Oncogene Addiction & Biomarkers: Who Should Be Tested and When ?

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HARVARD MEDICAL SCHOOL

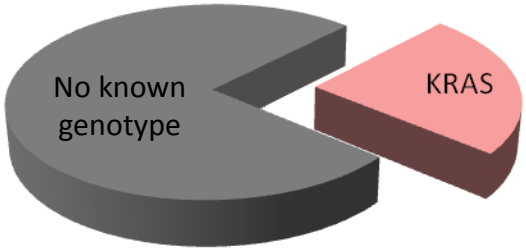
Conflicts of Interest

Consultant for: Astra Zeneca, Boehringer Ingelheim, Pfizer, Genentech, Roche, Sanofi-Aventis, Clovis Oncology, Chugai Pharmaceuticals, Merrimack Pharmaceuticals

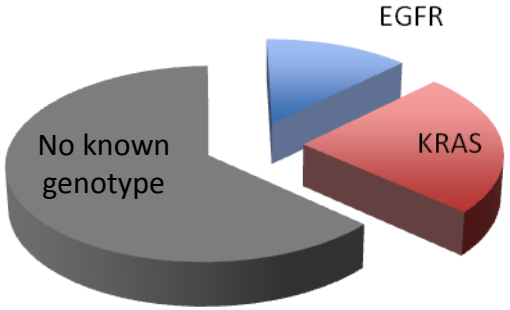
Stockholder in: Gatekeeper Pharmaceuticals

Other: LabCorp – post-marketing royalties from DFCI owned intellectual property on EGFR mutations

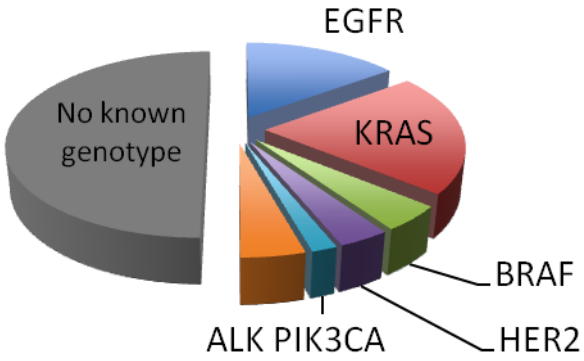
Evolution of Identification of Genomic Alterations in Lung Adenocarcinoma



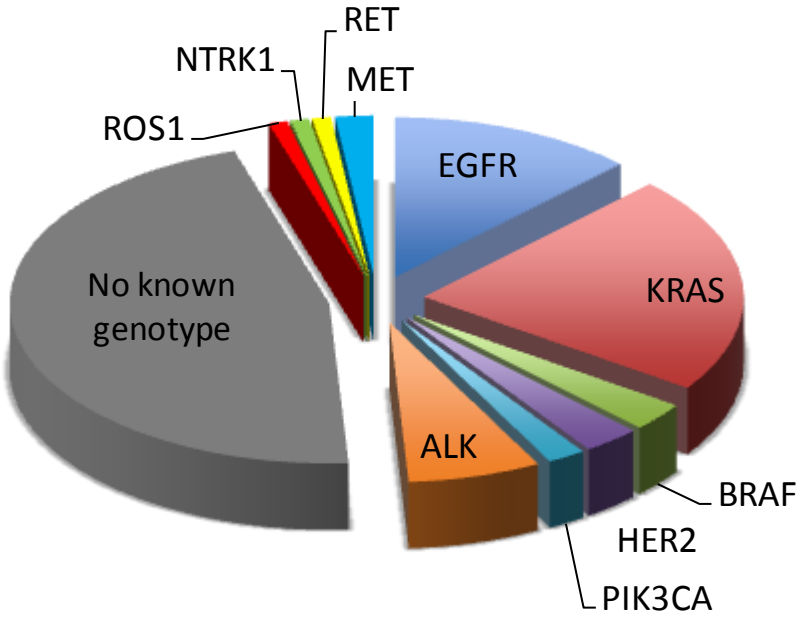
1984 - 2003



2004



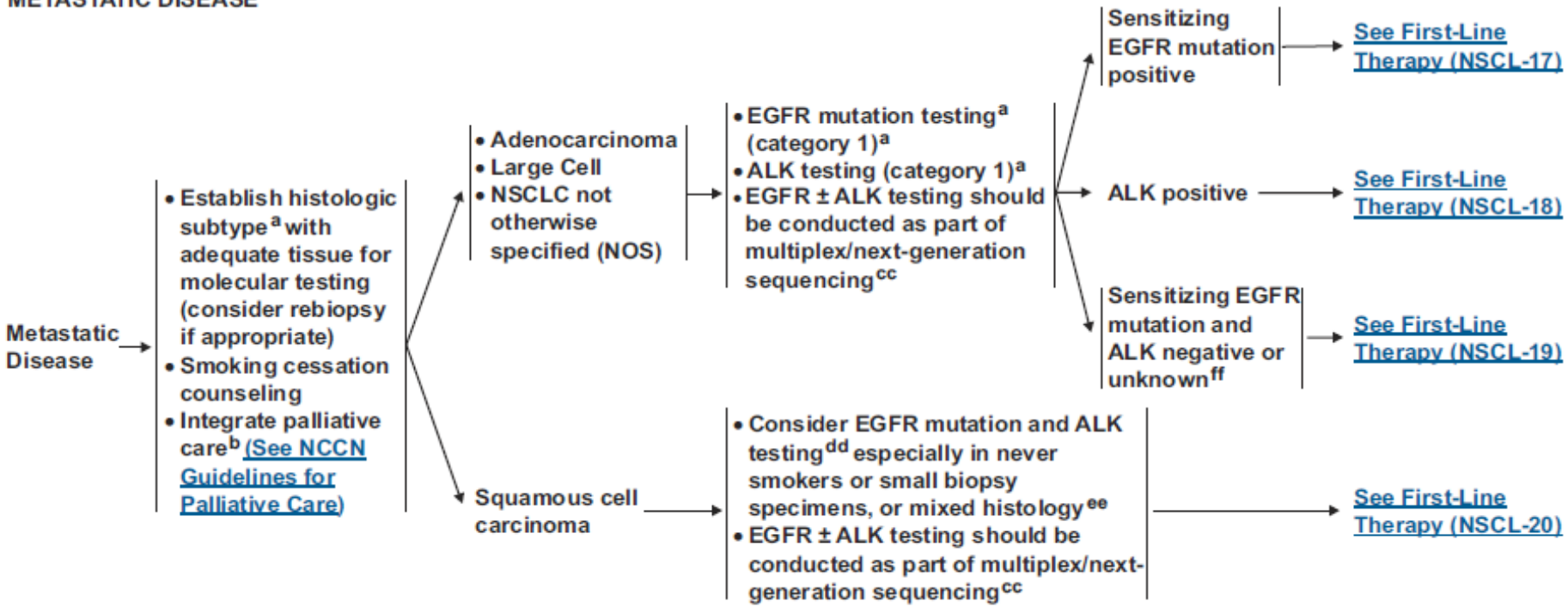
2009



2014

SYSTEMIC THERAPY FOR METASTATIC DISEASE

HISTOLOGIC SUBTYPE



[a] See Principles of Pathologic Review (NSCL-A).

[b] Temel JS, Greer JA, Muzikansky A, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. N Engl J Med 2010;363:733-742.

[cc] See Targeted Agents for Patients with Other Genetic Alterations (NSCL-H).

[dd] In patients with squamous cell carcinoma, the observed incidence of EGFR mutations is 2.7% with a confidence that the true incidence of mutations is less than 3.6%. This frequency of EGFR mutations does not justify routine testing of all tumor specimens. Forbes SA, Bharma G, Bamford S, et al. The catalogue of somatic mutations in cancer (COSMIS). Curr Protoc Hum Genet 2008;chapter 10:unit 10.11.

[ee] Paik PK, Varghese AM, Sima CS, et al. Response to erlotinib in patients with EGFR mutant advanced non-small cell lung cancers with a squamous or squamous-like component. Mol Cancer Ther Published on-line August 14, 2012.

[ff] Consider ROS1 testing; if positive, may treat with crizotinib. Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-870.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

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

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"The major recommendations are to use testing for EGFR mutations and ALK fusions to guide patient selection for therapy with an epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) inhibitor, respectively, in all patients with advanced-stage adenocarcinoma, regardless of sex, race, smoking history, or other clinical risk factors, and to prioritize EGFR and ALK testing over other molecular predictive tests."

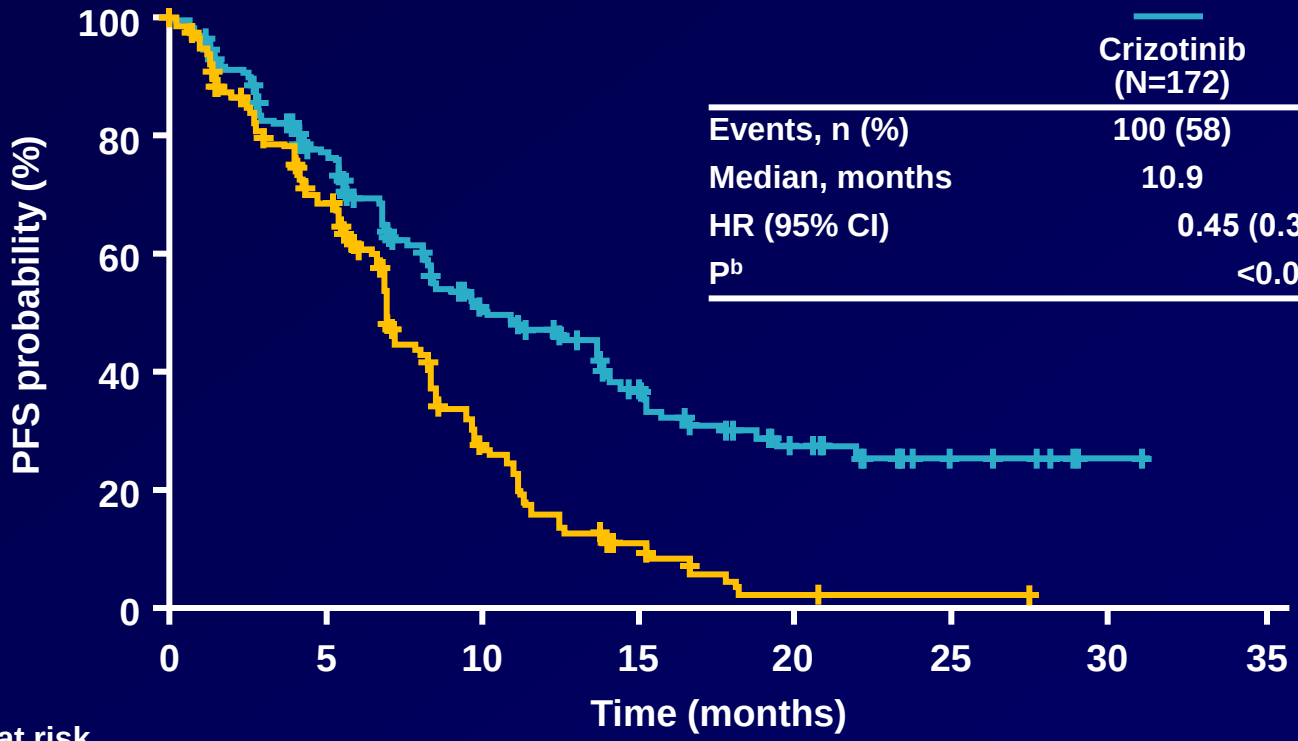
J Mol Diagn. 2013 Jul;15(4):415-53.

EGFR-TKI as standard 1st-line therapy for patients with *EGFR* mutations

Study	Drugs	N (*EGFR* mutation)	RR	Median PFS (months)
IPASS	**Gefitinib** vs carboplatin/paclitaxel	261	**71.2%** vs 47.3%	**9.5** vs 6.3
WJTOG 3405	**Gefitinib** vs cisplatin/docetaxel	172	**62.1%** vs 32.2%	**9.2** vs 6.3
NEJGSG002	**Gefitinib** vs carboplatin/paclitaxel	224	**73.7%** vs 30.7%	**10.8** vs 5.4
EURTAC	**Erlotinib** vs cisplatin/docetaxel	173	**58.1%** vs 14.9%	**9.7** vs 5.2
OPTIMAL	**Erlotinib** vs gemcitabine/carboplatin	154	**83.0%** vs 36.0%	**13.7** vs 4.6
LUX-Lung 3	**Afatinib** vs cisplatin/pemetrexed	345	**56.0%** vs 23.0%	**11.1** vs 6.9
LUX-Lung 6	**Afatinib** vs gemcitabine/cisplatin	364	**66.9%** vs 23.0%	**11.0** vs 5.6

Gefitinib EU Summary of Product Characteristics;
Mitsudomi et al. Lancet Oncol 2010;11:121–1128; Maemondo et al. N Engl J Med 2010;362:2380–2388;
Rosell et al. Lancet Oncol 2012;13:239–246; Zhou et al. J Clin Oncol 2012;30: Abs 7520;
Sequist et al. J Clin Oncol 2013;31:3327–3334; Wu et al. Lancet Oncol 2014;15:213–222

Crizotinib Superior to Pemetrexed-based Chemotherapy in Prolonging PFS



	Crizotinib (N=172)	Chemotherapy (N=172)
Events, n (%)	100 (58)	137 (80)
Median, months	10.9	7.0
HR (95% CI)	0.45 (0.35–0.60)	
P[b]	<0.0001	

No. at risk								
Crizotinib	172	120	65	38	19	7	1	0
Chemotherapy	171	105	36	12	2	1	0	0

Data cutoff: November 30, 2013
[a]Assessed by IRR
[b]1-sided stratified log-rank test

Mok et al. ASCO 2014

Factors Impacting choice of genomic tests

- Availability of test ?
- How quickly can you get a result ?
- Do you need to test for more than one genomic alteration ?
- Cost

Different Ways of Detecting Mutations

- **Allelotyping**
  - Assay to detect a specific predefined mutation
- **Sequencing**
  - Assay to analyze DNA changes including mutations in specific regions of a gene

Different Ways of Detecting Mutations

- **Allelotyping**
  - Assay to detect a specific predefined mutation
  - Advantage: Fast, sensitive
  - Disadvantage: Not comprehensive

- **Sequencing**
  - Assay to analyze DNA changes including mutations in specific regions of a gene
  - Advantage: Comprehensive
  - Disadvantage: lower sensitivity, slow

FDA Approved Allelotyping Tests for detection of EGFR Mutations

- Approved for selection of patients for first line erlotinib therapy
- Detects 41 EGFR mutations in exons 18 - 21

- Approved for selection of patients for first line afatinib therapy
- Detects 29 EGFR mutations in exons 18 - 21

Comparison of EGFR Allelotyping Tests

Cobas EGFR Test

Exon	Mutation Detected
18	G719 A/C/S
19	29 different deletions
20	T790M
	S768I
	5 different insertions
21	L858R

Therascreen EGFR Test

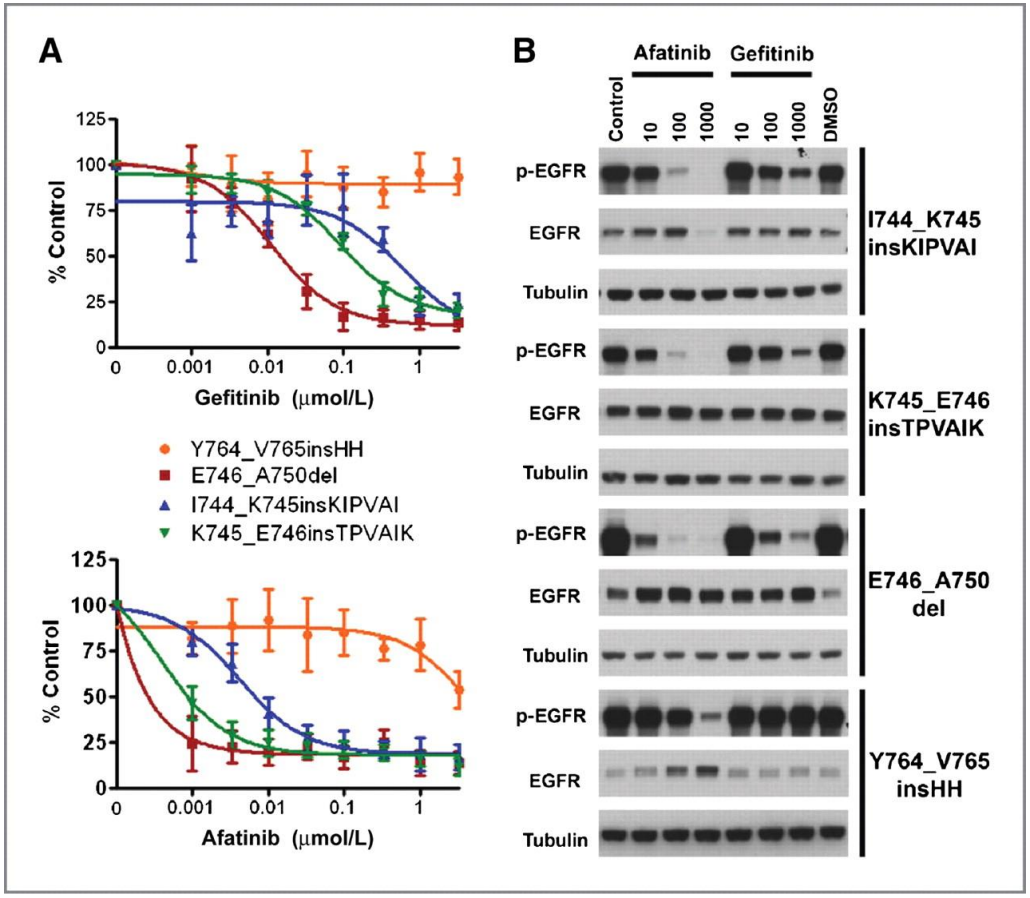
Exon	Mutation Detected
18	G719 A/C/S
19	19 different deletions
20	T790M
	S768I
	3 different insertions
21	L858R
	L861Q

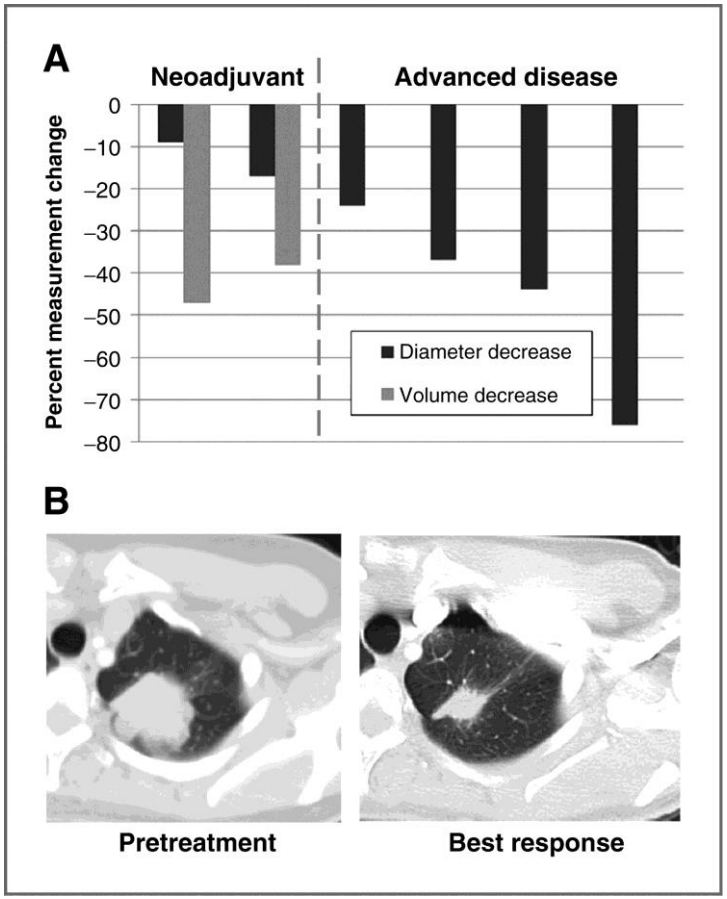
EGFR L861Q and response to EGFR TKIs

Author	#	EGFR	Drug	Response
Maheswaran	1	L861Q	G or E	SD:1
Sequist	1	L861Q	G	SD:1
Costa	1	L861Q	G	SD:1
Rizvi	1	L861Q	G	SD:1
De Pas	1	L861Q	E	PD:1
Wu	6	L861Q	G	PR:4 SD:1 PD:1
Ong	1	L861Q	E	PR:1
Maemondo	3	L861Q	G	PR:1 SD:1 PD:1

PR: 6/15 (40%); SD 6/15 (40%); PD 3/15 (20%)

Exon 19 Insertions are a rare EGFR TKI sensitive mutation

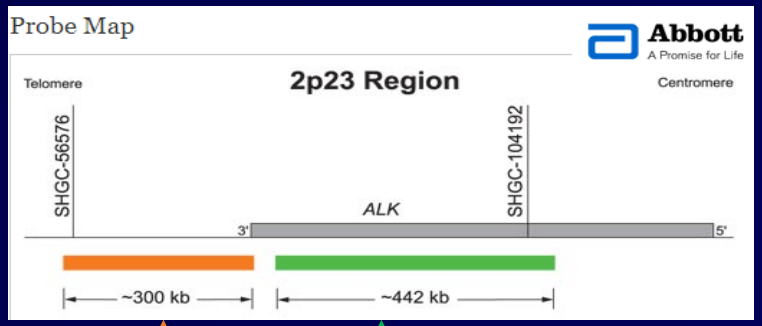




He M et al. Clin Cancer Res 2012;18:1790-1797

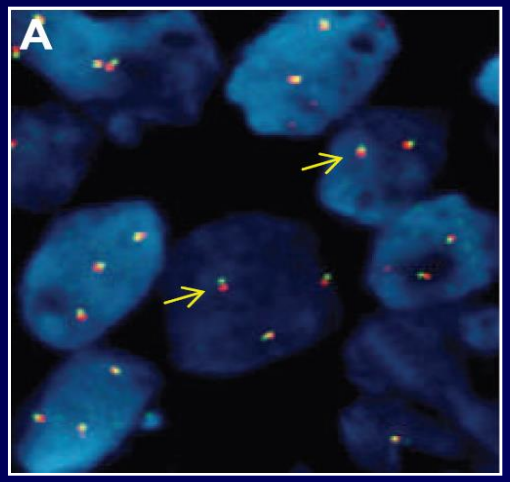
FISH detects the *ALK* gene rearrangement

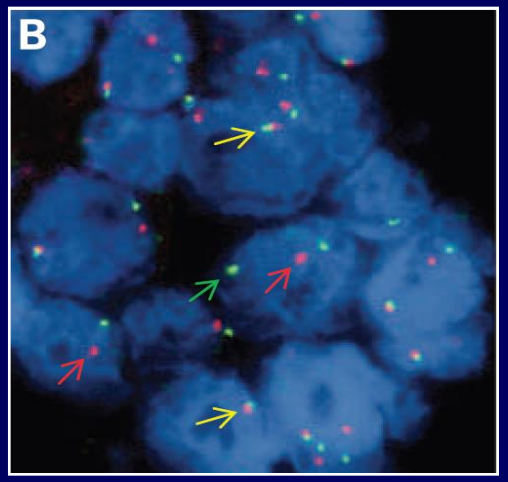
Vysis ALK Break-Apart FISH Probe Kit

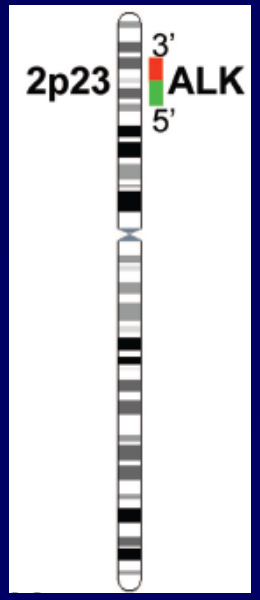


Spectrum orange probe telomeric of break site

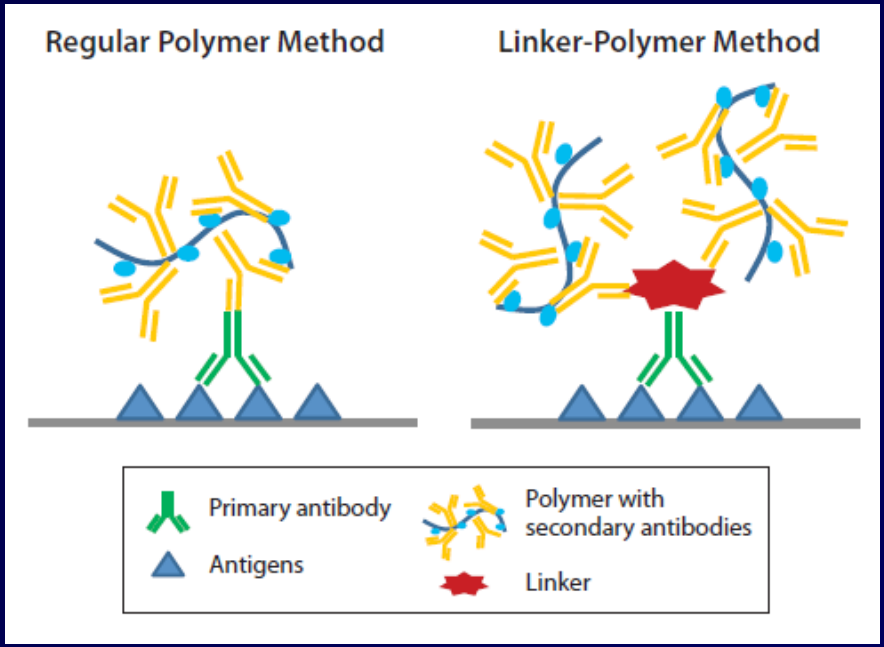
Spectrum green probe centromeric of break site

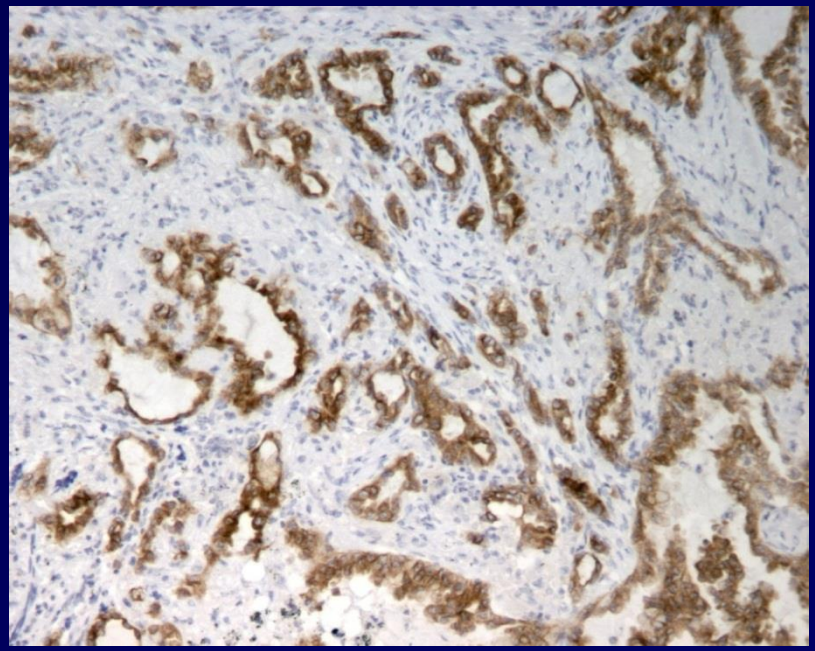






Immunohistochemistry detects ALK protein





Routinely used in pathology practice, fast and cost-effective

Identifies protein expression, not the gene rearrangement

IASLC ATLAS of ALK Testing in Lung Cancer, Chapter 4, 2013

Commercially available anti-ALK antibodies

Antibody Clone	Antibody Type	Commercial Vendor	Clinical Assay
ALK1	Mouse monoclonal	Dako	LDT
5A4	Mouse monoclonal	Novocastra/ Abcam	LDT
D5F3	Rabbit monoclonal	Cell Signaling	Ventana Kit LDT
ZAL4	Rabbit polyclonal	Invitrogen	LDT

IASLC ATLAS of ALK Testing in Lung Cancer, Chapter 4, 2013

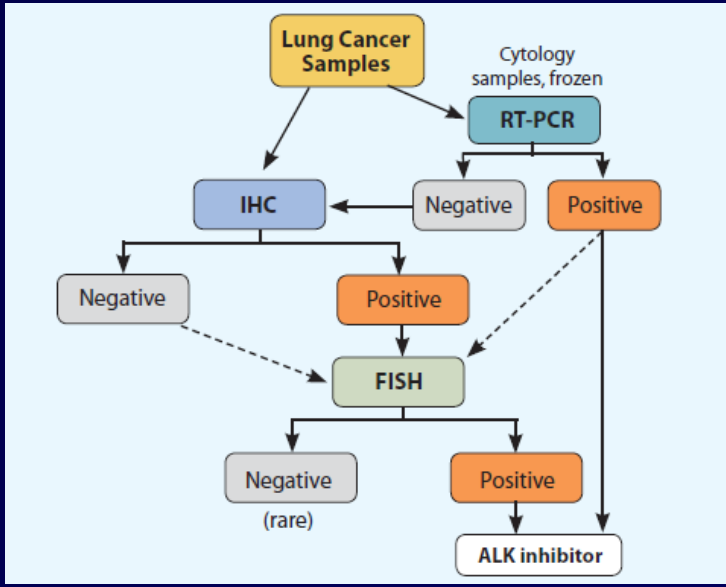
Reported sensitivity and specificity of ALK IHC compared with FISH

Antibody	Sensitivity	Specificity	No. of Studies
ALK 1 (with amplification)	67–100%	98–100%	6
5A4	93–100%	96–100%	10
D5F3	83–100%	95–100%	6
ZAL4	100%	90%	2

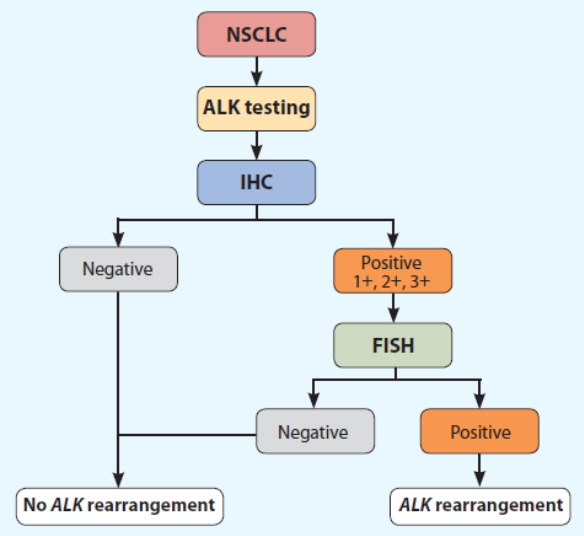
IASLC ATLAS of ALK Testing in Lung Cancer, Chapter 4, 2013

Example algorithms for ALK testing

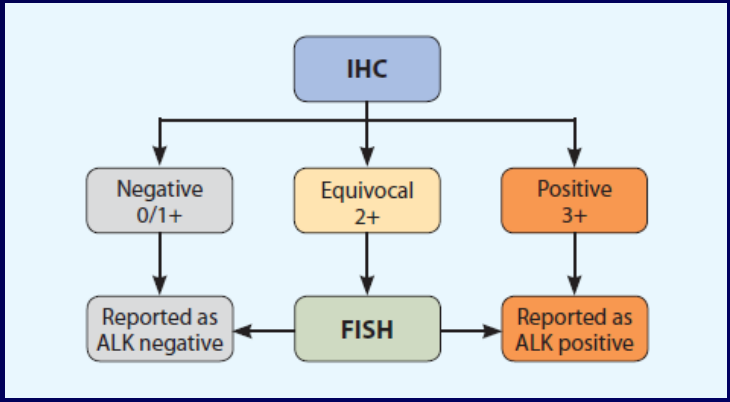
Japan



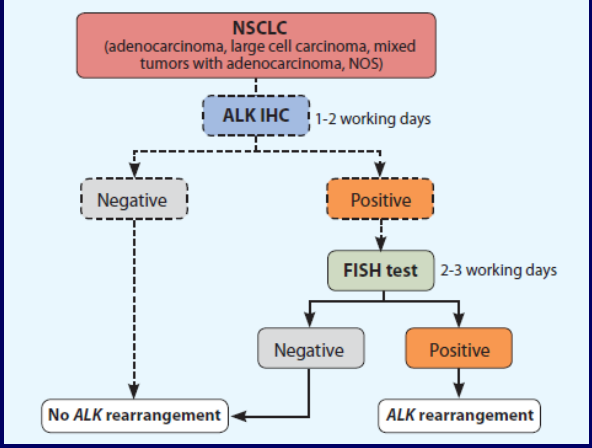
Europe



Korea



Italy



IASLC ATLAS of ALK Testing in Lung Cancer, Chapter 6, 2013

Genomic Alterations and Methods of testing

Gene	Alteration	Method	Lab
EGFR	Mutation	Sequencing	Molecular Pathology
KRAS	Mutation	Sequencing	Molecular Pathology
ALK	Rearrangement	FISH	Cytogenetics
FGFR1	Amplification	FISH	Cytogenetics

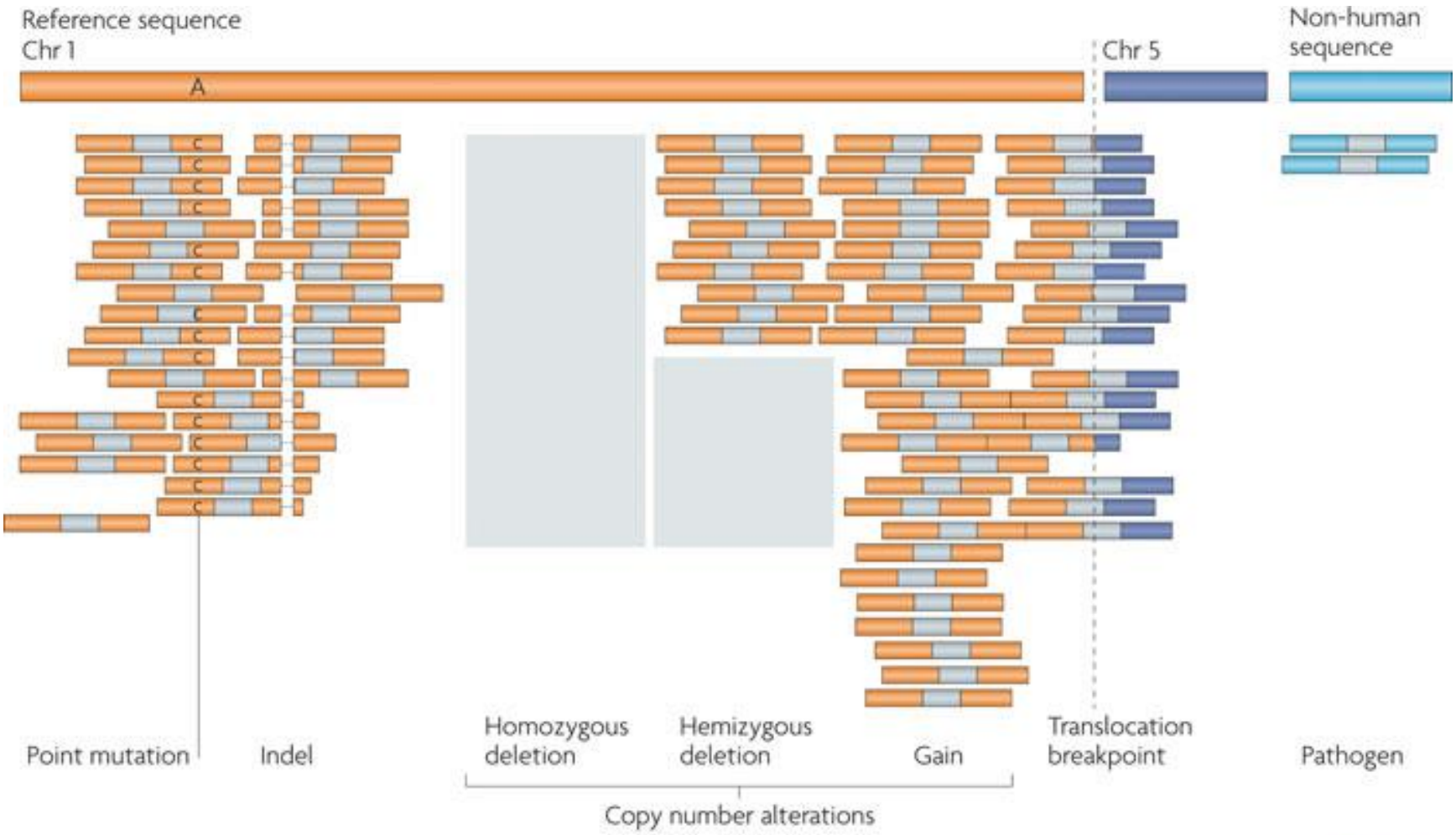
Genotyping can be multiplexed

FISH is difficult to multiplex

Need separate assays for
ALK, ROS, RET, MET, ERBB2, FGFR1
6 X $800 = $4800

Most NSCLC biopsies not amenable to 6 FISH tests & sequencing

Massively Parallel Sequencing



Meyerson M, Gabriel S, and Getz G, *Nature Reviews Genetics*, 2010

Comparison on Next Generation Sequencing Technologies

Technology	Percent of Genome Sequenced	Cost	Depth of Coverage
Whole Genome Sequencing	100%		
Whole Exome Sequencing	1% (25,000 genes)		
Targeted Sequencing	0.005% - 0.1% (100s – 1000s of genes)		

Nick Wagle

Sequencing-based Approaches to Profiling

	Targeted Sequencing	Whole Exome Sequencing	Combination of Both
What is tested?	Predefined set of exons, non-coding regions, selected translocations	All exons	All exons, plus predefined set of exons, non-coding regions, selected translocations
Starting Material	FFPE Slides	FFPE Slides	FFPE Slides
Cost			
Coverage	500-1000x	100-200x	100-200x exome 500-1000x targeted
Matched Normal?	*No**	Yes	Yes
Analytic / Interpretive Complexity			
Advantages	Lowest Cost High Sensitivity Translocations	All genes Research / Discovery	All genes High Sensitivity Translocations Research / Discovery
Disadvantages	Expandability Discovery	Sensitivity No Translocations	Cost

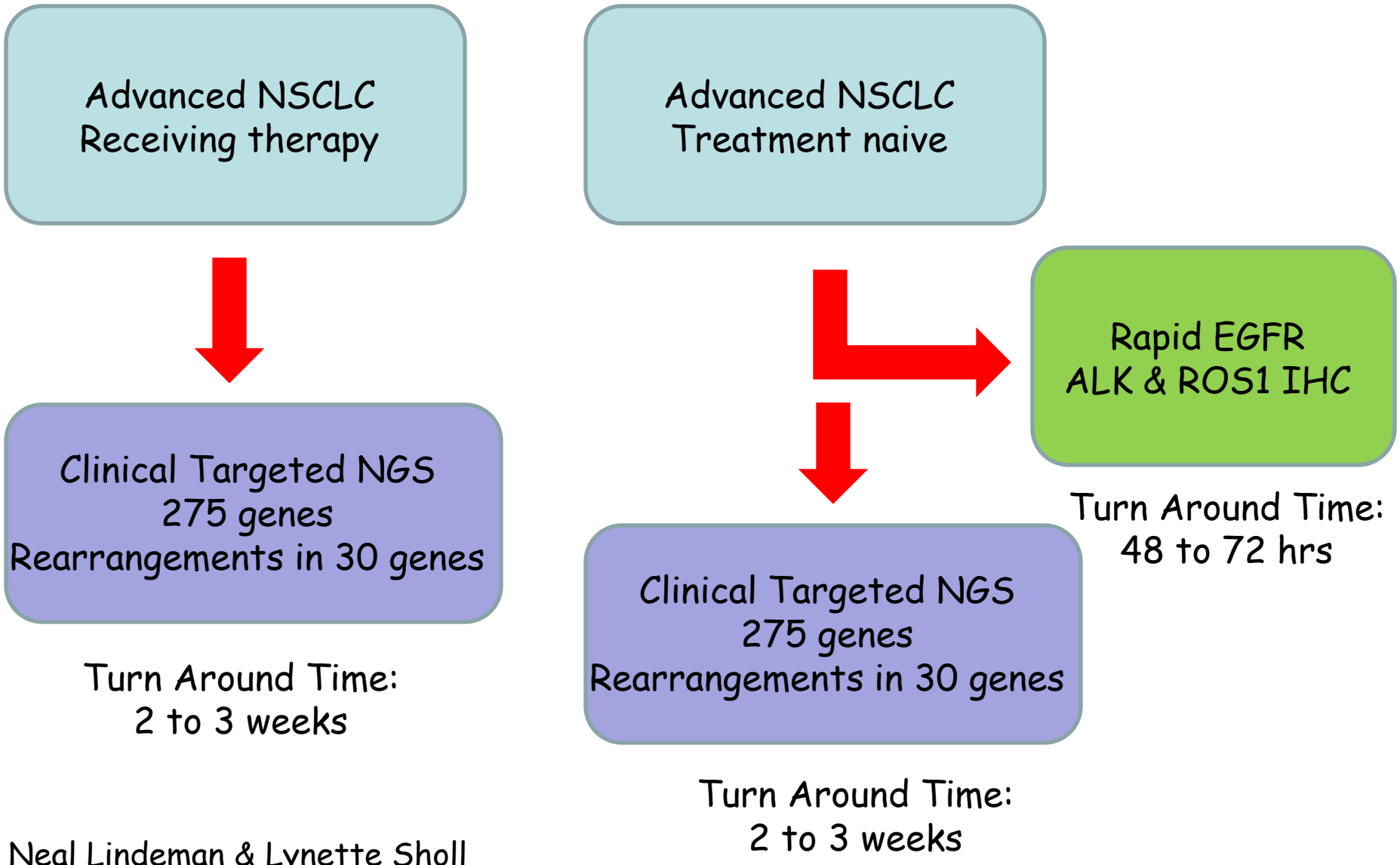
Oncopanel – DFCI/BWH Targeted Next Generation Sequencing – Launched summer 2013

ABL1	BUB1B	CTNNB1	EZH2	GPC3	MLH1	PDGFRA	REL	STK11
AKT1	CARD11	CUX1	FAM46C	GSTM5	MLL	PDGFRB	RET	SUFU
AKT2	CBL	CYLD	FANCA	H3F3A	MLL2	PHF6	RFWD2	SUZ12
AKT3	CBLB	DDB2	FANCC	HNF1A	MPL	PHOX2B	RHPN2	SYK
ALK	CCND1	DDR2	FANCD2	HRAS	MSH2	PIK3C2B	ROS1	TCF3
ALOX12B	CCND2	DICER1	FANCE	ID3	MSH6	PIK3CA	RPL26	TERC
APC	CCND3	DIS3	FANCF	IDH1	MTOR	PIK3R1	RUNX1	TERT
AR	CCNE1	DMD	FANCG	IDH2	MUTYH	PIM1	SBDS	TET2
ARAF	CD274	DNMT3A	FAS	IGF1R	MYB	PMS1	SDHAF2	TNFAIP3
ARID1A	CD58	EED	FBXW7	IGH@	MYBL1	PMS2	SDHB	TP53
ASXL1	CD79B	EGFR	FGFR1	IgK@	MYC	PNRC1	SDHC	TRA@
ATM	CDC73	EP300	FGFR2	IgL@	MYCL1	PRAME	SDHD	TRB@
ATRX	CDH1	EPHA3	FGFR3	IKZF1	MYCN	PRDM1	SETBP1	TRG@
AURKA	CDK1	EPHA5	FGFR4	IKZF3	MYD88	PRF1	SF1	TSC1
AURKB	CDK2	EPHA7	FH	JAK2	NBN	PRKAR1A	SF3B1	TSC2
AXL	CDK4	ERBB2	FKBP9	JAK3	NF1	PRKCI	SH2B3	U2AF1
B2M	CDK5	ERBB3	FLCN	KDM6B	NF2	PRKDC	SMAD2	VHL
BAP1	CDK6	ERBB4	FLT1	KDR	NFE2L2	PRPF40B	SMAD4	WRN
BCL2	CDK9	ERCC2	FLT3	KIT	NFKBIA	PRPF8	SMARCA4	WT1
BCL2L12	CDKN2A	ERCC3	FLT4	KRAS	NFKBIZ	PSMD13	SMARCB1	XPA
BCL6	CDKN2B	ERCC4	FUS	LMO1	NKX2-1	PTCH1	SMC1A	XPC
BCOR	CDKN2C	ERCC5	GATA3	MAP2K1	NOTCH1	PTEN	SMC3	XPO1
BCORL1	CEBPA	ESR1	GATA4	MCL1	NOTCH2	PTK2	SMO	ZNF708
BLM	CHEK2	ETV1	GATA6	MDM2	NPM1	PTPN11	SOCS1	ZRSR2
BMPR1A	CIITA	ETV4	GLI1	MDM4	NRAS	RAD21	SOX2	
BRAF	CREBBP	ETV5	GLI2	MECOM	NTRK3	RAF1	SRC	
BRCA1	CRKL	ETV6	GLI3	MEF2B	PALB2	RARA	SRSF2	
BRCA2	CRLF2	EWSR1	GNA11	MEN1	PARK2	RB1	STAG1	
BRD4	CRTC1	EXT1	GNAQ	MET	PAX5	RBL2	STAG2	
BRIP1	CRTC2	EXT2	GNAS	MITF	PDCD1LG2	RECQL4	STAT3	

Illumina HiSeq 2500

Neal Lindeman – Director; Center for Advanced Molecular Diagnostics

Algorithm for molecular testing of NSCLC at DFCI



Neal Lindeman & Lynette Sholl

NGS in the Clinic

- Targeted NGS has been adopted as our standard genotyping assay
  - Results appear in medical record

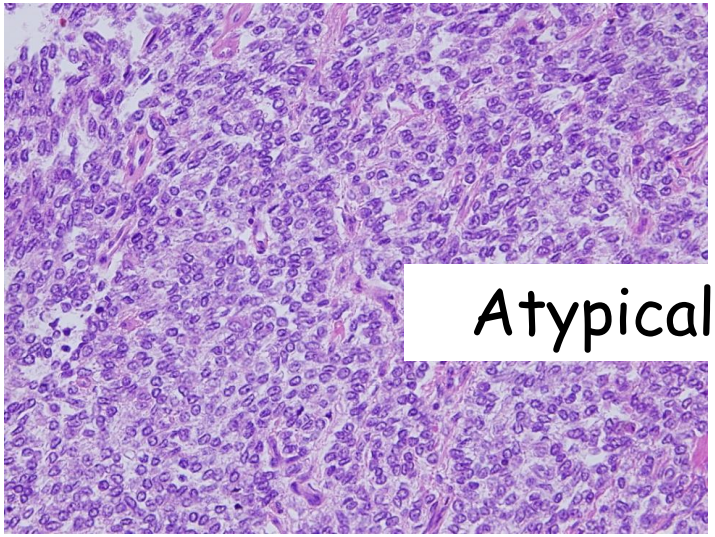
- Initial experience (ASCO 2014)
  - Ordered on 188 pts from 7/13 – 12/13
  - 51 (27%) insufficient
  - Median turnaround time was 24 days

Alteration type	Non-squamous (N = 117)		Squamous (N=17)	
	N	%	N	%
Point mutations				
EGFR	13	11	-	-
BRAF	6	5	1	6
PIK3CA	6	5	4	24
KRAS	41	35	1	6
Insertions/deletions				
EGFR	7	6	-	-
HER2	2	2	-	-
Rearrangements				
ALK	4	3	-	-
ROS1	-	-	-	-
RET	1	1	-	-
High amplification				
EGFR	3	3	-	-
HER2	-	-	-	-
MET	4	3	-	-
PIK3CA	1	1	2	12
FGFR1	1	1	-	-

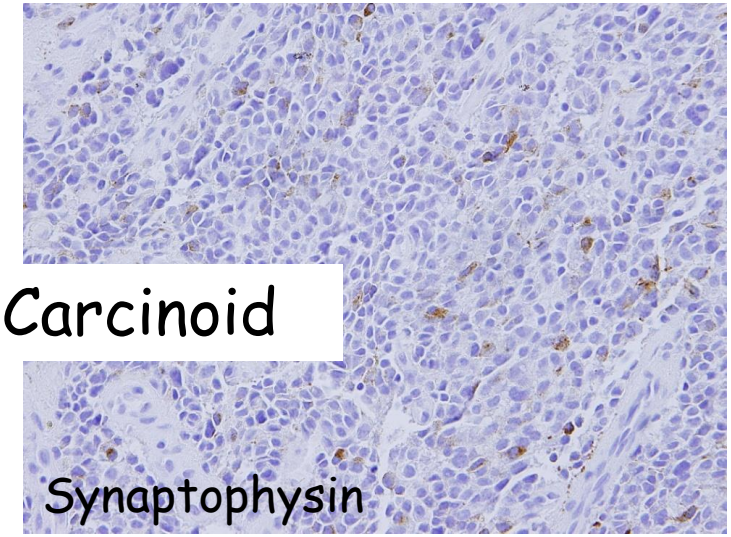
Oxnard et al. ASCO 2014

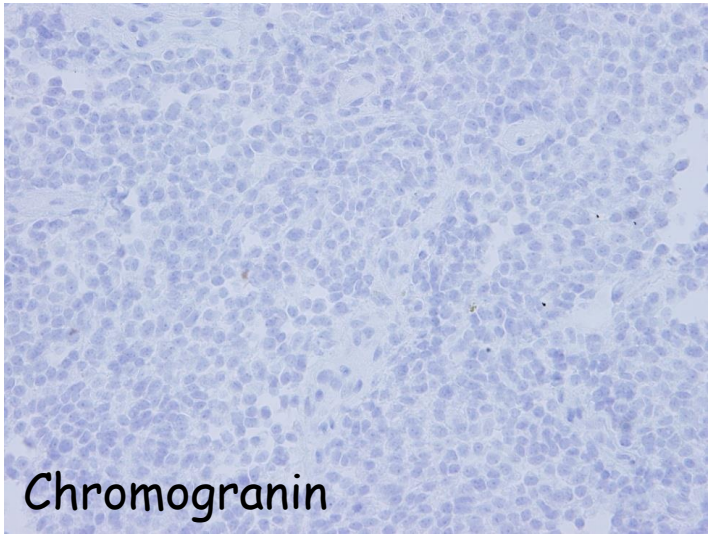
NGS can help guide clinical management

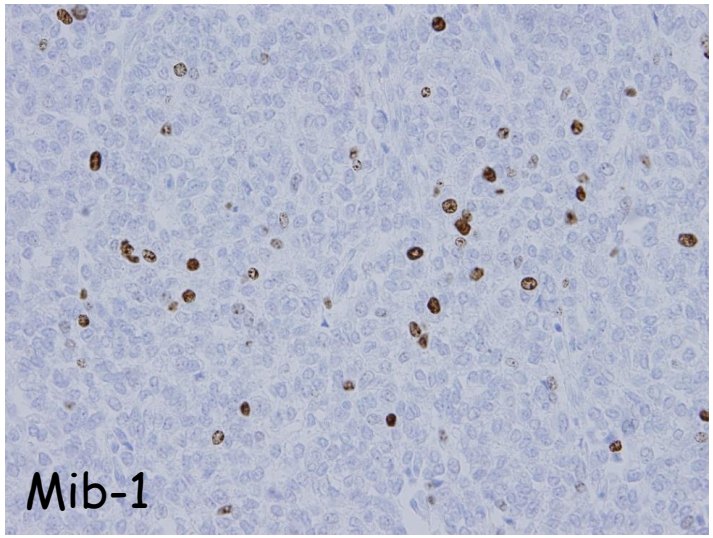
40 yo Chinese male; RUL mass resected in China – pathology consistent with SCLC. LNs negative. Seen at DFCI for opinion regarding chemotherapy.



Atypical Carcinoid

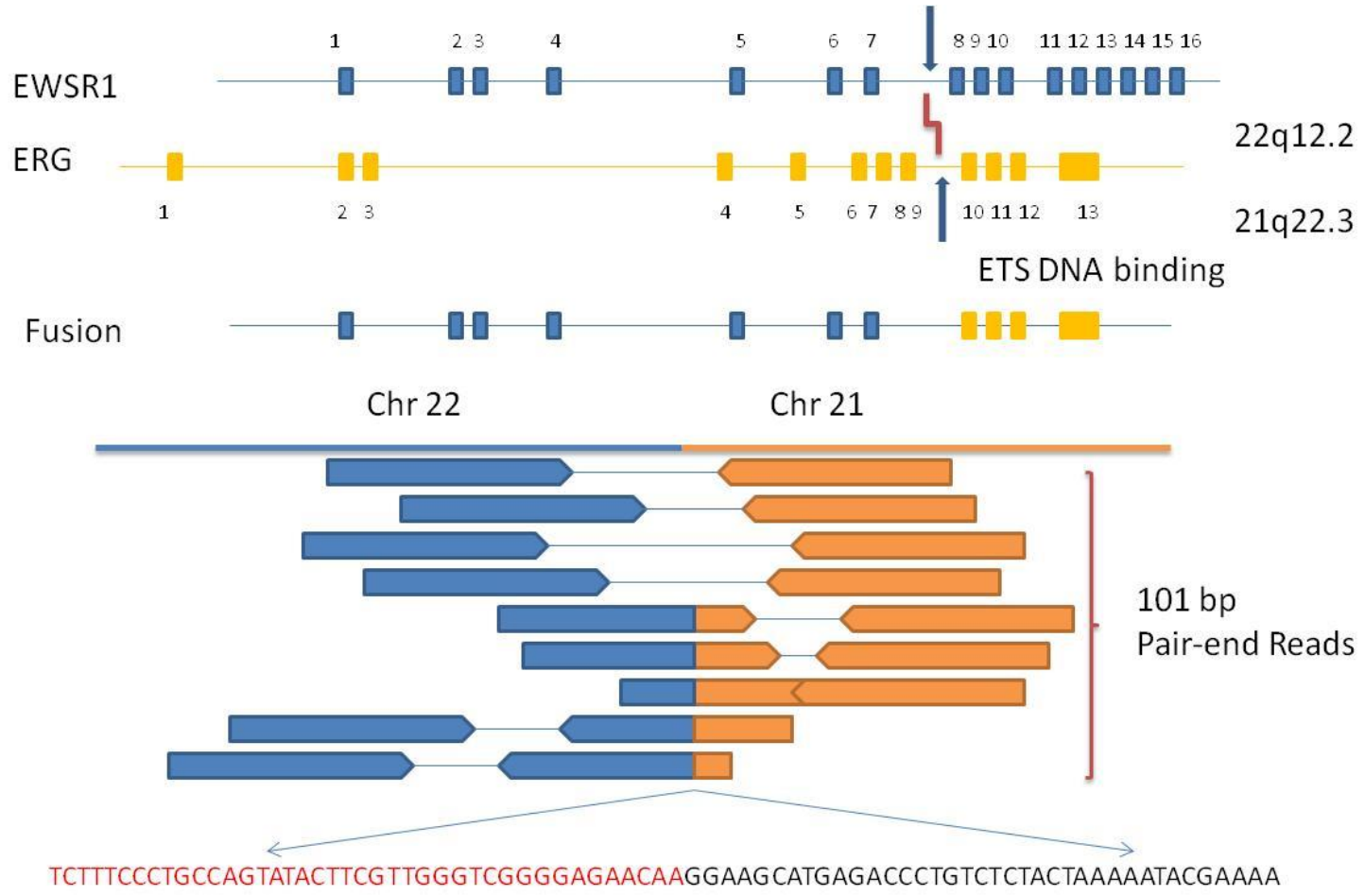






NGS can help guide clinical management

Treated with adjuvant cisplatin/etoposide X4. Chemotherapy completed in March 2013. Follow up PET/CT demonstrated progresion.

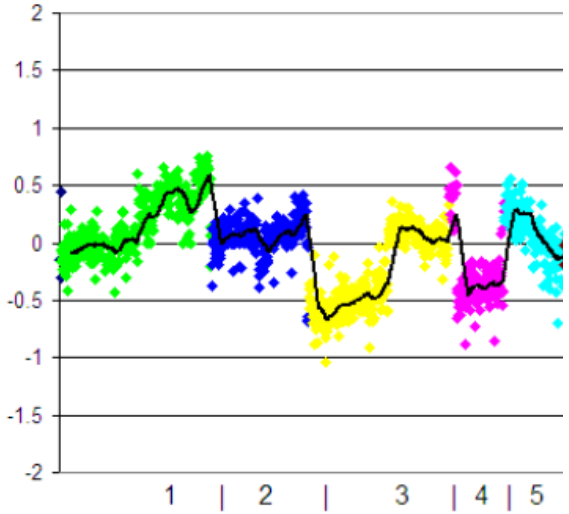


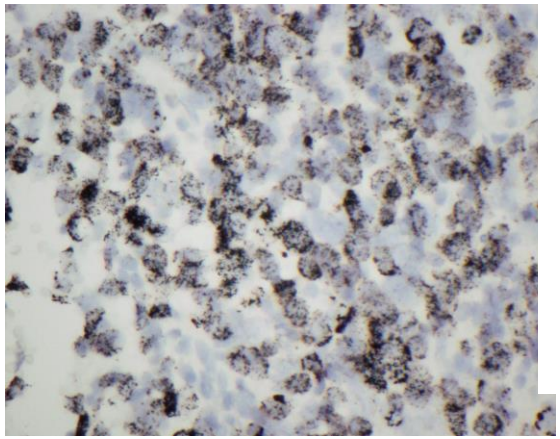
EWSR1-ERG Fusion gene

Kwok Wong, Lynette Sholl & Frank Kuo

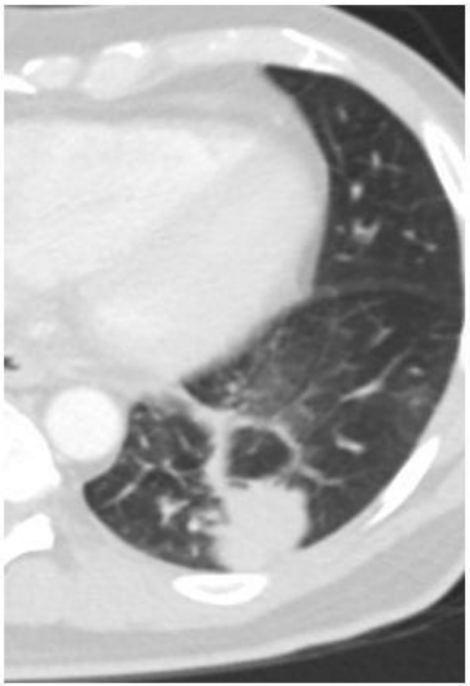
NGS can help guide clinical management

61 yo never smoker; s/p 1st line chemotherapy. Tumor pan "wild type" by

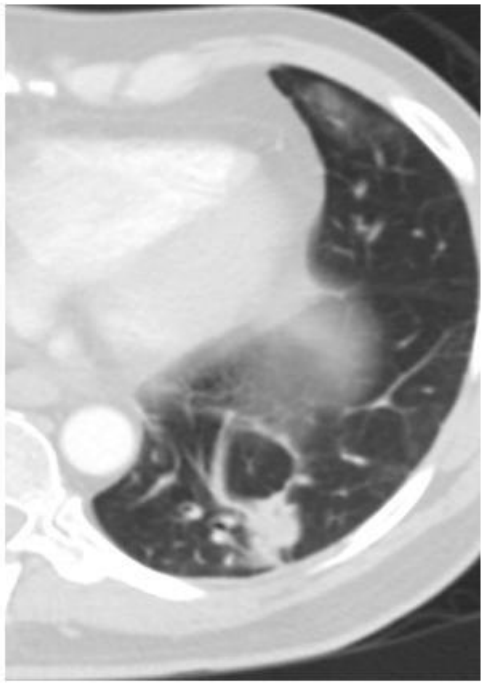




Baseline



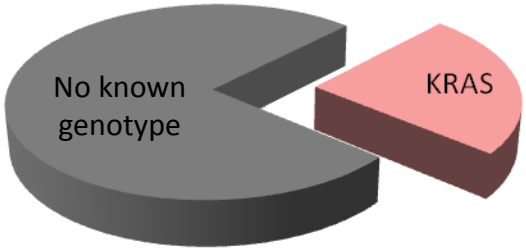
After 3 months on erlotinib



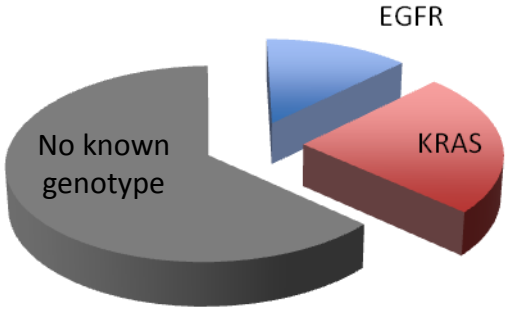
- Patient initiated second-line erlotinib at 150mg daily and had a response in lung mass
- Developed progression in brain after 5 months, but systemic response has been sustained now 9 months on erlotinib

Oxnard et al. ASCO 2014; Abstract 8090

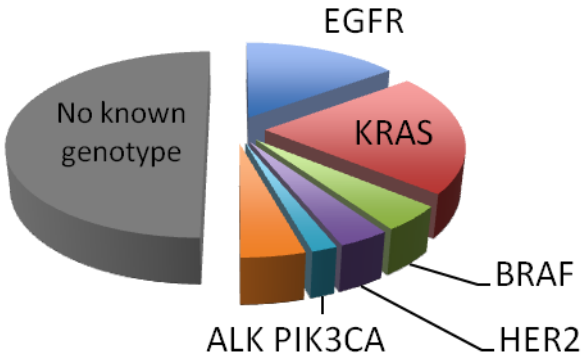
Evolution of Identification of Genomic Alterations in Lung Adenocarcinoma



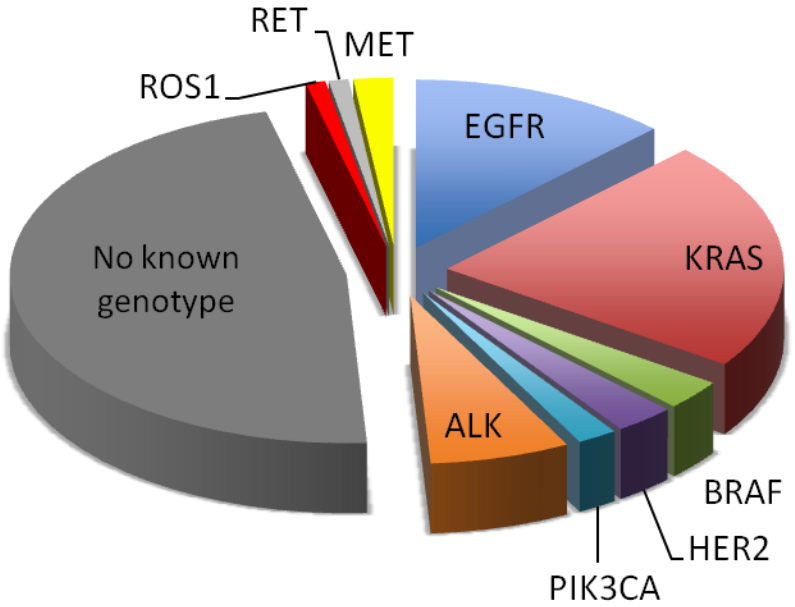
1984 - 2003



2004



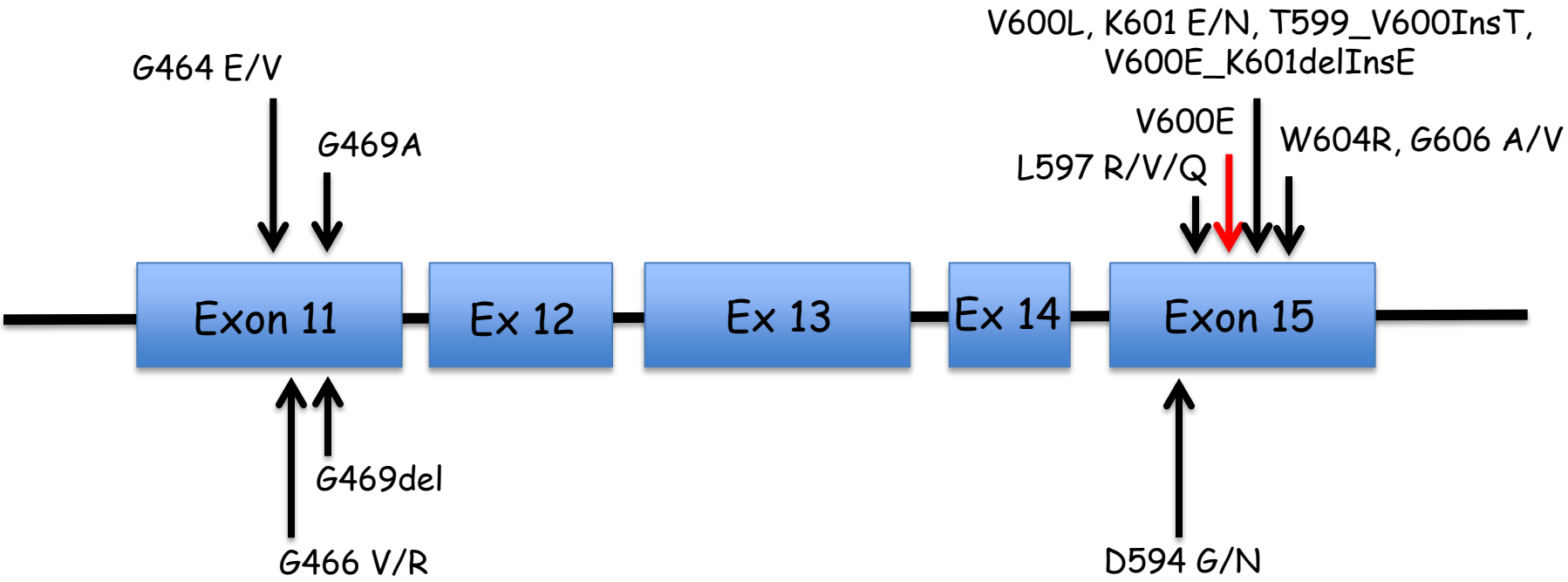
2009



2014

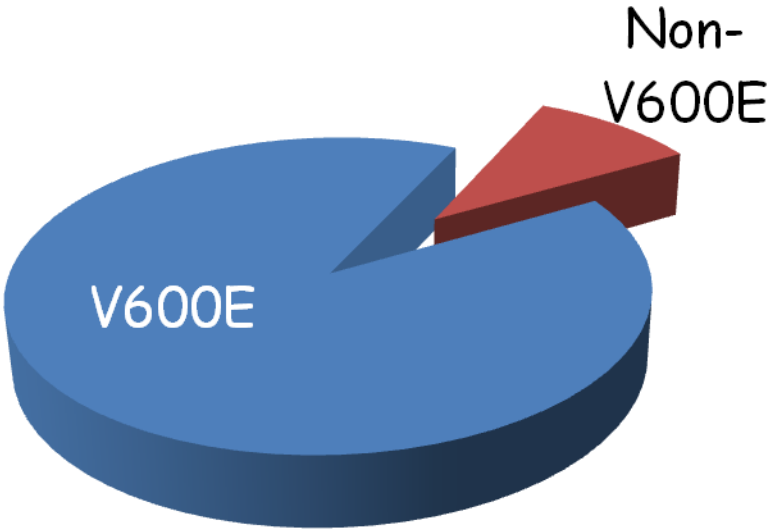
Summary of BRAF Mutations in NSCLC

Author	N	Histology	Stage	Testing
Paik	697	Adeno	I-IV	V600, D594, G469
Marchetti	1,046	Adeno & Squamous	I-IV	Exons 11 & 15
Cardarella	883	Adeno	I-IV	Exons 11 & 15

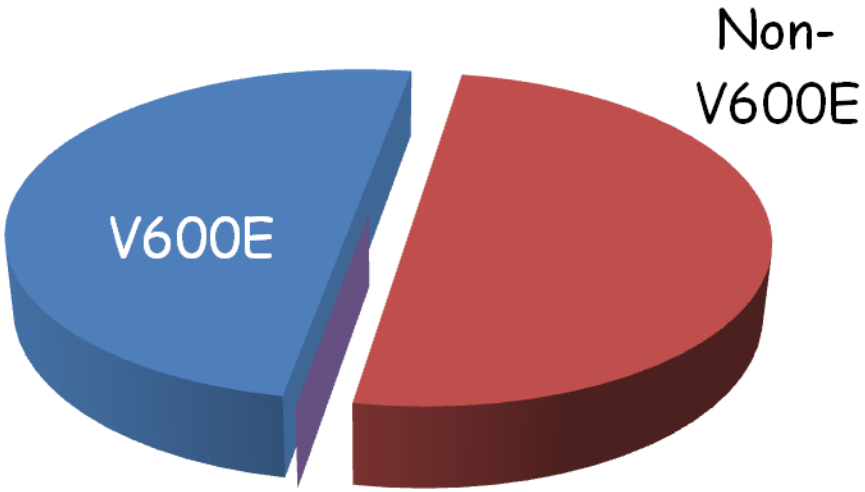


Paik et al., JCO 2011; Marchetti et al., JCO 2011; Cardarella et al., *CCR* 2013

Relative frequency of *BRAF* mutations in melanoma and lung adenocarcinoma



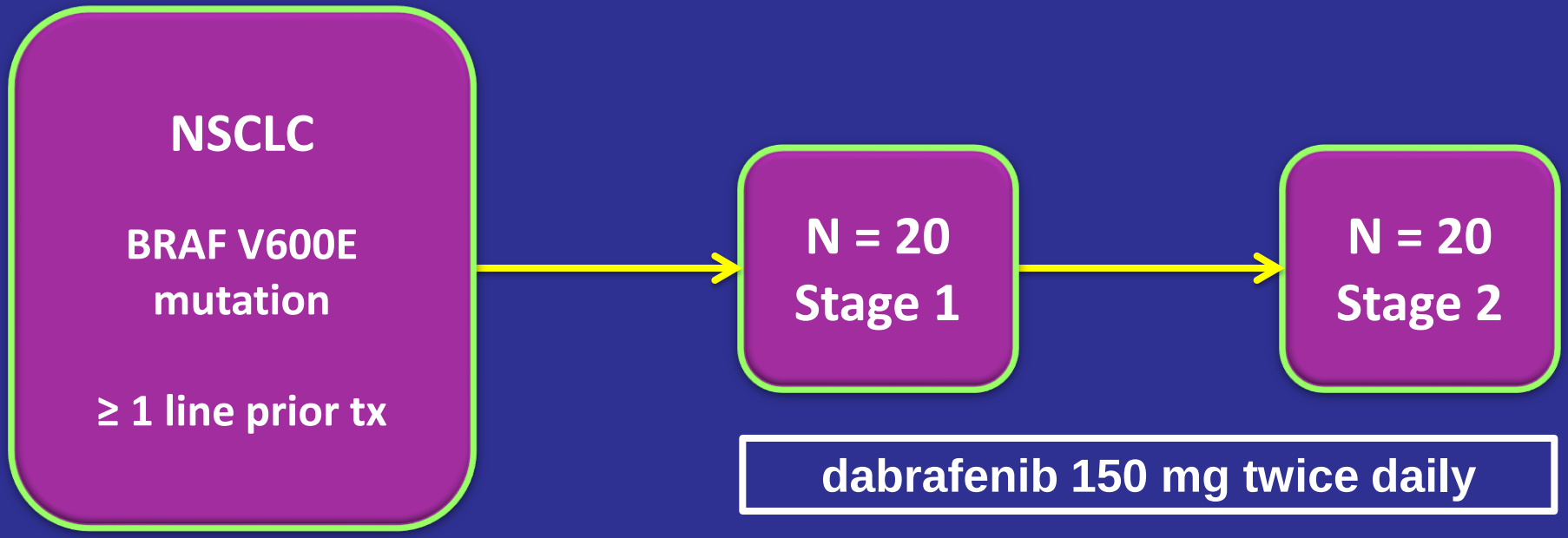
Melanoma



Lung Adenocarcinoma

Debrafenib in BRAF V600E NSCLC

- Single arm, phase 2, open label

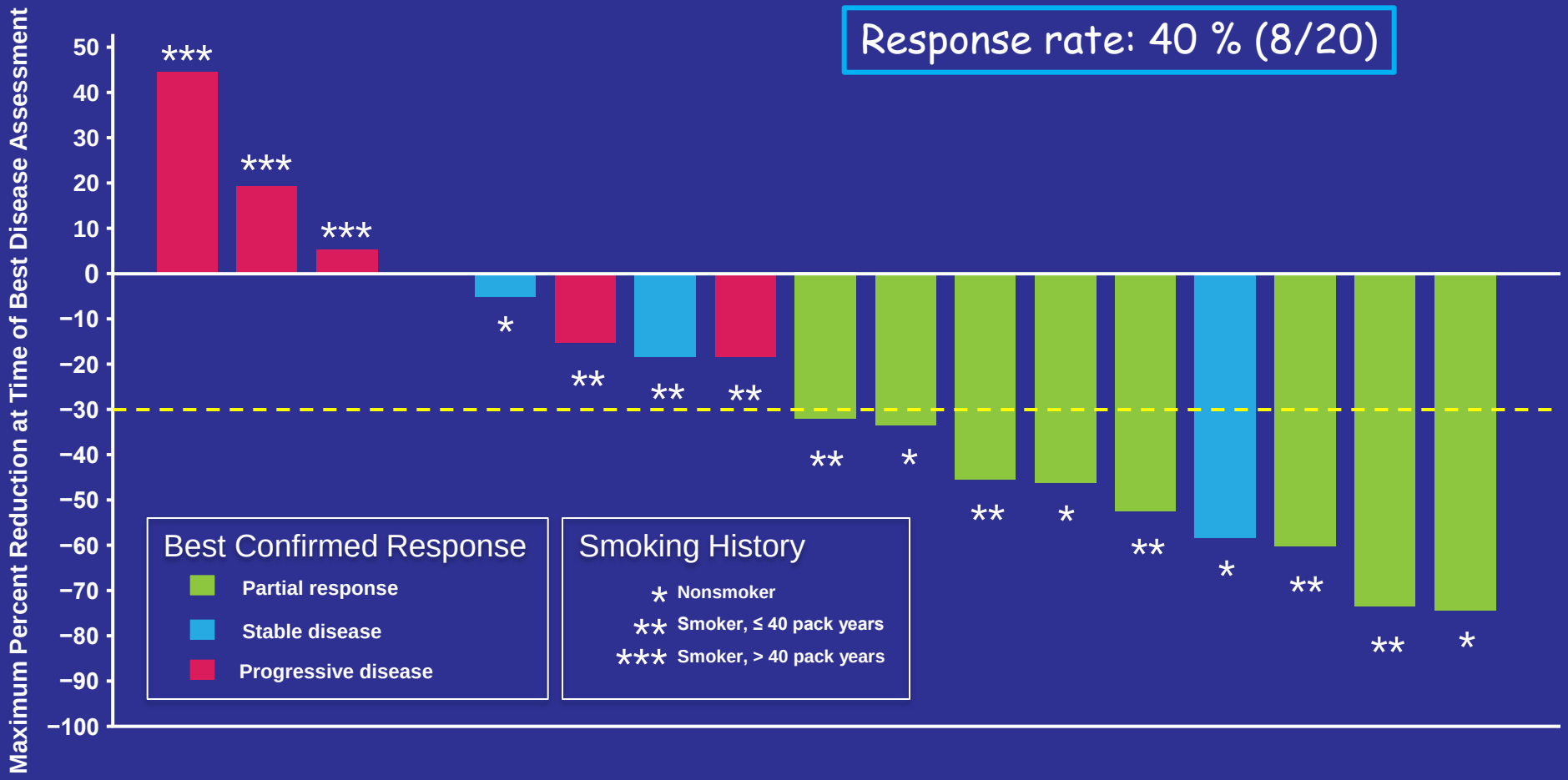


Primary objective: Investigator-assessed ORR

Secondary objectives: PFS, duration of response, overall survival (OS), safety, tolerability, and population pharmacokinetics

Green-Dahlberg 2-stage: H(0)—ORR ≤ 10% versus H(1)—ORR ≥ 30% (primary cohort)

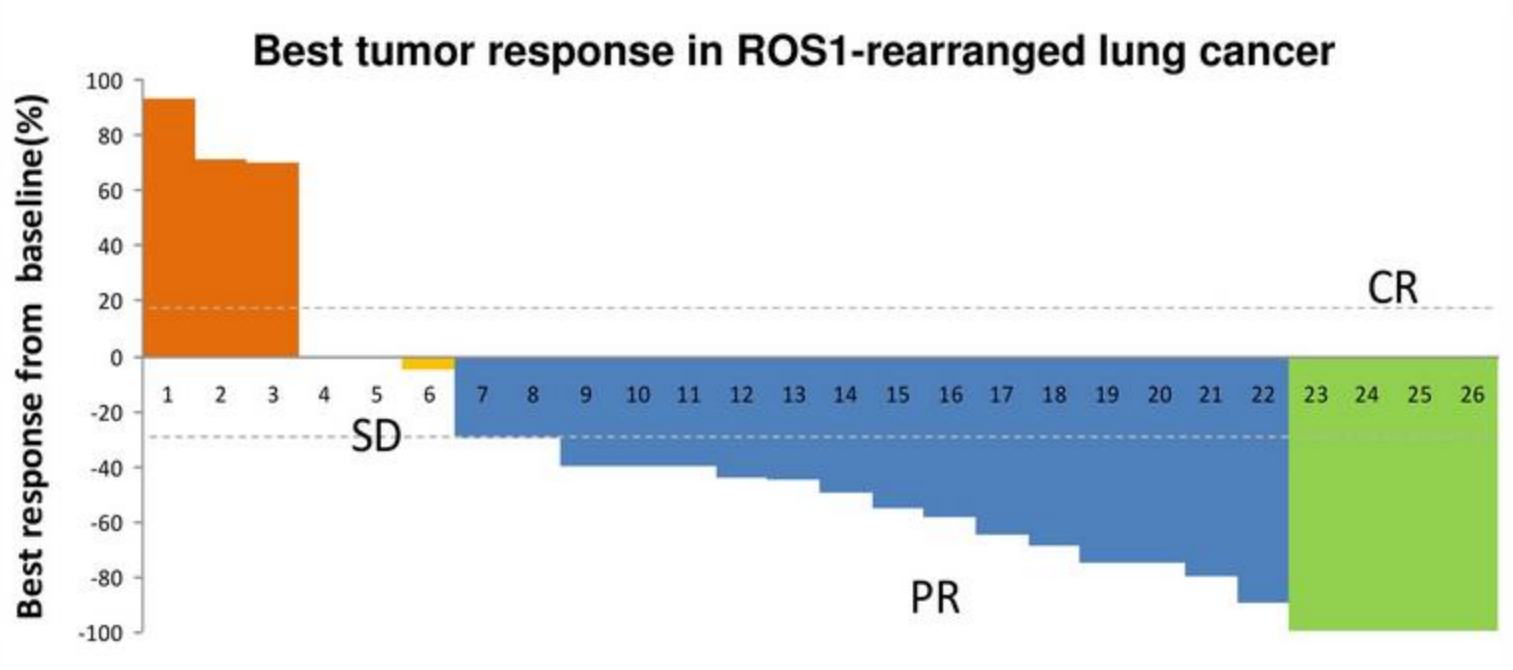
Maximum Reduction of Sum of Lesion Diameters by Best Confirmed Response for the First 20 Patients[a]



[a] 3 patients are not in the plot: 1 patient had PD on day 6 due to new lesion, target lesions were not assessed postbaseline; and 2 patients discontinued study treatment due to serious adverse events (SAEs) prior to postbaseline disease assessment.

ROS1 rearrangements

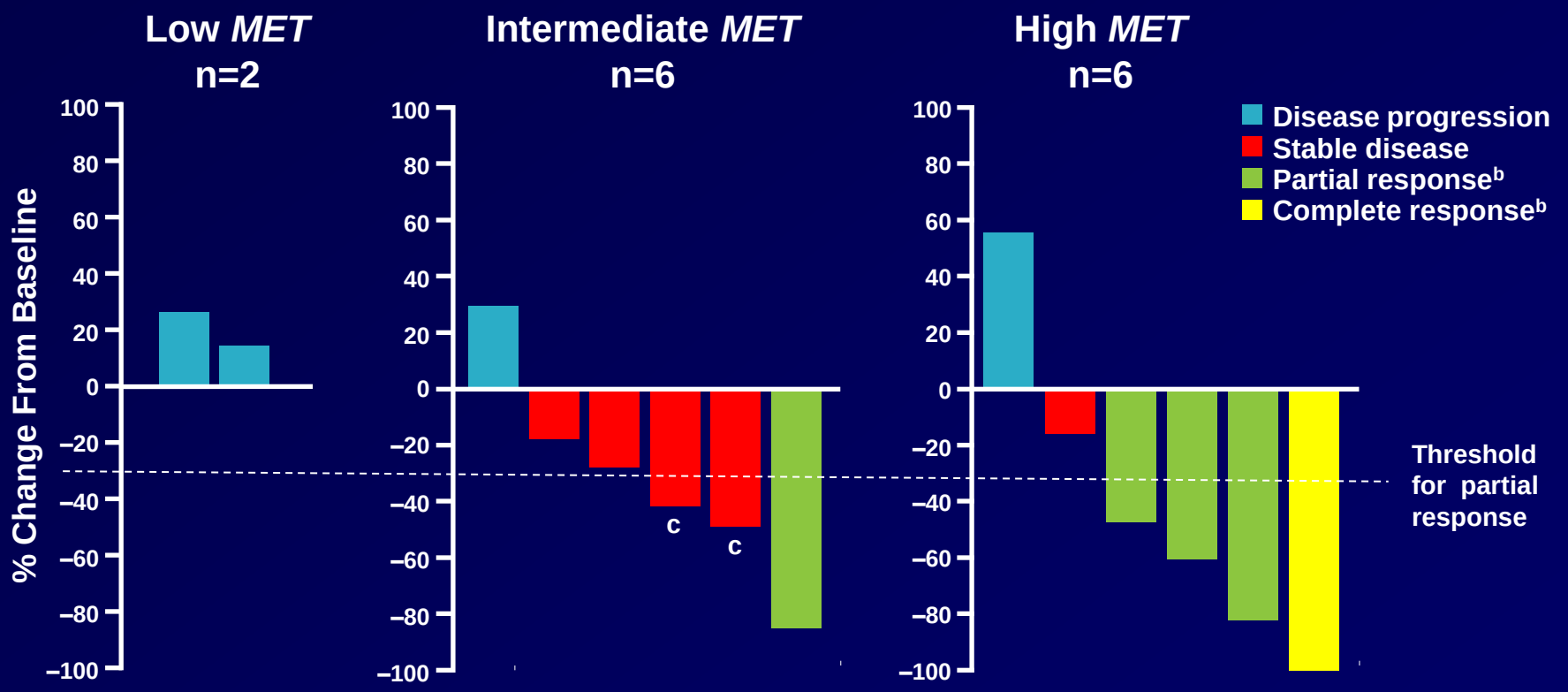
- European experience with ROS1
  - 28 FISH positive patients identified retrospectively from centers in 6 countries
  - 77% response rate to crizotinib



Mazieres et al, ASCO, 2014

Tumor Shrinkage Seen in Intermediate and High *MET* Cohorts

Best percent change from baseline in target tumor lesions[a] by patient



Camidge et al. ASCO 2014

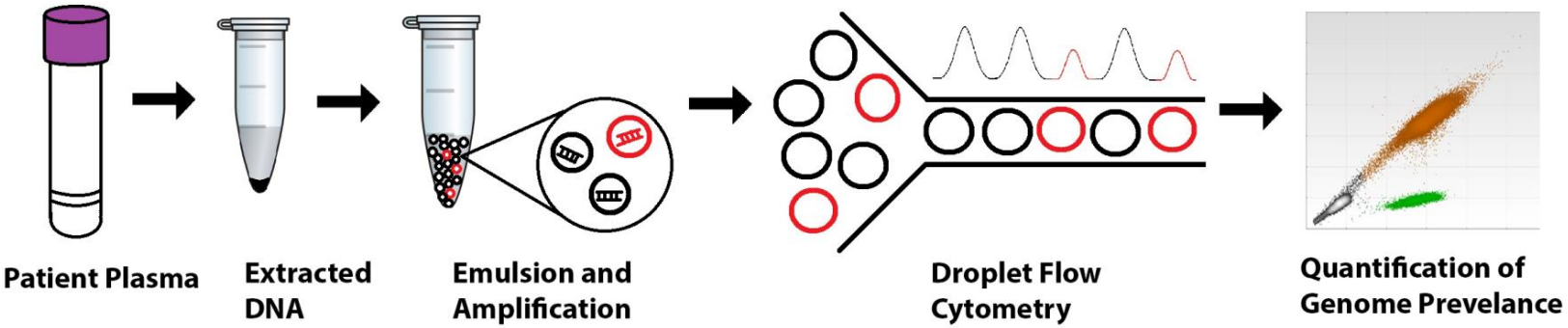
TARGETED AGENTS FOR PATIENTS WITH GENETIC ALTERATIONS

Genetic Alteration (ie, Driver event)	Available Targeted Agents with Activity Against Driver Event in Lung Cancer
EGFR mutations	erlotinib,[1] gefitinib,[2] afatinib[3]
ALK rearrangements	crizotinib[4]
HER2 mutations	trastuzumab,[5] afatinib[6]
BRAF mutations	vemurafenib,[7] dabrafenib[8]
MET amplification	crizotinib[9]
ROS1 rearrangements	crizotinib[10]
RET rearrangements	cabozantinib[11]

[1]Sequist LV, Joshi VA, Janne PA, et al. Response to treatment and survival of patients with non-small cell lung cancer undergoing somatic EGFR mutation testing. Oncologist 2007;12:90-98.
[2]Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science 2004;304:1497-1500.
[3]Sequist LV, Yang JC-H, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. J Clin Oncol 2013;31:3327-3334.
[4]Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small cell lung cancer. N Engl J Med 2010;363:1693-1703.
[5]Cappuzzo F, Bemis L, Varella-Garcia M. HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer. N Engl J Med 2006;354:2619-2621.
[6]Mazieres J, Peters S, Lepage B, et al. Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol 2013;31:1997-2003.
[7]Gautschi O, Pauli C, Strobel K, et al. A patient with BRAF V600E lung adenocarcinoma responding to vemurafenib. J Thorac Oncol 2012;7:e23-24.
[8]Planchard D, Mazieres J, Riely GJ, et al. Interim results of phase II study BRF113928 of dabrafenib in BRAF V600E mutation-positive non-small cell lung cancer (NSCLC) patients [abstract]. J Clin Oncol 2013;31(Suppl 15): Abstract 8009.
[9]Ou SH, Kwak EL, Siwak-Tapp C, et al. Activity of crizotinib (PF02341066), a dual mesenchymal-epithelial transition (MET) and anaplastic lymphoma kinase (ALK) inhibitor, in a non-small cell lung cancer patient with de novo MET amplification. J Thorac Oncol 2011;6:942-946.
[10]Bergethon K, Shaw AT, Ou SH, et al. ROS1 rearrangements define a unique molecular class of lung cancers. J Clin Oncol 2012;30:863-870.
[11]Drilon A, Wang L, Hasanovic A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov 2013; 3:630-635.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

Digital Droplet PCR for detection of tumor derived mutations in circulating free (cf) DNA



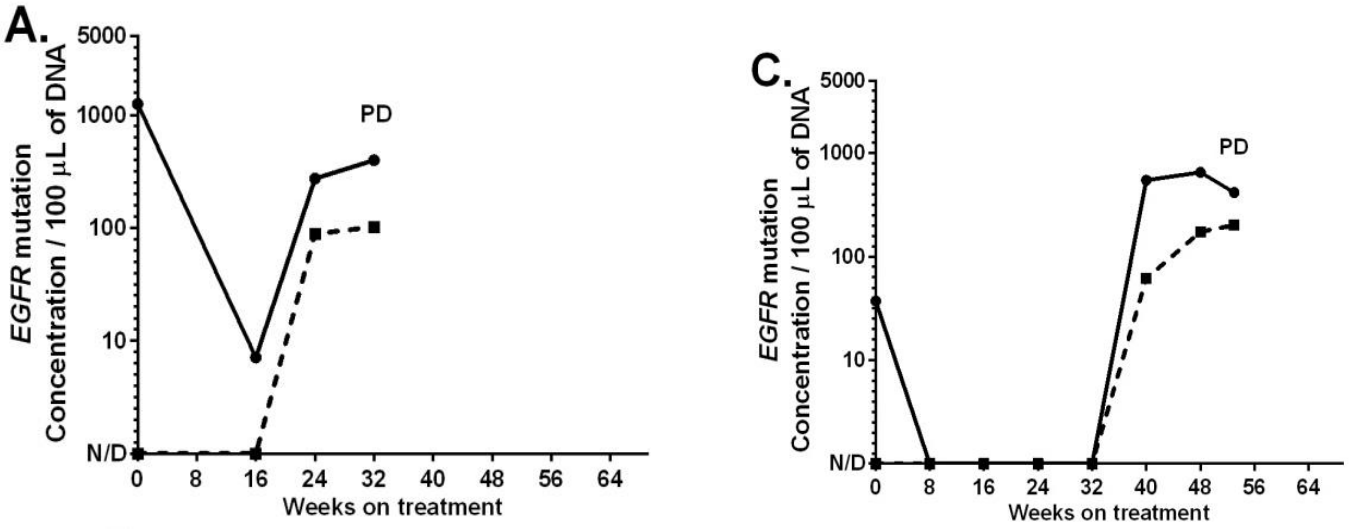
Oxnard et el. CCR 2014

Non-invasive disease monitoring

Stage IV NSCLC
EGFR mutant
Treatment naive

Erlotinib
150 mg

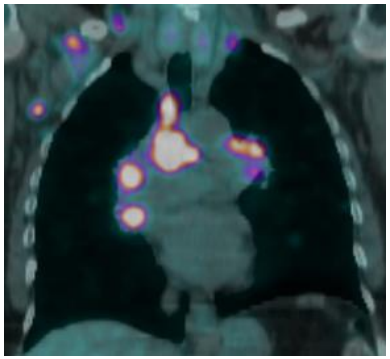
Biopsy at resistance
Circulating tumor cells
Plasma for cfDNA



Serial monitoring for EGFR activating and EGFR T790M resistance mutation in erlotinib treated EGFR mutant patients

Oxnard et al. CCR 2014

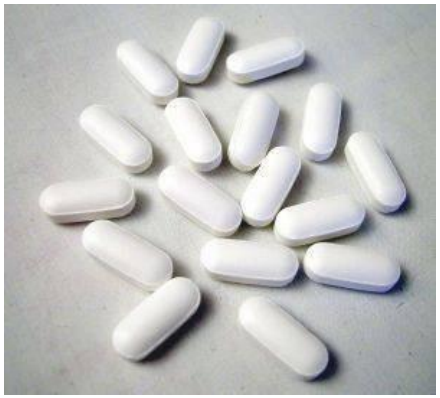
Case report: Acquired resistance



DAY 0:
CT shows marked progression on erlotinib, plasma drawn

DAY 1:
cfDNA genotyping detects 806 copies/ml of *EGFR* T790M

DAY 25:
Report from rebiopsy genotyping shows *EGFR* T790M

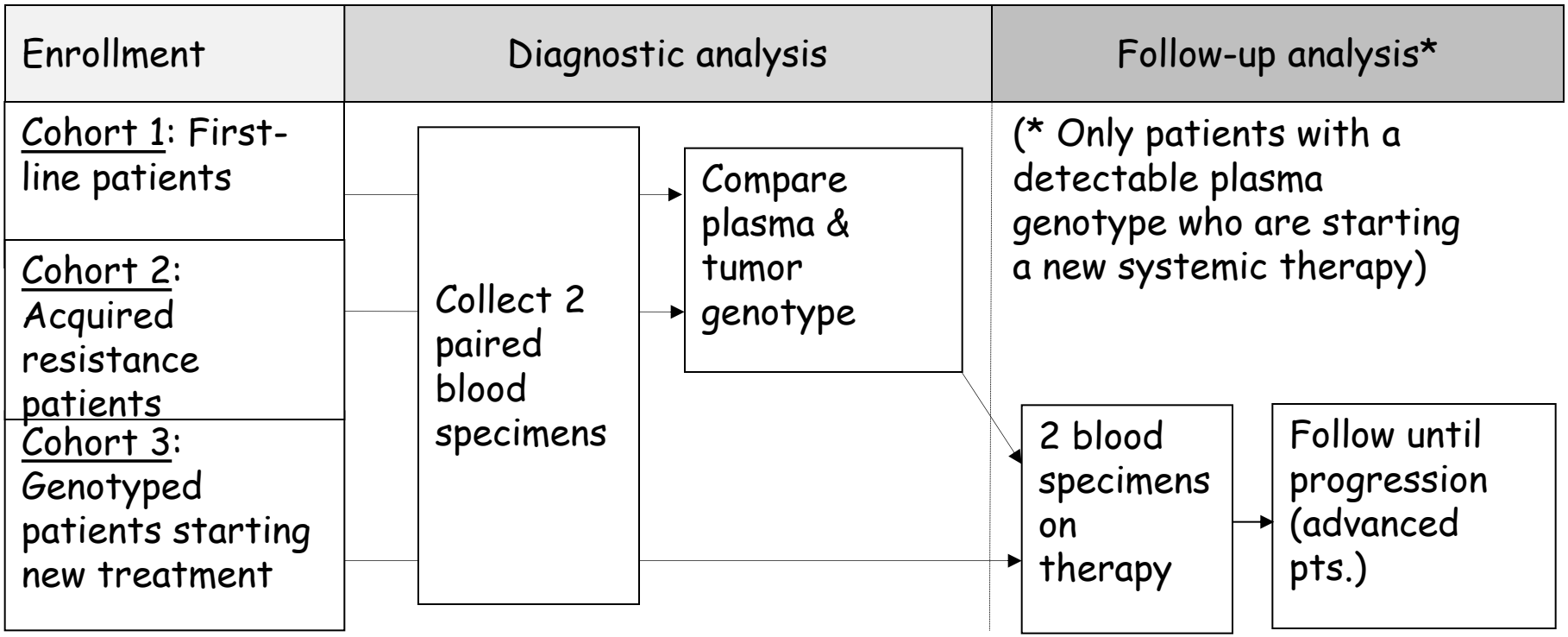


DAY 31:
Patient starts treatment with an investigational EGFR inhibitor

DAY 73:
CT demonstrates a radiographic response

Sacher et al, ASCO, 2014

Prospective validation – DFCI# 14-147 Schema



Same day registration and initial blood draw

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Plasma Genotyping report for

***EGFR* T790M/L858R/del19 and KRAS G12X/G13D**

Protocol #: 14-147

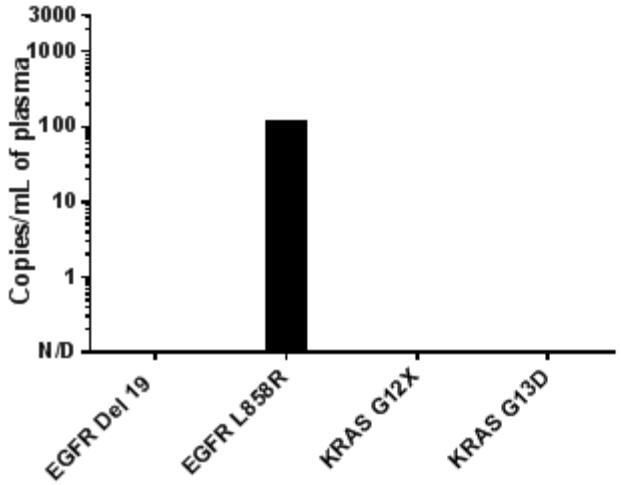
Specimen ID: S016-IA-Cohort 1A

Date drawn: 8/14/2014

DNA concentration: Not determined

Date report generated: 8/19/2014

Genotype	Tested (y/n)	Detected (y/n)	Concentration (copies/mL)
EGFR Ex Del 19	y	n	N/D
EGFR L858R	y	y	107
EGFR T790M	n	N/A	N/A
KRAS G12X	y	n	N/D
KRAS G13D	y	n	N/D



Adrian Sacher, Geoff Oxnard, Cloud Paweletz

Oncogene Addiction & Biomarkers: Who Should Be Tested and When ?

- Genomic testing can identify targetable drug sensitive alteration

- Who to test depends on
  - Test availability & infrastructure, speed & cost
  - Ideally test every non-squamous NSCLC

- Blood testing is the future and may be a rapid alternative

Acknowledgements

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Geoff Oxnard

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Jennifer Heng

Stacy Mach

TRL/Belfer

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Neal Lindeman

Lynette Sholl

Scott Rodig

Radiology

Ritu Gill

Thoracic Surgery

Mike Jaklitsch

Yolanda Colson

Scott Swanson

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