

ALK, MET inhibitors

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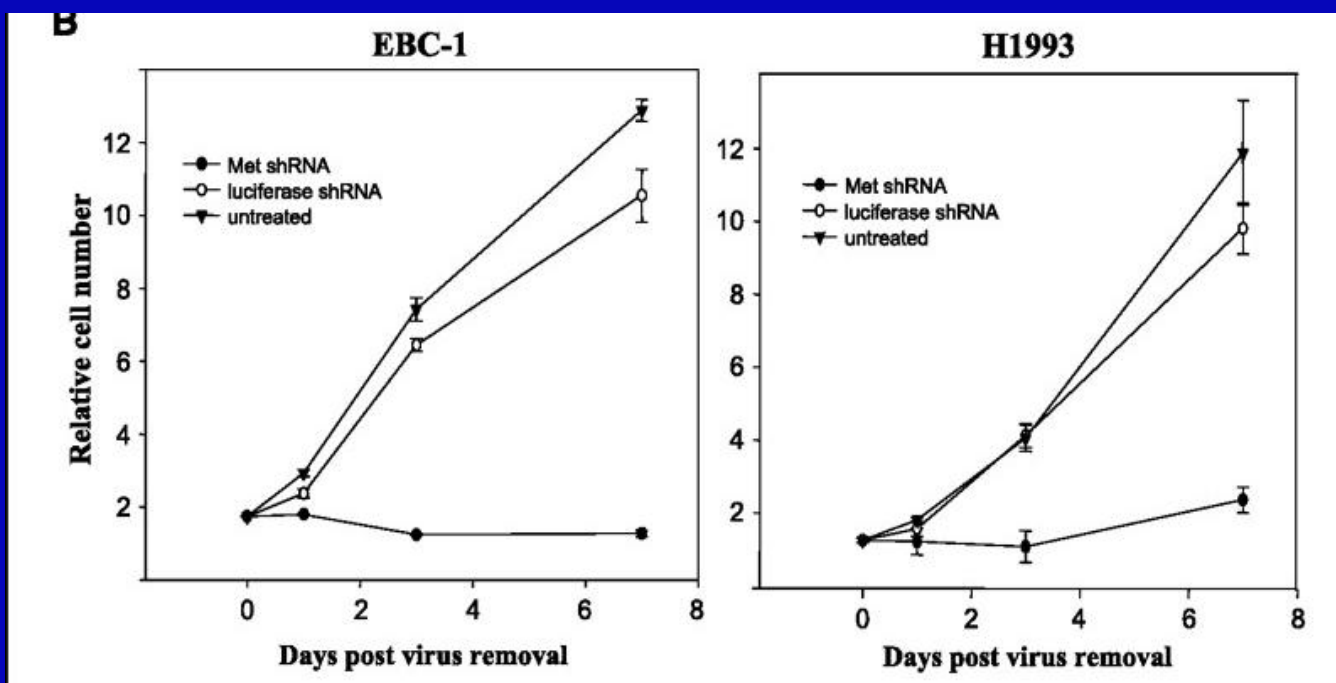
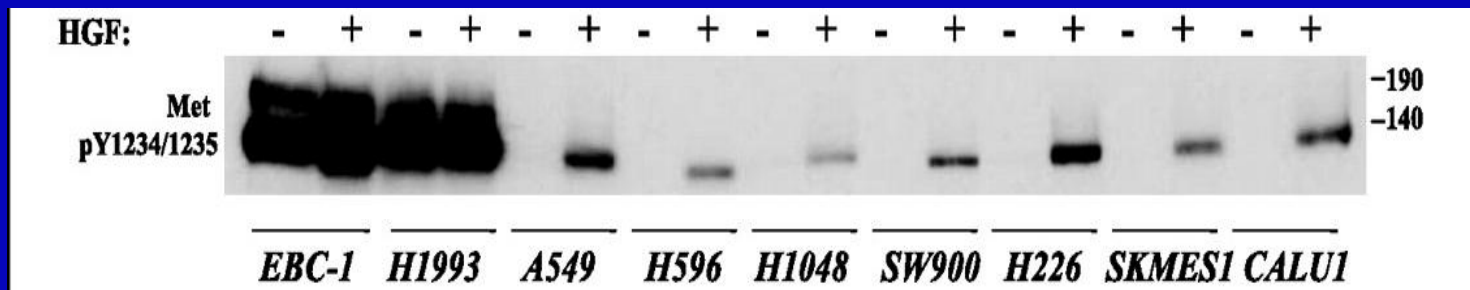
ARTICLES

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

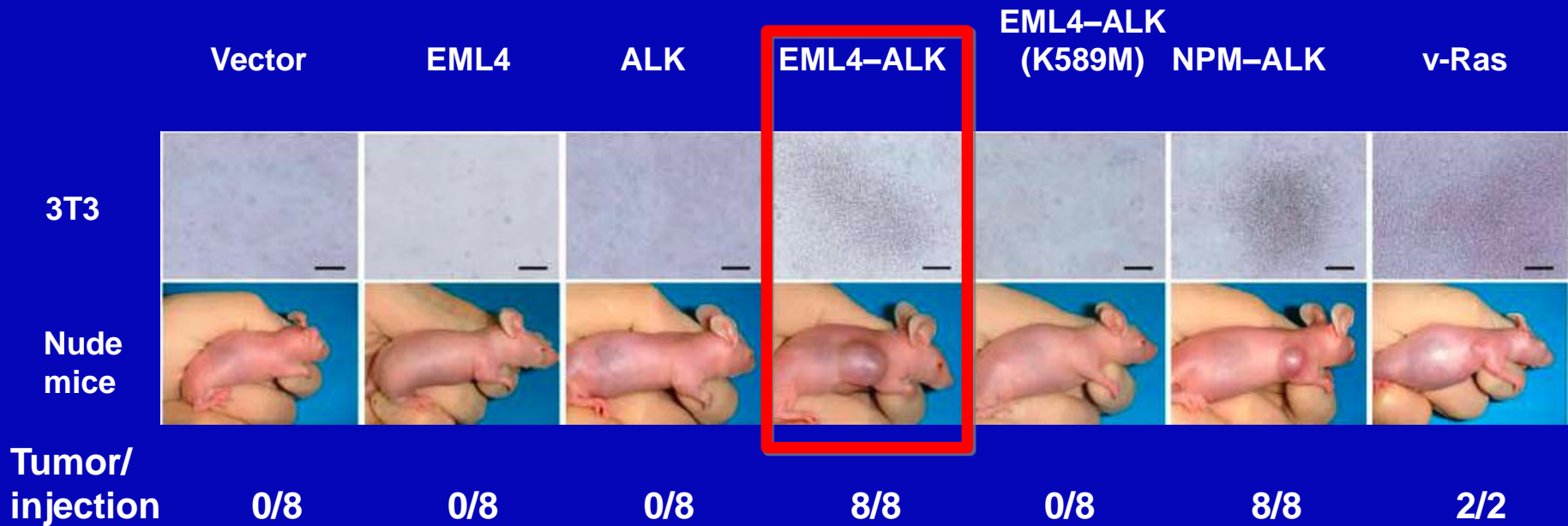
Manabu Soda^{1,2}, Young Lim Choi¹, Munehiro Enomoto^{1,2}, Shuji Takada¹, Yoshihiro Yamashita¹, Shunpei Ishikawa⁵, Shin-ichiro Fujiwara¹, Hideki Watanabe¹, Kentaro Kurashina¹, Hisashi Hatanaka¹, Masashi Bando², Shoji Ohno², Yuichi Ishikawa⁶, Hiroyuki Aburatani^{5,7}, Toshiro Niki³, Yasunori Sohara⁴,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

Improvement in the clinical outcome of lung cancer is likely to be achieved by identification of the molecular events that underlie its pathogenesis. Here we show that a small inversion within chromosome 2p results in the formation of a fusion gene comprising portions of the echinoderm microtubule-associated protein-like 4 (*EML4*) gene and the anaplastic lymphoma kinase (*ALK*) gene in non-small-cell lung cancer (NSCLC) cells. Mouse 3T3 fibroblasts forced to express this

Lung cancer cell lines with Met gene amplification are dependent on met for growth and survival



EML4-ALK is a potent “oncogenic” driver

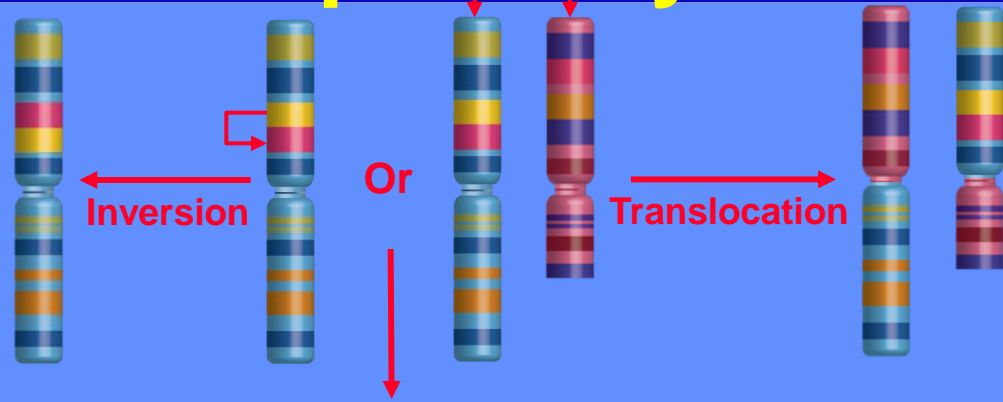


Inhibition of ALK leads to dramatic *in vivo* tumor regression

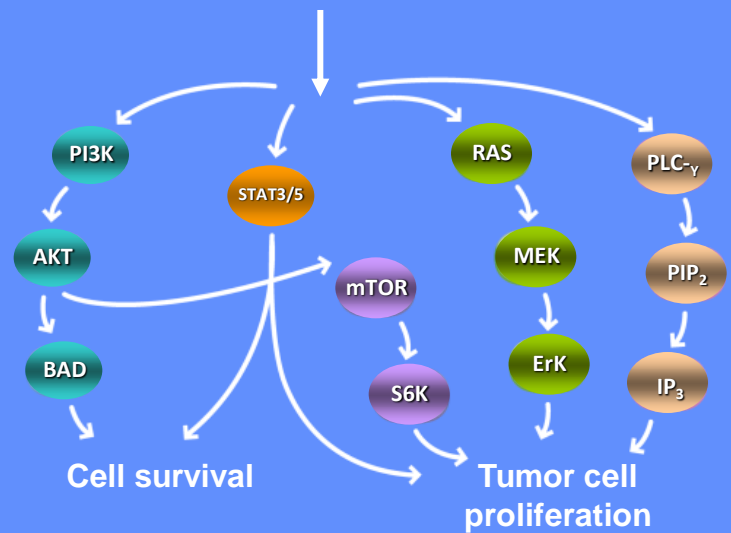
EML4 = echinoderm microtubule-associated protein-like 4;
NPM = nucleophosmin

Soda M et al. Nature 2007;448:561–567
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ALK pathway



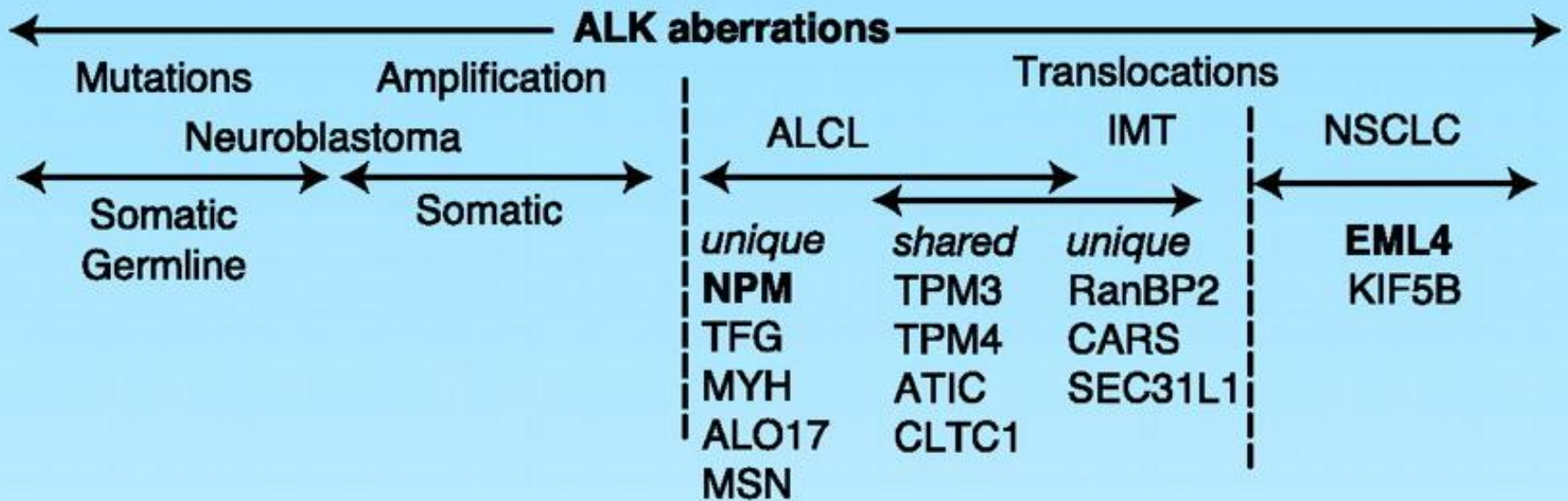
ALK ——— ALK fusion protein*



*Subcellular localization of the ALK fusion gene, while likely to occur in the cytoplasm, is not confirmed.^{1,2}

1. Inamura K et al. J Thorac Oncol 2008;3:13–17
 2. Soda M et al. Proc Natl Acad Sci U S A 2008;105:19893–19897
 Figure based on: Chiarle R et al. Nat Rev Cancer 2008;8(1):11–23;
 Mossé YP et al. Clin Cancer Res 2009;15(18):5609–5614; and Data on file. Pfizer Inc.

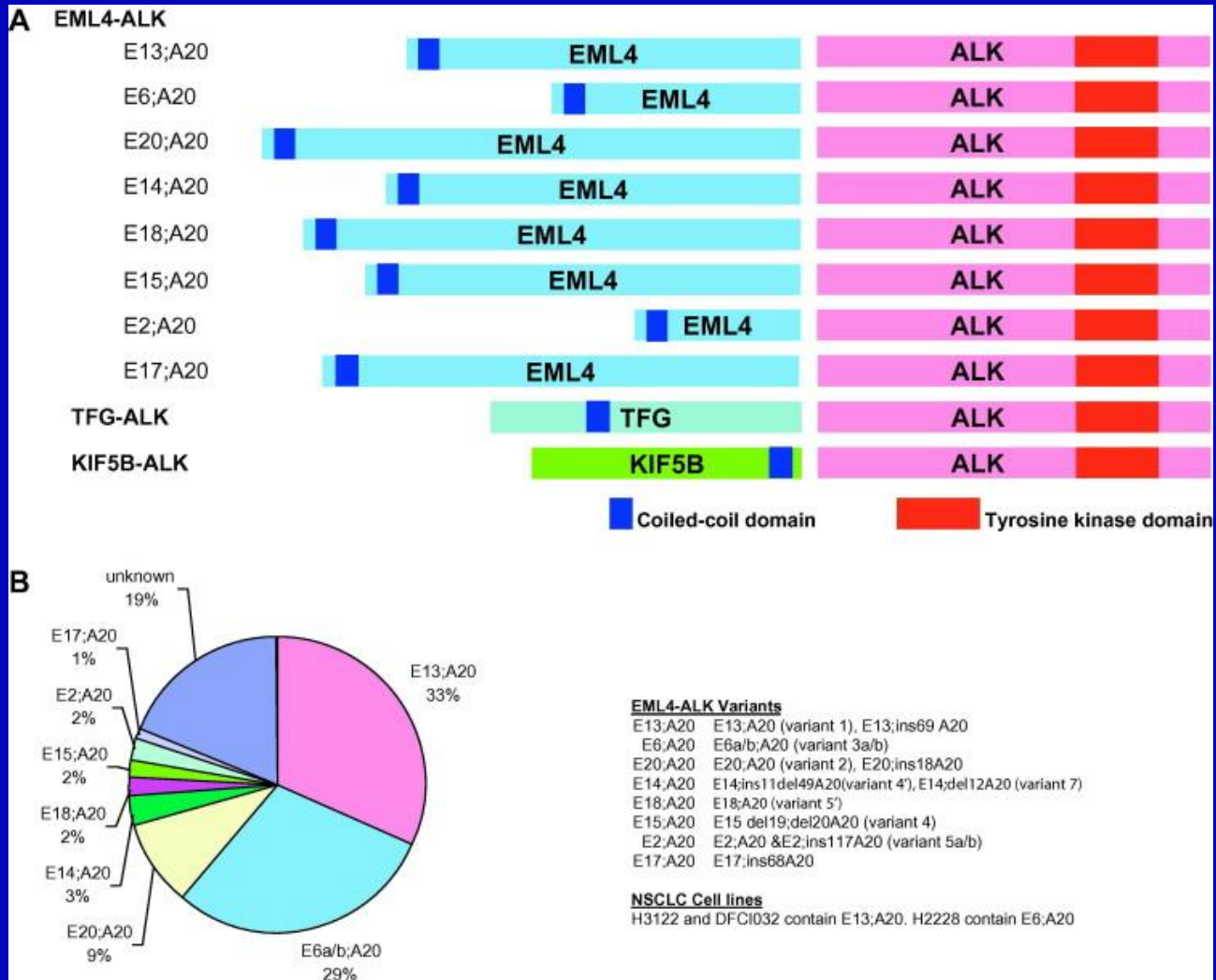
ALK aberrations in malignancies



Frequency of EML4-ALK fusion in NSCLC

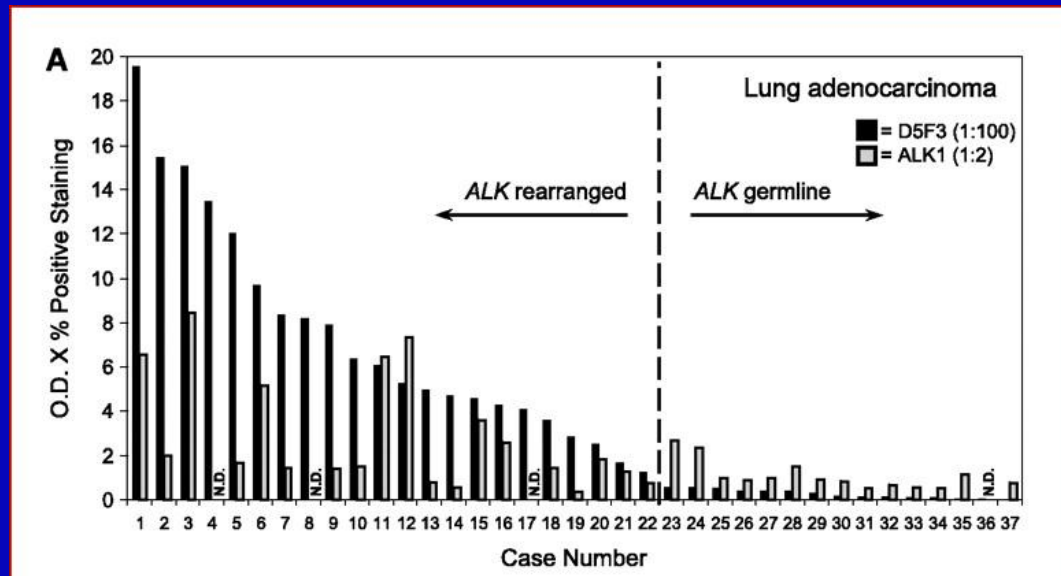
Soda, Nature 07	75	6.7%	RT-PCR
Koivunen, CCR 08	305	3%	RT-PCR, exon array
Takeuchi, CCR 08			
Adenocarcinoma	253	4.4%	Multiplex RT-PCT, sequencing
Other histologies	111	0%	
Shinmura, LC 08	77	2.6%	RT-PCR, sequencing
Perner, Neoplasia 08	603	2.6%	FISH
Martinelli, AJP 09	129	7.5%	RT-PCR
Takahashi, ASO 10	313	1.6%	RT-PCR, sequencing
Zhang, Mol Cancer 10	103	11.6%	RT-PCR, gene expression
Shaw, JCO 09	141	13%	FISH
(enriched population with 2/4 clinical characteristics associated with EGFR mutation)			

Variants of ALK fusion partners

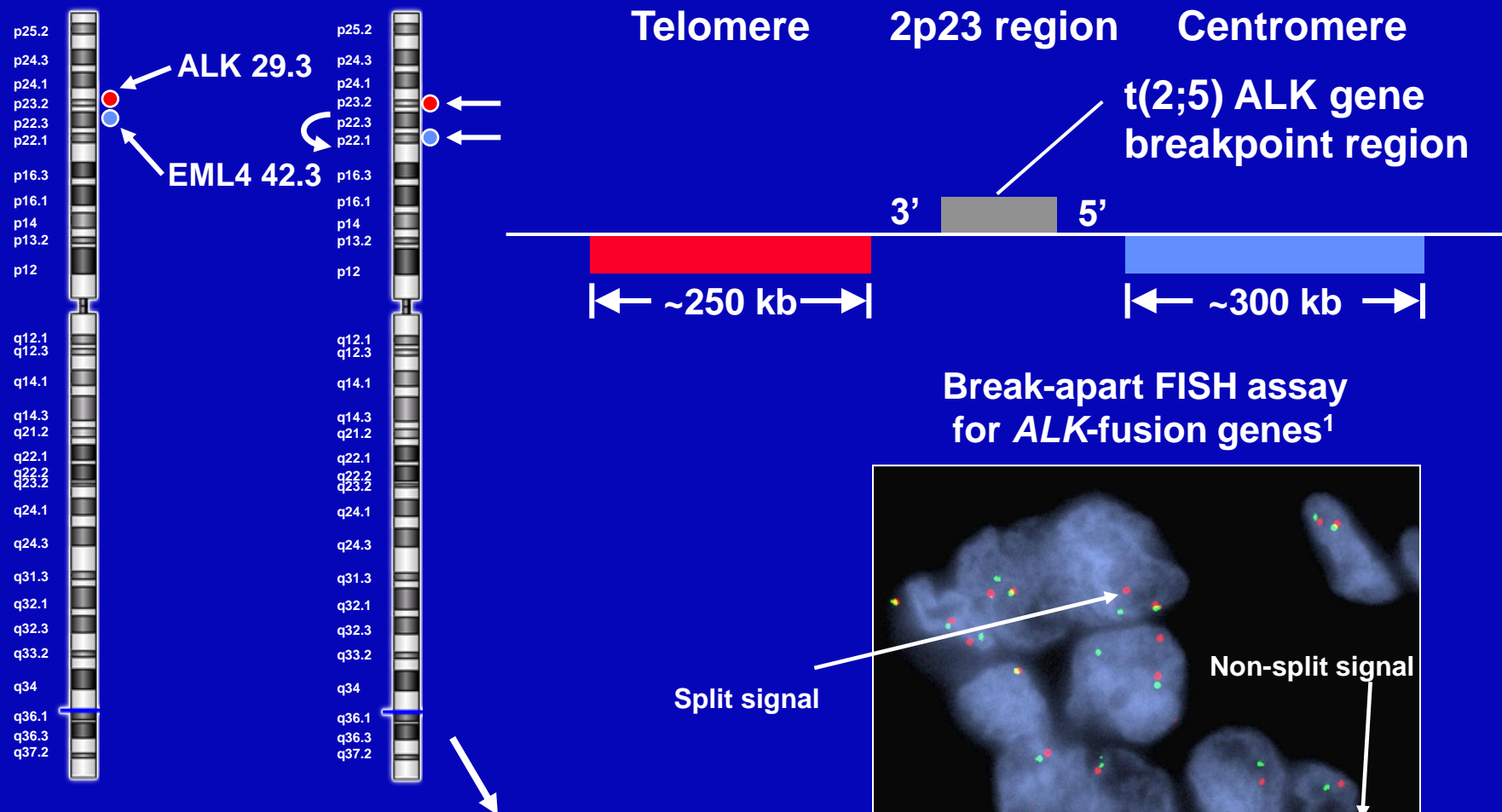


ALK immunohistochemistry

- Traditional antibodies not reliable
Rodig, CCR 2009; Martinelli, AJP 2009
- Intercalated antibody-enhance polymer (IAEP) method gave better results and allowed identification of KIF5B als new ALK fusion partner
Takuchi, CCR 2009
- Encouraging results with new monoclonal rabbit anti-human CD246 antibodies
Mino-Kenudson, CCR 2010



Break-apart FISH assay for *ALK* rearrangement



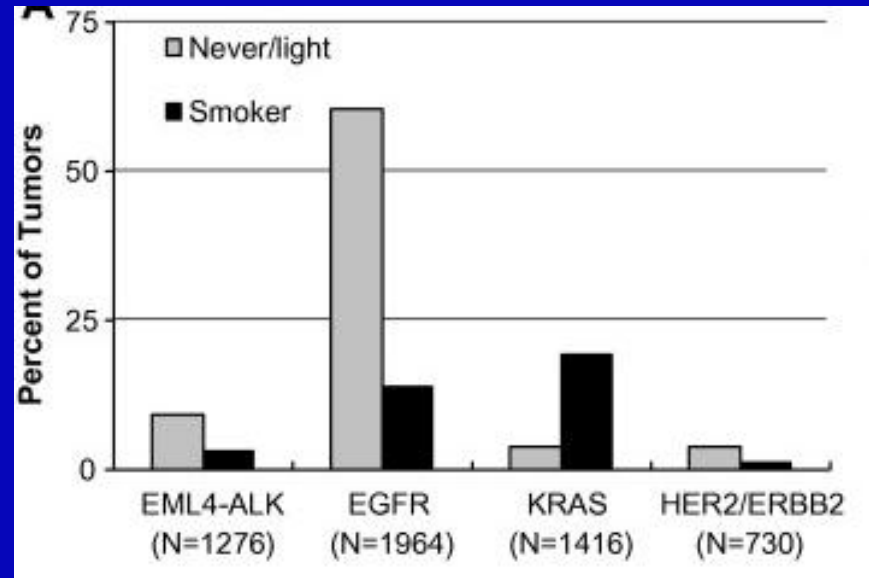
ALK break-apart FISH assay
[Courtesy John Iafrate, Massachusetts General Hospital]

Assay is positive if rearrangements can be detected in $\geq 15\%$ of cells

Clinical features of patients with EML4-ALK NSCLC

- More frequent in never or former light smokers

Sasaki, EJC 2010



- Predominant in younger patients
 - 36% (4/11) under 50 y/o (compared to 5% ALK-negative adenocarcinomas)

Inamura, Modern Path, 2009

- Median age 52 years vs 66 and 64 for mEGFR or WT tumors

Shaw, JCO 2009

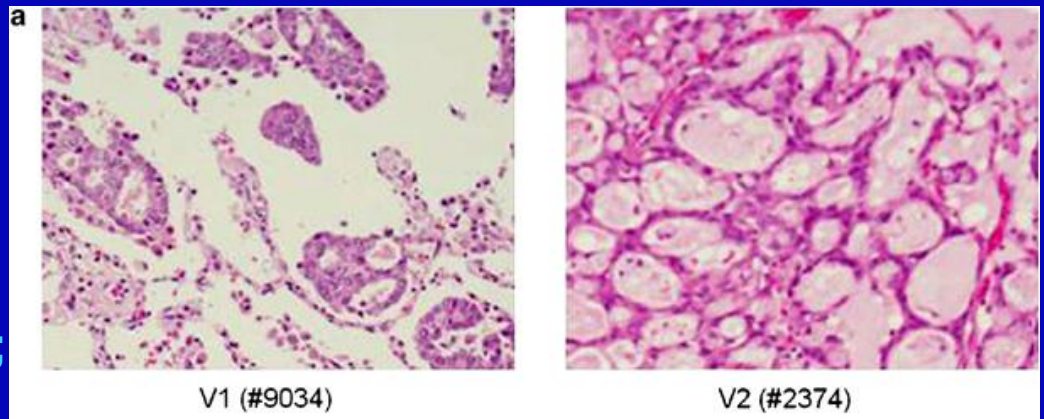
Clinical features of patients with EML4-ALK NCSLC

- Likely similar response to platin-based chemotherapy as WT tumors

Shaw, JCO 2009

- Predominantly found in adenocarcinoma. TTF1 pos., acinar histology, mutually exclusive with EGFR and KRAS mutations

Takeuchi, CCR 2008;
Inamura, Modern Path, 2009;
Takahashi, ASO 2010;
Zhang, Mol Cancer 2010



- Slow clinical course after surgery?

Murakami, Lung Cancer, 2010

Crizotinib selectivity profile

Upstate 102
kinase

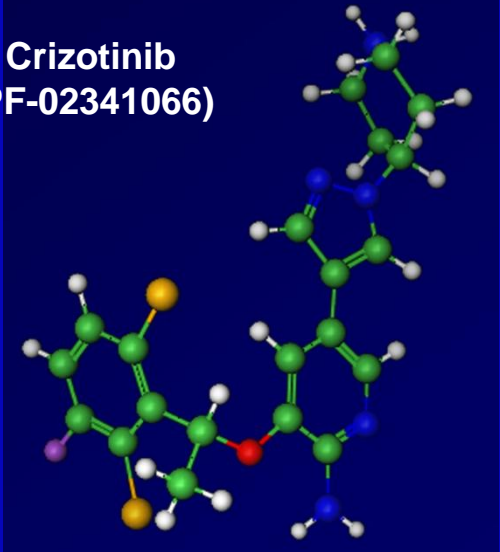
Kinase	% Inhibition
Met(h)	98
TrkA(h)	103
TrkB(h)	102
ALK(h)	100
TrkC(h)	100
Abl(T315I)(h)	98
Yes(h)	95
Lck(h)	95
Ros(h) (SKY1)	94
Axl(h)	93
Fes(h)	93
Lyn(h)	93
Arag(h)	91
Ros(h)	90
CDK2(cyclinE)(h)	87
Fms(h)	84
EphA2(h)	80
Bmx(h)	79
EphB2(h)	77
EphA(h)	73
Fyn(h)	68
IRK(h)	64
CDK7(cyclinMAT1)(h)	58
cSRC(h)	58
IGF-1R(h)	56
AuroraA(h)	54
Syk(h)	52
FGFR3(h)	50
PKCα(h)	50
BTK(h)	35
CDK1(cyclinB)(h)	25
p38α(h)	24
PRK2(h)	22
PAK-1β(h)	21
PKR(h)	21
Ros(h)	21
GSK3β(h)	18
Fes(h)	17
MAPK1(h)	17
ZAP-70(h)	17
Abl(h)	16
c-Raf-1(h)	16
PKD2(h)	15
RCKA4(h)	14
Rak3(h)	14
GSK3α(h)	11
CDK3(cyclinE)(h)	10
PDGFRα(h)	10
Rak1(h)	7
Src(h)	6
CHK1(h)	5
Erk4(h)	5
Rak2(h)	5
JNK1α1(h)	4
PKBα(h)	4
Btk(h)	3
CDK3(cyclinE)(h)	3
PKCβ(h)	3
PKCδ(h)	3
CDK2(cyclinA)(h)	2
PAK2(h)	2
PKCβ(h)	2
Pim-1(h)	1
PKCγ(h)	1
SAPKα(h)	1
CaMKIIβ(h)	0
Mkk7β(h)	0
CaMKI(h)	-1
CHK2(h)	-1
CRK2(h)	-1
JNK2(h)	-1
Mkk6(h)	-1
CK1δ(h)	-2
PKCα(h)	-2
MAPK2(h)	-3
MEK1(h)	-3
PKCβ(h)	-3
PKCε(h)	-3
PKCθ(h)	-3
PKCζ(h)	-3
PKCδ(h)	-3
PKCβ(h)	-3
PKCβ(h)	-3
MSK1(h)	-6
PDGFRβ(h)	-6
PKCδ(h)	-6
SAPPK(h)	-6
MAPKAPK2(h)	-7
PKA(h)	-7
AMPK(h)	-9
CDK6(cyclinD3)(h)	-9
CKI(h)	-9
SAPPKα(h)	-9
JNK3(h)	-10
PKR(h)	-10
IKKα(h)	-11
NEK2(h)	-11

13 kinase
"hits" <100X
selective for
c-MET

Cellular selectivity on 10 of
13 relevant hits

Kinase	IC ₅₀ (nM)	Selectivity ratio
c-MET	8	-
ALK	20	2X
RON	298	34X
Axl	294	34X
Tie-2	448	52X
Trk A	580	67X
Trk B	399	46X
Abl	1,159	166X
IRK	2,887	334X
Lck	2,741	283X
Sky	>10,000	>1,000X
VEGFR2	>10,000	>1,000X
PDGFRβ	>10,000	>1,000X

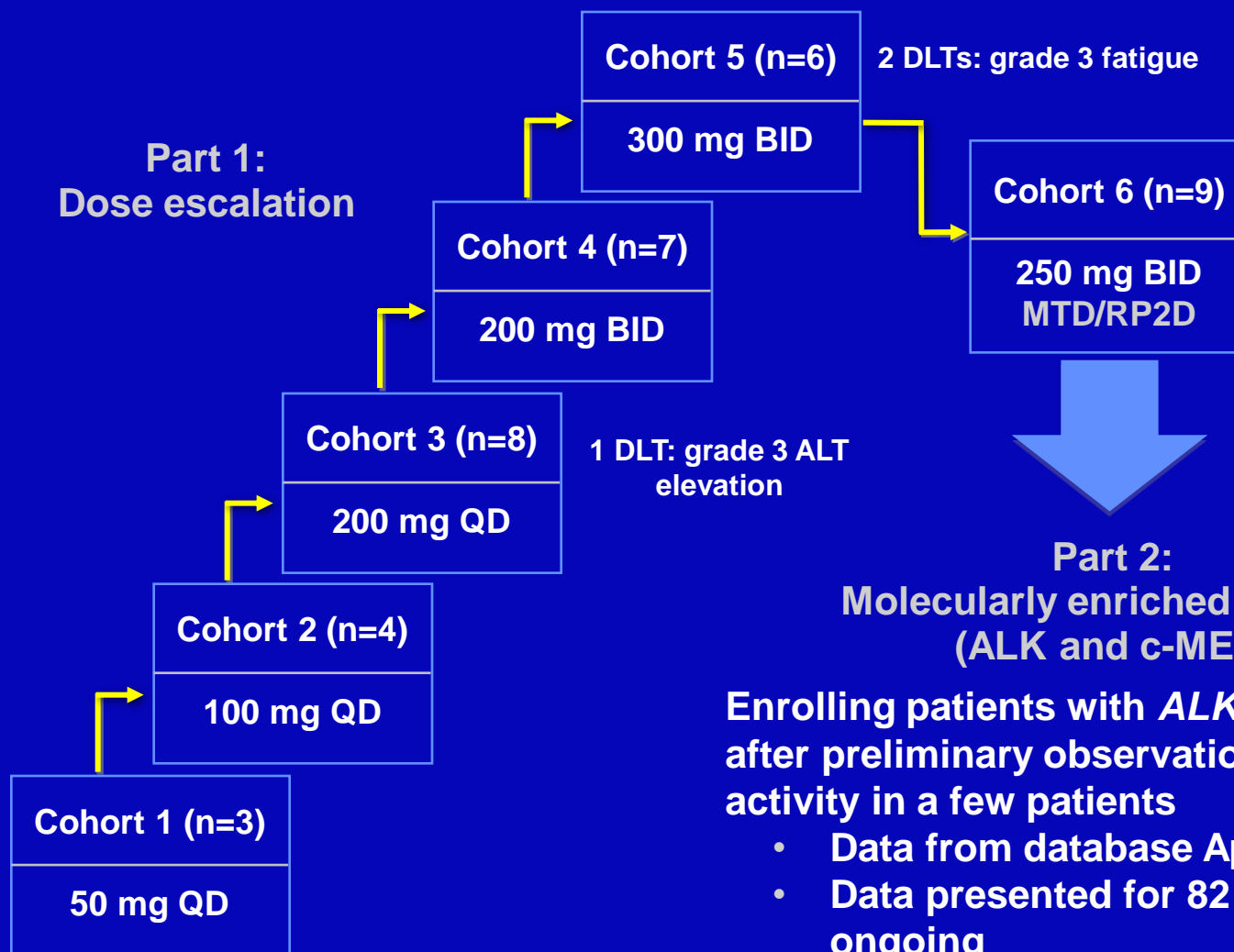
Crizotinib
(PF-02341066)



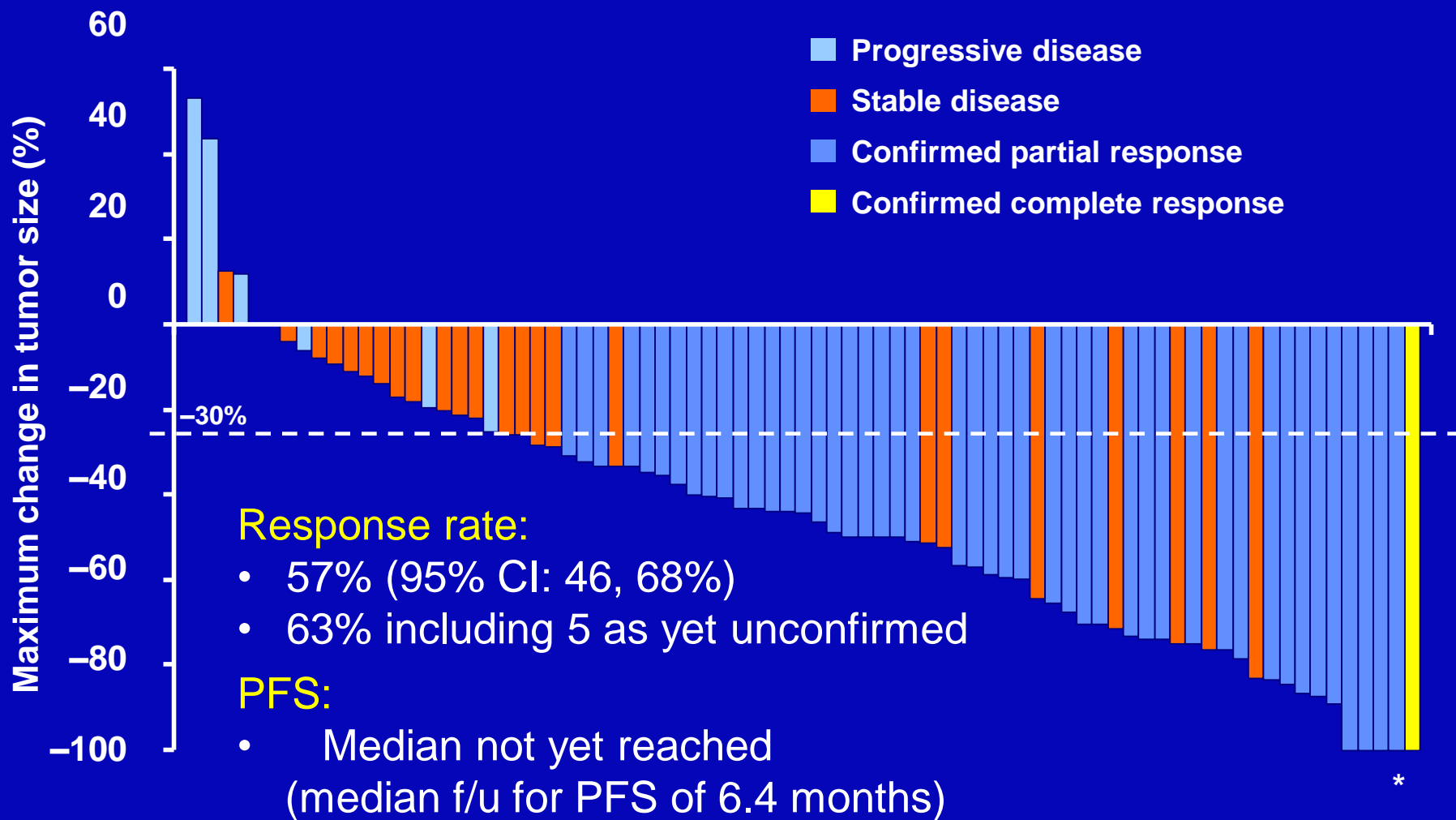
Selectivity findings

- **Crizotinib – ALK and c-MET inhibition at clinically relevant dose levels**
- **Crizotinib – low probability of pharmacologically relevant inhibition of any other kinase at clinically relevant dose levels**

Crizotinib: First-in-human/Patient Trial



Responses to Crizotinib for patients with *ALK*-positive NSCLC



*Partial response patients with 100% change have non-target disease present

Current Crizotinib Clinical Trials

PROFILE 1007

Key entry criteria

- Positive for ALK by central laboratory
- 1 prior chemotherapy (platinum-based)

R
A
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D
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M
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Z
E

N=318

Crizotinib 250 mg BID (n=159)
administered on a continuous
dosing schedule

**Pemetrexed 500 mg/m² or
docetaxel 75 mg/m² (n=159)**
infused on day 1 of a 21-day cycle

PROFILE 1005

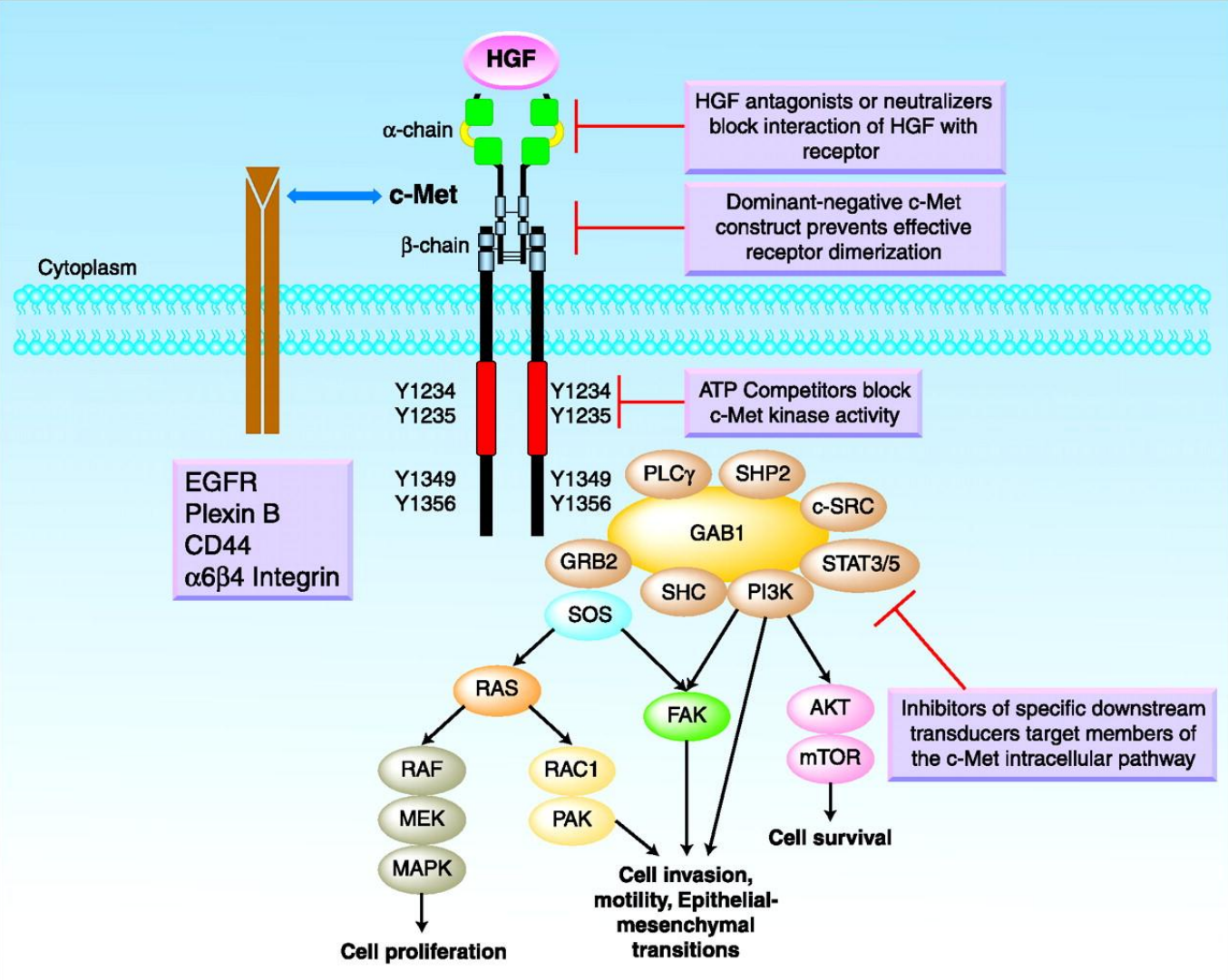
Key entry criteria

- Positive for ALK by central laboratory
- Progressive disease in Arm B of study A8081007
- >1 prior chemotherapy

N=250

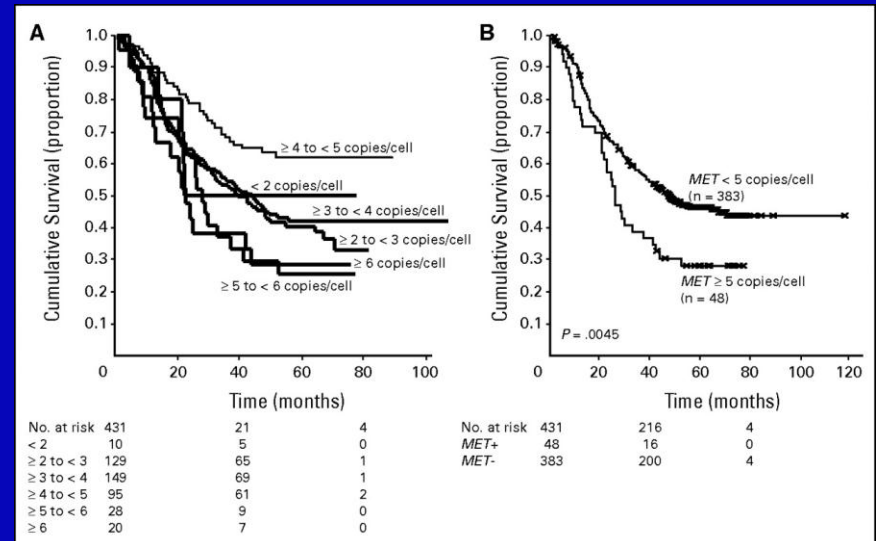
Crizotinib 250 mg BID (N=250)
administered on a continuous
dosing schedule

Targeting MET



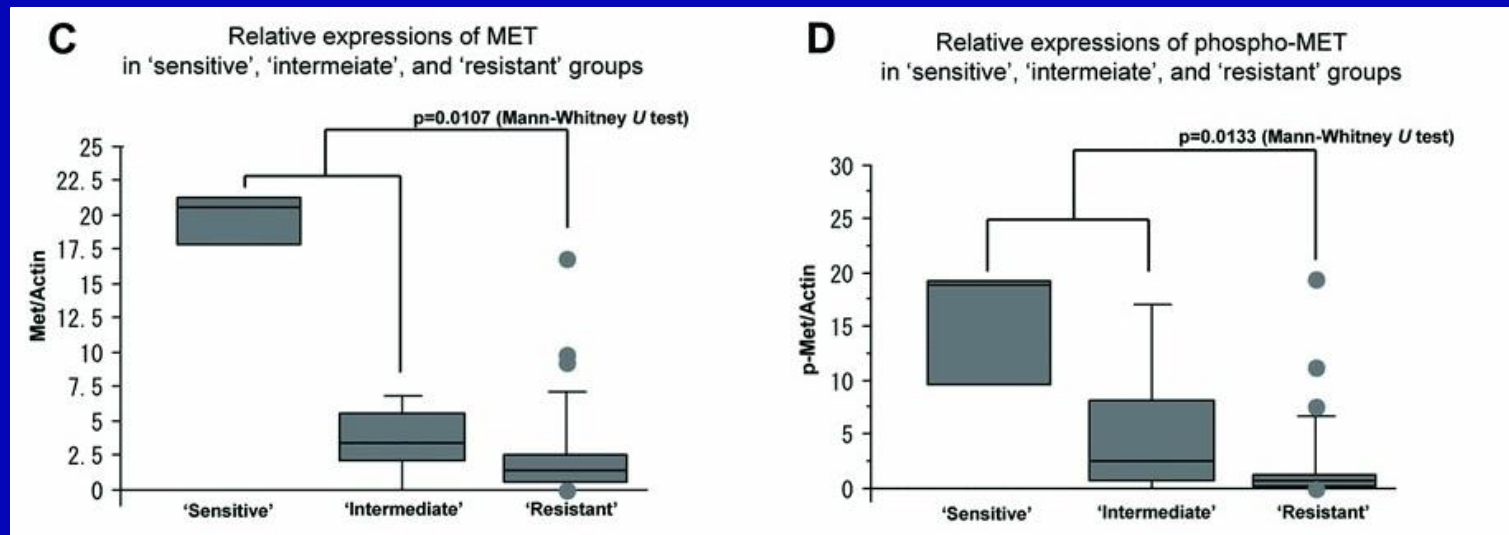
Why targeting MET and HGF in NSCLC: Met amplification

- NSCLC cell lines with met amplification depend on MET for growth and survival
Lutterbach, CR 2007
- MET amplification occurs in 21% of EGFR TKI resistant NSCLC, but is otherwise a rare event (3%)
Bean, PNAS 2007
- Increased MET copy number associated with worse prognosis in resected NSCLC. Amplification in 4%
Cappuzzo, JCO 2009



Molecular predictor of sensitivity to MET inhibitor in lung cancer cell line

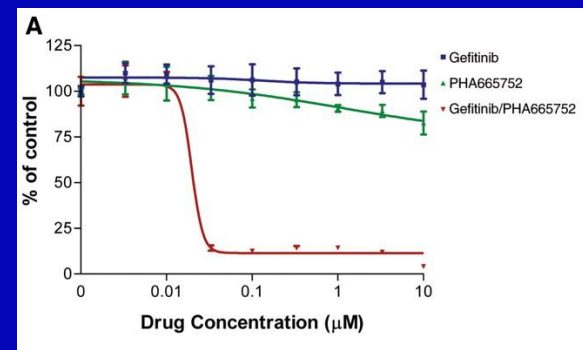
- PHA665752
- 41 cell lines
- 8 sensitive cell lines, incl. 4 with MET amplification
- High phosphoMET associated with sensitivity, especially in RAS mutated cell lines



Why targeting MET and HGF in NSCLC: EGFR TKI resistance (and vice versa)

- MET amplification and resistance to EGFR TKIs:
 - Combination of gefitinib and MET inhibitor

Engleman, Science 2007



- Association of HGF production (with MET amplification) and resistance to EGFR TKIs

Turke, Cancer Cell 2010; Kasahara, CCR 2010

- TAK 701 antibody against HGF reverses gefitinib resistance in HGF producing cell line

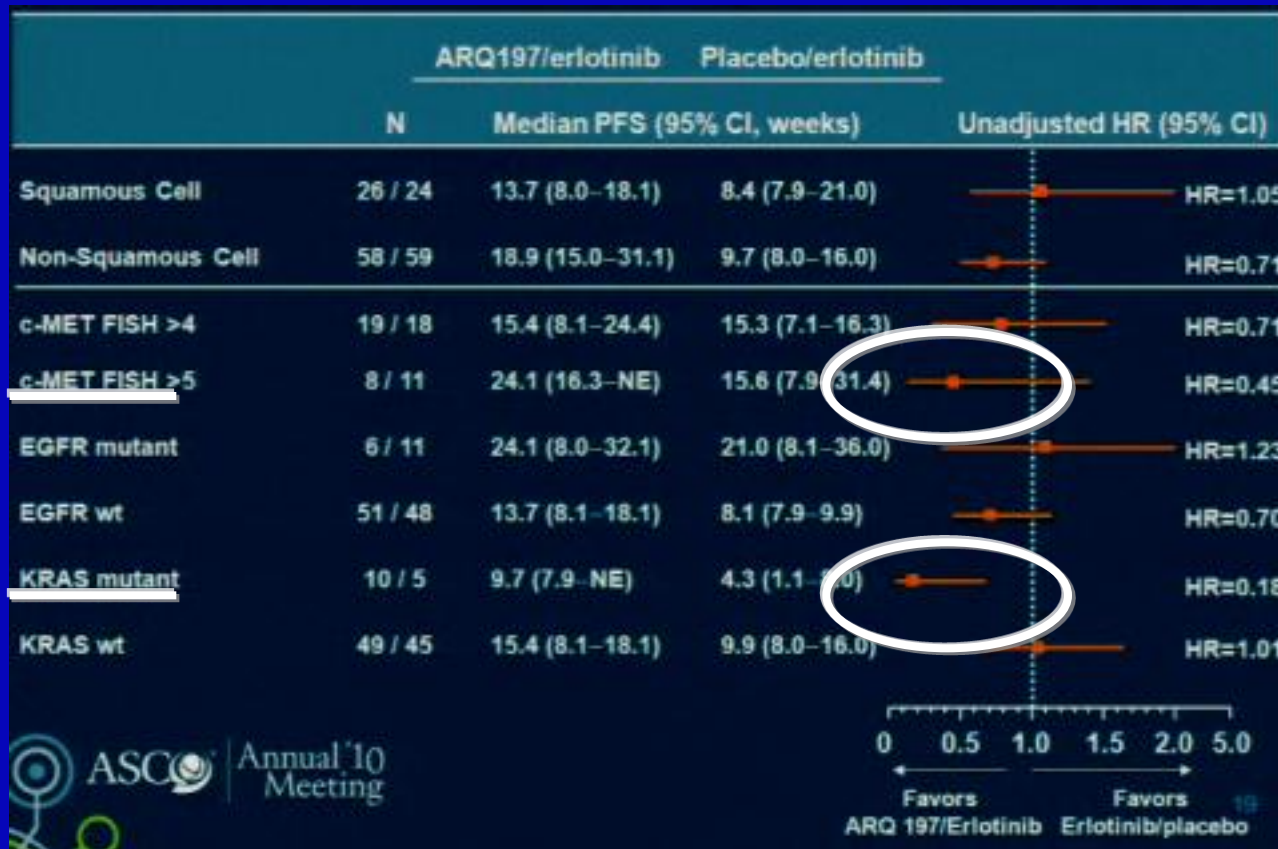
Okamoto, Mol Cancer Ther 2010

- Acquired MET resistance by switch to EGFR-dependency

McDermott, CR 2010

Oral met inhibitors in NSCLC

- Phase II trial comparing erlotinib plus ARQ 197 to erlotinib plus placebo in second line
Schiller, ASCO 2010



Summary

- **Adenocarcinoma of the lung with an ALK fusion gene is a newly defined separate entity of lung cancer for which a specific therapeutic agent is available**
 - **Clinical nature?**
 - **Best diagnostic method?**
 - **First line therapy?**
 - **Resistance mechanisms?**
- **Adenocarcinoma with MET amplification provide another opportunity to develop targeted approaches**
 - **There is preclinical evidence suggesting combined therapy with EGFR TKIs may be of advantage**

Treatment-related Adverse Events in ALK-positive NSCLC ($\geq 10\%$)

Adverse event	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Total n (%)
Nausea	43 (52)	1 (1)	0	0	44 (54)
Diarrhea	38 (46)	1 (1)	0	0	39 (48)
Vomiting	35 (43)	1 (1)	0	0	36 (44)
Visual disturbance*	34 (42)	0	0	0	34 (42)
Constipation	18 (22)	2 (2)	0	0	20 (24)
Peripheral edema	13 (16)	0	0	0	13 (16)
Dizziness	12 (15)	0	0	0	12 (15)
Decreased appetite	11 (13)	0	0	0	11 (13)
Fatigue	8 (10)	0	0	0	8 (10)

*Changes in light/dark accommodation (no abnormalities on ophthalmologic exam)

N=82