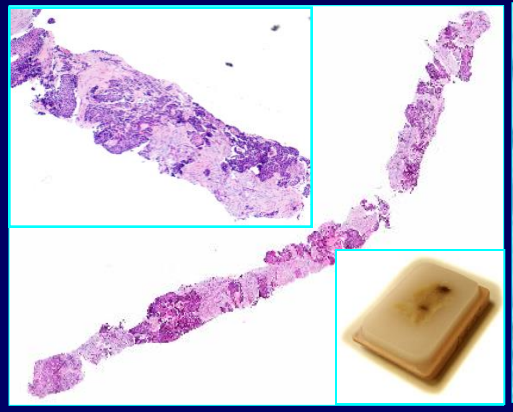


Alcohol-fixed

Histology

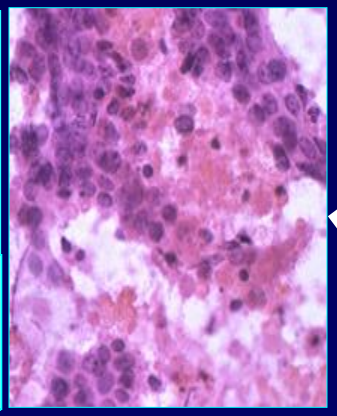


Core Needle Biopsy (CNB)



Formalin-fixed and Paraffin-embedded (FFPE)

Fine Needle Aspiration (FNA)



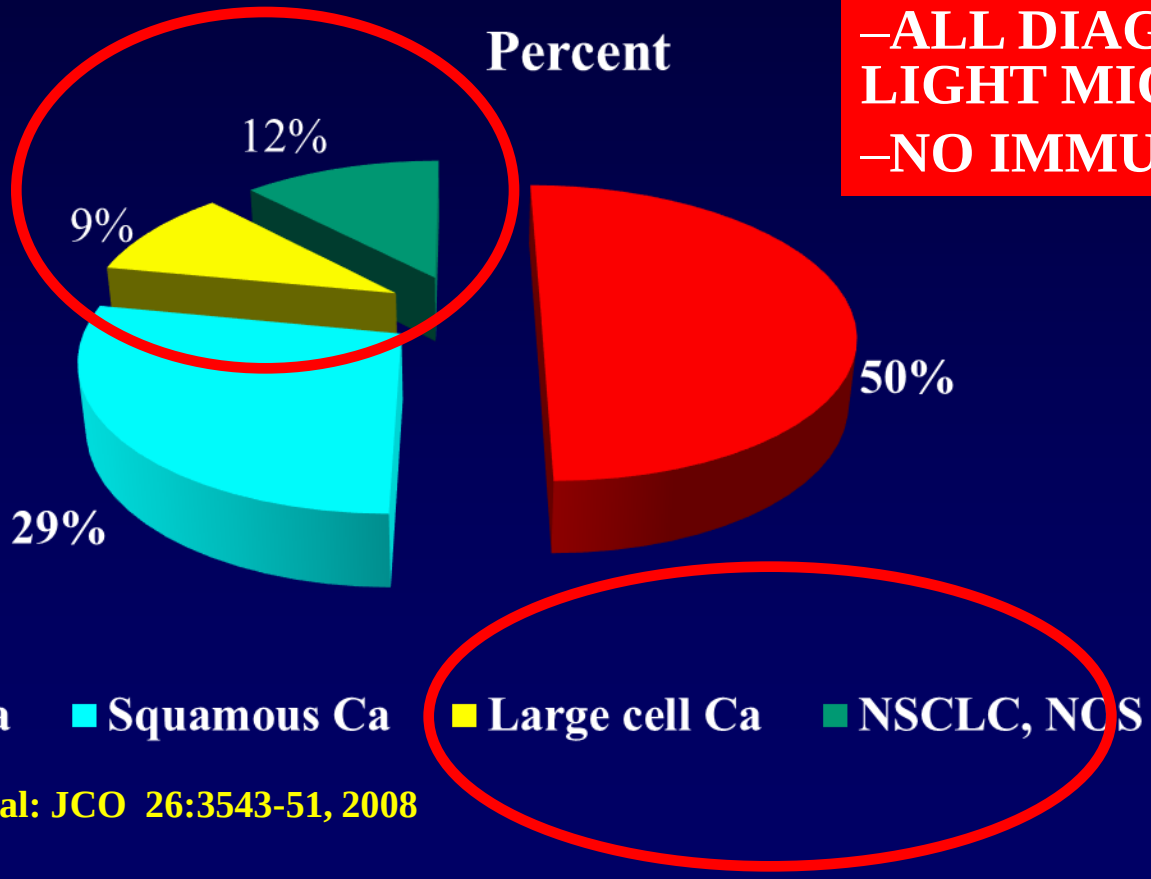
Alcohol-fixed

Alcohol-fixed – Cell Block

New Therapies in Advanced NSCLC

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

PHASE III STUDY COMPARING CISPLATIN PLUS GEMCITABINE WITH CISPLATIN & PEMETREXED IN ADVANCED NSCLC



–ALL DIAGNOSES – BY LIGHT MICROSCOPY
–NO IMMUNOSTAINS

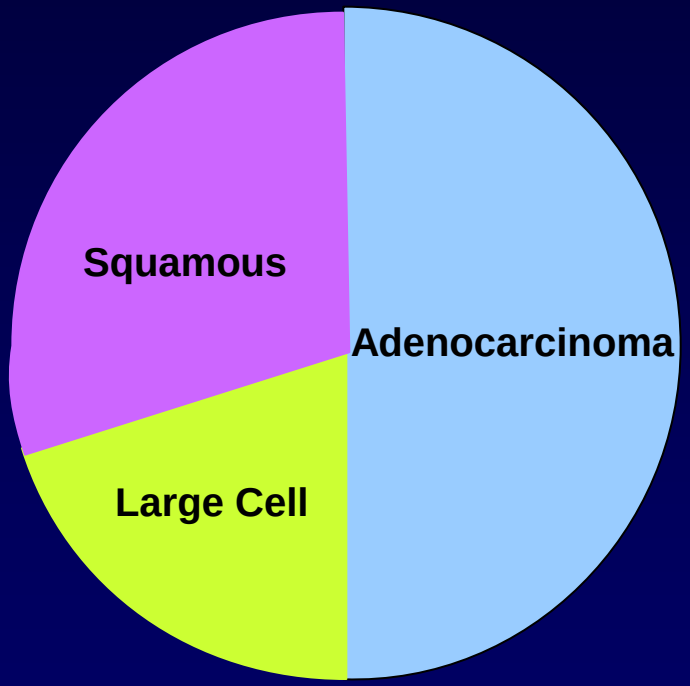
Scagliotti G, et al: JCO 26:3543-51, 2008

IMMUNOHISTOCHEMICAL MARKERS

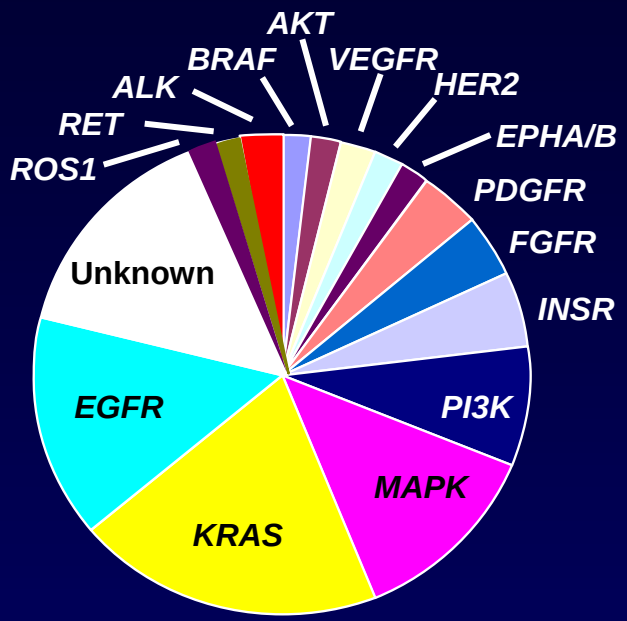
- **ADENOCARCINOMA (ONE MARKER)**
  - **TTF-1 (best), Napsin, PE-10**
- **SQUAMOUS CARCINOMA (ONE MARKER)**
  - **p63 (best), p40, CK5/6, 34βE12**
  - **Desmocolin-3 (need more testing)**
- **Cocktails – nuclear/cytoplasmic antibodies**
  - **Adenoca – TTF-1/Napsin**
  - **Squamous – p63/CK5/6**

Molecular Testing for NSCLC - 2012

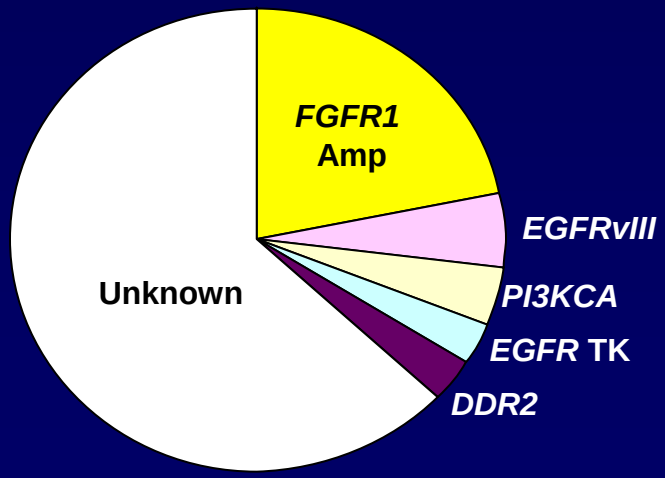
Traditional



Adenocarcinoma



Squamous Cell Ca



Adapted from W. Pao and N Girard, Lancet Oncol, 2011

ORIGINAL ARTICLE

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

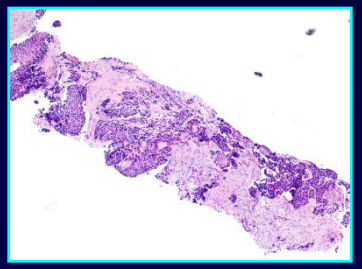
Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

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

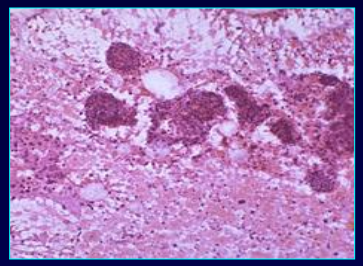
Journal Thoracic Oncol , April 2013

Diagnostic Algorithm for Small Biopsy and Cytology Specimens

Tumor Positive

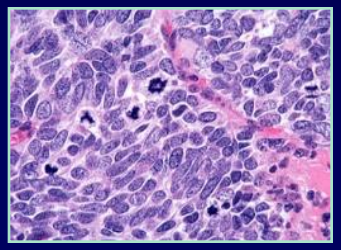


Biopsy



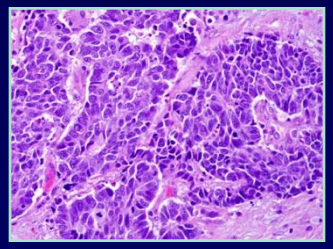
Cytology

SCLC



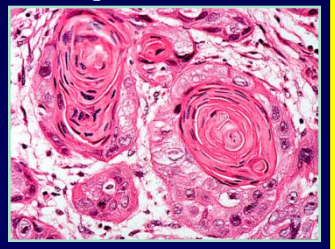
Morphology

LCNEC



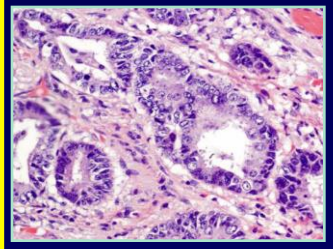
Morphology
IHC NE (+)

Squamous



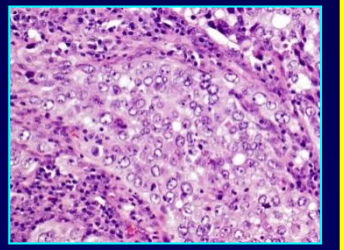
Morphology
IHC p63/p40 (+)

Adenoca



Morphology
IHC TTF1 (+)

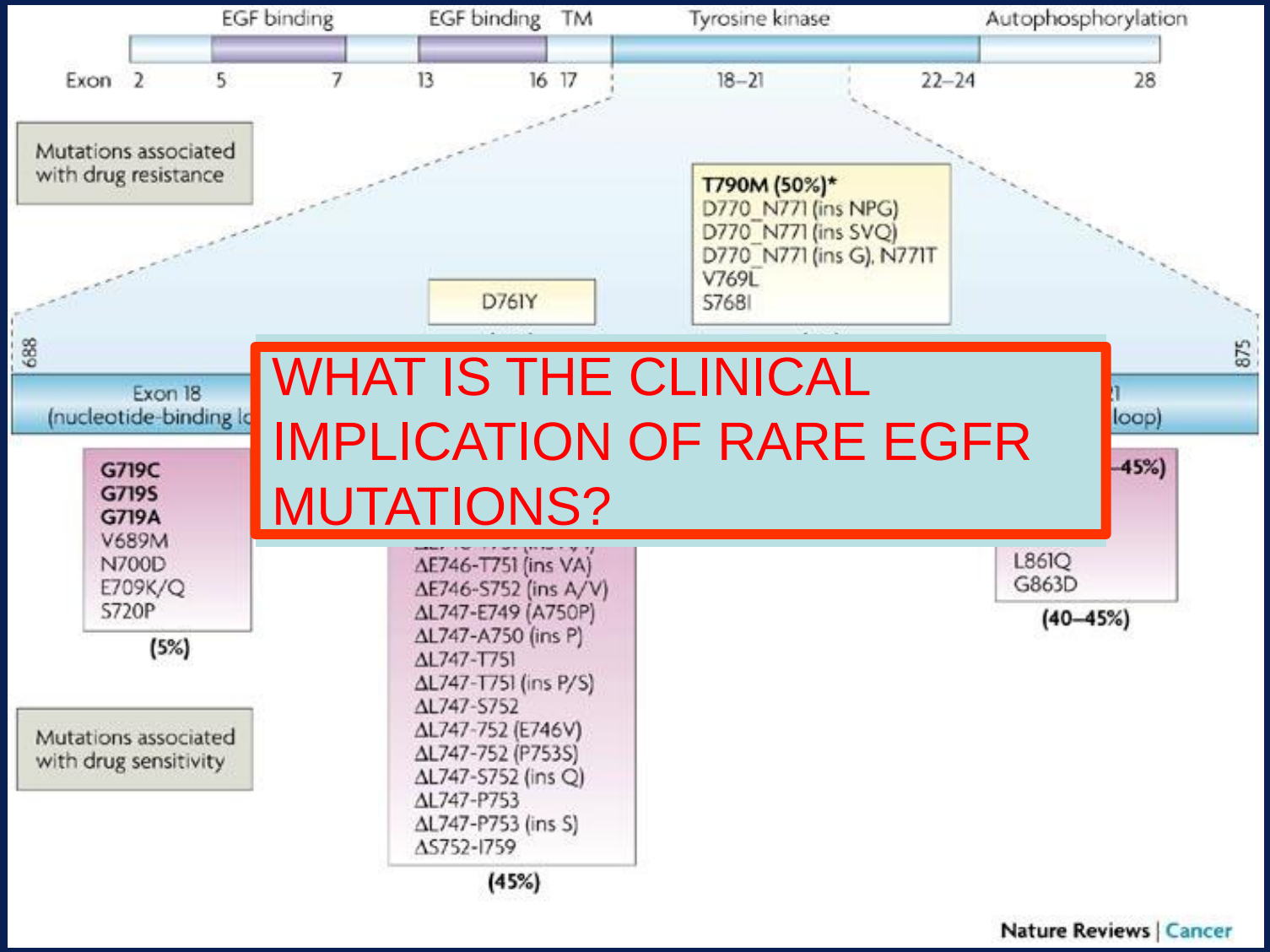
NSCLC-NOS



Morphology
IHC (-)

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

EGFR kinase domain mutations



Mutation Tests with Increased Sensitivity

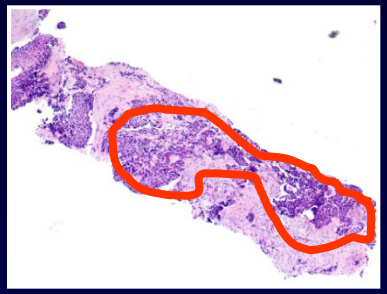
Method	Sensitivity	Mutations Identified
Direct sequencing	25%	Known and new
PCR-SSCP	10%	Known and new
TaqMan PCR	10%	Known only
Loop-hybrid mobility shift assay	7.5%	Known only
Cycleave PCR	5%	Known only
PCR-RLFP (fragment length analysis)	5%	Known only
MassARRAY genotyping	5%	Known only
LNA-PCR clamp	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

NSCLC Molecular Diagnosis

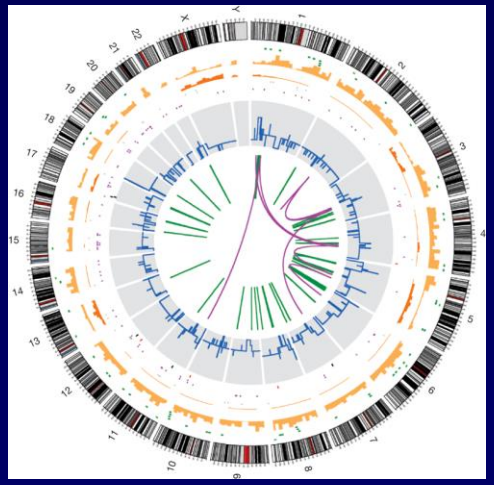
Tumor (CNB)



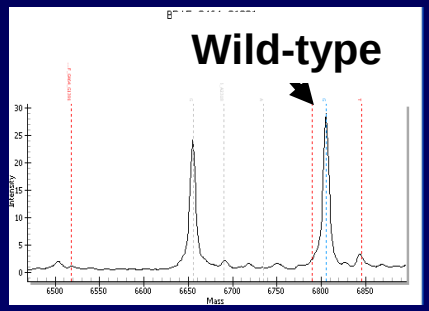
FFPE DNA Extraction

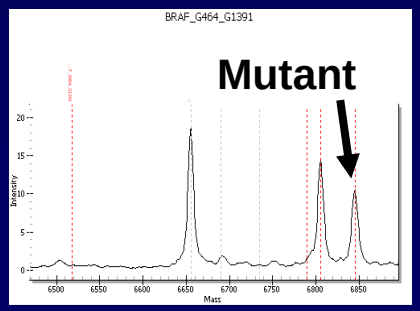
Multiplex PCR
~20ng DNA/multiplex reaction

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



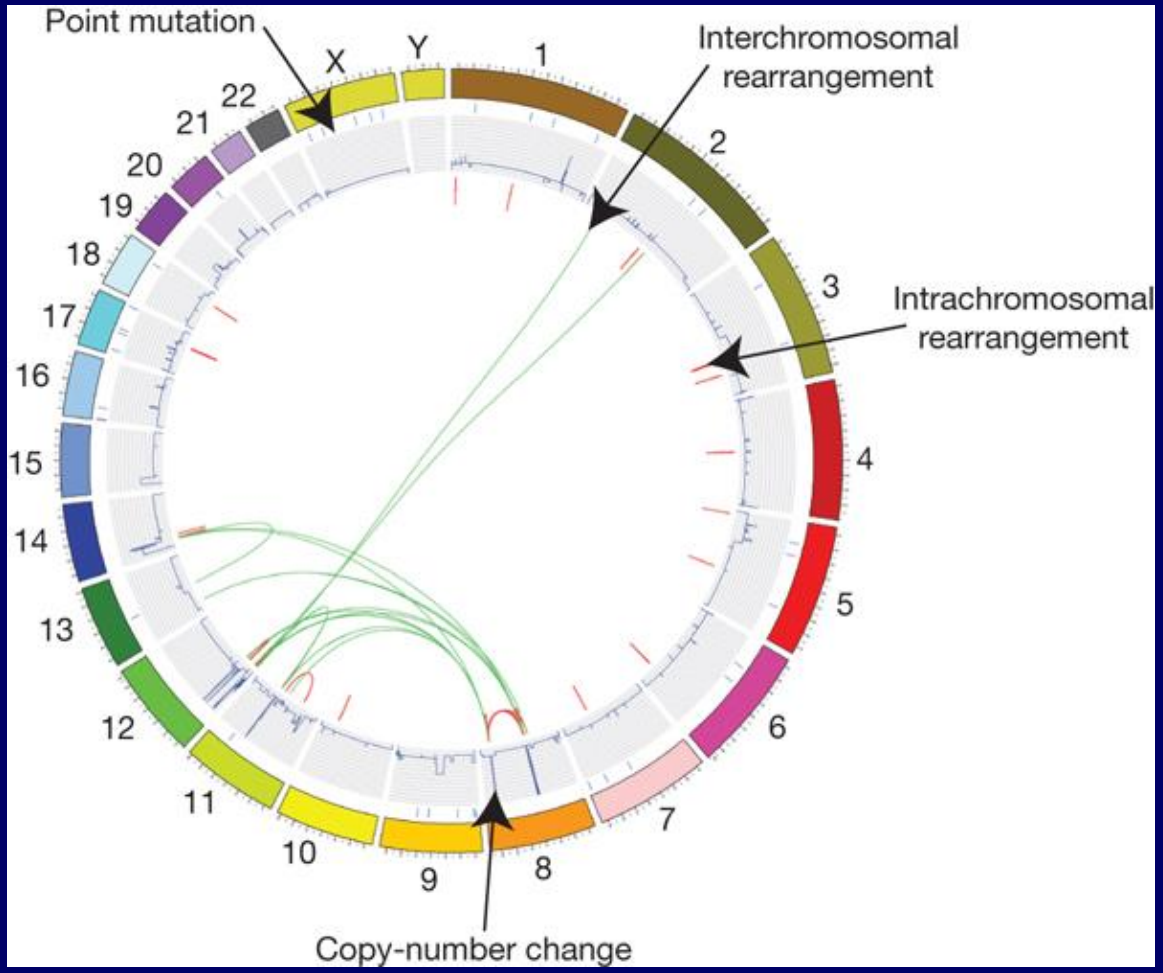
Sequenom™ (*BRAF*: G464-G1391)





~10% Sensitivity

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



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

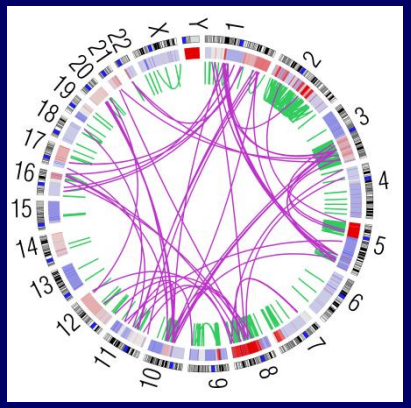
- Mutation detection
- DNA copy number detection
- Translocations/gene fusions
- RNA-seq: gene expression, alternative splicing

Characteristics:

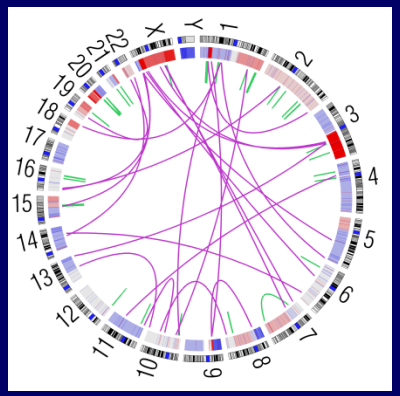
- High coverage: multiple (~500x) reads of the same sequence to gain confidence in result
- Critical when ratio of neoplastic to non-neoplastic cells is low
- Allows signal to be sifted from the noise
- Examination of reads in both directions to rule out artifacts
- Confirm or rule out sequence variant using an additional method (e.g. Sanger)

Squamous cell lung cancer: complexity revealed by whole genome sequencing

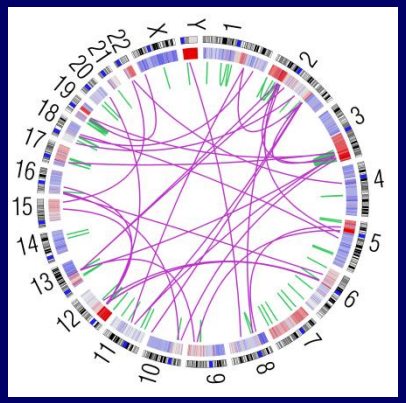
LUSC-66-2756



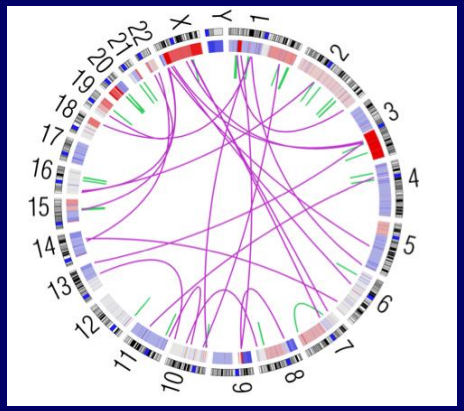
LUSC-34-2600



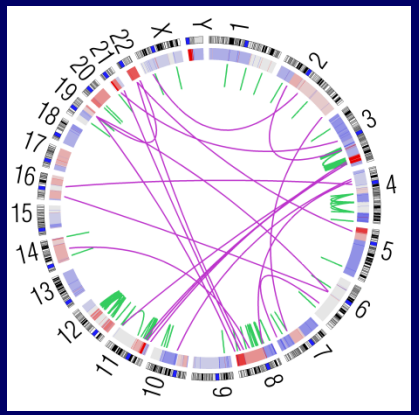
LUSC-43-3394



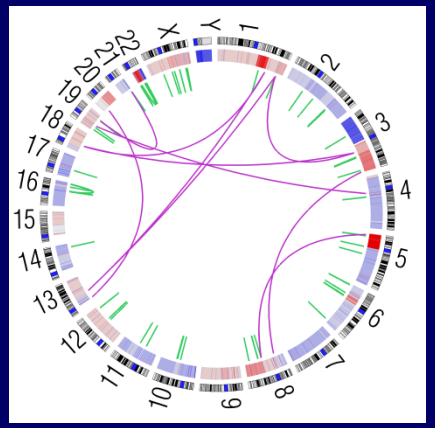
LUSC-34-2609



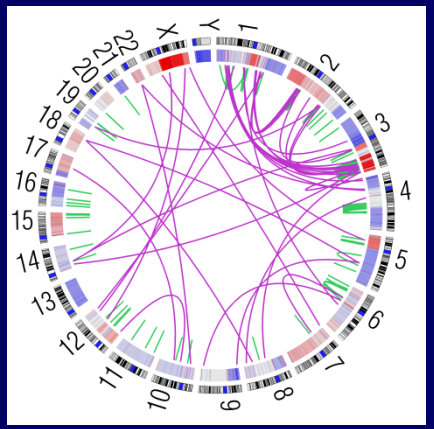
LUSC-56-1622



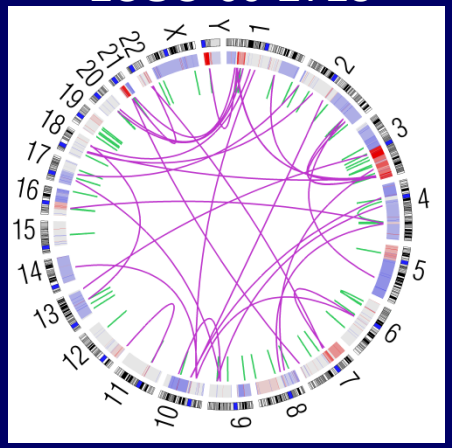
LUSC-60-2695



LUSC-60-2711



LUSC-60-2713

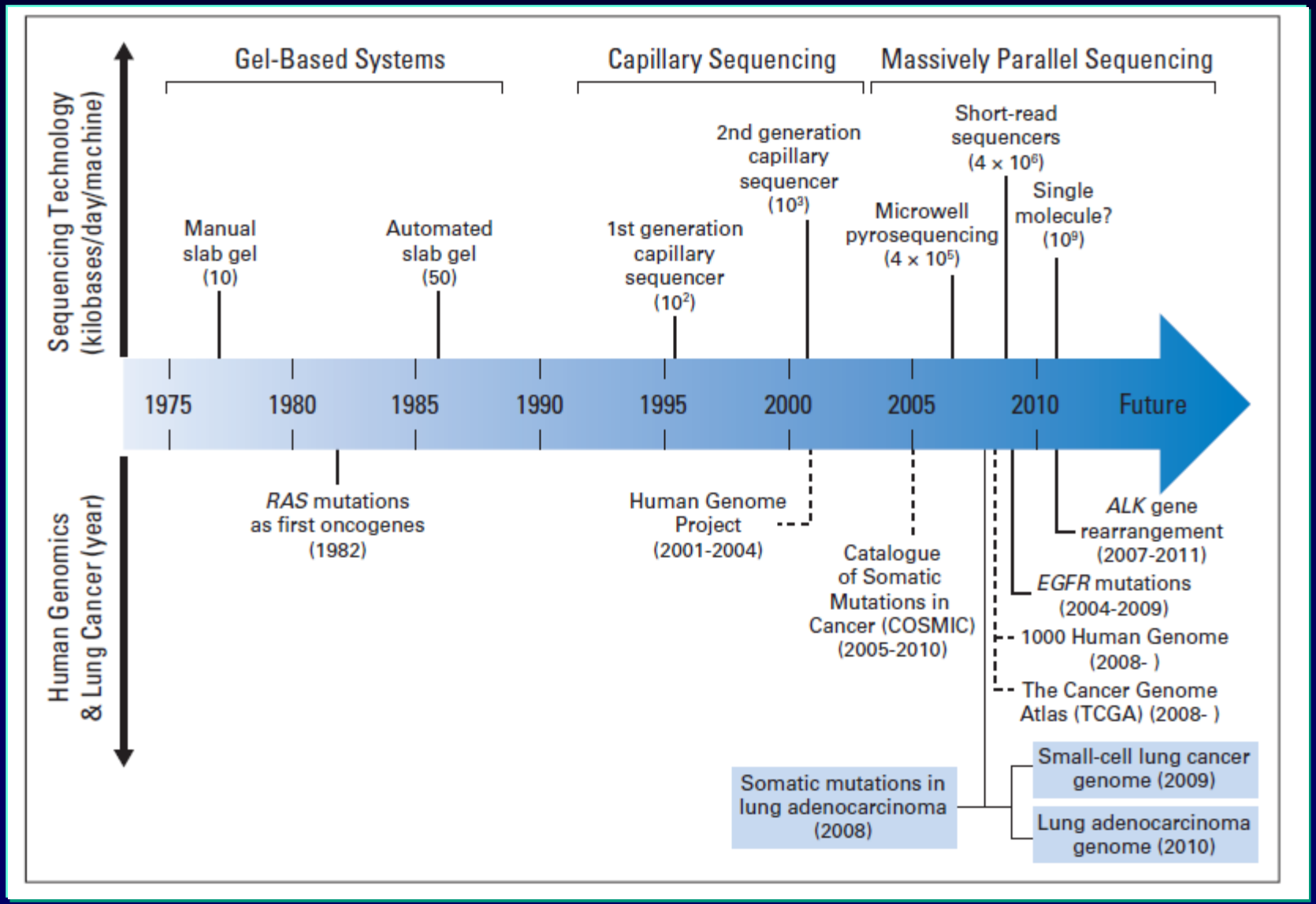


- **Only NGS allows the multiplexed nature required to obtain the information we need with the specimens that we can obtain**
- **Test development, reporting, and incorporation into clinical practice will require continued development and refinement.**

BIOINFORMATIC CHALLENGE !

Modified from G. Riely, Santa Monica 2013

Advances in Sequencing Methodologies and Human Lung Cancer Genomics



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

Next Generation of Sequencing

Illumina HiSeq 2000

300 – 600 Gigabases
6 – 11 days

Illumina MiSeq

1.5 Gigabases
1 day

Ion Torrent PGM

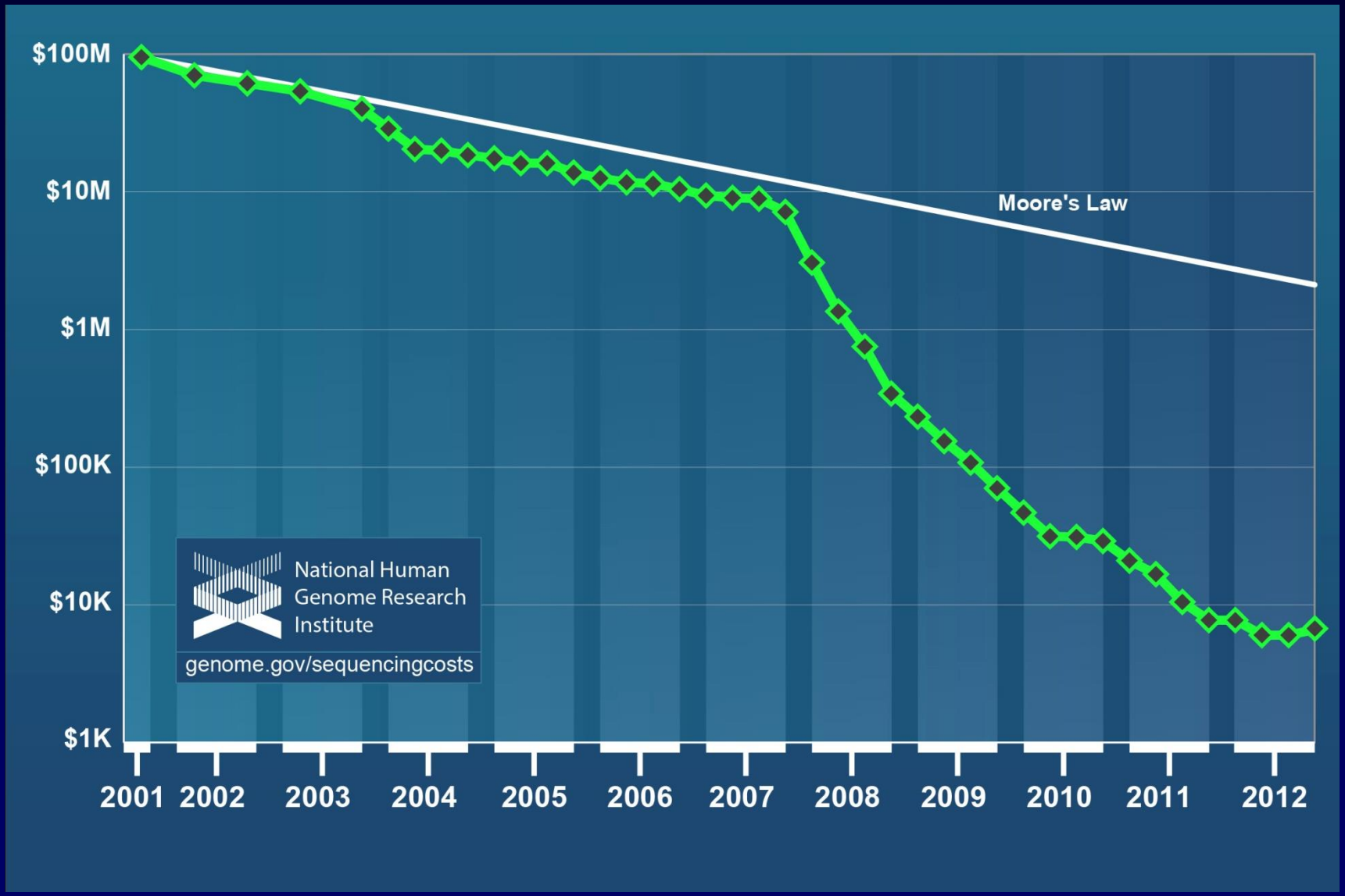
1 Gigabase
6 hours

Illumina HiSeq 2500

Ion Torrent Proton

Human Genome in a Day

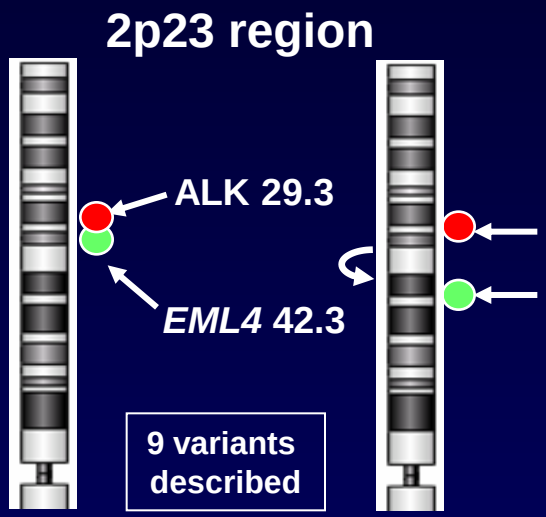
Cost of Genome Sequencing



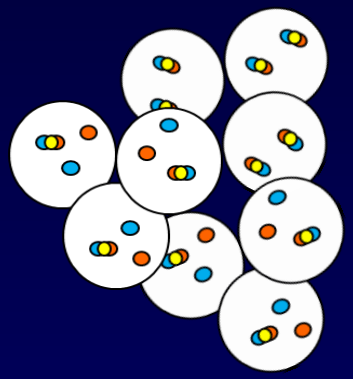
Courtesy of G. Riely

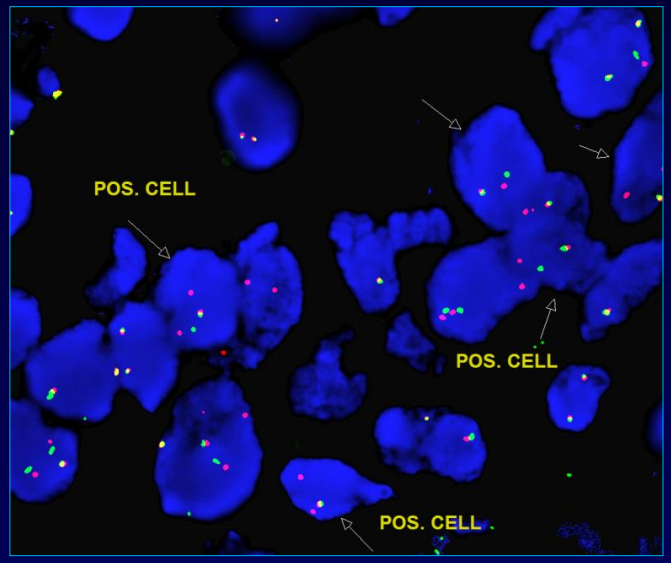
EML4-ALK Fusion in NSCLC

FISH Test: "Break-apart Probe"

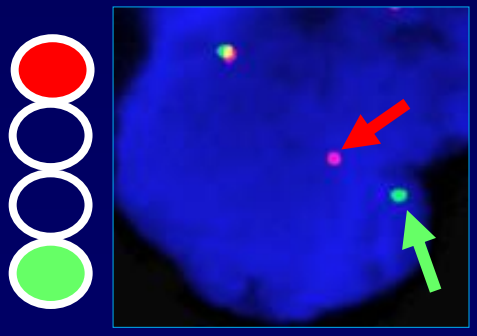


Positive Case:
>15% Cells Positive
(50-100 cells)

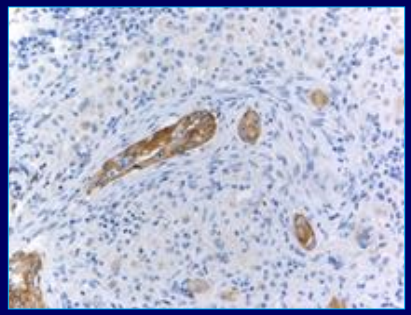




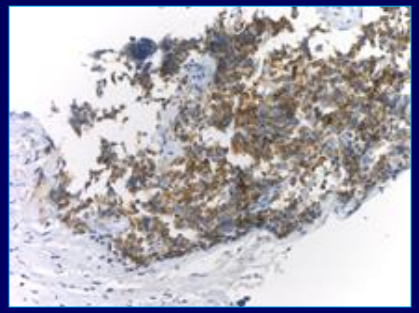
Positive Cell:
Two signals separation



ALK Immunohistochemistry (Clone D5F3)



EML4-ALK Fusion (+)

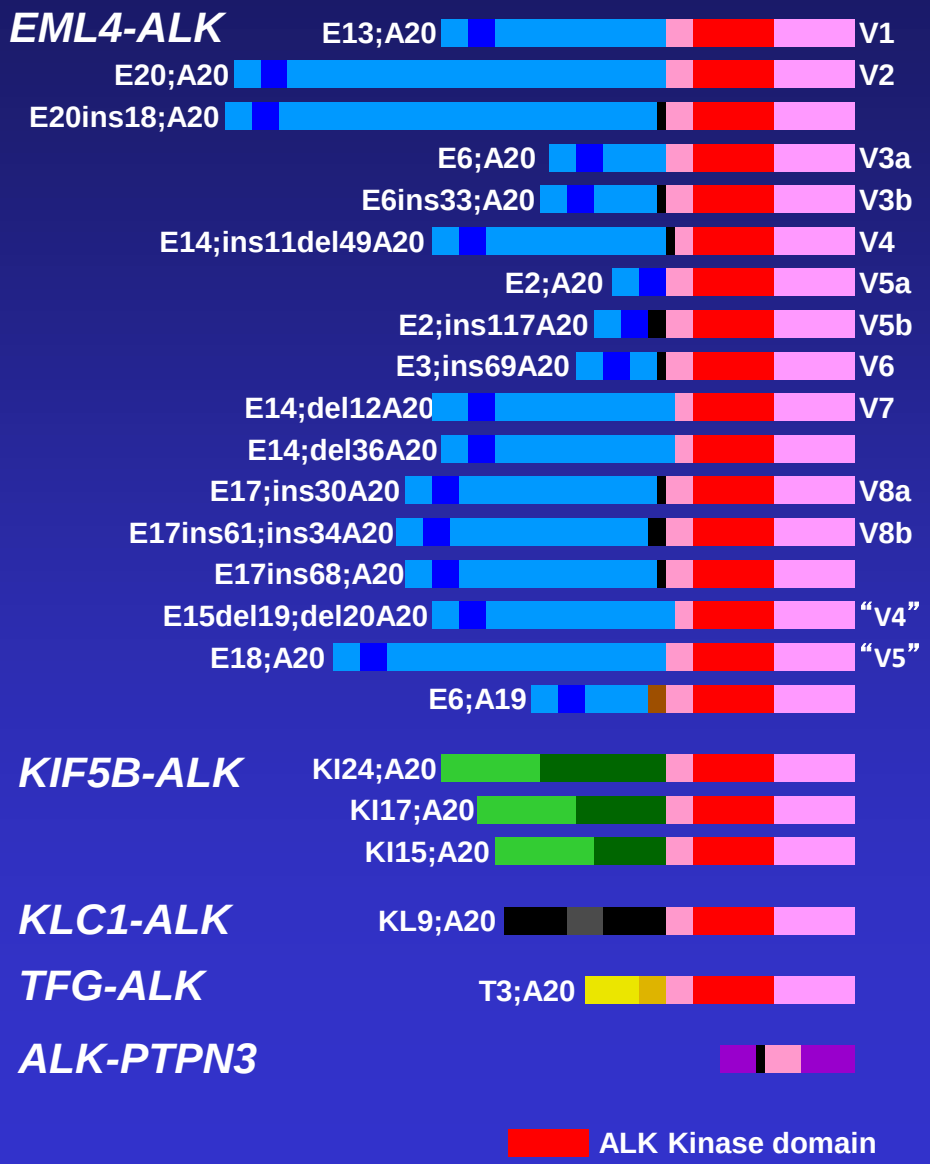


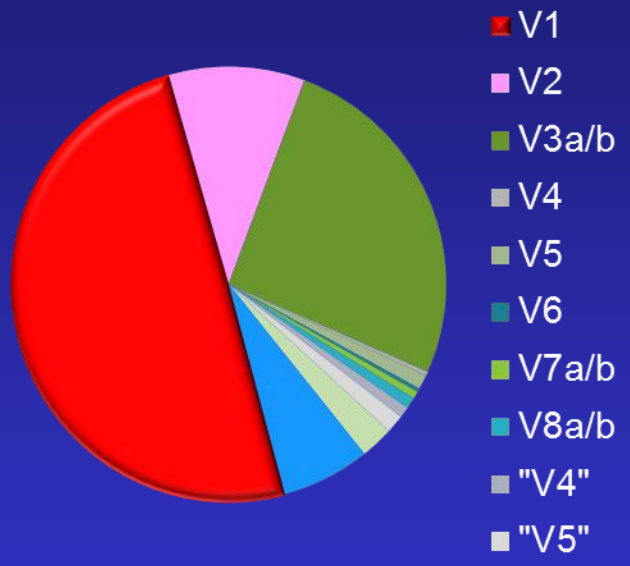
EML4-ALK Fusion (-)

Courtesy of Dr. Y. Yatabe

- **Biopsy:**
  - FFPE
- **Cytology:**
  - Cell blocks (FFPE)

EML4-ALK Fusion Variants in NSCLC





Ou et al., Oncologist 2012; 2012;17:1351-75

Detection of ALK by IHC

D5F3 **ALK1**

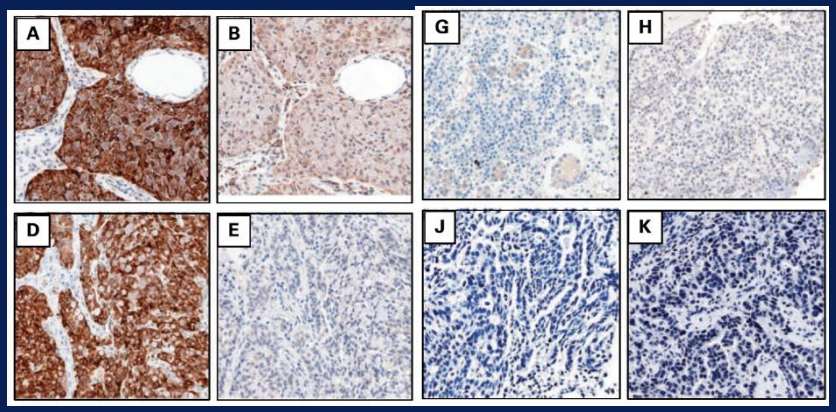


Table 1. Interpretation of IHC staining on lung adenocarcinoma

	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

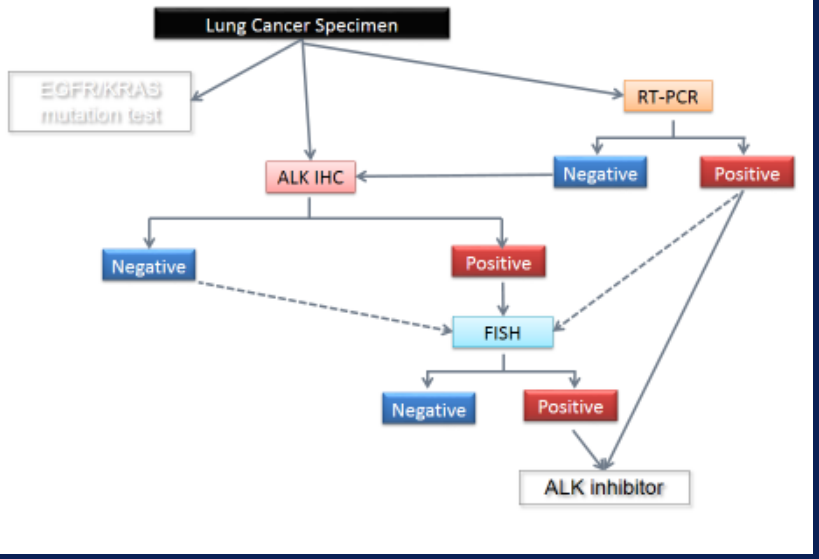
Jong-Mu Sun, MD, PhD, Yoon-La Choi, MD, PhD,† Jae-Kyung Won, MD,‡ Fred R. Hirsch, MD, PhD,§ Jin Seok Ahn, MD, PhD,* Myung-Ju Ahn, MD, PhD,* and Keunchil Park, MD, PhD**

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†*

NEW JAPANESE GUIDELINES FOR ALK-TESTING



Courtesy Dr. Yatabe

Screening of Anaplastic Lymphoma Kinase Rearrangement by Immunohistochemistry in Non-small Cell Lung Cancer

Correlation with Fluorescence In Situ Hybridization

Jin Ho Paik, MD, PhD, Gheeyoung Choe, MD, PhD,* Hyojin Kim, MD,* Ji-Young Choe, MD,* Hyun Ju Lee, MD,* Choon-Taek Lee, MD, PhD,† Jong Seok Lee, MD, PhD,† Sanghoon Jheon, MD, PhD,‡ and Jin-Haeng Chung, MD, PhD**

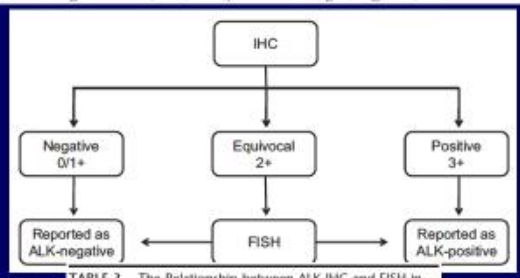


TABLE 3. The Relationship between ALK IHC and FISH in All Cases

	ALK FISH		
ALK IHC	(+)	(−)	Total (%)
0	0	586 (91.6)	586 (91.6)
1+	0	16 (2.5)	16 (2.5)
2+	6 (0.9)	10 (1.6)	16 (2.5)
3+	22 (3.4)	0	22 (3.4)
Total	28 (4.4)	612 (95.6)	640 (100)

IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ALK, ana-

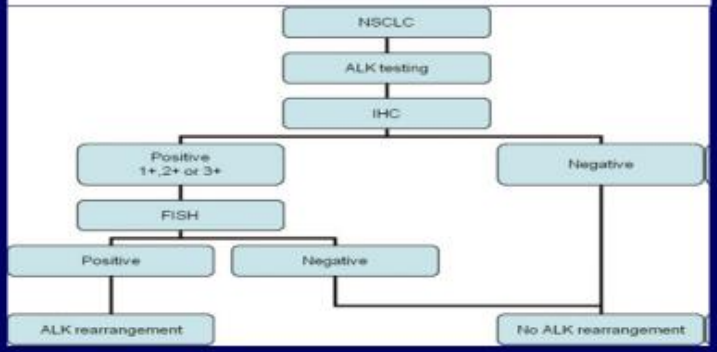
Journal of Thoracic Oncology • Volume 6, Number 3, March 2011

Virchows Arch
DOI 10.1007/s00428-012-1281-4

REVIEW AND PERSPECTIVES

EML4-ALK testing in non-small cell carcinomas of the lung: a review with recommendations

Erik Thunnissen · Lukas Bubendorf · Manfred Dietel · Göran Elmberger · Keith Kerr · Fernando Lopez-Rios · Holger Moch · Wlodzimierz Olszewski · Patrick Pauwels · Frédérique Penault-Llorca · Giulio Rossi



NOT ALL “DRIVERS” ARE MUTATIONS/ FUSIONS

Detailed genomic analysis of squamous cell lung cancers has identified several new potential therapeutic targets

Gene	Event Type	Frequency
FGFR1	Amplification	20-25%
FGFR2	Mutation	5%
PIK3CA	Mutation	9%
PTEN	Mutation/Deletion	18%
CCND1	Amplification	8%
CDKN2A	Deletion/Mutation	45%
PDGFRA	Amplification/Mutation	9%
EGFR	Amplification	10%
MCL1	Amplification	10%
BRAF	Mutation	3%
DDR2	Mutation	4%
ERBB2	Amplification	2%

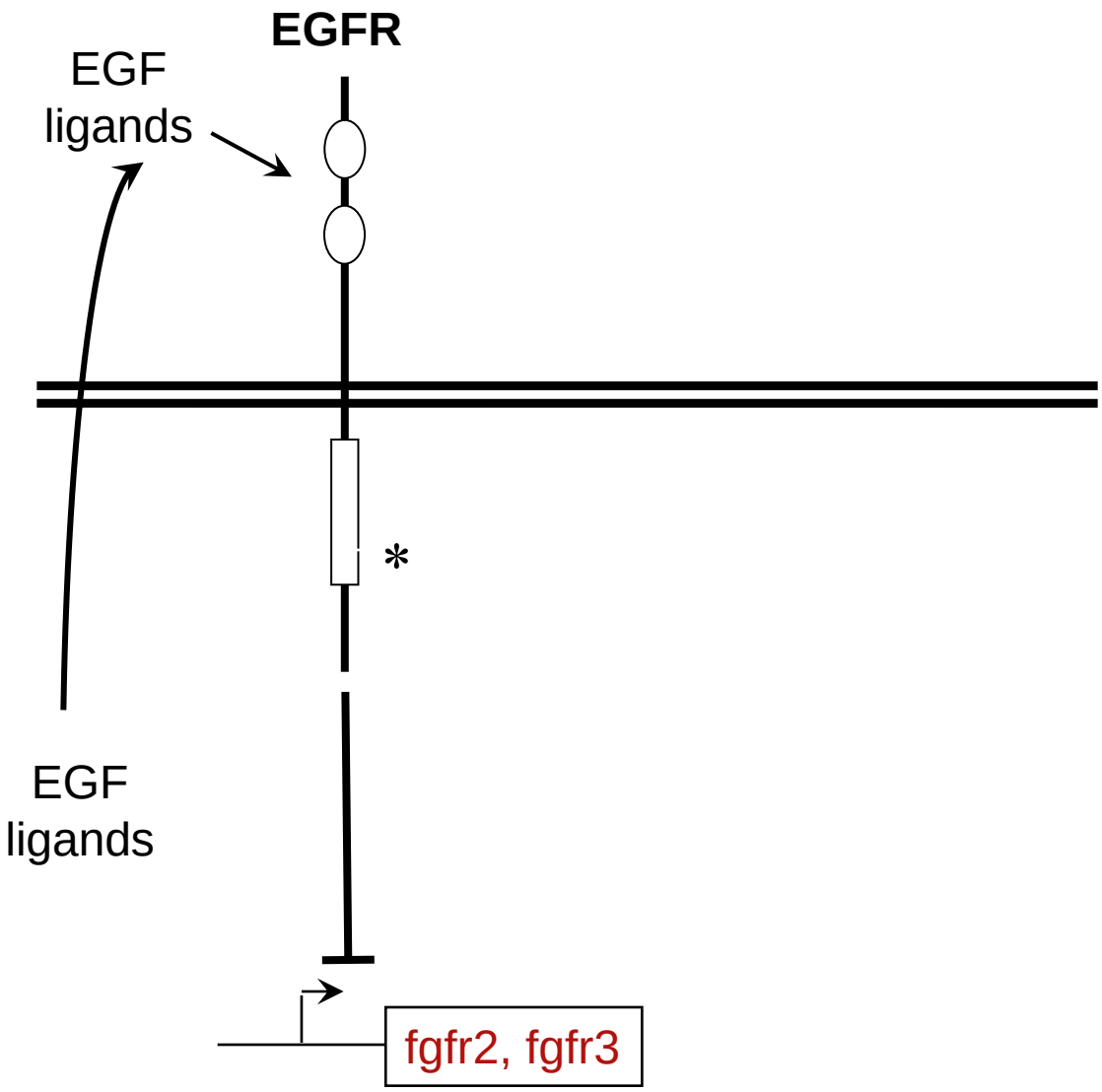
In 63% of lung SCCs we can now identify a possible therapeutic target

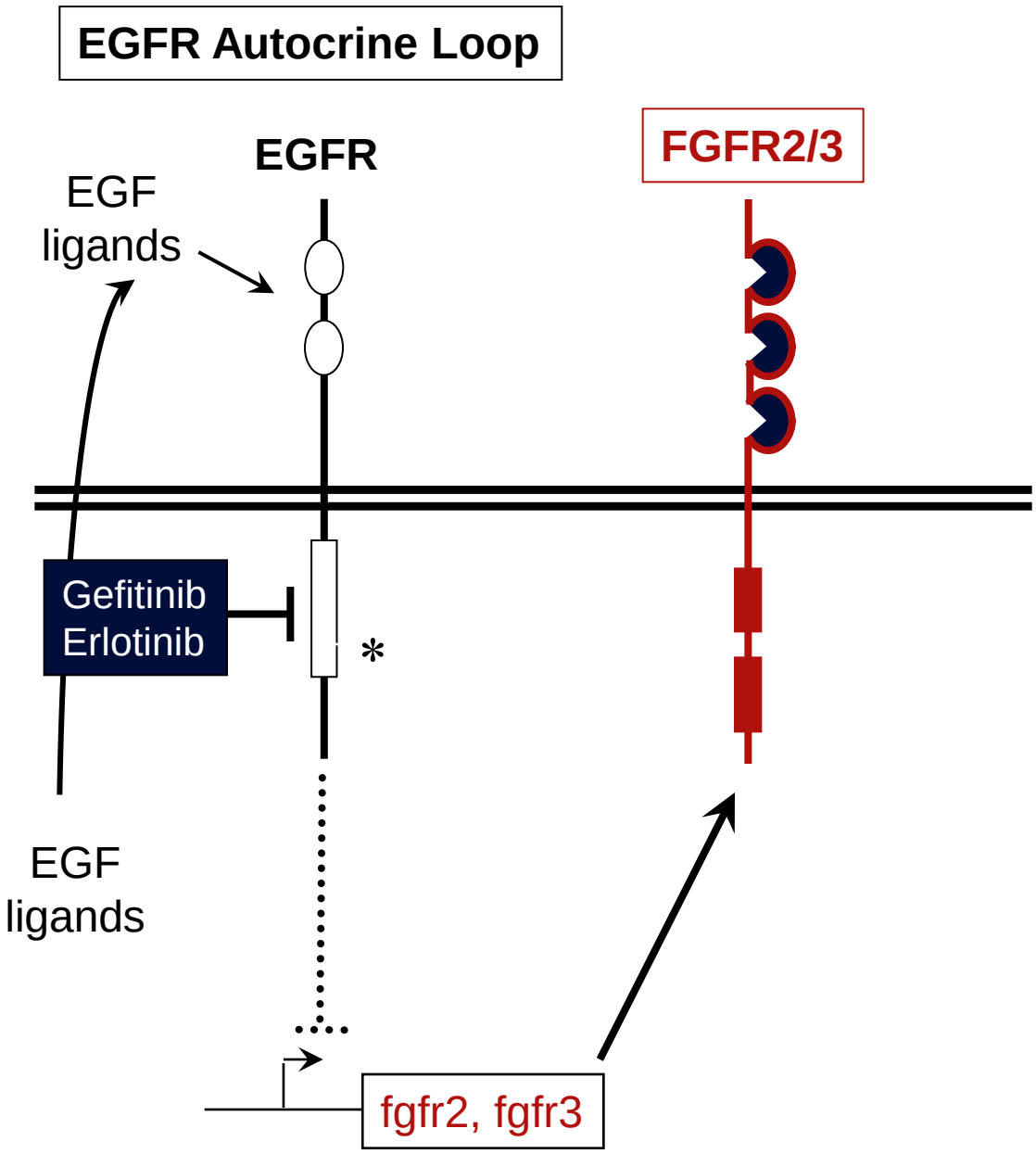
Targets need to be validated in pre-clinical models

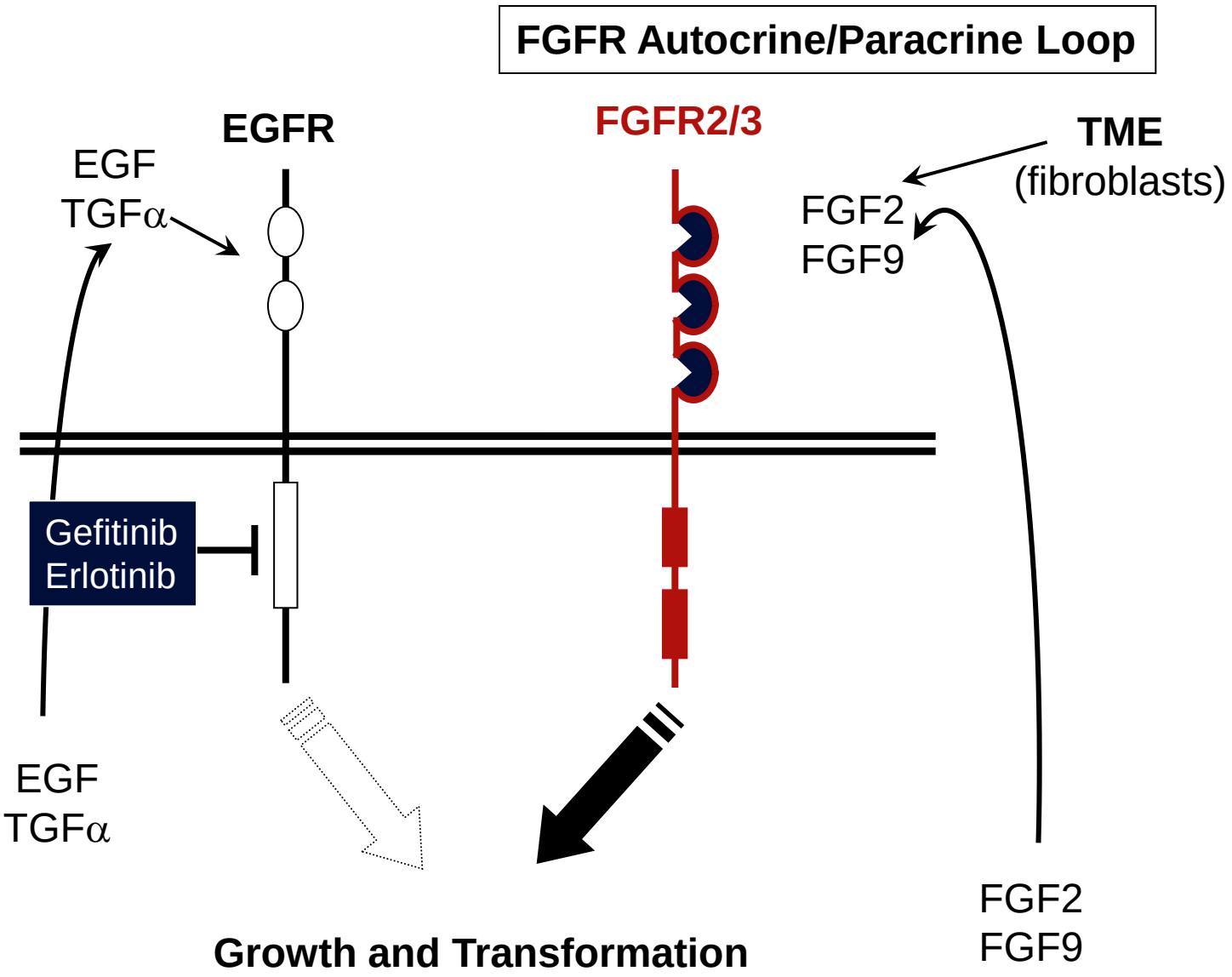
FGFR1/2, PIK3CA and DDR2 inhibitor trials are planned or ongoing

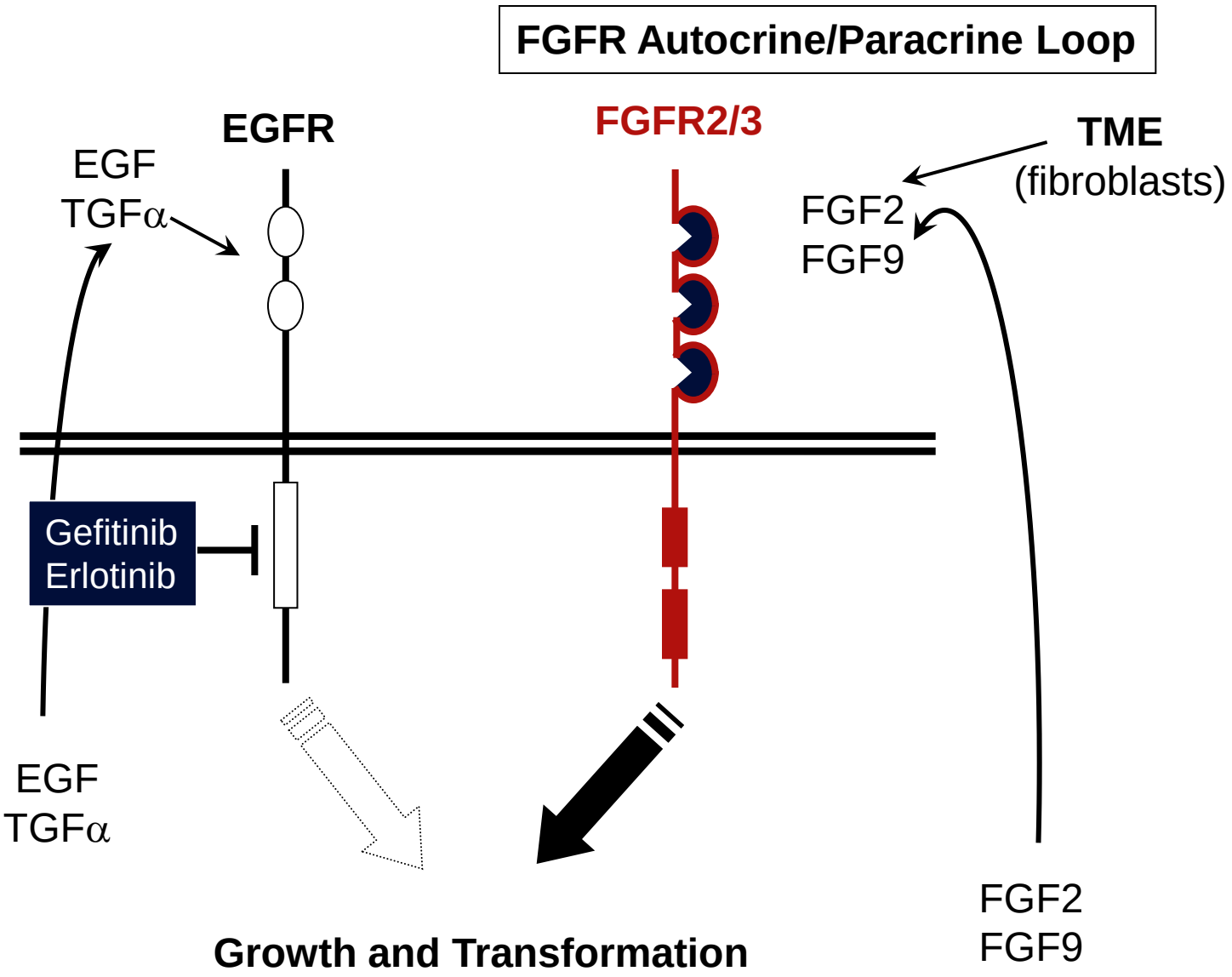
Peter Hammerman et al. WCLC 2011

EGFR Autocrine Loop




EGFR Autocrine Loop
EGFR
FGFR2/3
EGF
ligands
Gefitinib
Erlotinib
*
EGF
ligands
fgfr2, fgfr3


FGFR Autocrine/Paracrine Loop
EGFR
FGFR2/3
TME
(fibroblasts)
EGF
TGFα
FGF2
FGF9
Gefitinib
Erlotinib
EGF
TGFα
FGF2
FGF9
Growth and Transformation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FGF2
FGF9
Growth and Transformation

PD-L1 Status and Predictive Biomarkers in NSCLC Patients Treated With MPDL3280A: Efficacy

Elevated baseline PD-L1 expression is associated with response to MPDL3280A

PD-L1 Status	N = 53	
	ORR[a]	PD Rate[b]
IHC 3 (n = 6)	83% (5/6)	17% (1/6)
IHC 2 and 3 (n = 13)	46% (6/13)	23% (3/13)
IHC 1/2/3 (n = 26)	31% (8/26)	38% (10/26)
All patients [c] (N = 53)	23% (12/53)	40% (21/53)

[a] ORR includes investigator-assessed unconfirmed and confirmed PR by RECIST v1.1.
[b] PD rate indicates patient with best response with progressive disease.
[c] Includes patients with IHC 0/1/2/3 and 7 patients with unknown diagnosis.

Gettinger et al. IASLC WLCL 2013. Abstract 1653, Presentation MO19.09.

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

Pathology Consideration for Good Practice

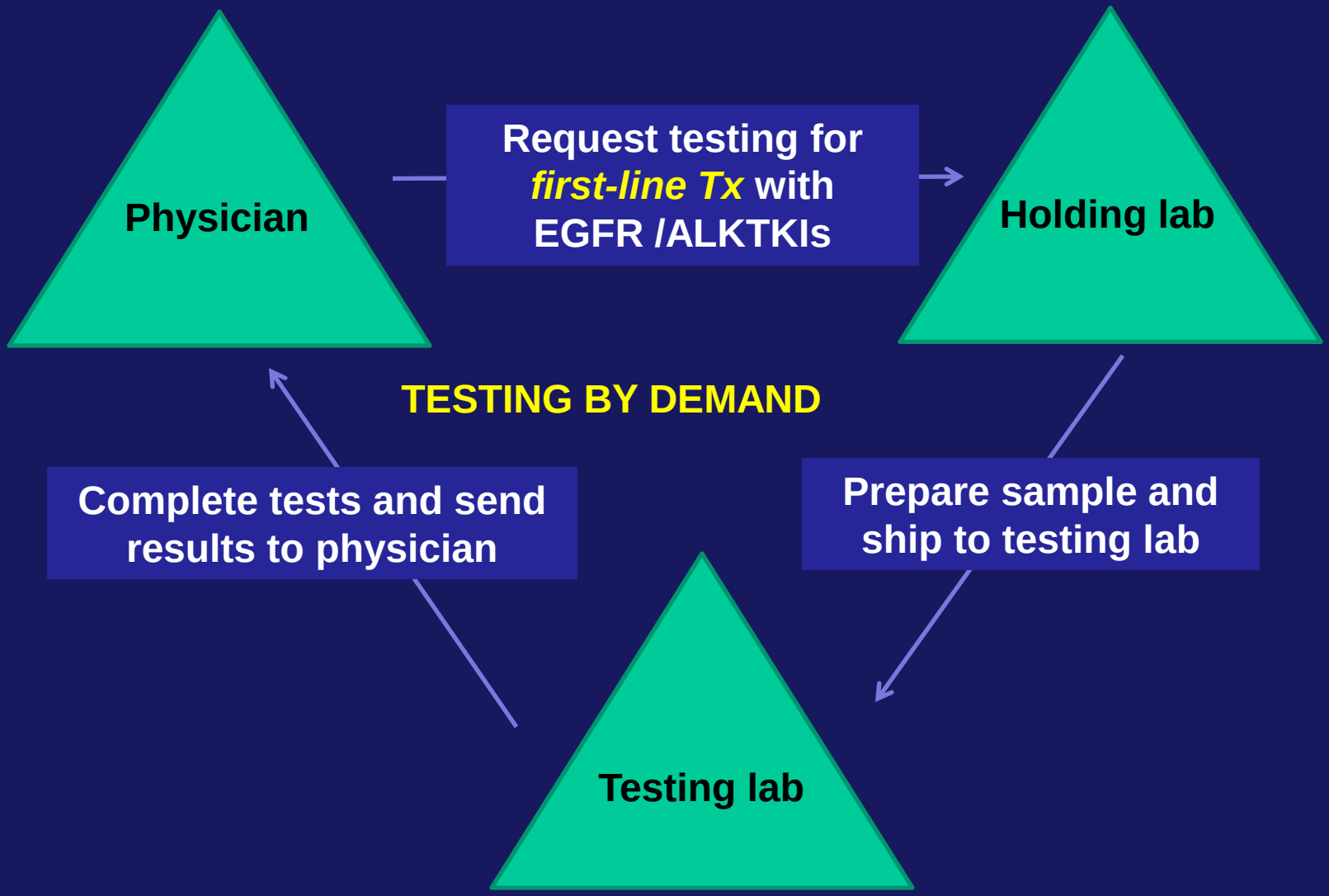
2. Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.
3. To guide therapy for patients with advanced lung adenocarcinoma, each institution should develop a multidisciplinary team that coordinates the optimal approach to 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

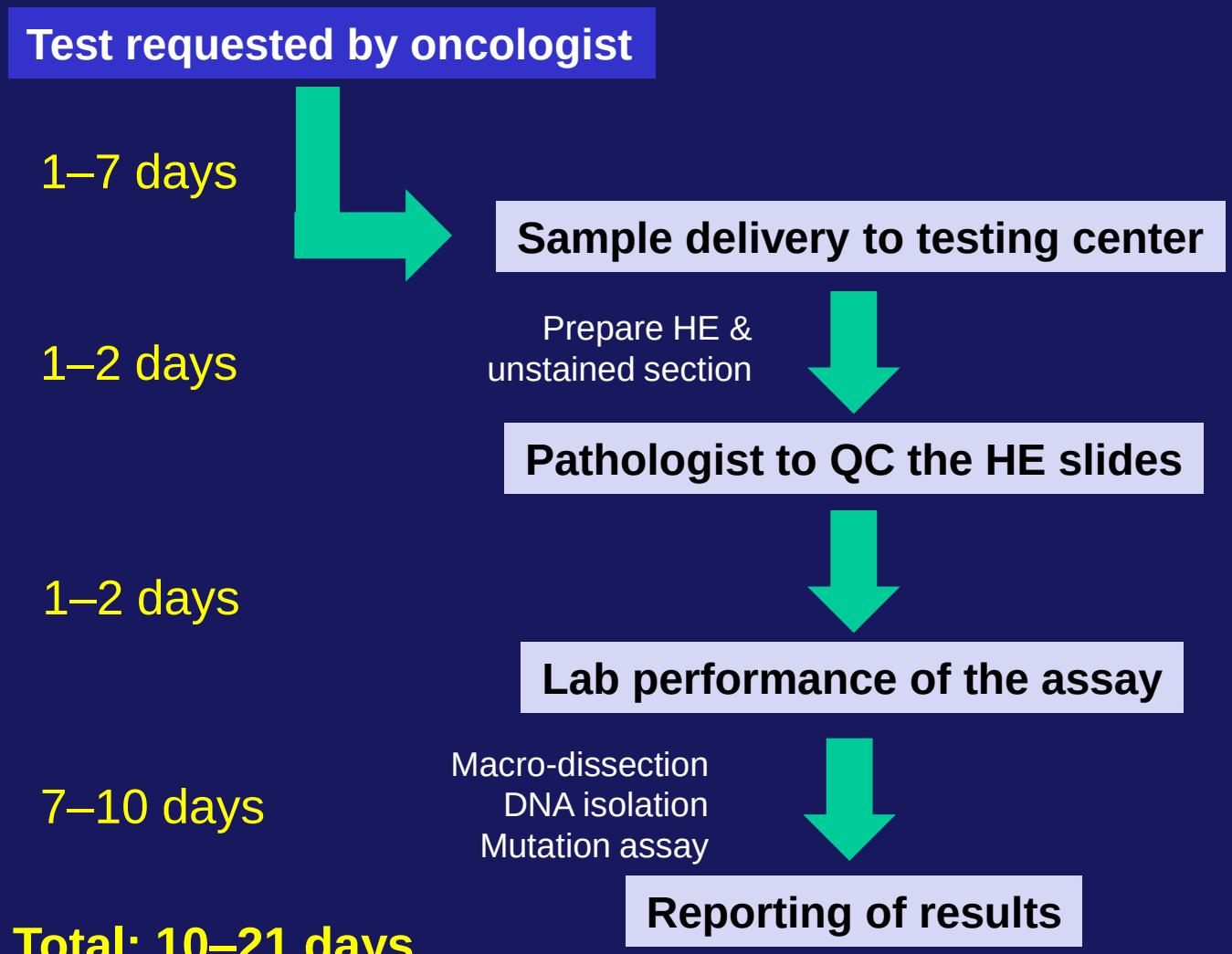
When should testing be ordered?



Tx, treatment

Courtesy Dr. Tsao

Turn Around Time for EGFR Testing



Total: 10–21 days (average 14 days)

QC, quality check

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

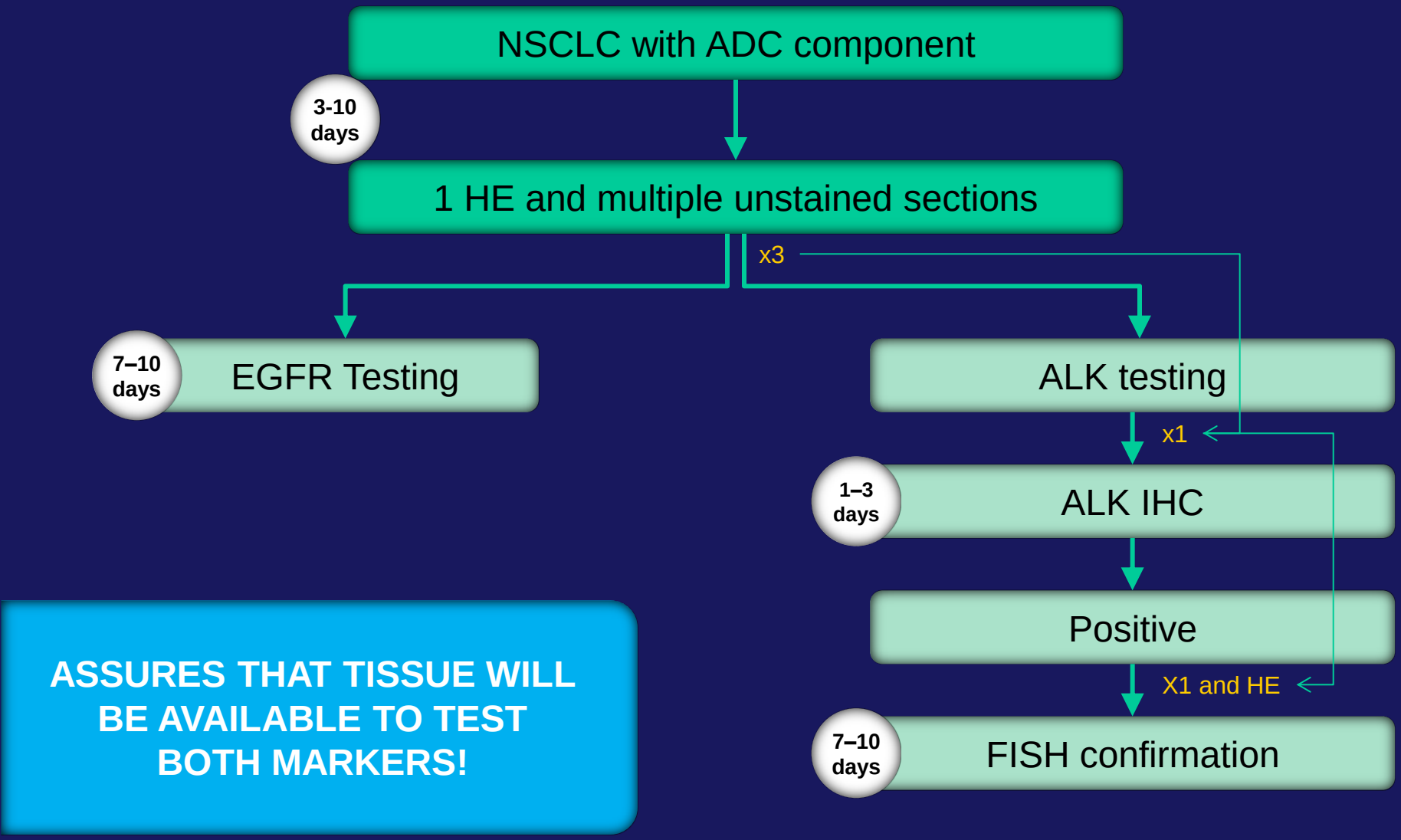
Reporting of results

Total: 10–21 days (average 14 days)

Reflex testing by pathologist at time of diagnosis

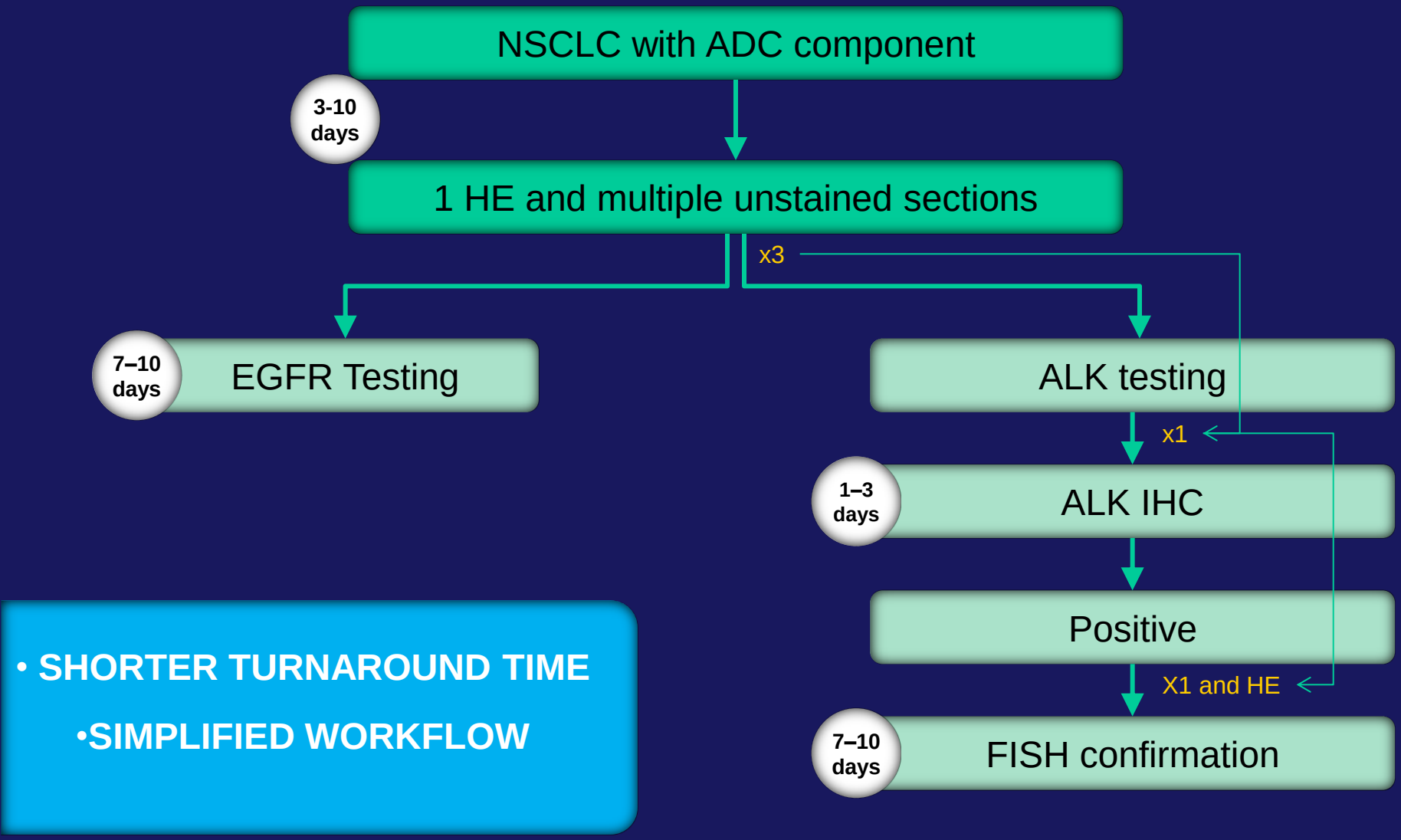
Result available at consultation

Optimal Sample Preparation for EGFR and ALK Testing



ASSURES THAT TISSUE WILL BE AVAILABLE TO TEST BOTH MARKERS!

Parallel EGFR and ALK Testing (OPTIMAL)



- **SHORTER TURNAROUND TIME**
- **SIMPLIFIED WORKFLOW**

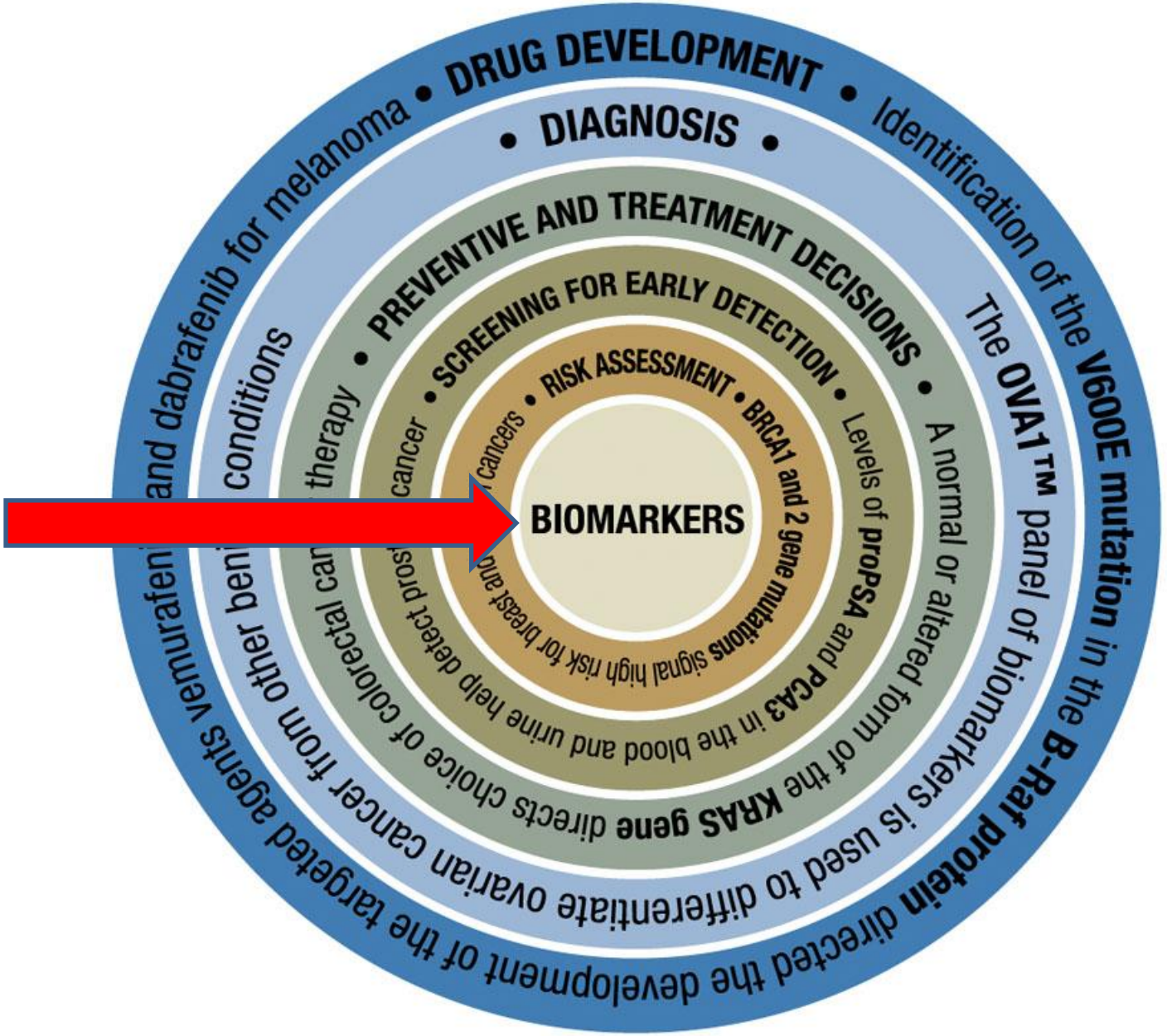
Conclusions

1. Presently only EGFR and ALK testing have utility in treatment decision making in non-squamous NSCLC

2. Individual centers should develop a multidisciplinary approach to implement molecular testing algorithms

3. Reflex testing at diagnosis is the ideal approach for routine molecular pathology practice

4. Next generation sequencing can resolve much of the complexity of molecular testing



AACR : CANCER REPORT 2013

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