



ASAN
Medical Center



University of Ulsan

Overview of ALK and ROS1 rearranged Lung Cancer

Chang-Min Choi

Department of Pulmonary and Critical Care Medicine,
Asan Medical Center, University of Ulsan College of Medicine

EGFR mutation / EML4-ALK Translocation

A drug that works — for some Researchers try to solve mystery of lung cancer medicine



By Raja Mishra
GLOBE STAFF

CONCORD — Early last year, Kate Robbins started her death journal. It was meant to guide her two children after lung cancer killed her. Robbins poured out advice: on dating, on morality, on family. The things a mother explains to her teens.

She also memorialized quiet, poignant moments. One rainy day, watching 10-year-old Hillary board the school bus, Robbins began weeping. She wrote simply, "You looked really pretty today." The kids would read the journal years later, she hoped, and feel their mother's love.

Advanced lung cancer patients rarely live one year. Robbins had months left. Then, last autumn, she began taking an experimental drug called Iressa, as did hundreds of other patients in the United States. Most continued to worsen.

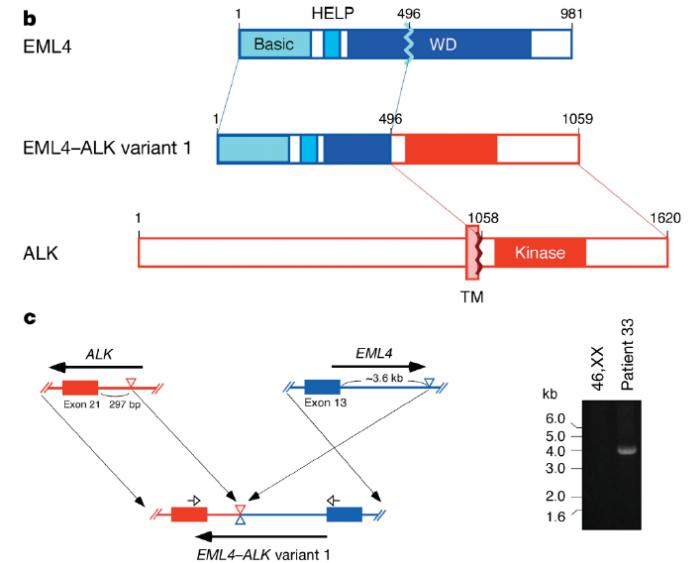
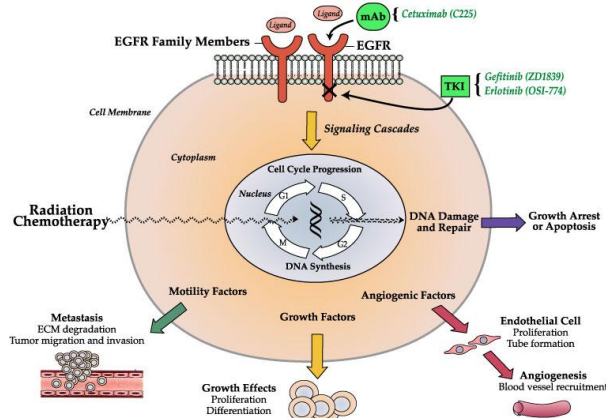
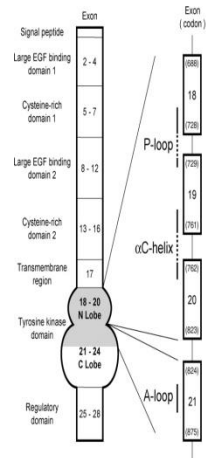
But a small group, including Robbins, thrived.



출처: USA투데이

위신에 소개된 환자의 사례는 드라마틱하다. 미국 워싱턴 주에 사는 맨디 힐(47)은 2009년 3월 의사로부터 말기폐암이라는 청천벽력 같은 소리를 들었다. 평생 담배를 입에 물지도 않았지만 몇 달 밖에 살 수 없다는 말에 절망했다. 항암치료와 방사선 치료를 받았지만 구질질 때문에 음식을 먹을 수 없었고 체중은 11kg 빠졌다. 머리카락은 남지 않았고 눈은 할했다. 정상적으로 숨을 쉬며 계단을 오를 수가 없었다. 목소리도 나오지 않았다. 힐은 지난 해 9월 마지막 지푸라기를 잡는 심정으로 크리조티닙 임상시험에 참여했다가 기적을 보게 됐다. 1주 만에 증세가 사라졌고 2주째 목 소리가 정상으로 되돌아온 것. 3주째에는

조깅을 다시 시작할 수 있었고 지금은 두 딸이 속한 팀에서 축구 감독을 하고 있다(사진 참조).

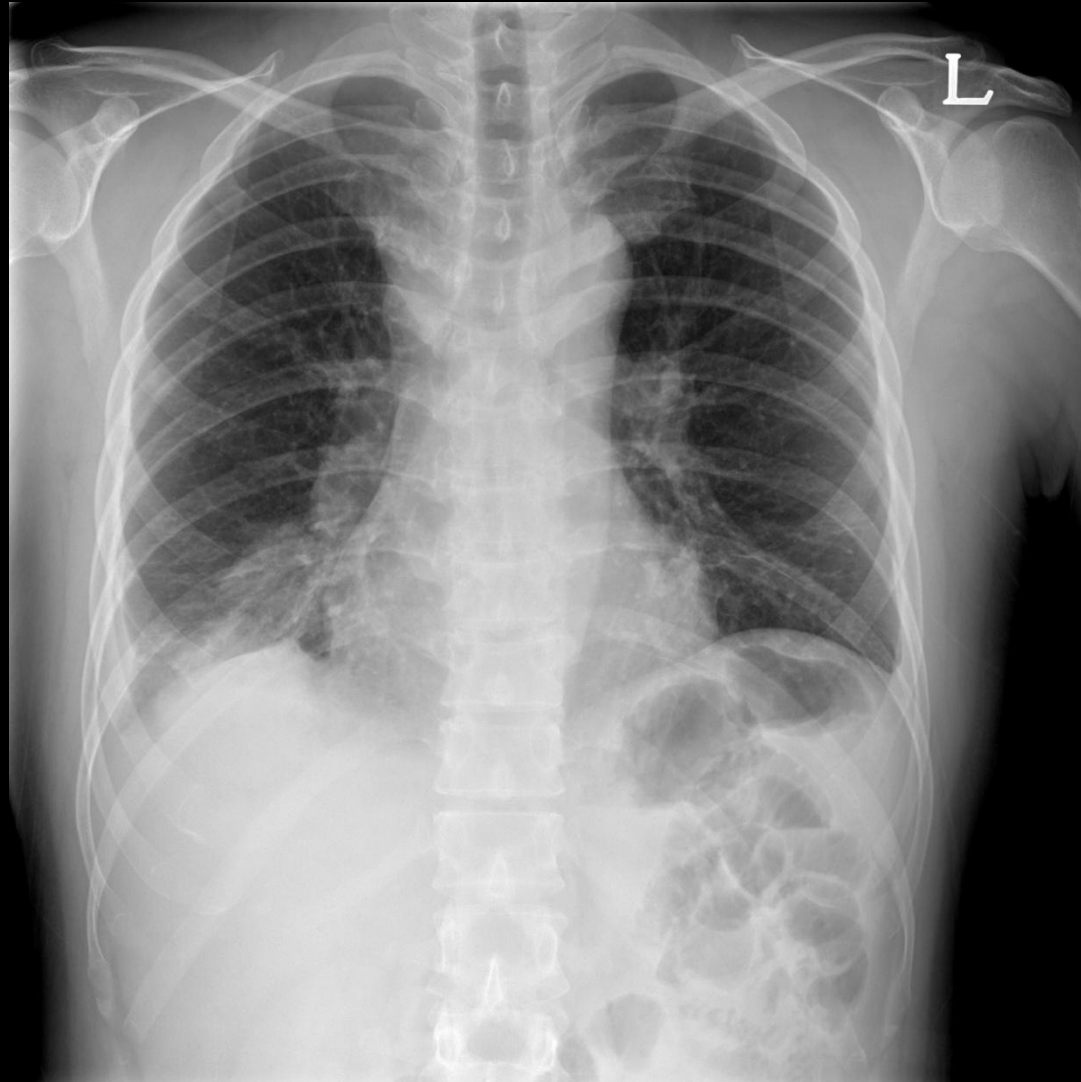


중례 요약

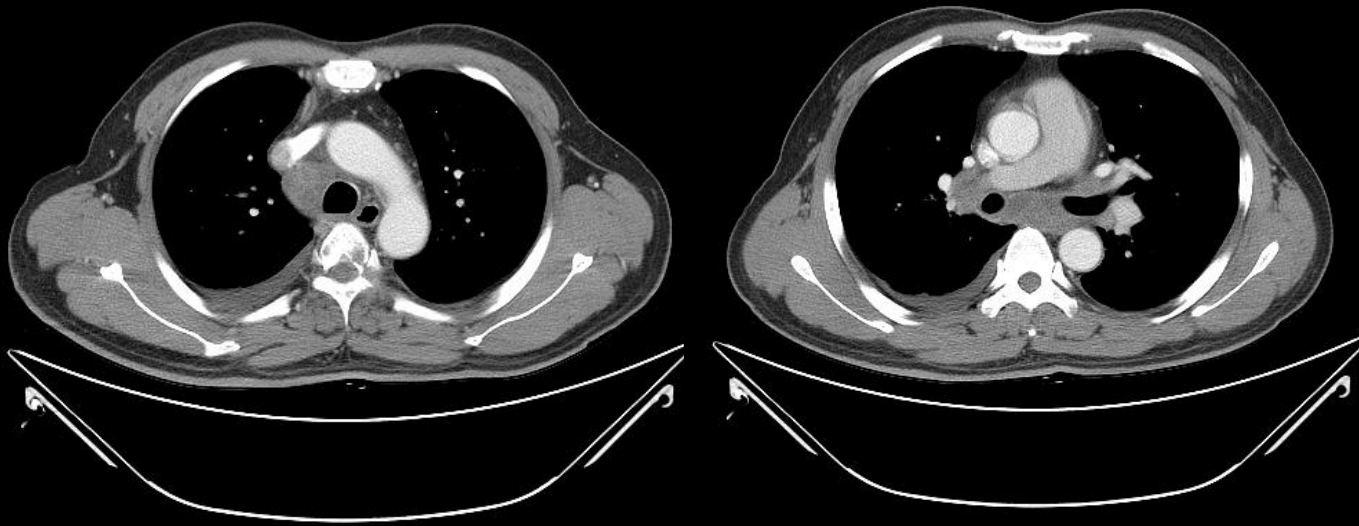
- 51세 남자
- 한달 전 시작된 기침
- 폐렴으로 진단하고 항생제 치료하였으나 호전되지 않음
- CT에서 폐암 의심하에 기관지내시경 시행하려 하였으나 허리 통증과 기침으로 인해 실패 후 전원됨
- Light ex-smoker 1/5 pack X 5 years (1PY)
- 객담(+) 객혈(-) 흉통(-)



Initial Radiographs

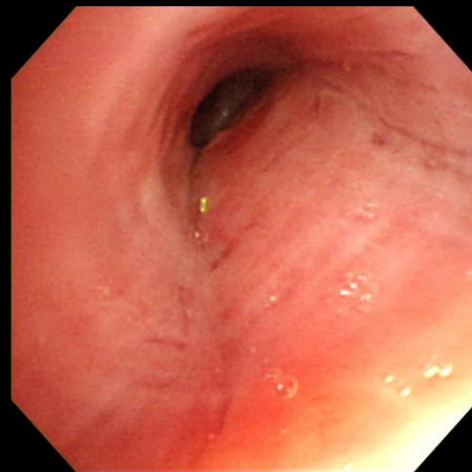
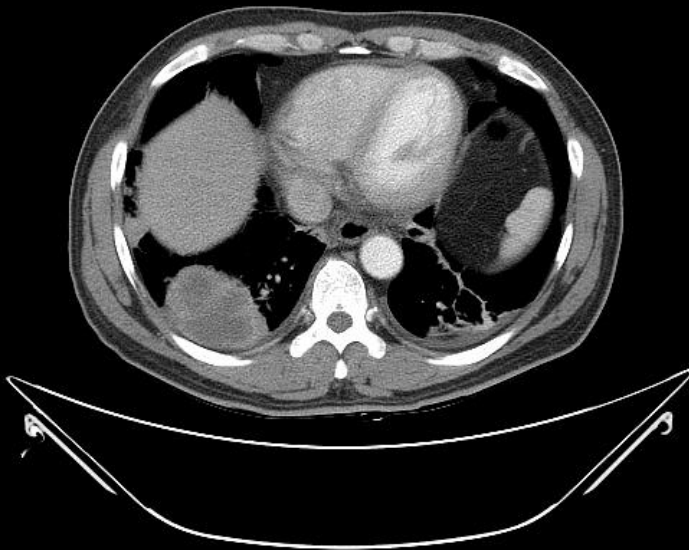


Initial CT Findings

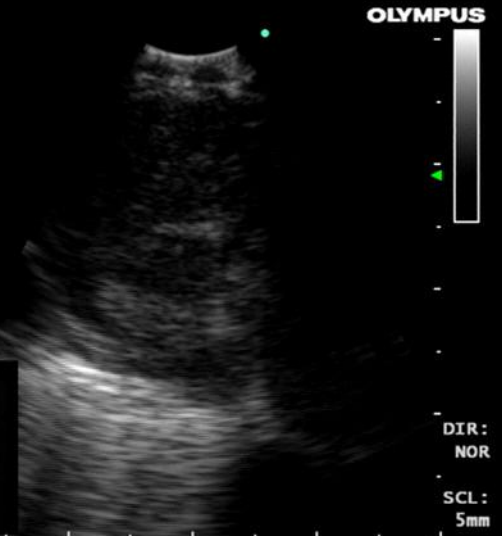
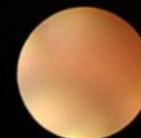


Stage T4N3M1b

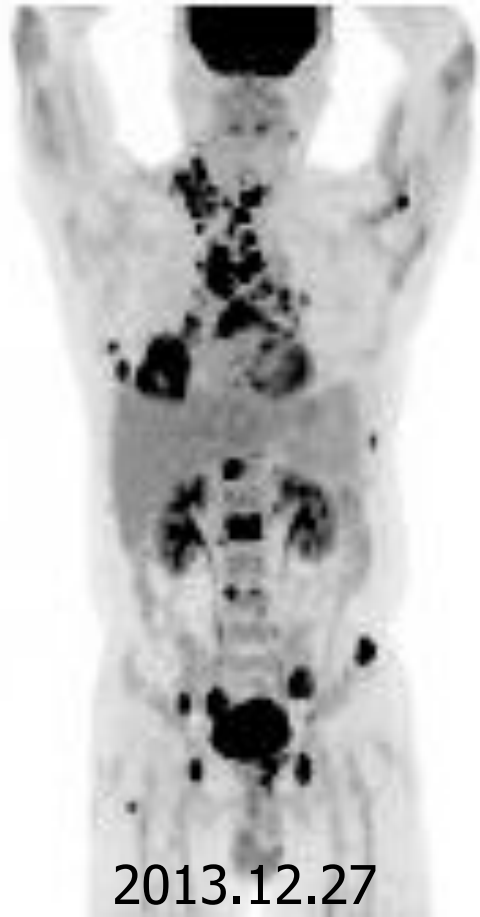
Bone, Neck LN



ID:
NAME:
AGE:
DOB: SEX:
12/27/2012
11:30:38
10MHz 4cm
G:15/19 I:L1
C:5/8 FC:3
L.DEN:x2.0
TX:100%
MEDIA
T/B:NONE



PET/CT Findings – Iressa PET study



Baseline PET SUV
Max=11.6, Peak 9.1



Gefitinib
1weeks



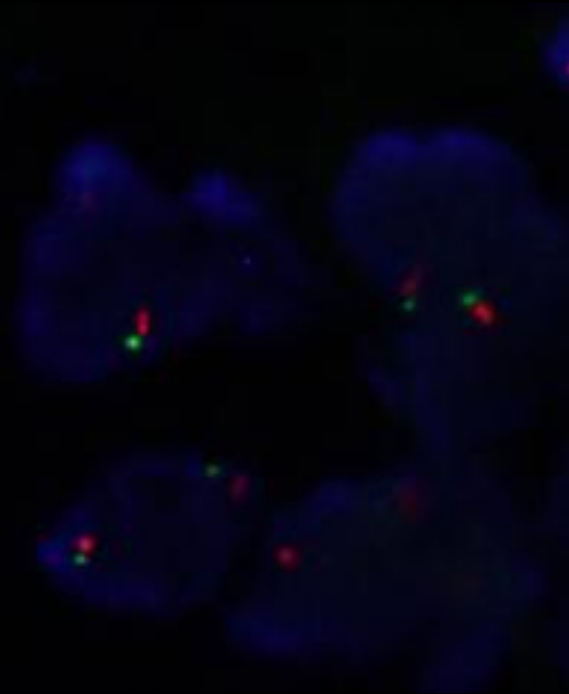
Baseline PET SUV
Max=12.9, Peak 10.9

PeakSUV change(%)
-19.8%



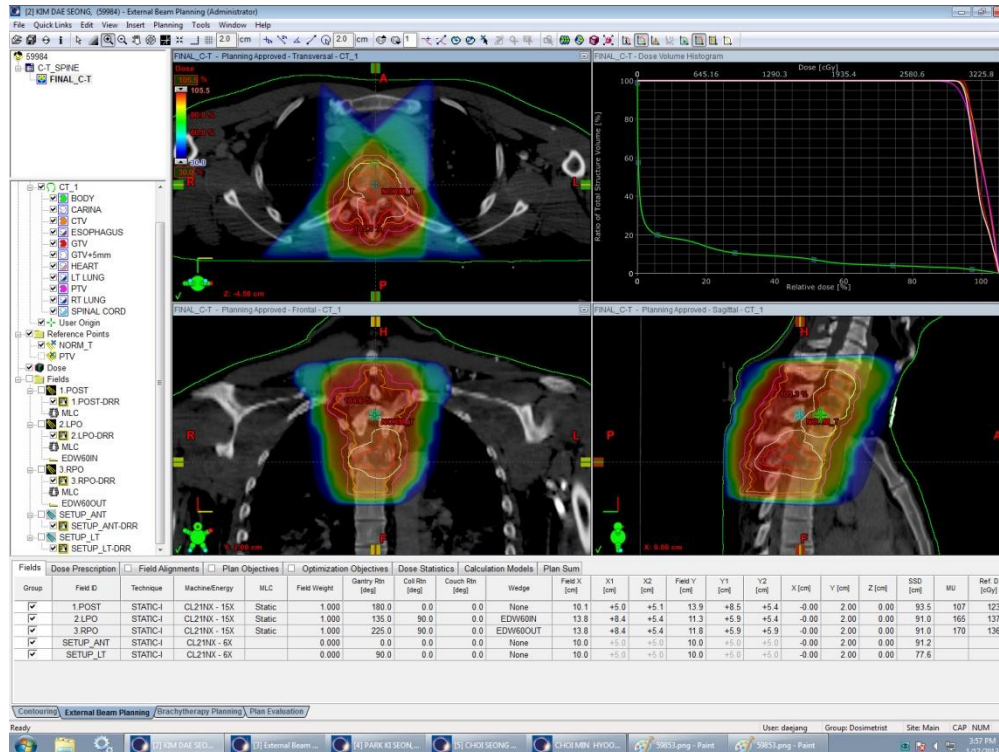
Biopsy Finding

- Metastatic Adenocarcinoma, poorly differentiated
- TTF-1(+) ALK IHC (+)
- No EGFR & K-RAS mutation
- ALK FISH (+) positive 24%



Hospital Course

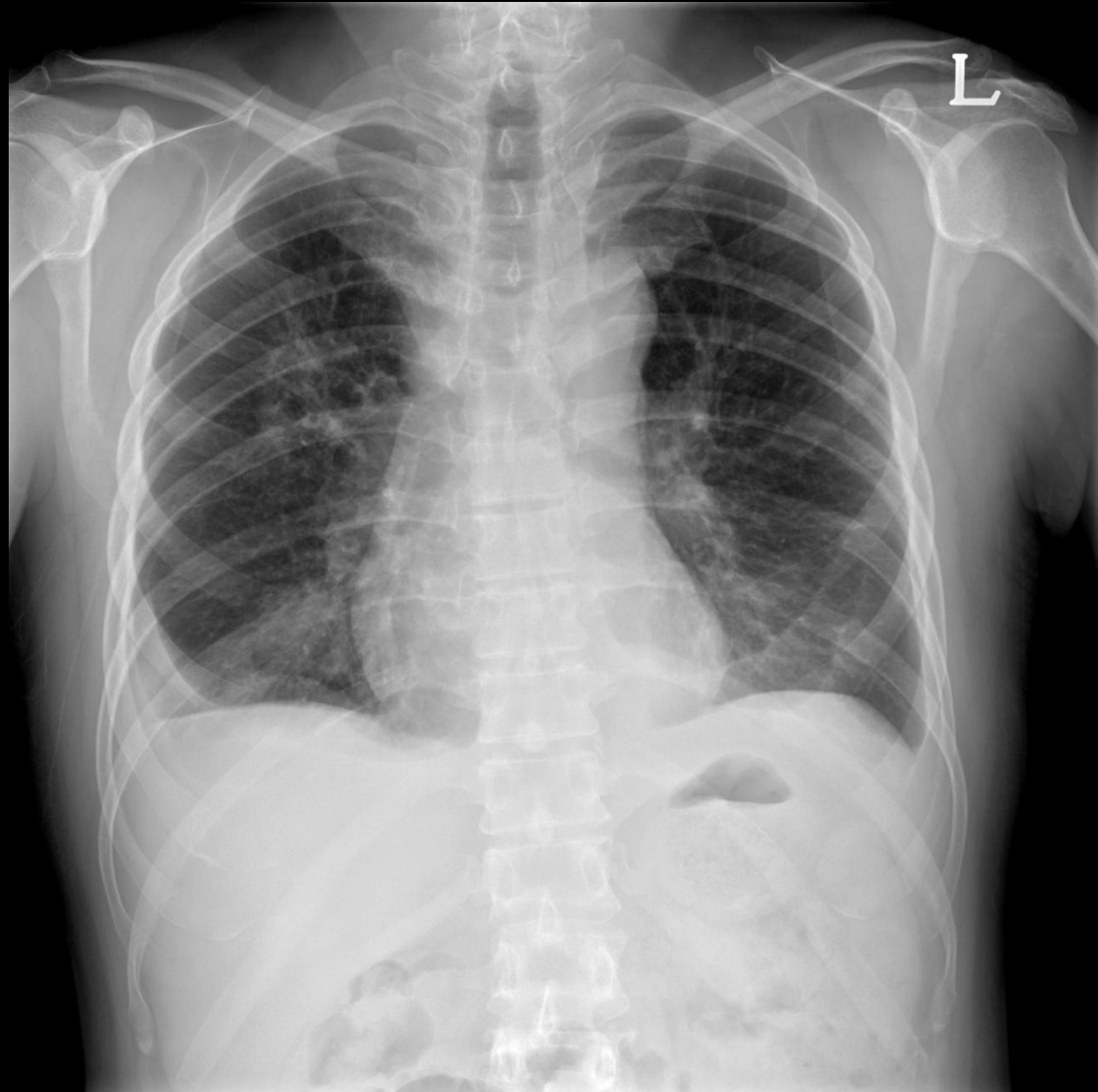
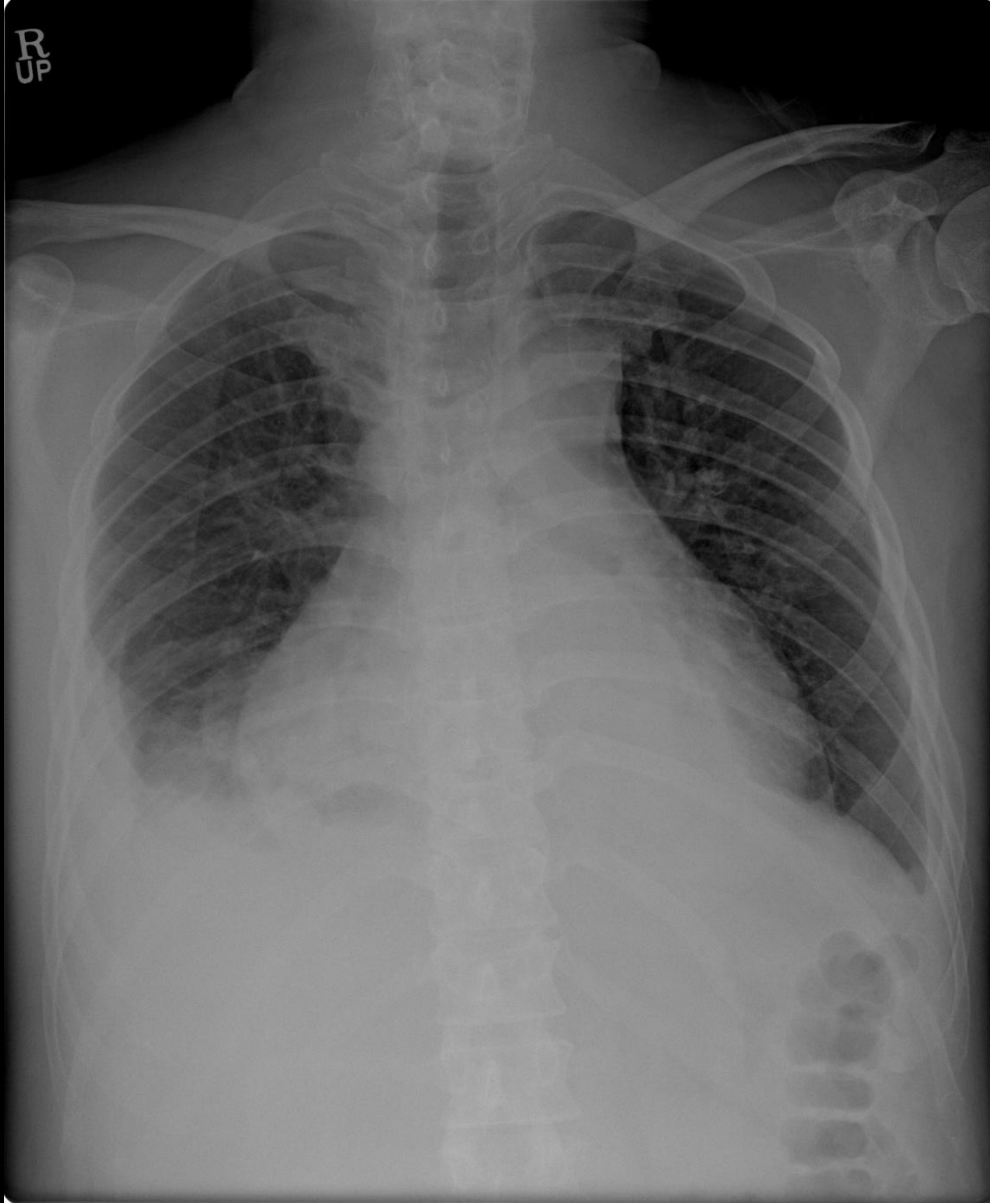
- Palliative Radiation on Spine d/t severe pain
21Gy (10Fx)
- 2013.1.21 Alimta + Cisplatin start



Hospital Course

- 1 weeks later,
Develop Sudden orthopnea & general weakness
Nausea, Poor oral intake
 - Refer to ER
LFT AST/ALT 1604/1501 Cr 2.13
BP 83/69 mmHg, PR 119/min RR 25/min Saturation 95%
-

Radiographs



Pericardial Effusion

- Malignant cell 50%
- Pericardiocentesis



PET/CT Findings – Crizotinob



2013.01.08



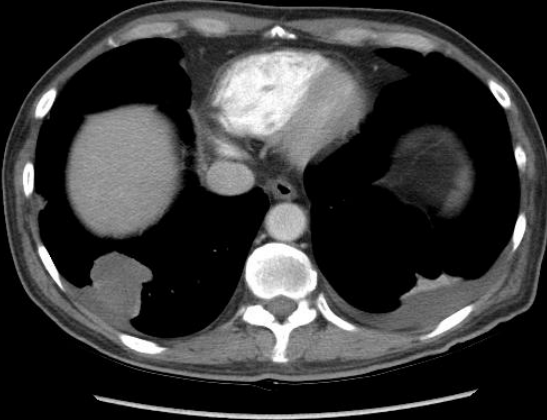
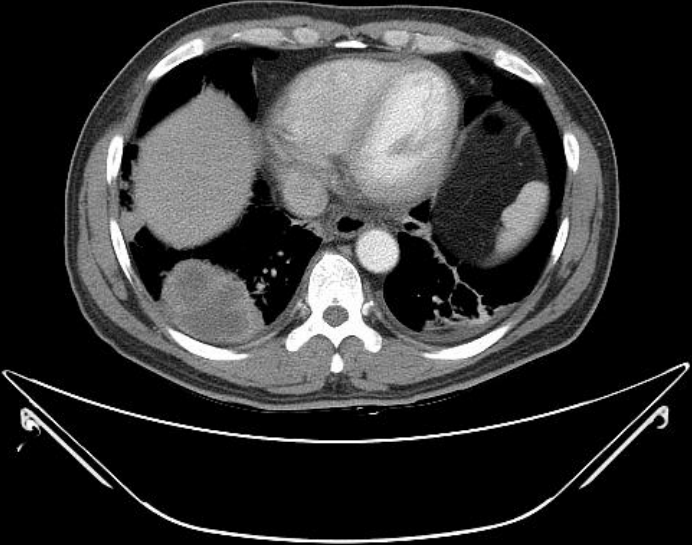
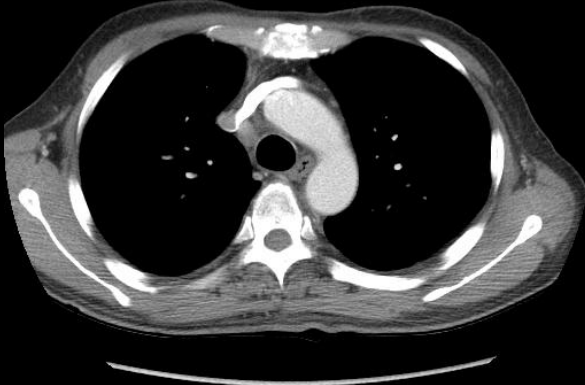
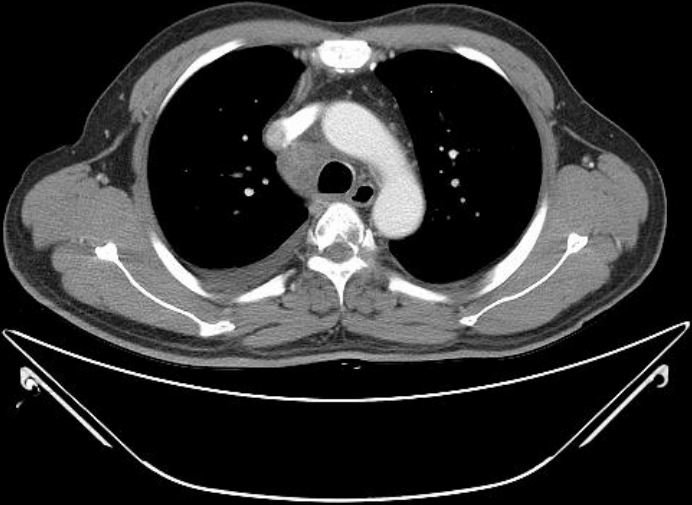
Crizotinob
1 weeks



2013.02.18



CT Findings



FDA approval

Xalkori Approval History BETA

FDA approved: Yes (First approved August 26th, 2011)

Brand name: Xalkori

Generic name: crizotinib

Company: Pfizer Inc.

Treatment for: [Non-Small Cell Lung Cancer](#)

[Xalkori](#) (crizotinib) is an oral first-in-class anaplastic lymphoma kinase (ALK) inhibitor for the treatment of patients with advanced non-small cell lung cancer (NSCLC) whose tumors are ALK-positive.

- **Crizotinib reportedly carries a \$9,600 per month price tag, plus the cost of the genetic testing from Abbott, and together this could be **prohibitive for many**.
'Financial Toxicity !'**
-

Identification of the transforming *EML4-ALK* fusion gene in non-small-cell lung cancer

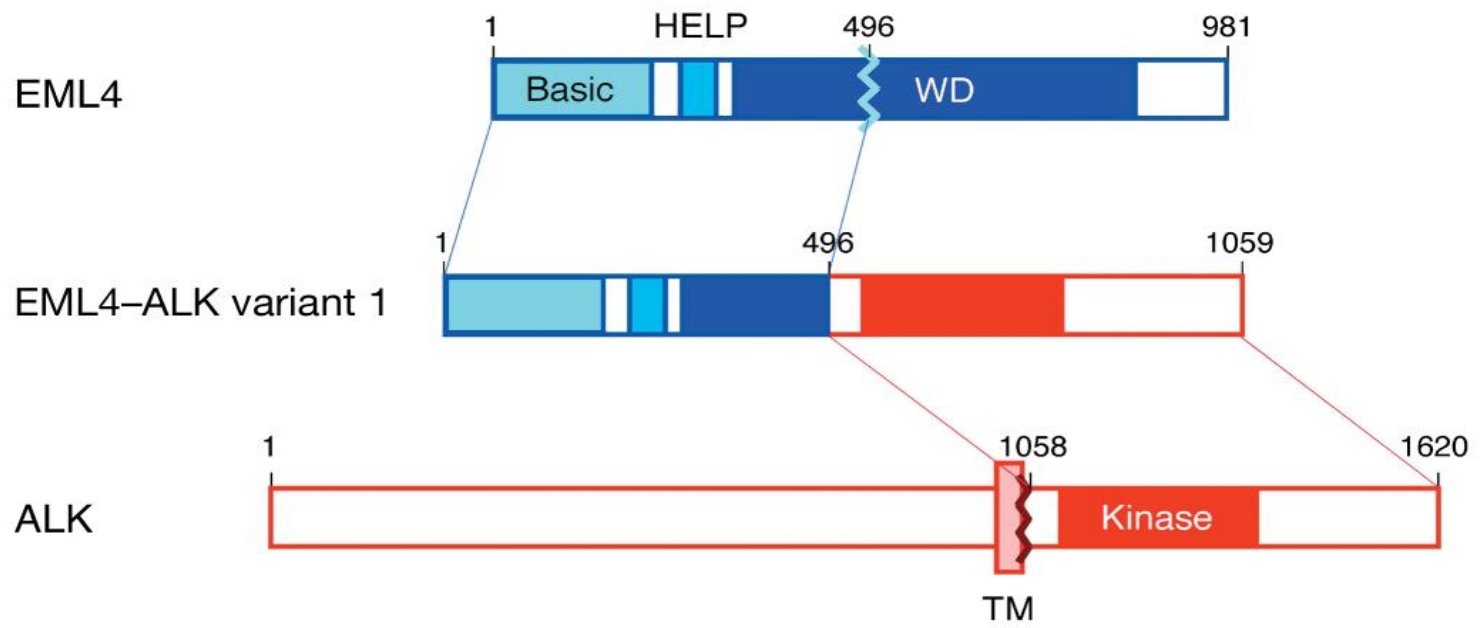
Manabu Soda^{1,2}, Young Lim Choi¹, Munehiro Enomoto^{1,2}, Shuji Takada¹, Yoshihiro Yamashita¹, Shunpei Ishikawa⁵, Shin-ichiro Fujiwara¹, Hideki Watanabe¹, Kentaro Kurashina¹, Hisashi Hatanaka¹, Masashi Bando², Shoji Ohno², Yuichi Ishikawa⁶, Hiroyuki Aburatani^{5,7}, Toshiro Niki³, Yasunori Sohara⁴, Yukihiro Sugiyama² & Hiroyuki Mano^{1,7}

NATURE | Vol 448 | 2 August 2007

EML4: echinoderm microtubule-associated protein-like 4

ALK: anaplastic lymphoma kinase

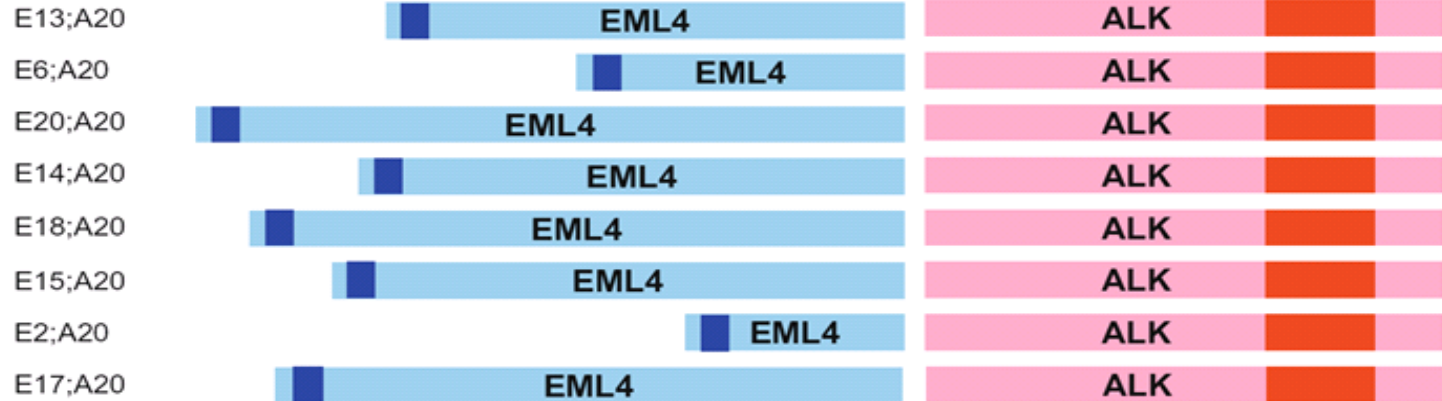
- **Detected in a 62 years old Japanese male with resected adenocarcinoma**
 - **Retroviral cDNA expression library – from RNA extracted from tumor tissue**
-



Chromosome 2p21 (EML4) --- Chromosome 2p23(ALK): separate by 12 megabp

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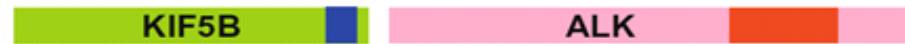
EML4-ALK



TFG-ALK

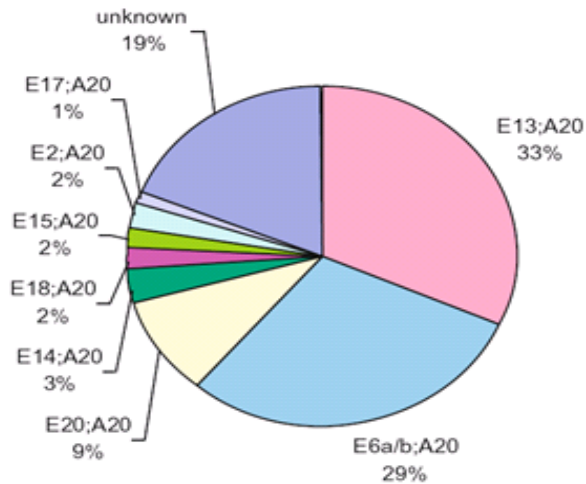


KIF5B-ALK



■ Coiled-coil domain

■ Tyrosine kinase domain



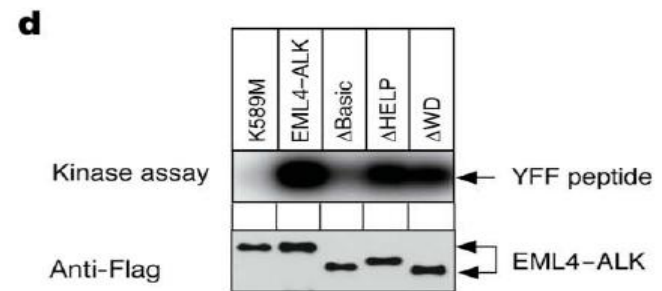
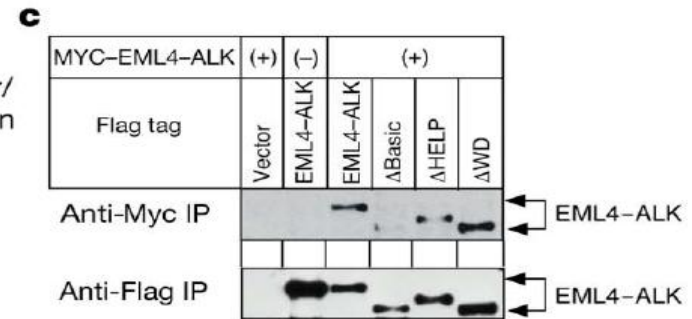
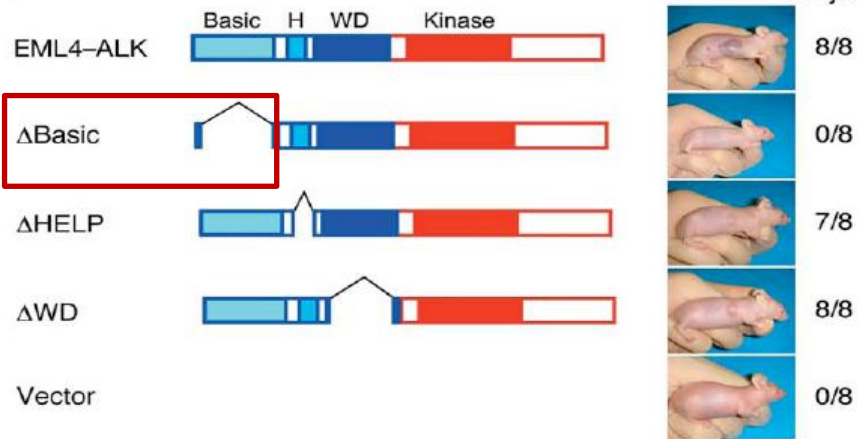
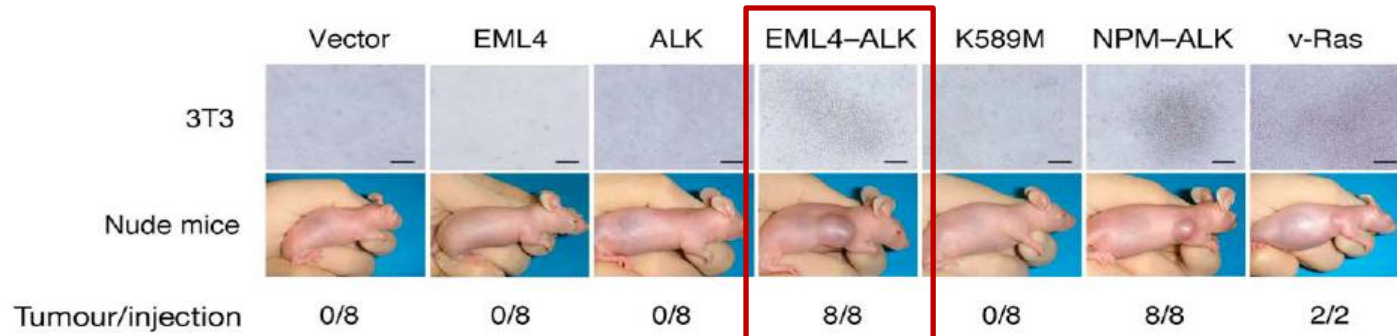
EML4-ALK Variants

E13;A20 E13;A20 (variant 1), E13;ins69 A20
 E6;A20 E6a/b;A20 (variant 3a/b)
 E20;A20 E20;A20 (variant 2), E20;ins18A20
 E14;A20 E14;ins11del49A20(variant 4'), E14;del12A20 (variant 7)
 E18;A20 E18;A20 (variant 5')
 E15;A20 E15 del19;del20A20 (variant 4)
 E2;A20 E2;A20 & E2;ins117A20 (variant 5a/b)
 E17;A20 E17;ins68A20

NSCLC Cell lines

H3122 and DFCl032 contain E13;A20. H2228 contain E6;A20

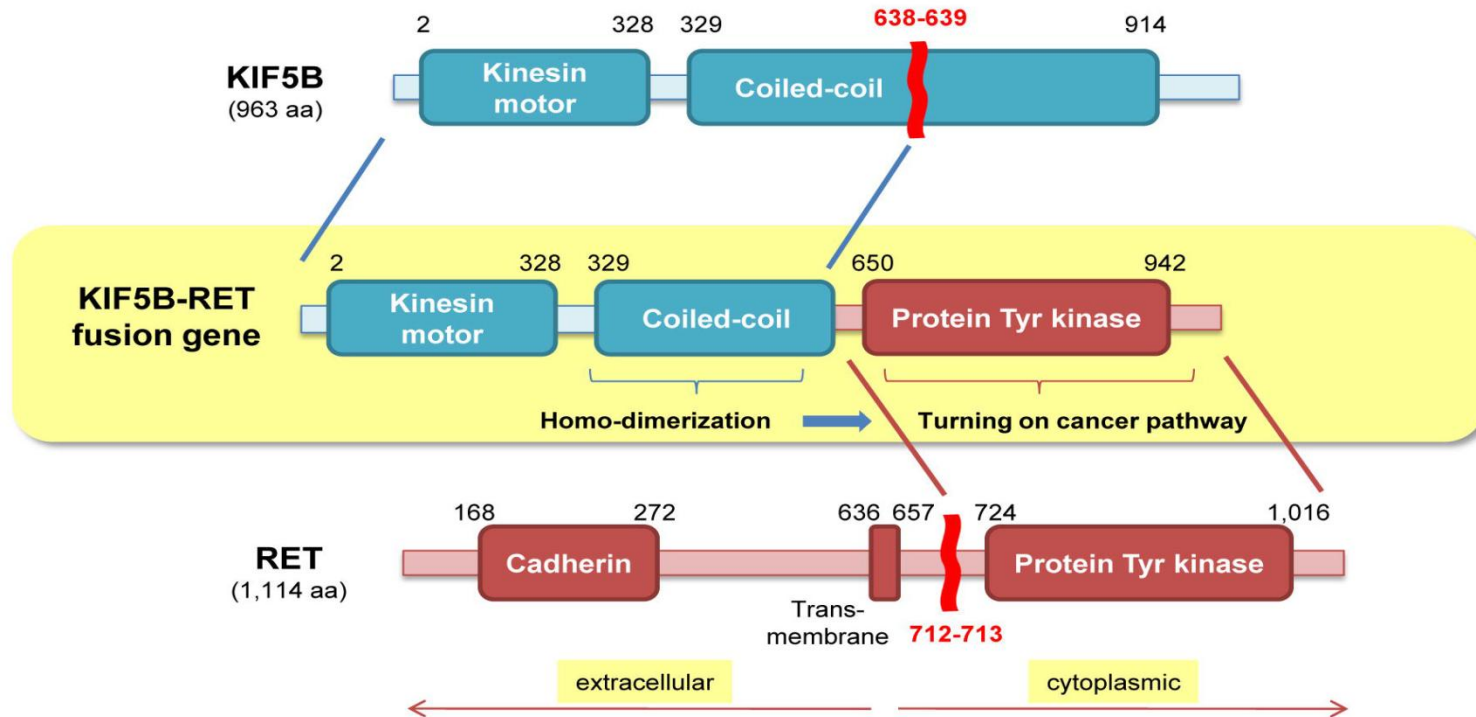
Transforming activity of EML4-ALK





Fusion of *KIF5B* and *RET* transforming gene in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing

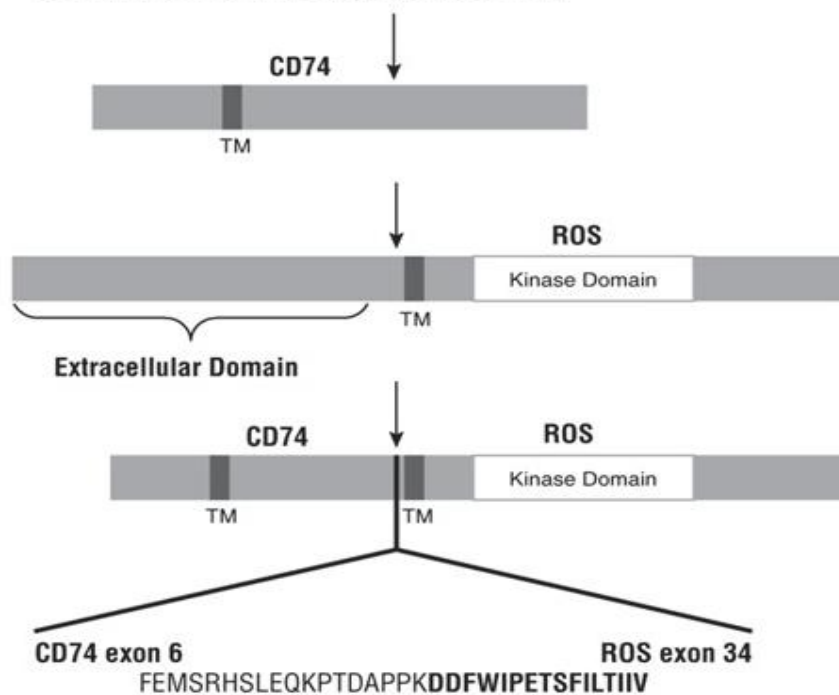
Young Seok Ju, Won-Chul Lee, Jong-Yeon Shin, et al.



ROS1 Rearrangements Define a Unique Molecular Class of Lung Cancers

Kristin Bergethon, Alice T. Shaw, Sai-Hong Ignatius Ou, Ryohei Katayama, Christine M. Lovly, Nerina T. McDonald, Pierre P. Massion, Christina Siwak-Tapp, Adriana Gonzalez, Rong Fang, Eugene J. Mark, Julie M. Batten, Haiquan Chen, Keith D. Wilner, Eunice L. Kwak, Jeffrey W. Clark, David P. Carbone, Hongbin Ji, Jeffrey A. Engelman, Mari Mino-Kenudson, William Pao, and A. John Iafrate

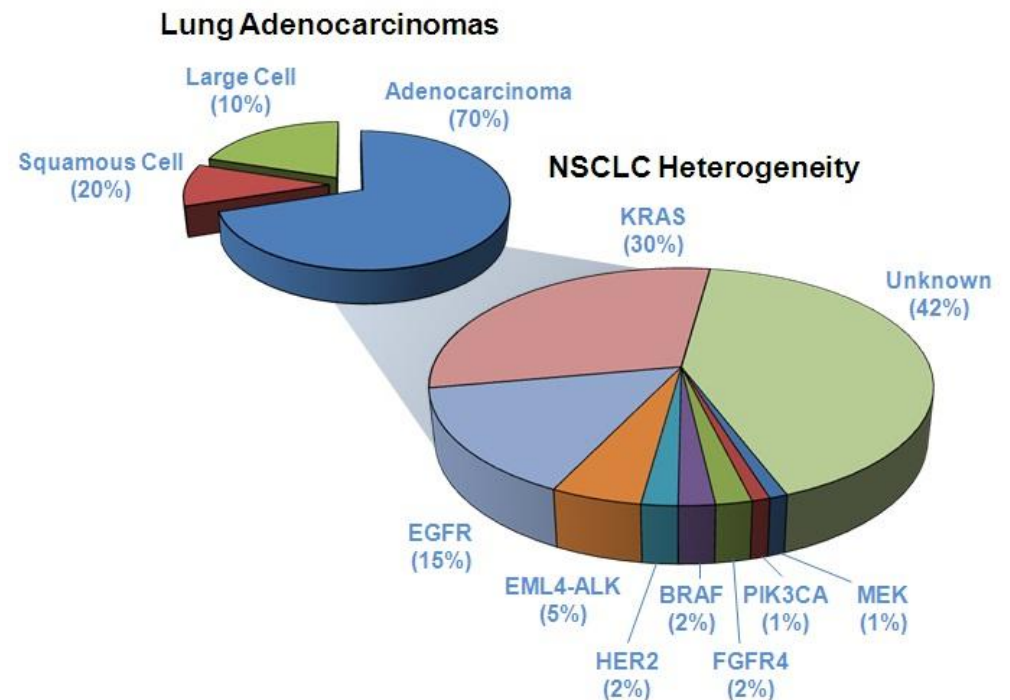
CD74-ROS Fusion in patient CS042



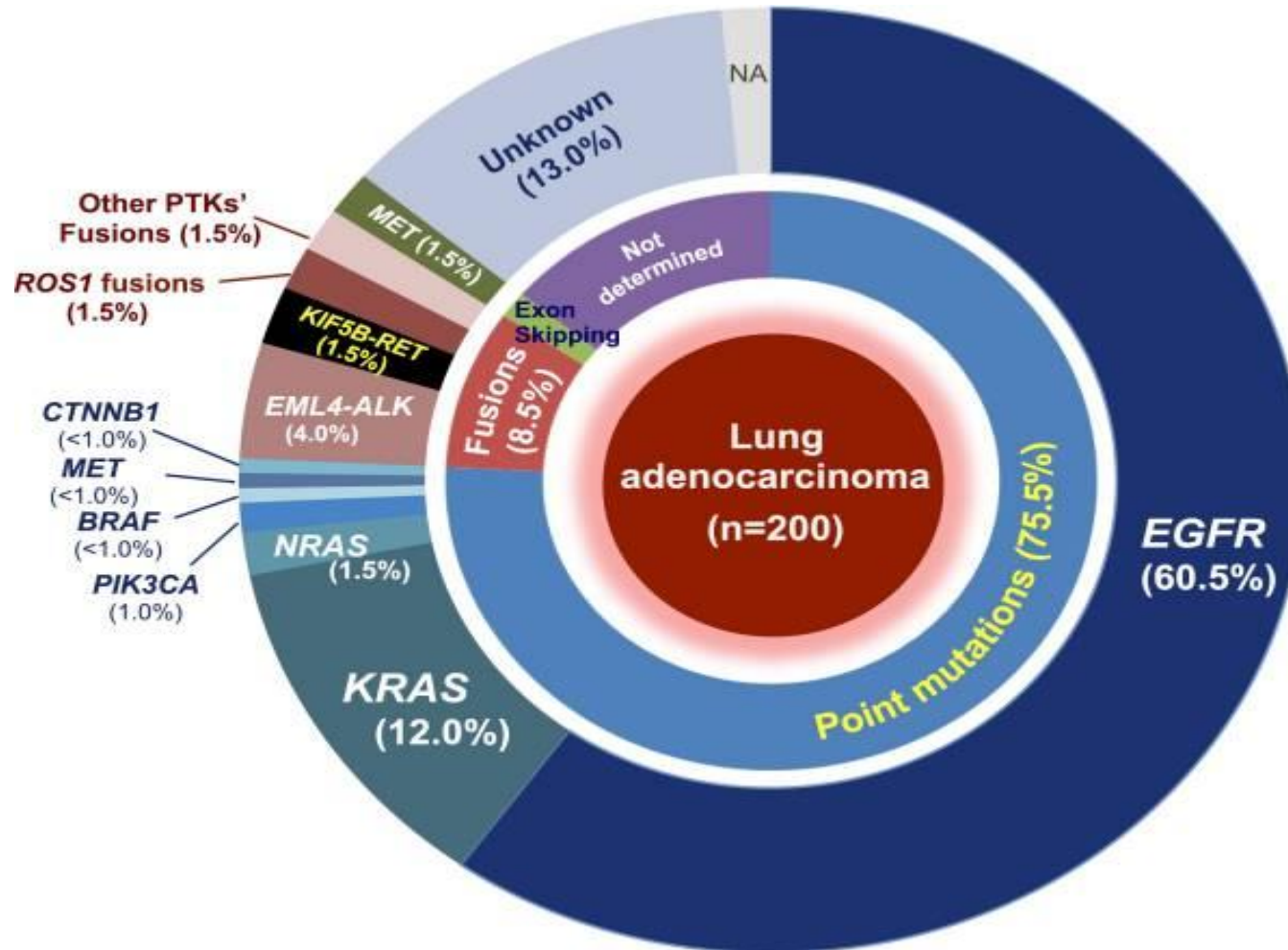
RET, ROS1 and ALK fusions in lung cancer

Takeuchi K, Soda M, Togashi Y, et al.

- in 1,529 lung cancers
- 44 ALK-fusion-positive
- 13 ROS1-fusion-positive
- 14 RET-fusion-positive



Mutation/Fusion in Korean Adenocarcinoma



Female: 109 (54.5%)
Never-smoker: 116 (58.0%)

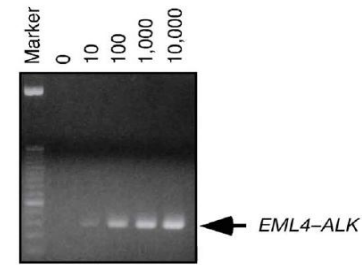
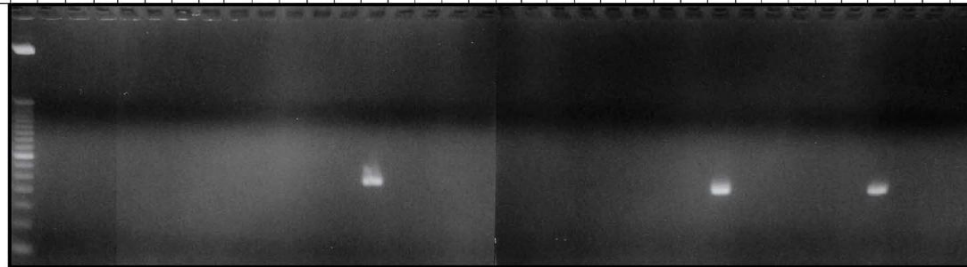
Diagnosis of EML4-ALK

- **RT-PCR**
 - **FISH (Fluorescent in situ hybridization)**
 - **IHC**
-

RT-PCR

- Rapid, Sensitive
- Need multiplex RT-PCR
- Formalin fixed paraffin embedded tissue: difficult for RT
- False positive?

Sex	F	M	M	M	F	M	M	F	F	F	M	M	M	F	M	M	M	M	M	M	M	M	M	M	F	M	F	F	M	F	M	F	M			
Pathology		S	S	A	S	A	A	A	A	S	S	A	A	B	S	A	A	S	S	A	S	A	S	A	A	A	A	S	A	A	A	S				
EGFR mutation																																				
KRAS mutation																																				
Smoking habit		○	○	○	○	○	○			○	○	○			○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○				
Patient	Marker	NTC	46,XX	2	6	7	12	13	14	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42



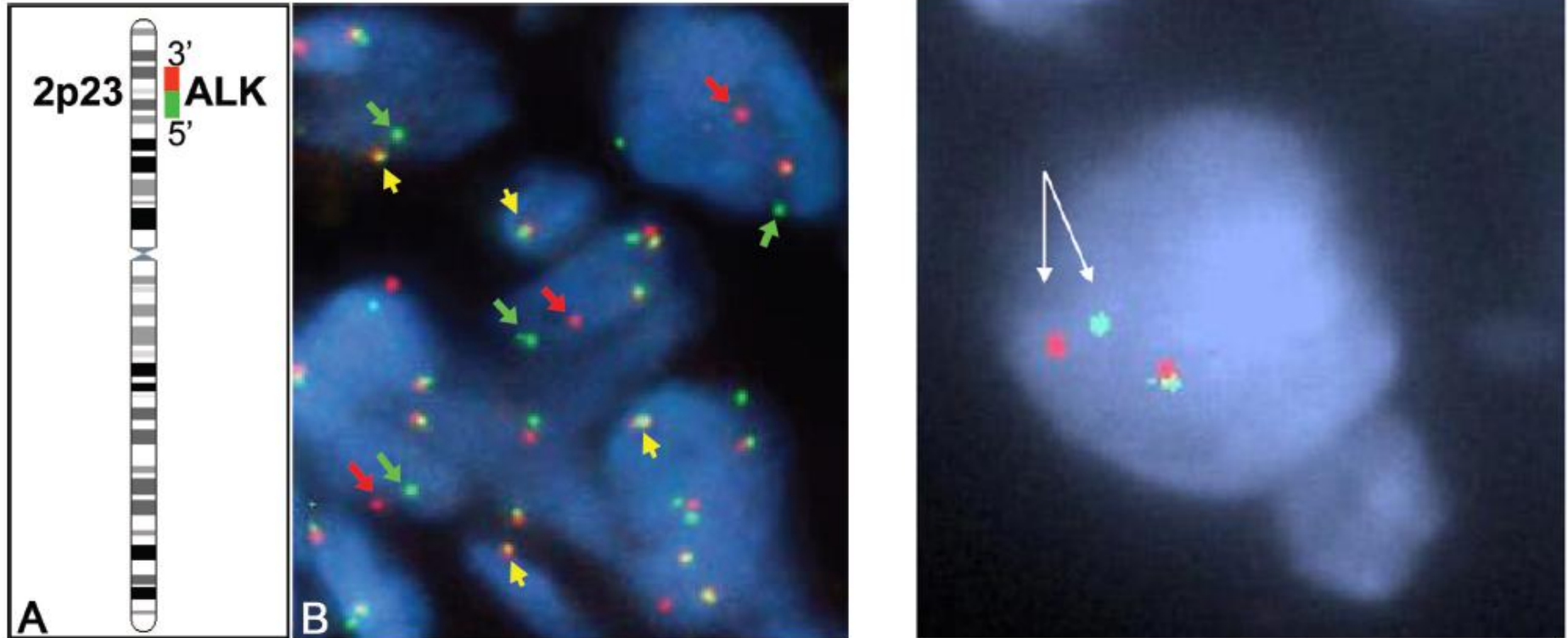
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FISH

- Vysis ALK Break Apart FISH Probe Kit (Abbott Molecular, Inc.)
- Approved by FDA concurrently with crizotinib.
- Less than \$250 per patient



FISH in an EML4-ALK positive tumor



A split of red and green probes

Cannot distinguish between the different EML4-ALK fusion variants

Positive criteria

- Split; ≥ 2 signal diameters
- Negative; $< 5/50$ or $< 10\%$
- Positive; $> 25/50$ or 50%
- Second reader on 10-50%
 - Positive; $> 15/100$ ($> 15\%$)

No. of Adjacent or Fused Signals	No. of Single Orange Signals	No. of Single Green Signals	Cell Classification
≥ 1	0	0	Negative
≥ 1	0	≥ 1	Negative
≥ 0	≥ 1	≥ 1	Positive
≥ 1	≥ 1	0	Positive

Scientific
background ?

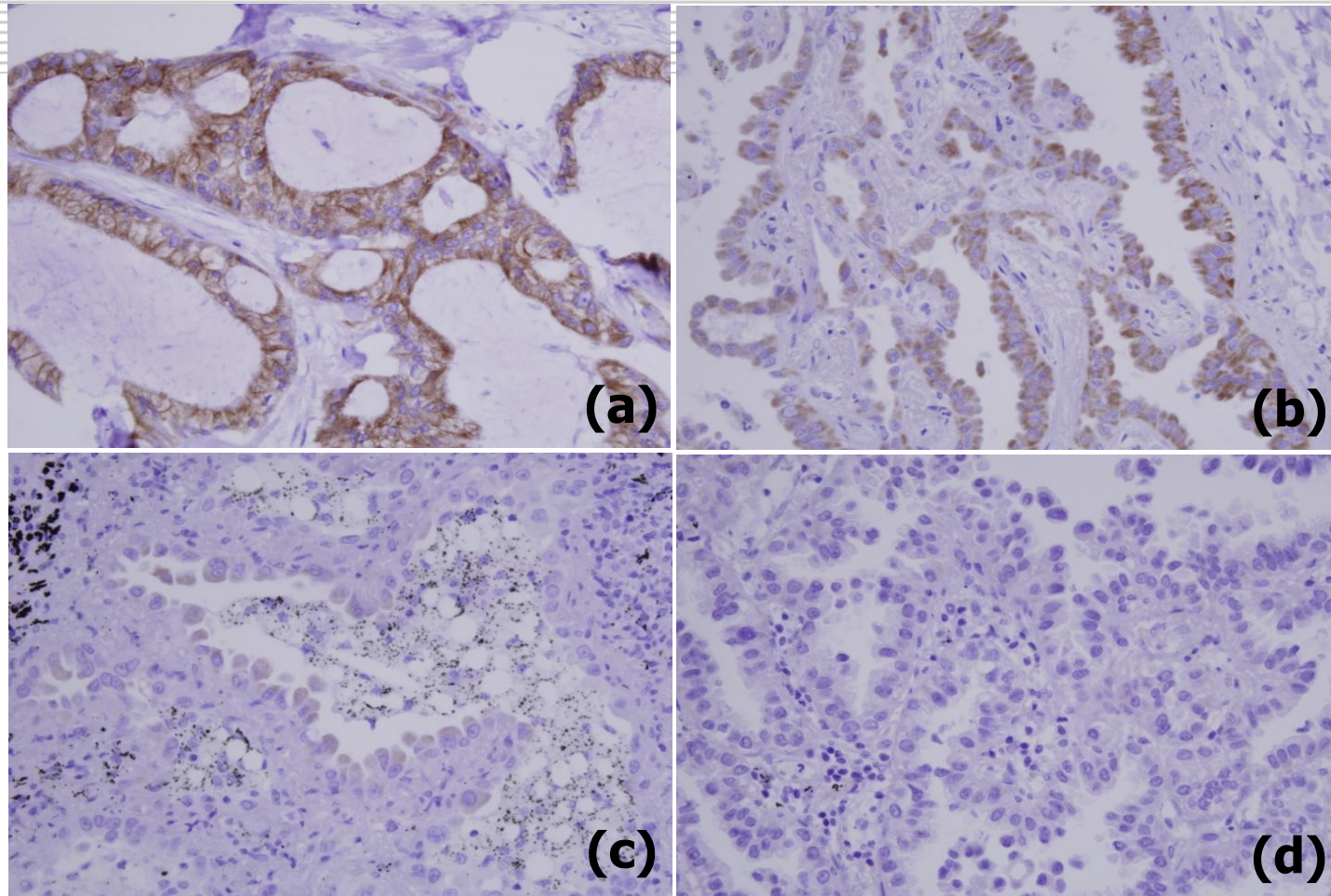
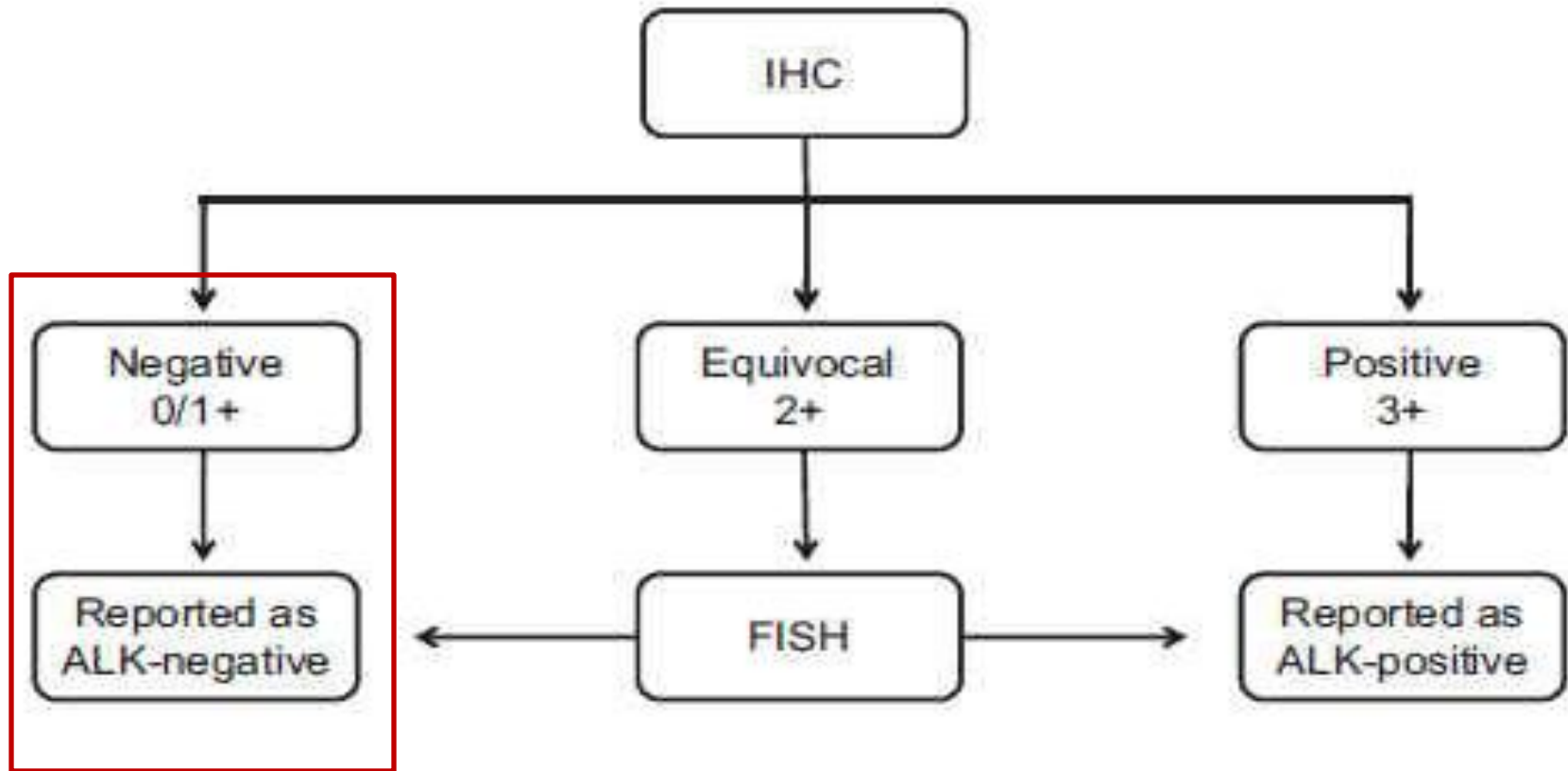


Figure 1. Immunohistochemical staining patterns for ALK by three grading system. (a) Diffuse staining with strong intensity (+++), (b) diffuse staining pattern with moderate or weak intensity (++), (c) focal staining with weak intensity (+), and (d) no staining (-).

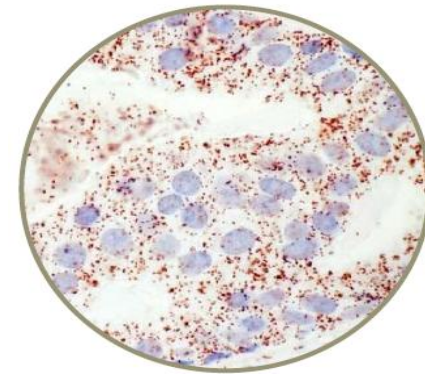
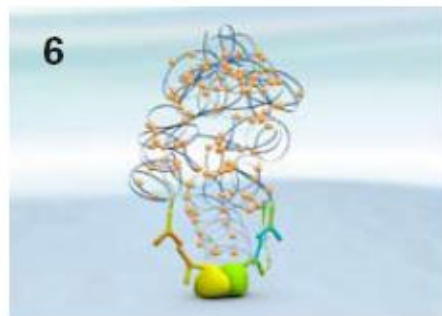
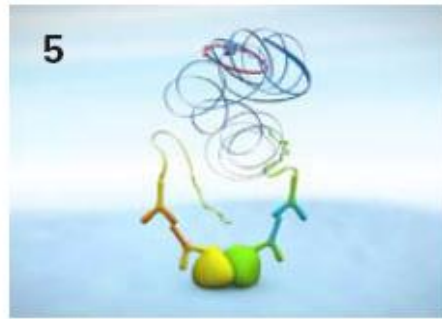
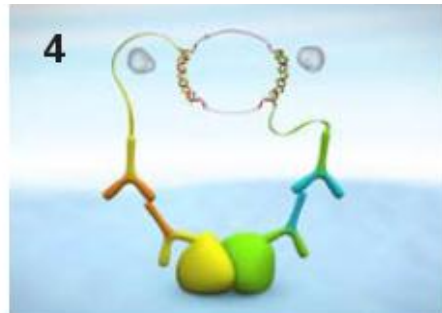
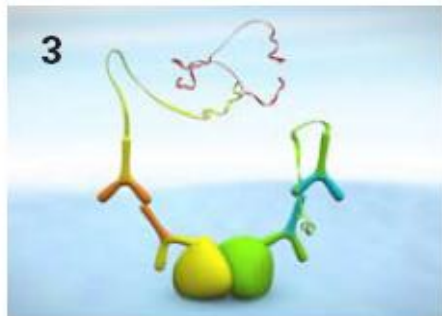
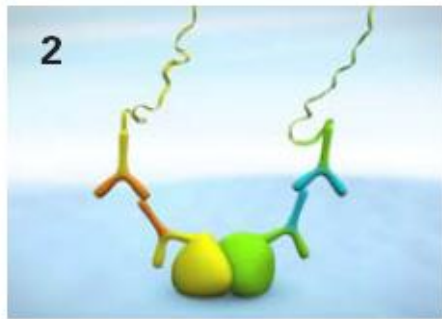
IHC grade	EML4-ALK FISH		Total
	Positive	Negative	
0	1	338	339
1	4	19	23
2	5	7	12
3	13	2	15
Total	23	366	389

Exact Cochran-Armitage trend test : $p < 0.001$

ALK IHC vs FISH

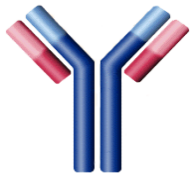
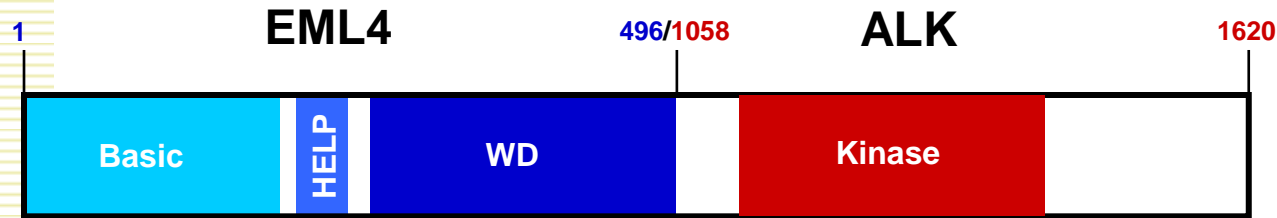


Duo-links®



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Antibodies for Duo-links®



EML4 antibody

epitope: 1-63 a.a of EML4

Abcam (Ab57297), Mouse

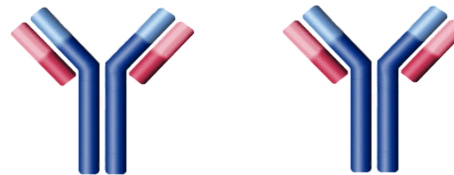
Recommended dilutions

WB: Use at a concentration of 1-5 µg/ml.

IF/ICC: Use at a concentration of 1-5 µg/ml.

IHC-P: Use at a concentration of 1-5 µg/ml.

Cellular localization: Cytoplasmic



p-ALK (Tyr1604) antibody

Novus (NB110-55453), Rabbit

Recommended dilutions

IHC: 1:100-1:250, IP: 1:20, IB: 1:5000

ALK antibody

epitope: C-terminus of ALK

Novus (NB120-4061), Rabbit

Recommended dilutions

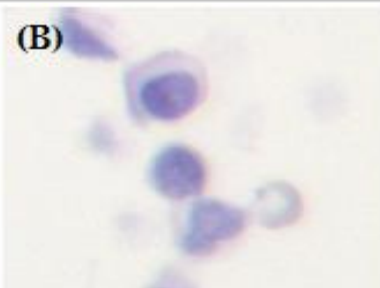
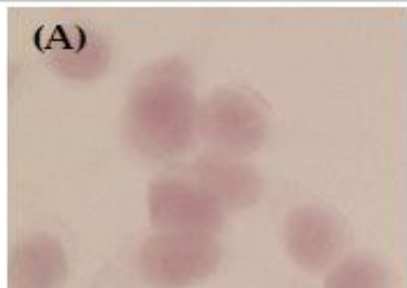
IHC, IHC-P, IHC-F: 1:50-1:100

Cellular localization: cell Membrane

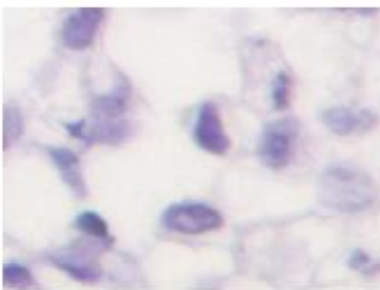
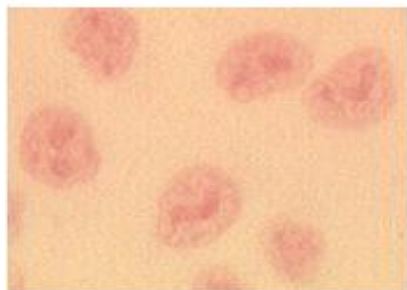
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Slide-mounted

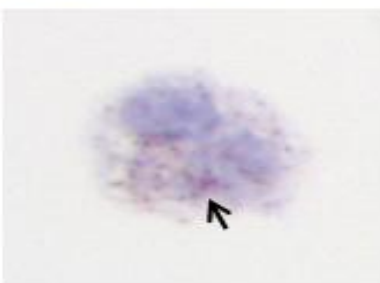
Paraffin-embedded



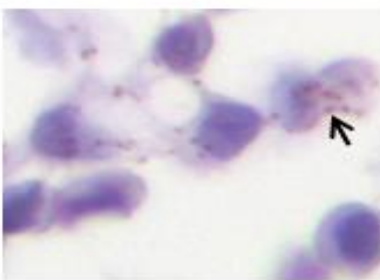
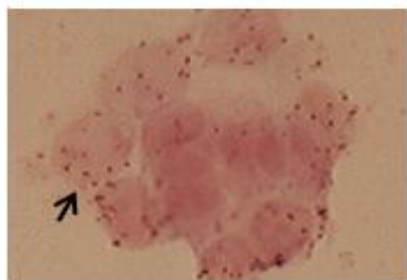
PC-9



H460



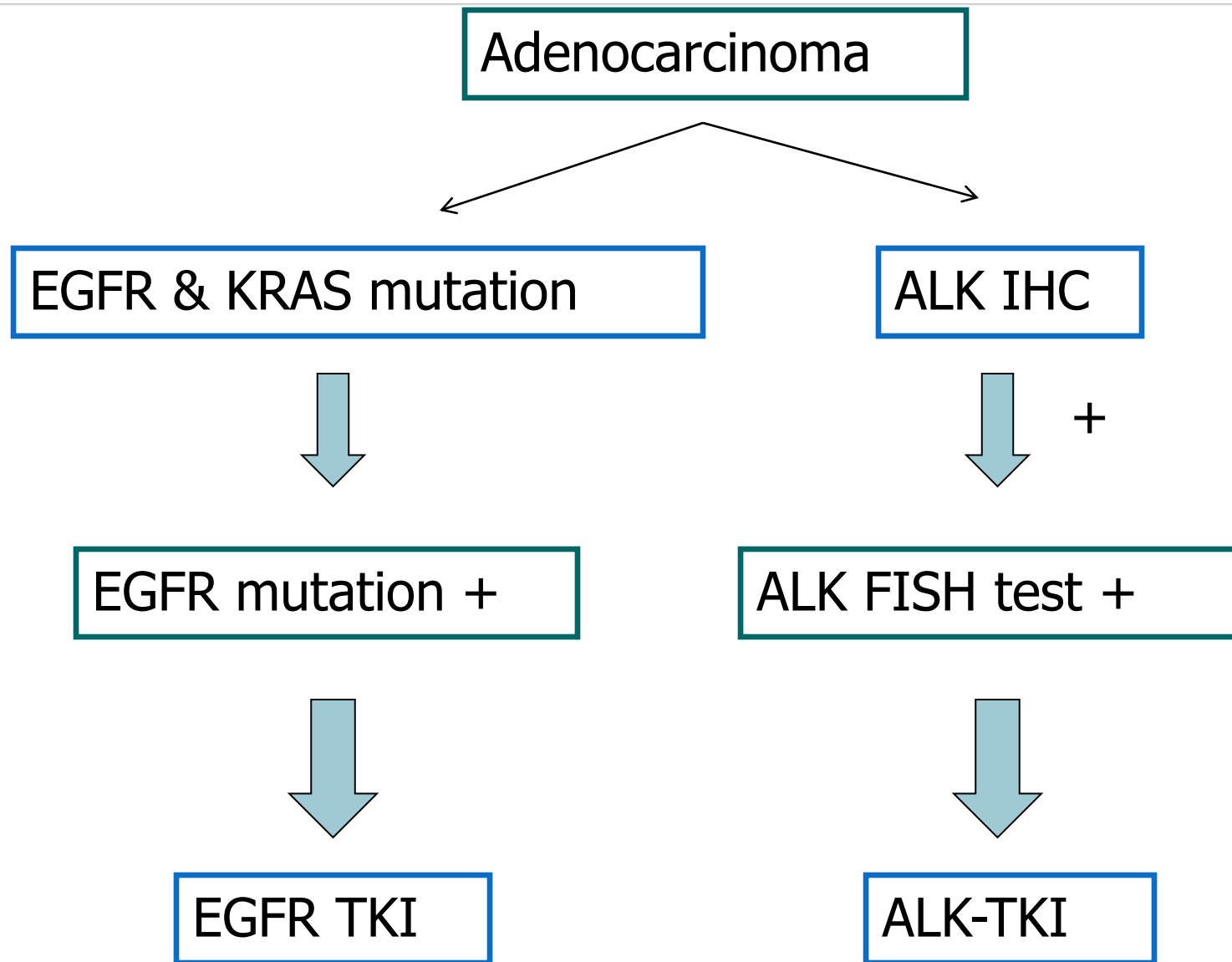
H2228 (V3)



H3122 (V1)

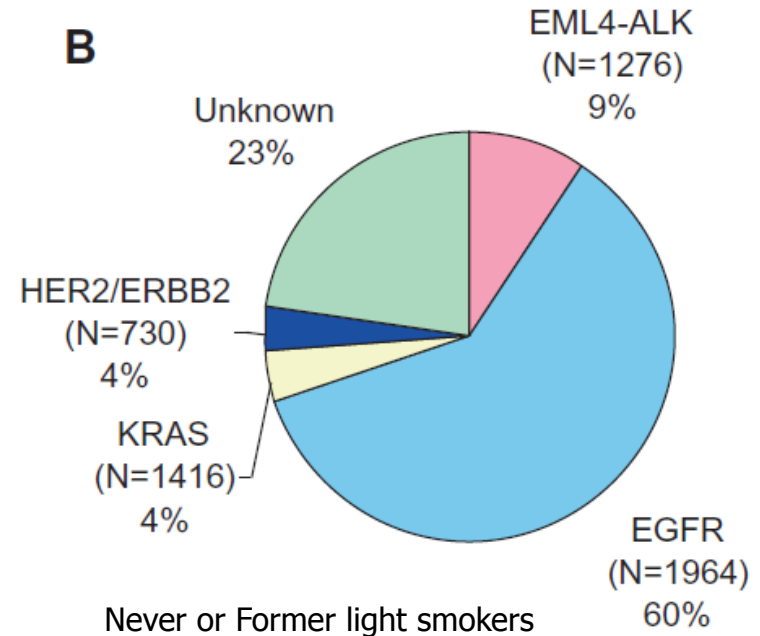
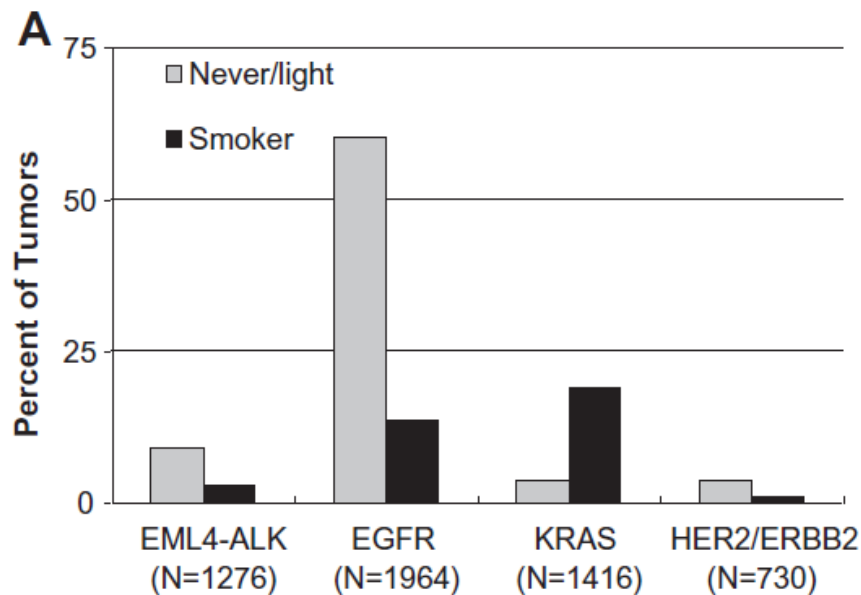
**ALK-
Cell lines**

**ALK+
Cell lines**



Clinical Features of EML4-ALK

- Unique clinical subgroup of NSCLC patients
- EML4-ALK and EGFR mutations are mutually exclusive.
- Occur in younger patients
- More advanced NSCLC



Mutually exclusive with EGFR, KRAS

Table 3. Mutation Analysis of Screened Patients With Non-Small-Cell Lung Cancer

Analysis	Genotype		
	<i>ALK</i>	<i>EGFR</i>	WT/WT
<i>ALK</i> rearrangement			
Positive	19	0	0
Total	19	31	91
<i>EGFR</i> mutation			
Positive	0	31	0
Total	19	31	74
<i>KRAS</i> mutation*			
Positive	0	0	6
Total	11	10	23

Abbreviation: WT, wild type.

**KRAS* mutation testing was not performed on all patients because of limited amounts of tissue.

Age/Sex

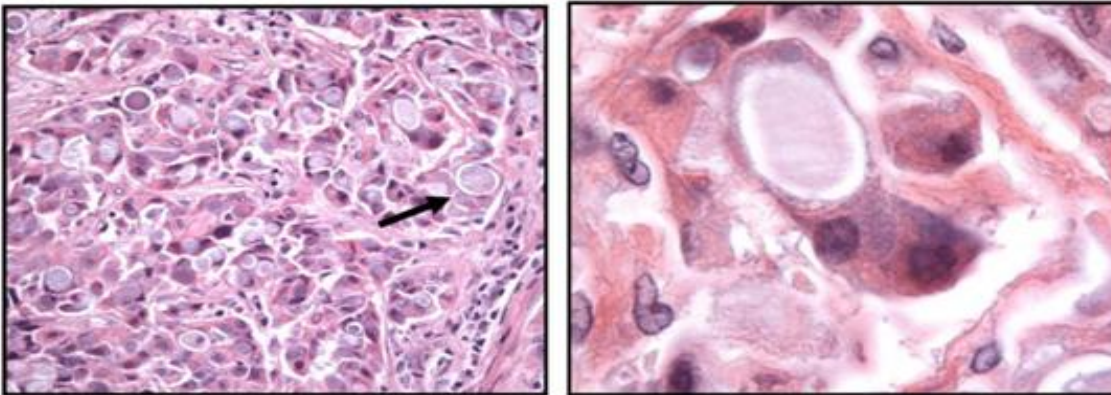
Characteristic	Genotype					
	<i>ALK</i> (n = 19)		<i>EGFR</i> (n = 31)		WT/WT (n = 91)	
	No.	%	No.	%	No.	%
Age, years						
Median	52		66		64	
Range	29-76		36-90		29-87	
Sex						
Male	11	58	8	26	29	32
Female	8	42	23	74	62	68

- Younger
- Not female-dominant

.....

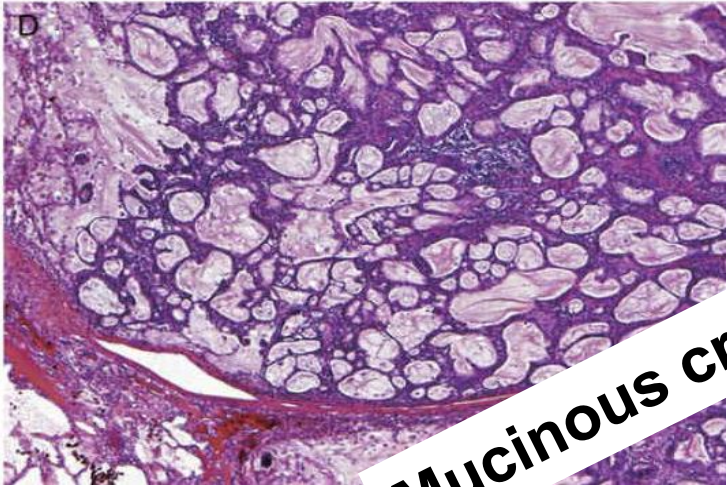
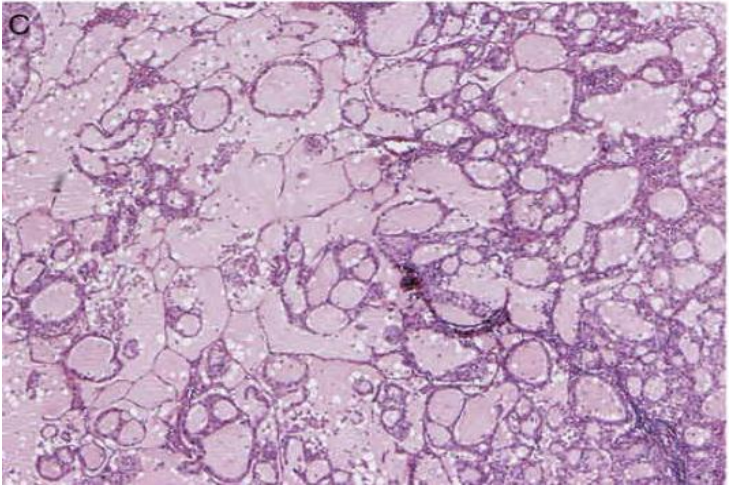
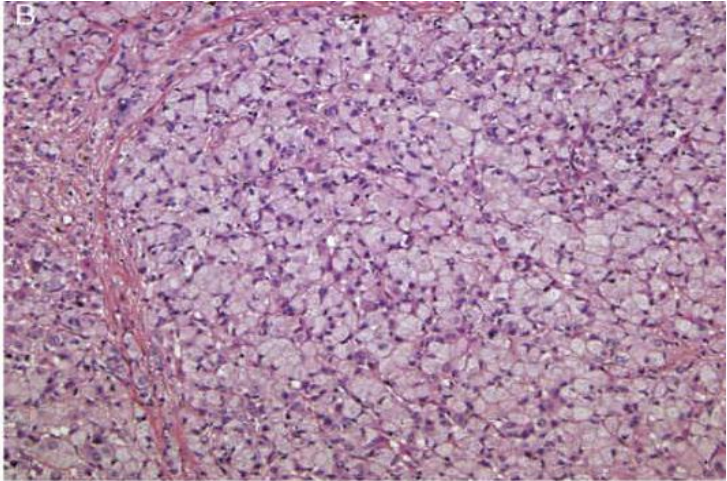
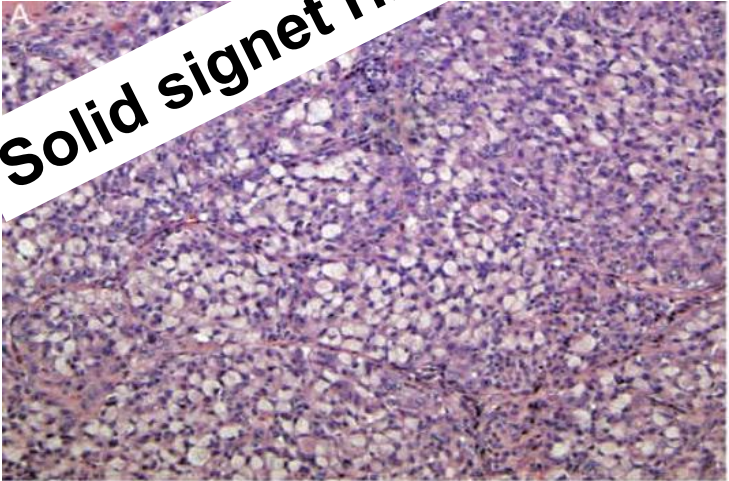
Pathology

- adenocarcinoma including acinar to solid type, mostly signet-ring cell nest with mucin
- squamous and mucoepidermoid: rarely found
- acinar adenocarcinoma: Asian patients
- signet ring cell : Western patients



Two common patterns

Solid signet ring cell

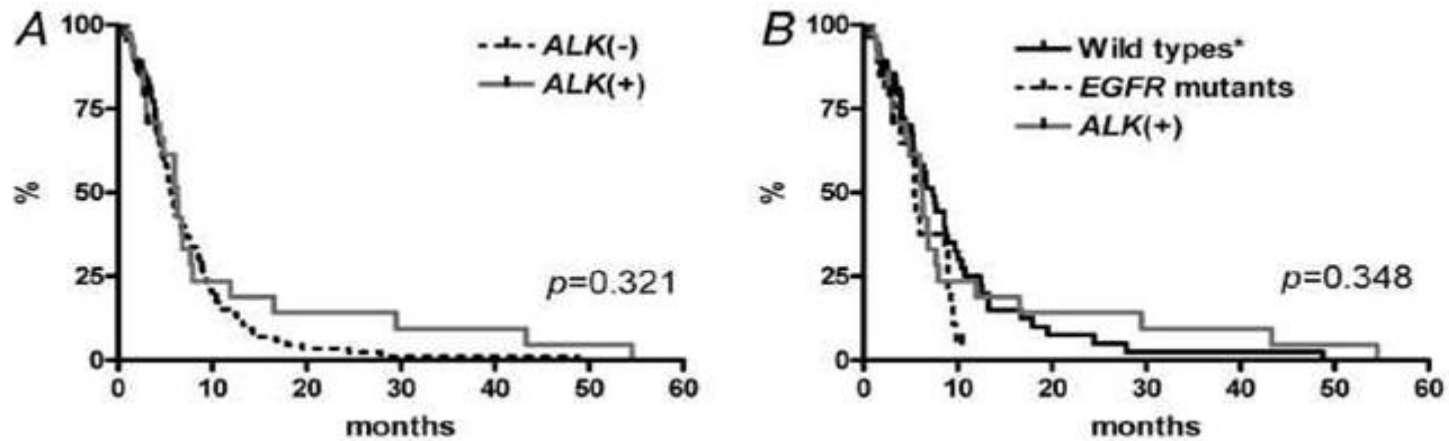


Mucinous cribriform



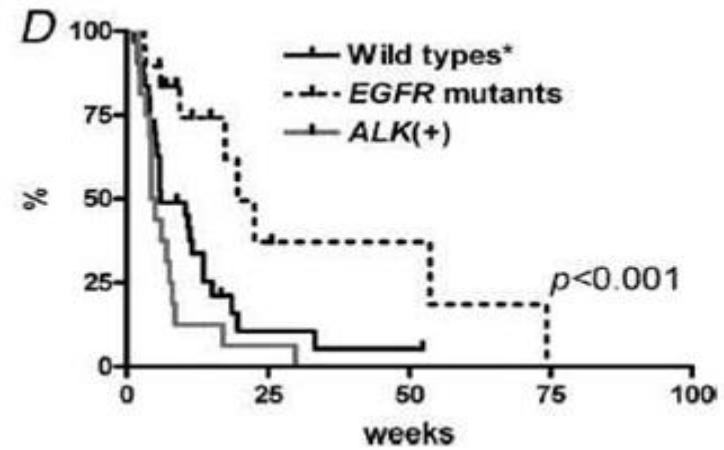
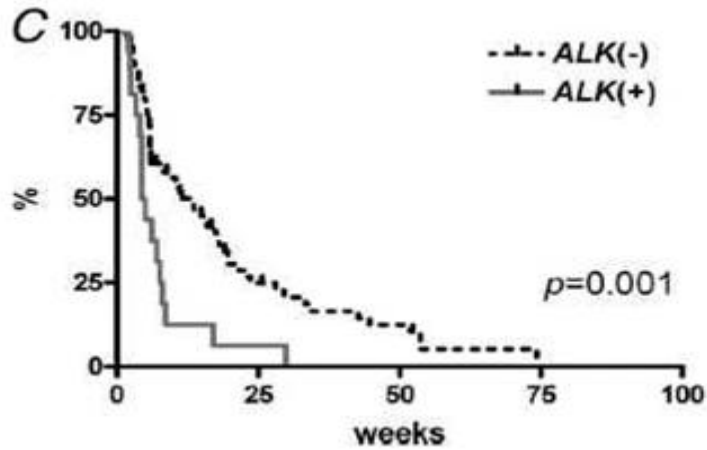
Treatment of ALK+ lung cancer

Platinum-based chemotherapy



similar to other types of NSCLC

EGFR-TKI

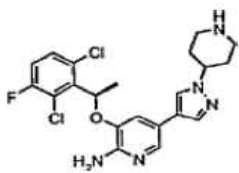
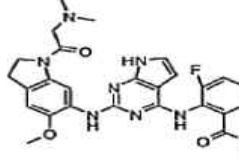
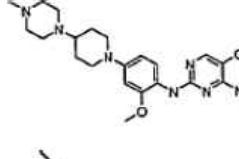
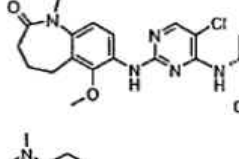
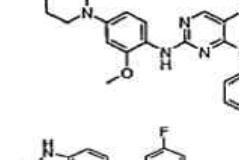
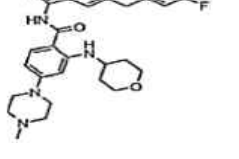


worse outcome

Effective drugs

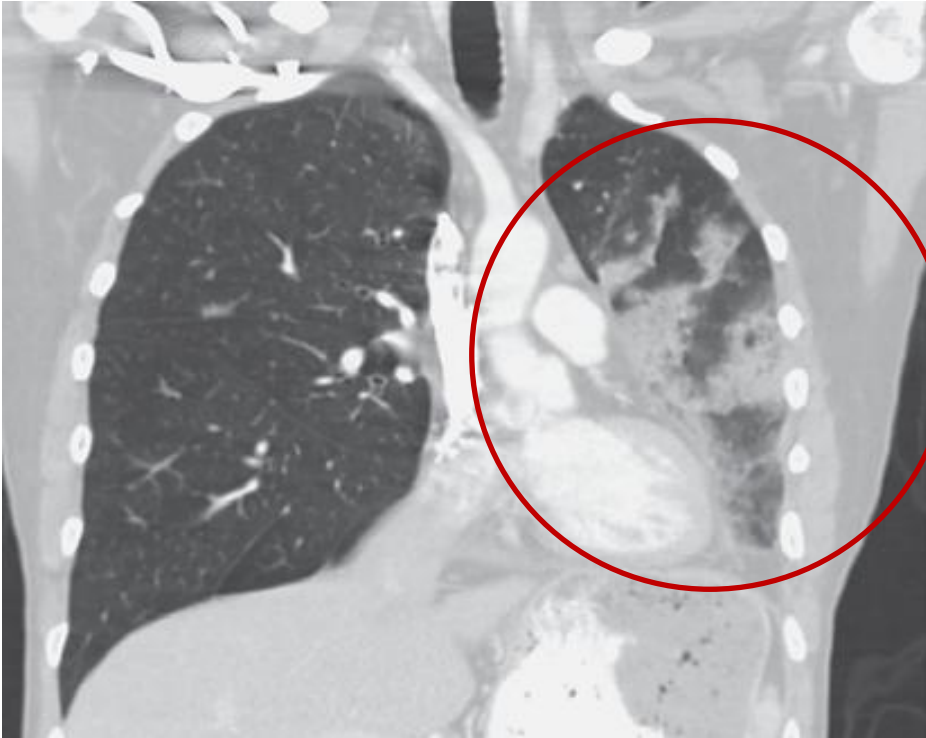
- **ALK inhibitors**
 - **HSP90 inhibitors**
-

ALK inhibitors

Compound	Biochemical structure	IC ₅₀ (nmol/L)	Phase of development	Other targets
<u>Crizotinib</u>		24	Phase III	C-MET
GSK1838705A		0.5	Preclinical	IGF-1R
<u>TAE684</u>		<10	Preclinical	IR
CEP-14083		11	Preclinical	IR
<u>AP26113</u>		3.2	Preclinical	IGF-1R
NMS-E628		55	Preclinical	IGF-1R, Aurora kinase-2
...				

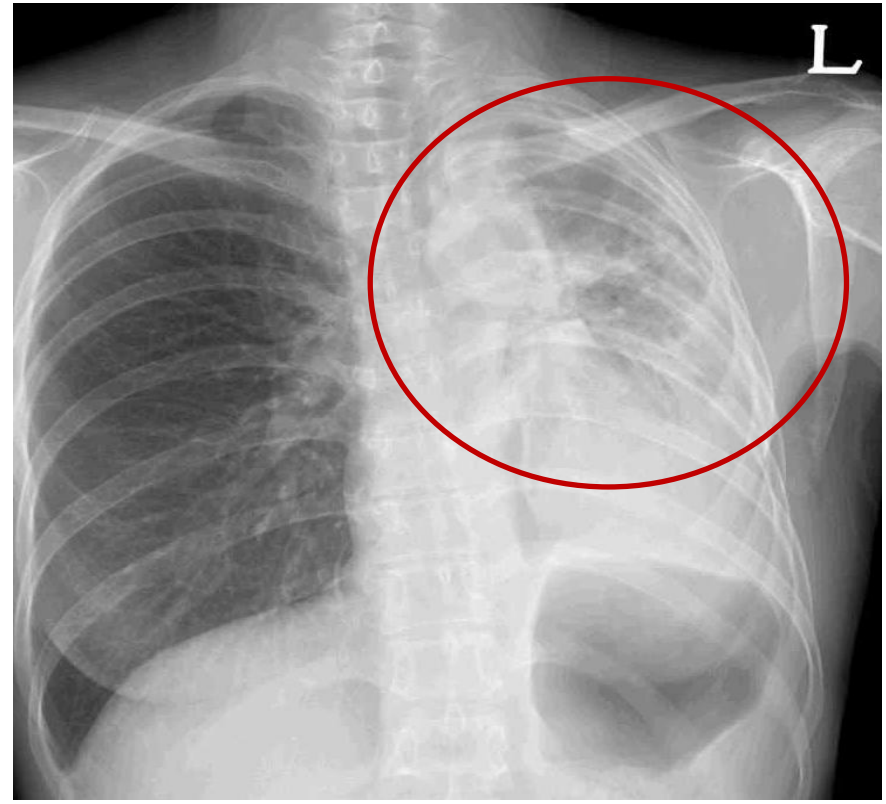
} 2nd generation

Effect of crizotinib



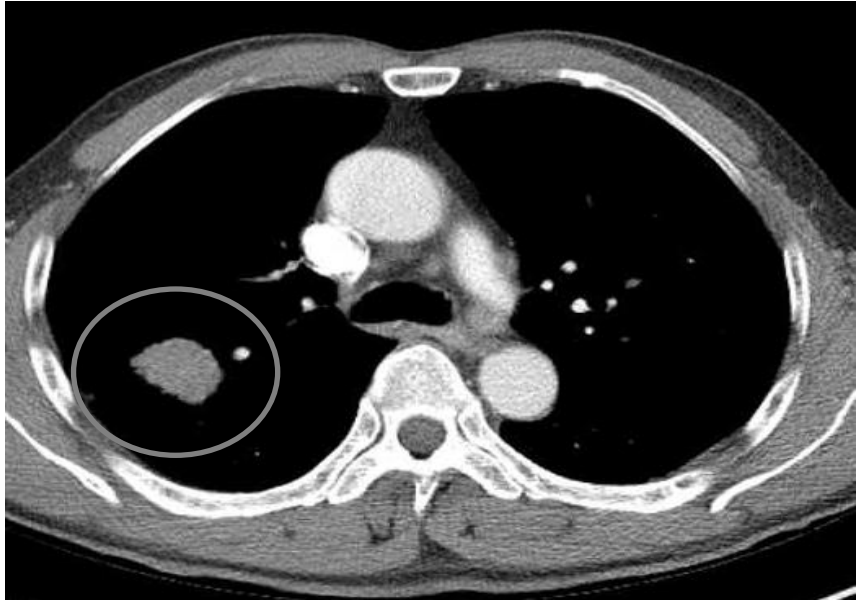
AFTER 2 CYCLES

F/34, adenocarcinoma, non-smoker

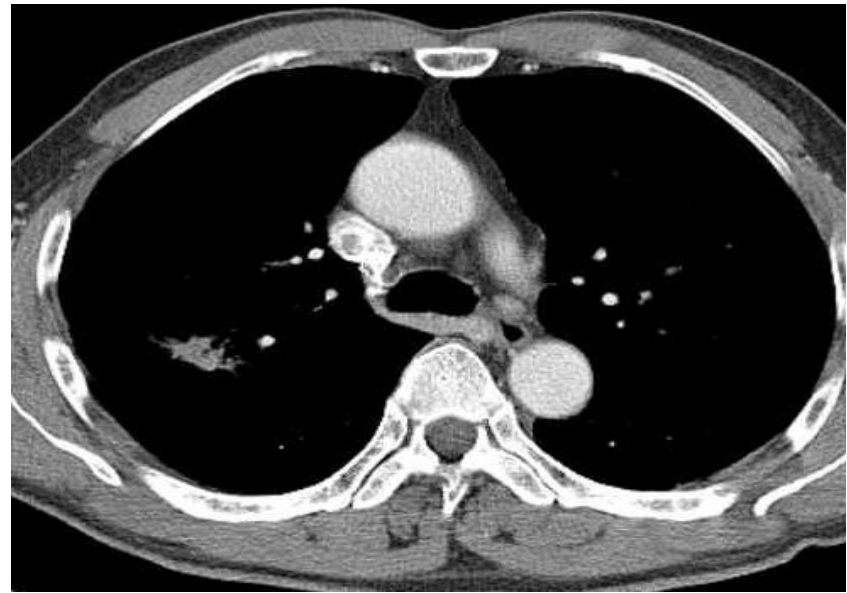


1 MONTH LATER

M/55, adenocarcinoma, smoker



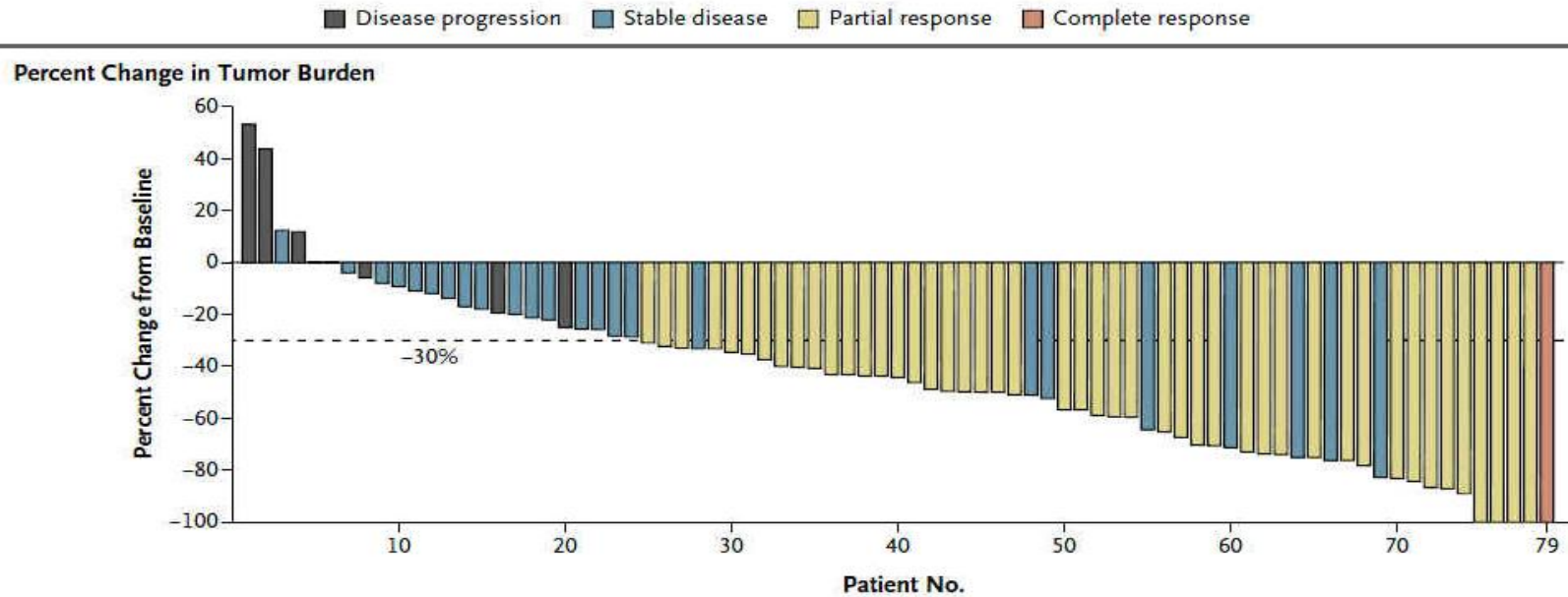
Jun-2011



Aug-2011

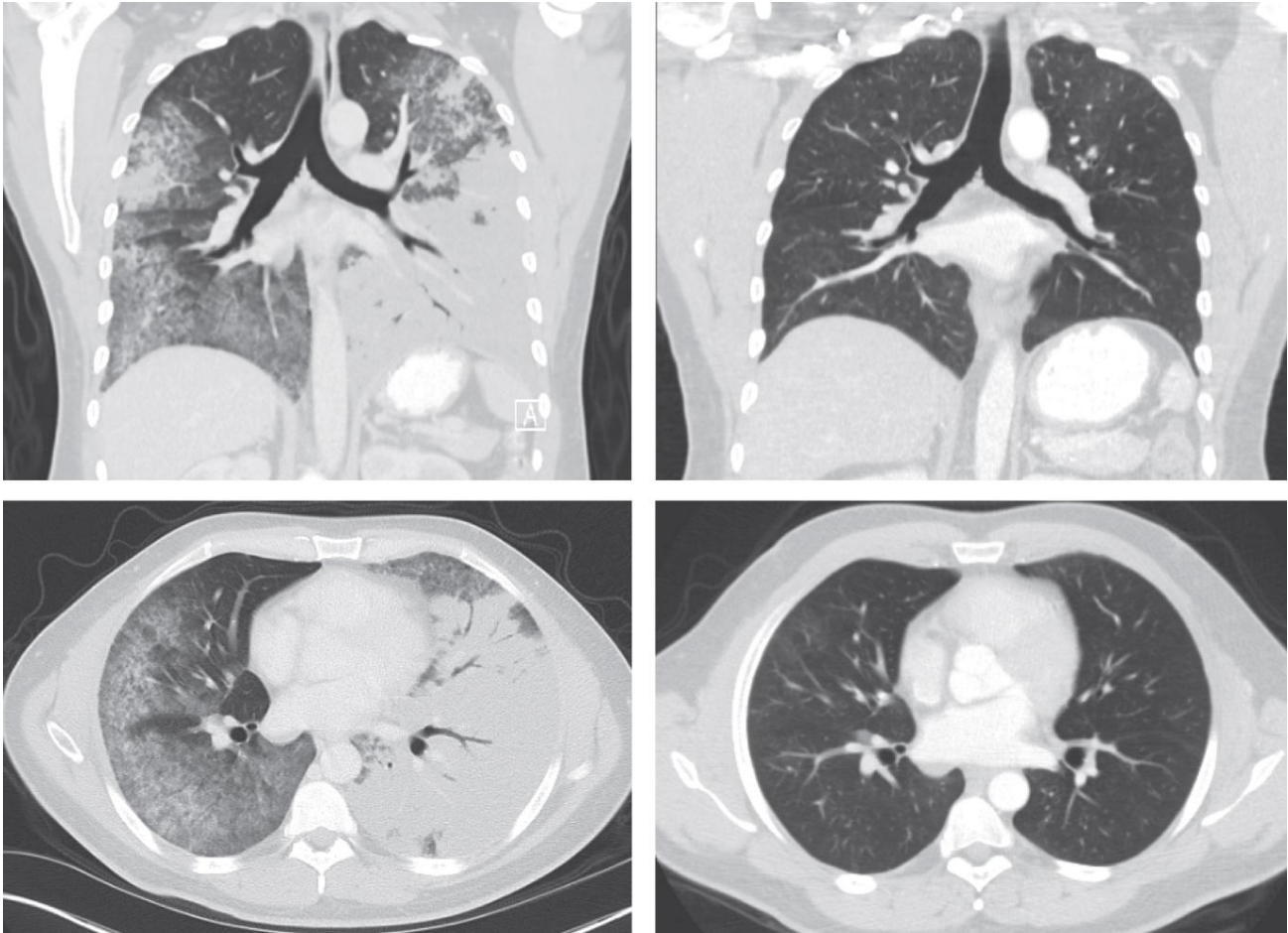
.....

Results of a clinical trial with crizotinib

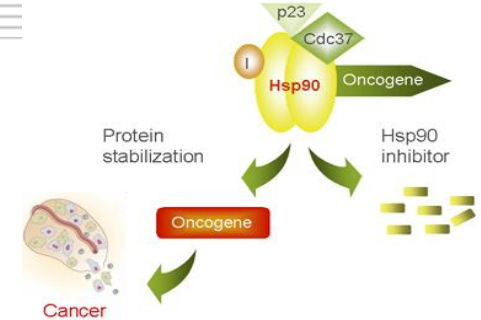


- Response rate; 57% (47/82)
- Stable; 33% (27/82)
- 6-month PFS; 72%

Crizotinib; also active to ROS1

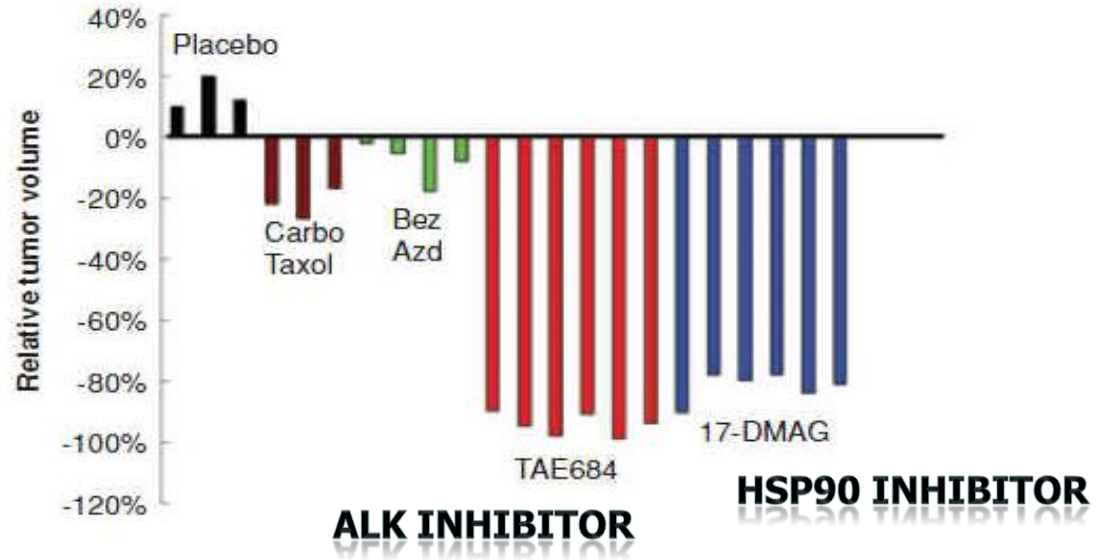
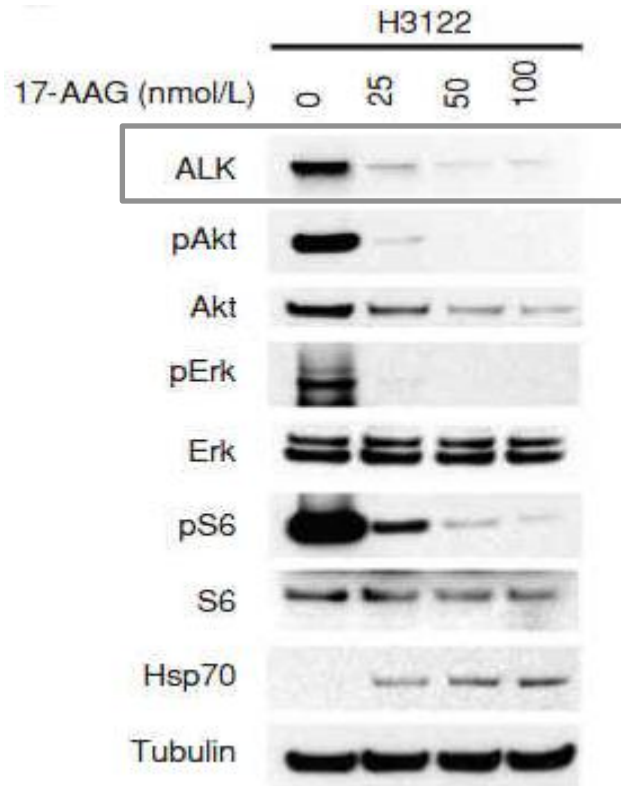


HSP90

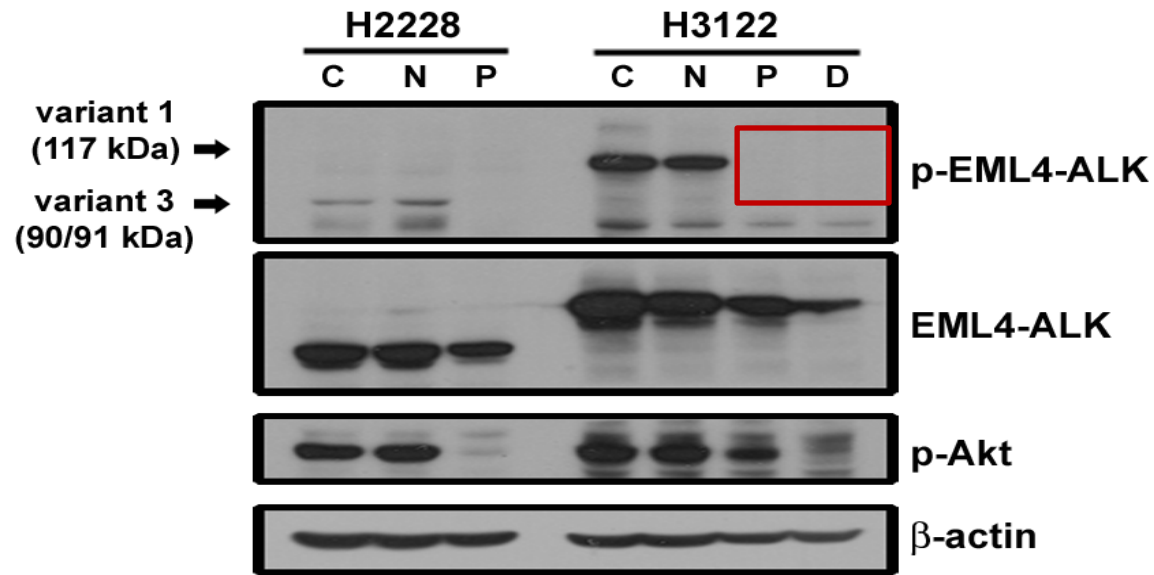


- A cellular chaperone for conformational maturation and stability of more than 200 client proteins
 - Oncoproteins; Her2 in breast cancer, c-KIT in GIST, Mutant EGFR, **EML4-ALK** in NSCLC, Bcr-Abl in CML
 - Hierarchy of client sensitivity; HER2 > mutant EGFR > Raf-1 > Akt > mutant BRAF > wild-type EGFR
-

Effect of HSP90 inhibitor



ALK modulation



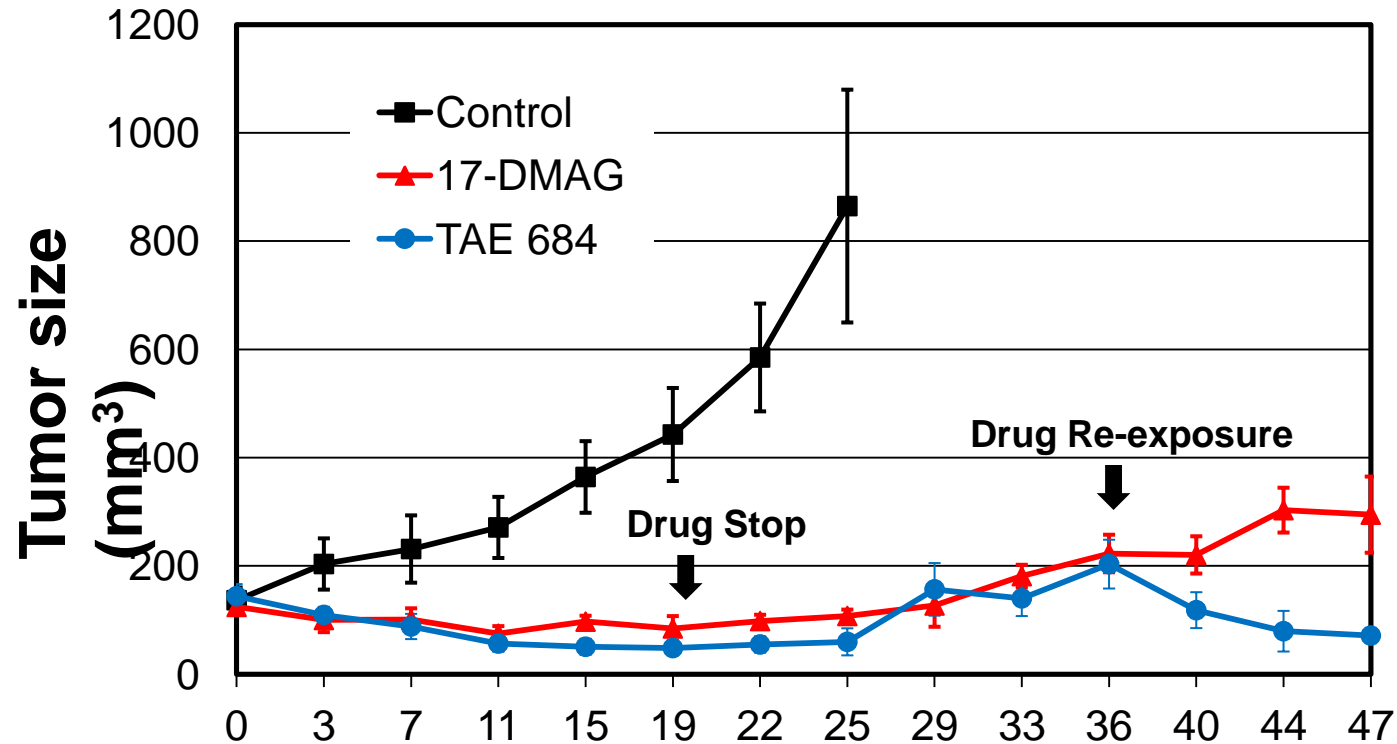
C, control; N, NPS-1034; P, PF-02341066; D, 17-DMAG

Dose: 1 μ M

Time: 24 hr

Crizotinib *HSP90 inhibitor*

Effect on xenograft model



17-DMAG: 10mg/kg, daily (5 day/week), IP
TAE 684: 15 mg/kg, daily (5 day/week), Oral



AUY922A2206 Korean IM

Renaissance Hotel, Seoul

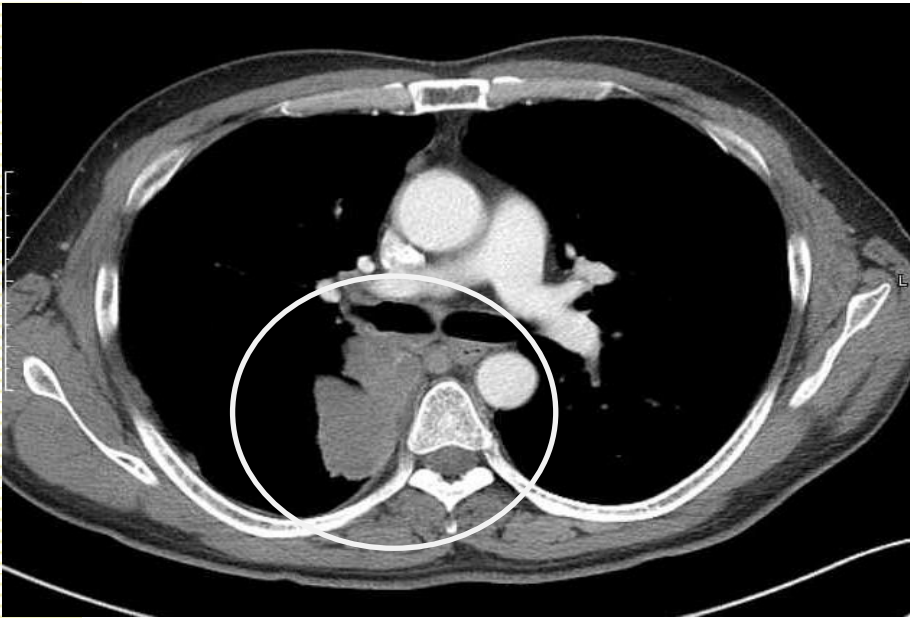
Mikhail Akimov, MD, PhD Clinical Program Leader

Emin Avsar, MSc, PhD Clinical Trial Head

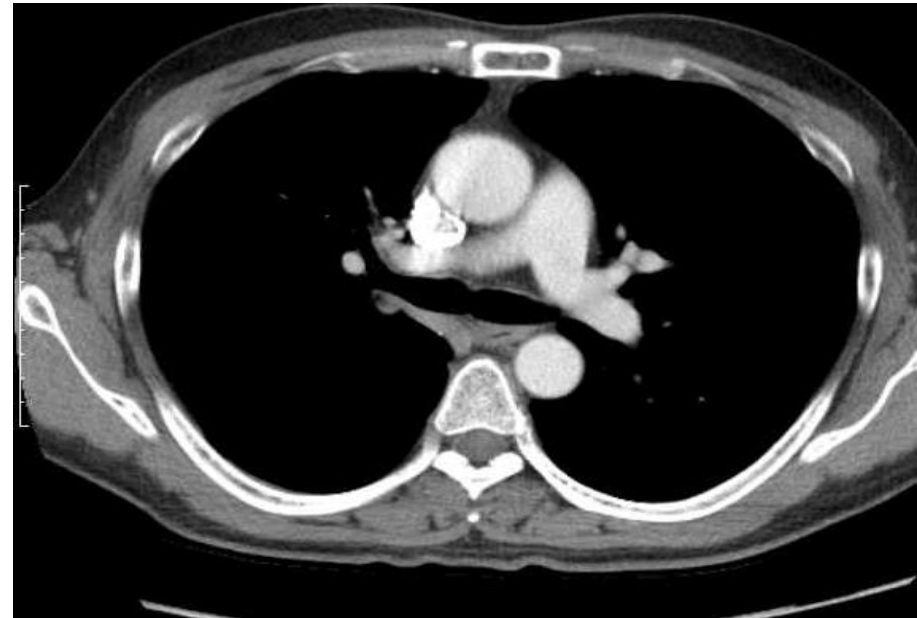
January 19th, 2010



M/51, adeno, non-smoker



2011.10.6



2011.11.18

F/62, adeno, non-smoker



2011. 8. 25



2011. 10. 7

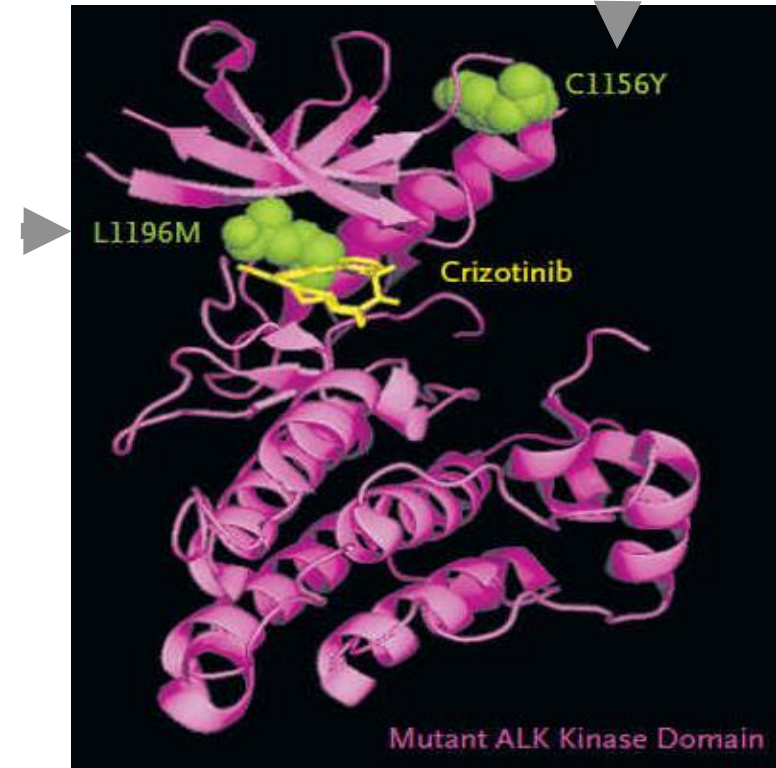
Unique side effect

- blurred vision, flashing, and delayed dark/light accommodation
- 'Trails' from lights in peripheral vision in low light conditions (e.g. dawn and dusk)



Resistance to crizotinib

- 28/M, non-smoker
- EML4-ALK+ by RT-PCR
- Response to crizotinib
- After 5 months, resistance developed
- Secondary mutation+



Secondary mutations

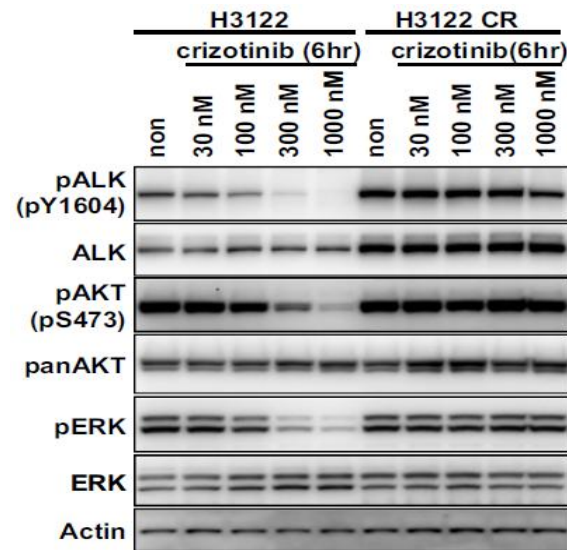
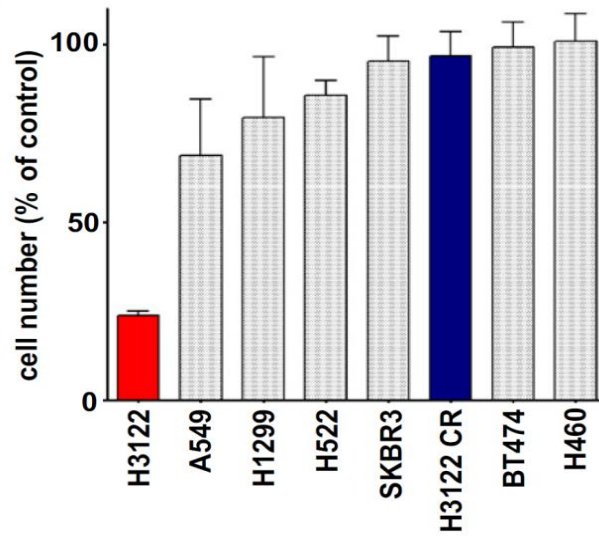
Table 1. Therapeutic interference. Compilation of ALK kinase domain amino acid substitutions associated with acquired resistance to crizotinib.

Alteration	Location within the kinase domain	ALK fusion	References
T1151I	N-terminal to the α C-helix	Not reported	(2)
L1152R	N-terminal to the α C-helix	<i>EML4-ALK E6;A20</i>	(26)
C1156Y	N-terminal to the α C-helix	<i>EML4-ALK E13;A20</i>	(22)
F1174L*	C-terminal to the α C-helix	<i>RANBP2-ALK</i>	(27)
L1196M	Gatekeeper mutation	<i>EML4-ALK E13;A20</i> ⁺	(2, 23, 24)
G1202R	Solvent front	Not reported	(2)
S1206Y	Solvent front	Not reported	(2)
G1269A	ATP-binding pocket	<i>EML4-ALK E6;A19</i> (n = 1) <i>EML4-ALK E6;A20</i> (n = 1)	(24)

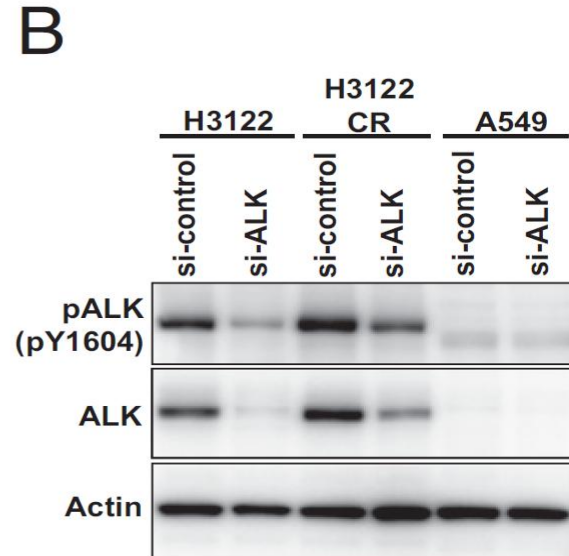
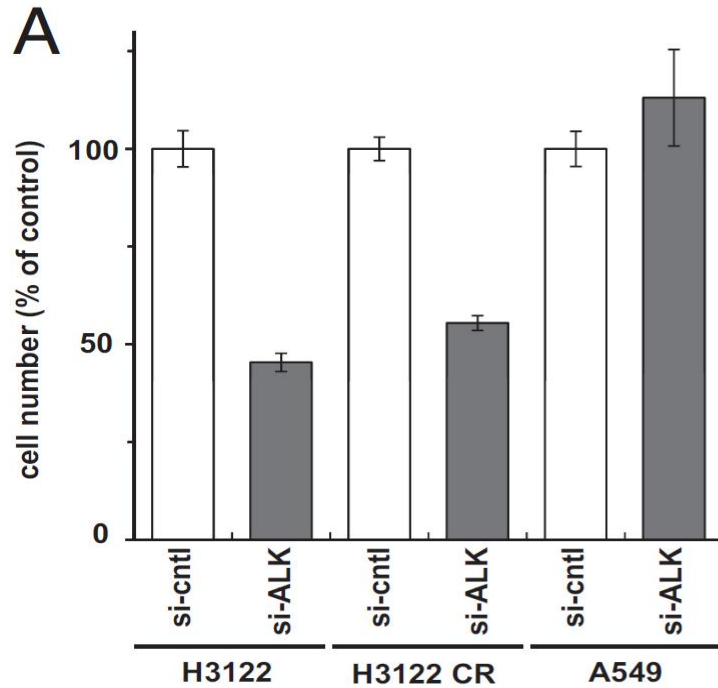
More complicated than EGFR mutant cells

Resistance to crizotinib

- In vitro model (H3122)
- **L1196M** secondary mutation in resistant cell line to crizotinib

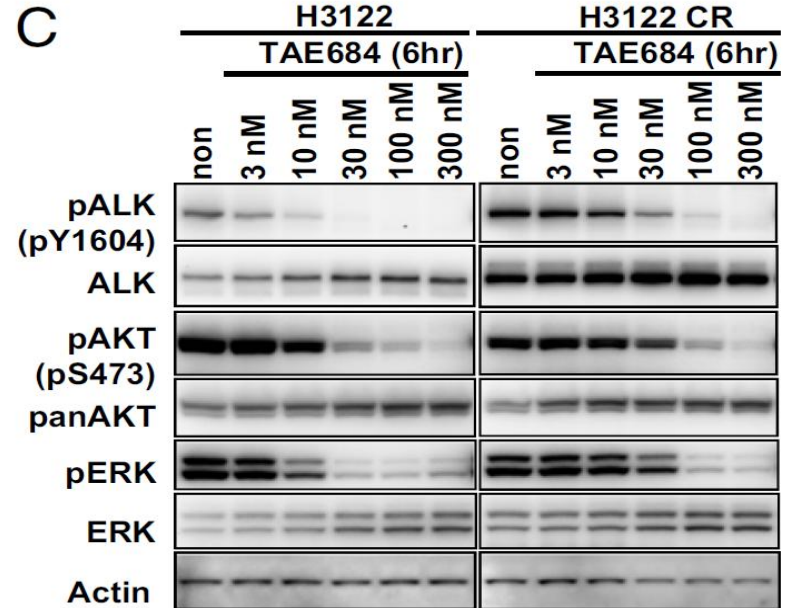
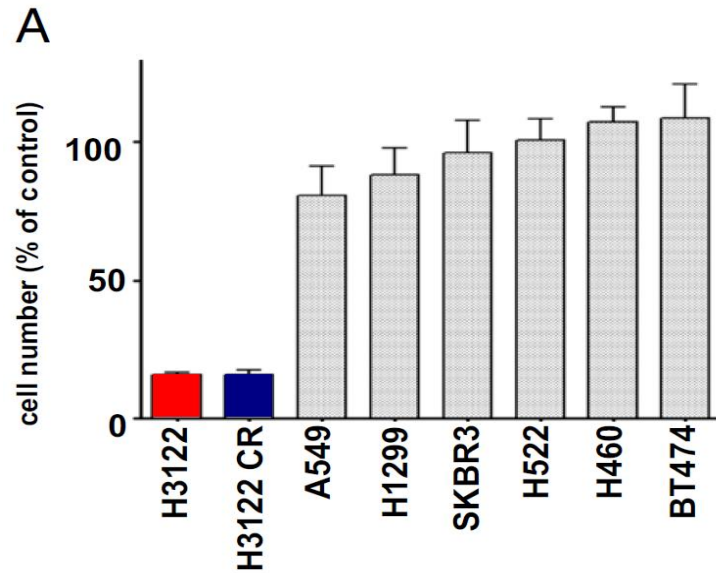


Response to siALK



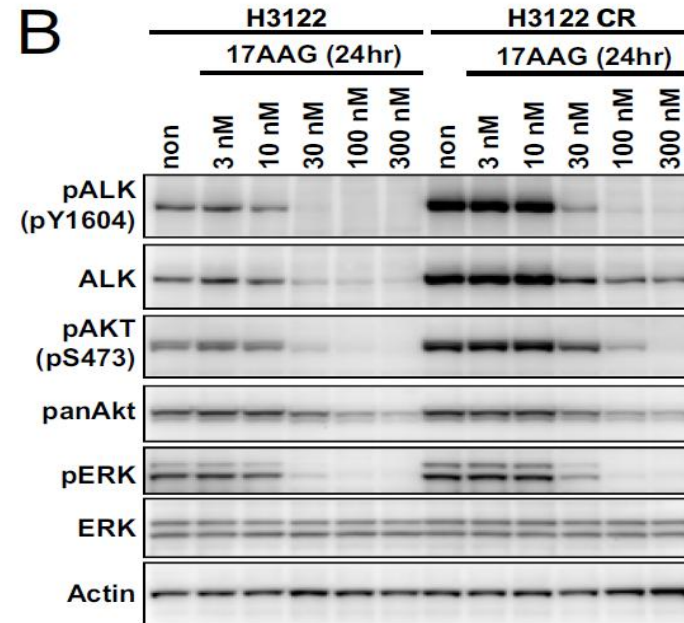
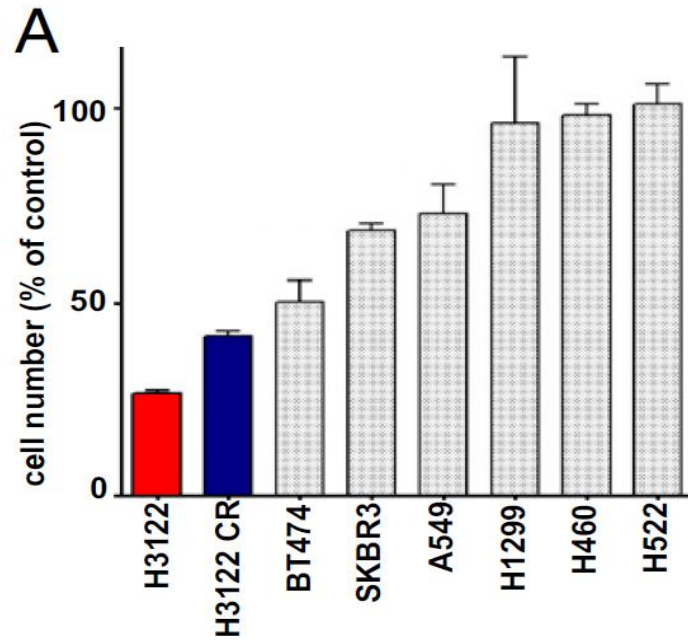
Addiction to ALK despite 2nd mutation

Response to 2nd-generation ALKi



Still sensitive to TAE684, AP26113

Response to HSP90 inhibitor



Also sensitive to HSP90 inhibitor

Possible resistant mechanisms

- **Genetic alteration of the drug target, ALK**
(point mutation and/or gene amplification)
 - **Alternate oncogenes**
(KRAS, EGFR mt, etc)
 - **Activation of bypass signaling**
(EGFR, KIT, etc)
 - **Small cell transformation, EMT ??**
-

Table 2. Molecular analysis of re-biopsy samples of evaluable patients

Patient #	Mechanisms of Resistance				
	ALK FISH	ALK FISH CNG	ALK kinase domain sequence ^a	EGFR	KRAS
4	Positive	Negative	L1196M	WT ^a	WT ^a
5	Positive	Negative	L1196M	WT ^a	WT ^a
6	Positive	Negative	G1269A*	WT ^{a,b}	WT ^{a,b}
7	Positive	Positive	G1269A	WT ^b	WT ^b
8	Positive	Positive	WT	WT ^a	WT ^a
9a	Negative	Negative	NA	L858R ^{a,b}	WT ^{a,b}
9b	Positive	Negative	WT*	WT ^b	WT ^b
10 [¶]	Positive/Negative [§]	Negative	WT	ND	G12C ^{§b}
11	Positive	Negative	WT	WT ^a	G12V ^a
12	Negative	Negative	WT	WT ^b	WT ^b
13 [¶]	Positive	Negative	WT*	ND	ND
14	Positive	Negative	WT	WT ^a	WT ^a

[¶]Patients with intrinsic resistance

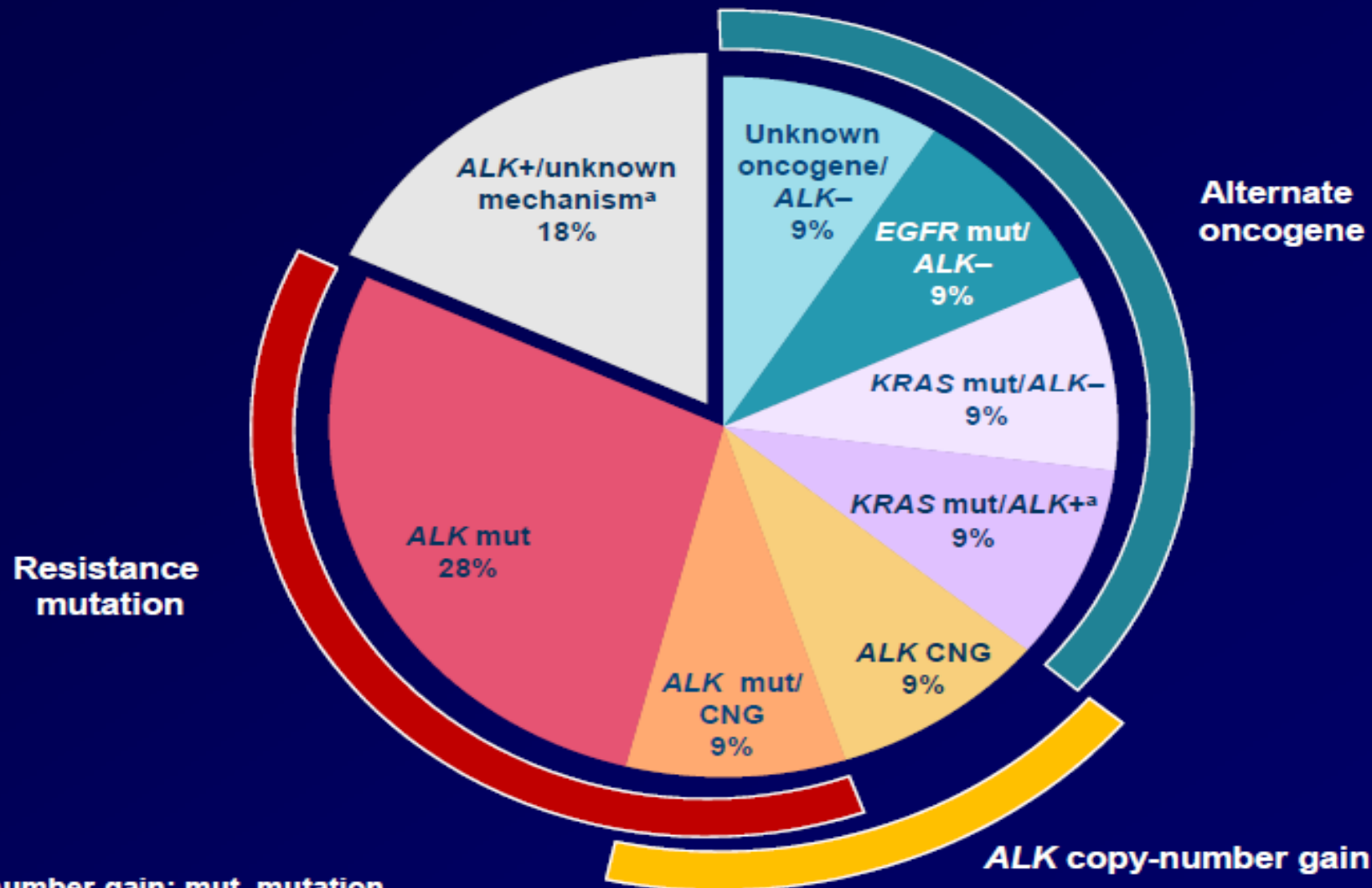
^aDirect sequencing

^bSNaPshot[®] includes *APC, AKT1, BRAF, CTNNB1, EGFR, FLT3, JAK2, KIT, KRAS, MAP2K1 (MEK1), NOTCH1, NRAS, PIK3CA,*

*Heterozygous for rs3795850 ALK SNP

[§]From patient-derived cell line with loss of *ALK* gene rearrangement by FISH or RT-PCR. The FFPE tissue was *ALK* FISH +.

Crizotinib Resistance Mechanisms

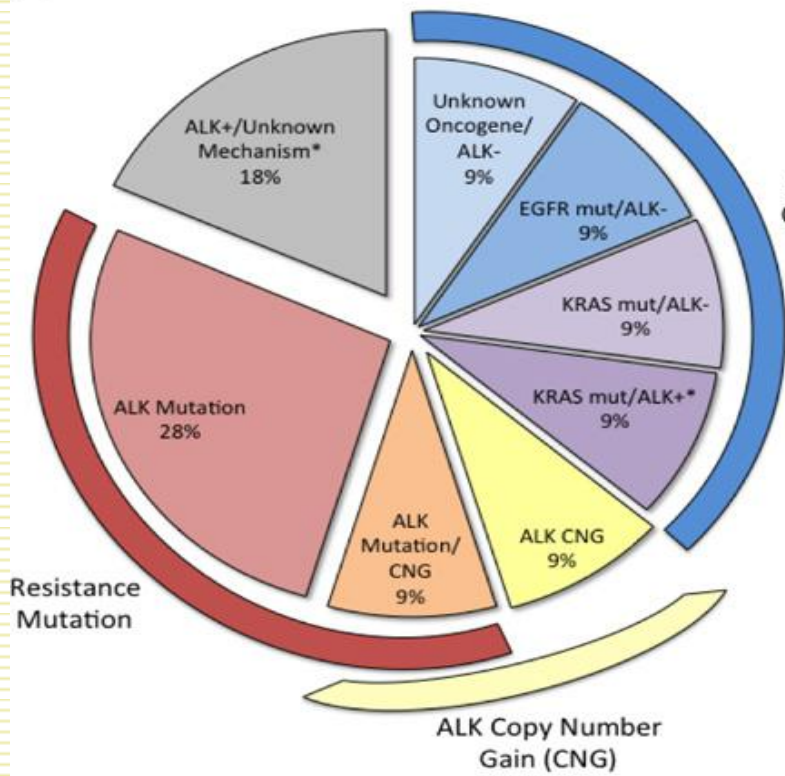


CNG, copy-number gain; mut, mutation

^aOne patient had intrinsic resistance within this category

Doebbele RC, Pilling AB, Aisner DL, et al. *Clin Cancer Res* 2012;18:1472-1482

A



B

