Molecular Pathology of Solid Tumors:
Cancer Profiling for Targeted Treatment Prediction

Targeted Treatment and Molecular Biomarkers in Lung Cancer

Ming Sound Tsao, MD, FRCPC
Choksi Chair in Lung Cancer Translational Research
Princess Margaret Hospital
University of Toronto

Uppsula, September 25, 2012

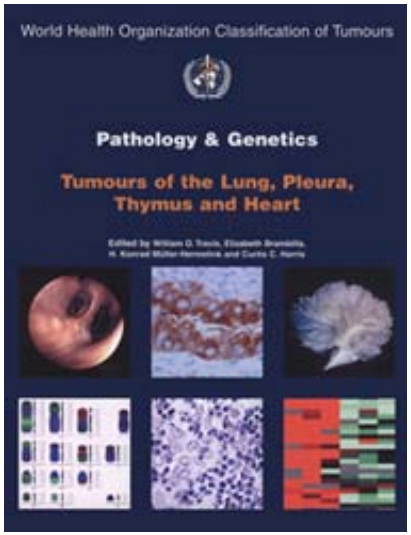
Disclosures

- Honoraria for Consultancies:
  - Lilly Canada (Lung cancer histopathology)
  - AstraZeneca Canada (EGFR mutation testing)
  - Boehringer-Ingelheim Canada (EGFR mutation testing)
  - Roche Oncology (EGFR TKI biomarkers)
  - Pfizer (Targeted therapy and ALK testing)
- Research grants:
  - Pfizer Canada
  - Ventana Medical Systems
  - Roche Oncology

Topics for Discussion

1. Recent advances in lung cancer treatment and diagnosis
2. Targeted therapy and predictive biomarkers for adenocarcinoma
3. Molecular profile of squamous cell carcinoma
4. Primary tumor xenograft as research model in targeted therapy

2004 WHO Classification of Malignant Lung Cancer



- **Squamous cell carcinoma**
- **Small cell carcinoma**
  - combined
- **Adenocarcinoma**
  - mixed type (>80%)
  - Acinar type
  - Papillary type
  - Bronchioloalveolar carcinoma
  - Solid type
- **Large cell carcinoma**
  - LCNEC (neuroendocrine)
  - etc.
- **Adenosquamous carcinoma**
- **Sarcomatoid carcinoma**
- **Carcinoid tumour**
  - Typical
  - Atypical
- **Salivary gland tumors**
  - Mucoepidermoid
  - Adenoid cystic
  - Epithelial-myoepithelial
- **Mesenchymal tumours**
  - Epithelioid hemangioendothelioma
  - Etc.

Before 2004

- **Histological classification underwent minor revisions (1982, 91, 97, 2004)**
- **Most important to distinguish small cell from non-small cell carcinoma**
- **Distinction between major subtypes of NSCLC, i.e. adeno, squamous, large cell is not crucial**
- **Use of non-specific term such as “Non-small cell Not Otherwise Specified (NOS)” has been acceptable**

Landmark Discoveries and Studies Leading to Paradigm Shift in Lung Cancer Diagnosis

EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy

J. Guillermo Paez,[1,2*] Pasi A. Jänne,[1,2*] Jeffrey C. Lee,[1,3*]
Sean Tracy,[1] Heidi Greulich,[1,2] Stacey Gabriel,[4] Paula Herman,[1]
Frederic J. Kaye,[5] Neal Lindeman,[6] Titus J. Boggon,[1,3]
Katsuhiko Naoki,[1] Hidefumi Sasaki,[7] Yoshitaka Fujii,[7]
Michael J. Eck,[1,3] William R. Sellers,[1,2,4†]
Bruce E. Johnson,[1,2†] Matthew Meyerson[1,3,4†]

SCIENCE April 29, 2004

Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib

Thomas J. Lynch, M.D., Daphne W. Bell, Ph.D., Raffaella Sordella, Ph.D., Sarada Gurubhagavatula, M.D., Ross A. Okimoto, B.S., Brian W. Brannigan, B.A., Patricia L. Harris, M.S., Sara M. Haserlat, B.A., Jeffrey G. Supko, Ph.D., Frank G. Haluska, M.D., Ph.D., David N. Louis, M.D., David C. Christiani, M.D., Jeff Settleman, Ph.D., and Daniel A. Haber, M.D., Ph.D.

NEW ENGLAND JOURNAL OF MEDICINE, MAY 20, 2004

The NEW ENGLAND JOURNAL of MEDICINE

Erlotinib in Previously Treated Non–Small-Cell Lung Cancer

Frances A. Shepherd, M.D., José Rodrigues Pereira, M.D., Tudor Ciuleanu, M.D., Eng Huat Tan, M.D., Vera Hirsh, M.D., Sumitra Thongprasert, M.D., Daniel Campos, M.D., Savitree Maoleekoonpiroj, M.D., Michael Smylie, M.B., Ch.B., Renato Martins, M.D., Maximiliano van Kooten, M.D., Mircea Dediu, M.D., Brian Findlay, M.D., Dongsheng Tu, Ph.D., Thomas Johnston, Andrea Bezjak, M.D., Gary Clark, Ph.D., Pedro Santabárbara, M.D., Ph.D., and Lesley Seymour, M.D., Ph.D., for the National Cancer Institute of Canada Clinical Trials Group*

NCIC CTG BR.21 Trial

(July 14, 2005)

Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

Tony S. Mok, M.D., Yi-Long Wu, M.D., F.A.C.S., Sumitra Thongprasert, M.D., Chih-Hsin Yang, M.D., Ph.D., Da-Tong Chu, M.D., Nagahiro Saijo, M.D., Ph.D., Patrapim Sunpaweravong, M.D., Baohui Han, M.D., Benjamin Margono, M.D., Ph.D., F.C.C.P., Yukito Ichinose, M.D., Yutaka Nishiwaki, M.D., Ph.D., Yuichiro Ohe, M.D., Ph.D., Jin-Ji Yang, M.D., Busyamas Chewaskulyong, M.D., Haiyi Jiang, M.D., Emma L. Duffield, M.Sc., Claire L. Watkins, M.Sc., Alison A. Armour, F.R.C.R., and Masahiro Fukuoka, M.D., Ph.D.

IPASS

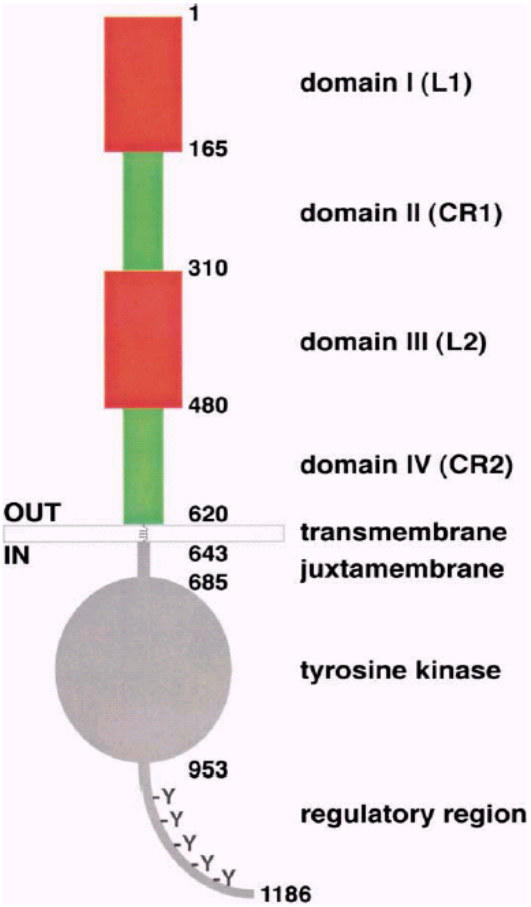
(September 3, 2009)

Personalized Cancer Therapy Based on Biomarkers or Molecular Pathology Information

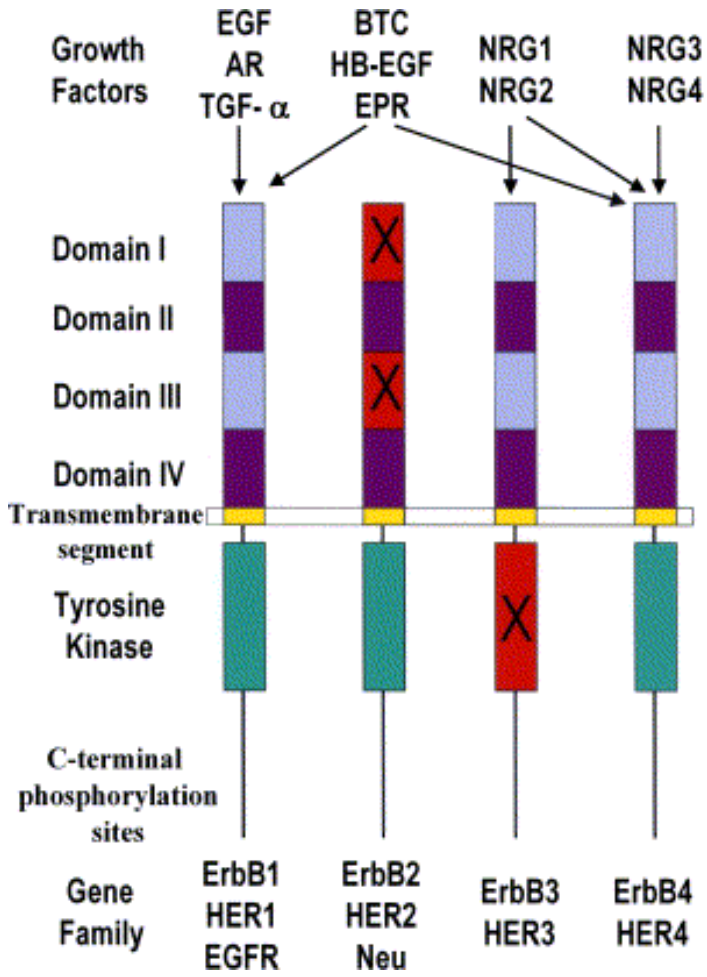
New Therapies in NSCLC

Drug (Target)	**Selection Marker**	**Histology Selection**
Gefitinib (EGFR)	**EGFR mutation (1st line)**	**Adenocarcinoma**
Erlotinib (EGFR)		
Crizotinib (ALK)	**ALK rearrangement**	
Bevacizumab (VEGFR) **Pemetrexed** (Folate Pathway)	**Histology**	**Non-squamous carcinomas**
Cetuximab (EGFR)	**EGFR protein by IHC (potential)**	**NSCLC**

Epidermal Growth Factor Receptor (EGFR/HER/ErbB)



Burgess AW, et al. Molec Cell 2003;12:541-52



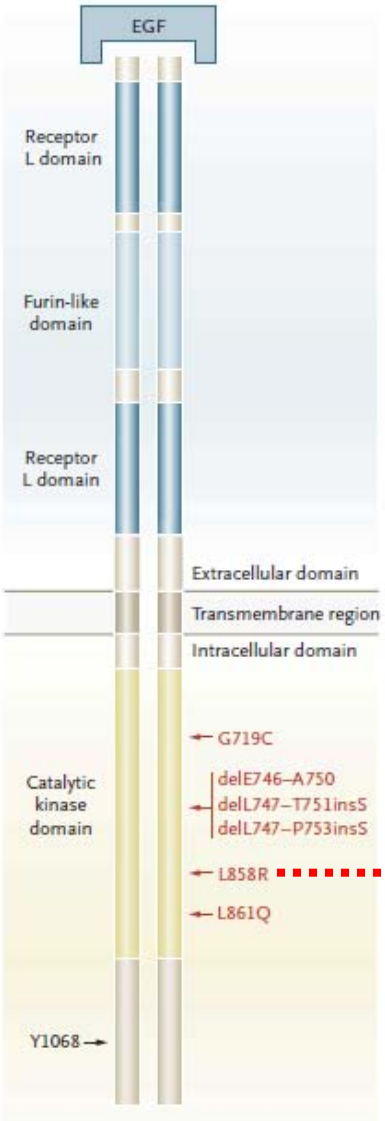
Roskoski Jr, BBRC 2004;319:1-11

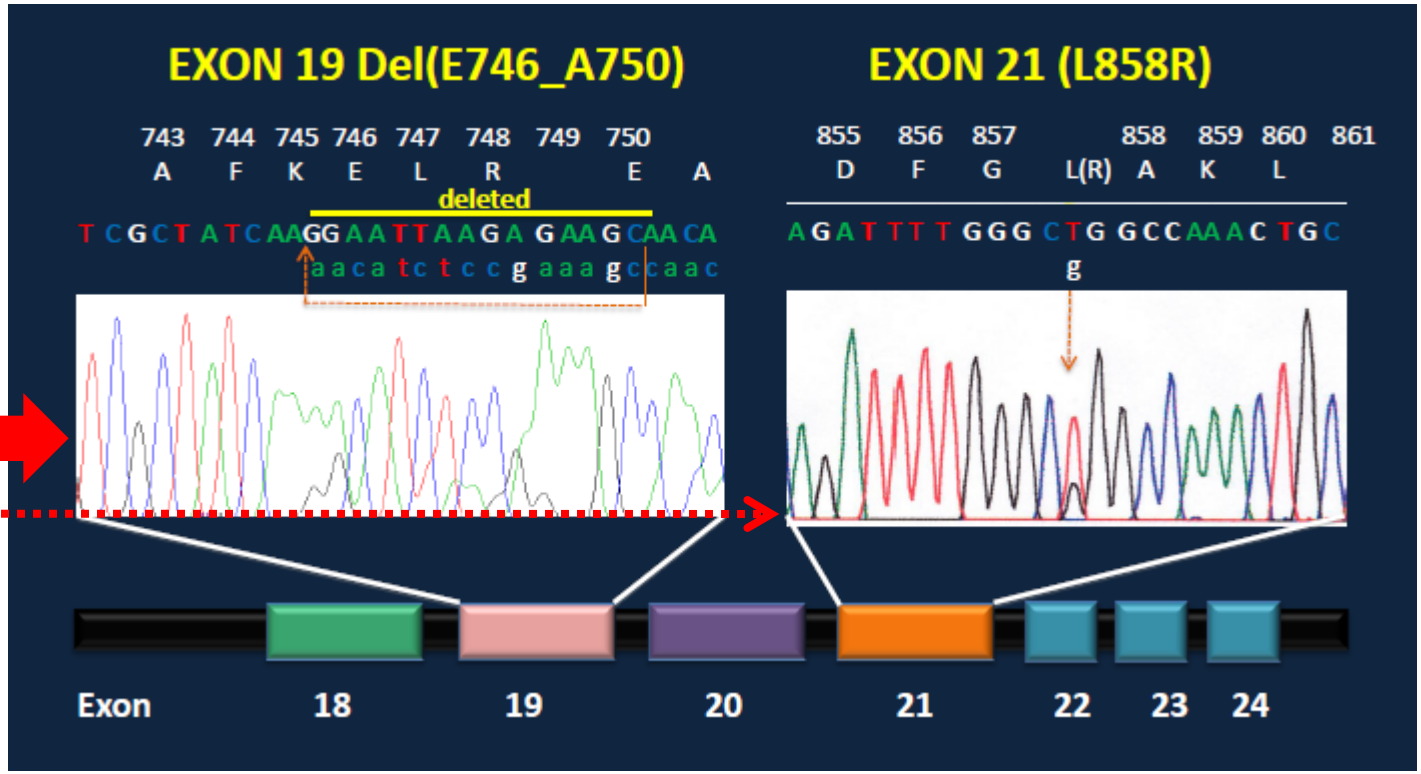
High Frequency of EGFR Expression in Non-small Cell Lung Cancer

	SQCC	ADC	LCC
Rusch (1997)	94%	57%	63%
Fontanini (1998)	57%	35%	23%
Hsieh (2000)	92%	53%	60%
Hirsch (2003)	82%	46%	33%

SQCC: squamous cell ca, ADC: adenoca; LCC: large cell ca

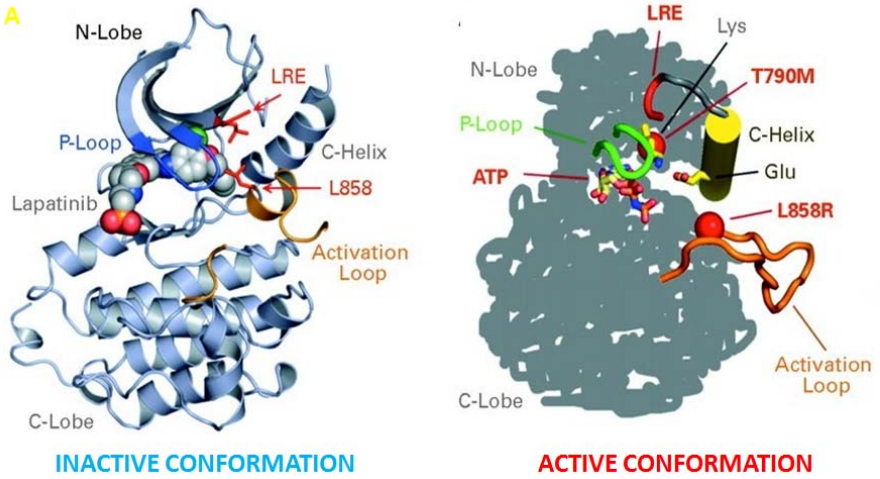
EGFR TK Domain Mutations

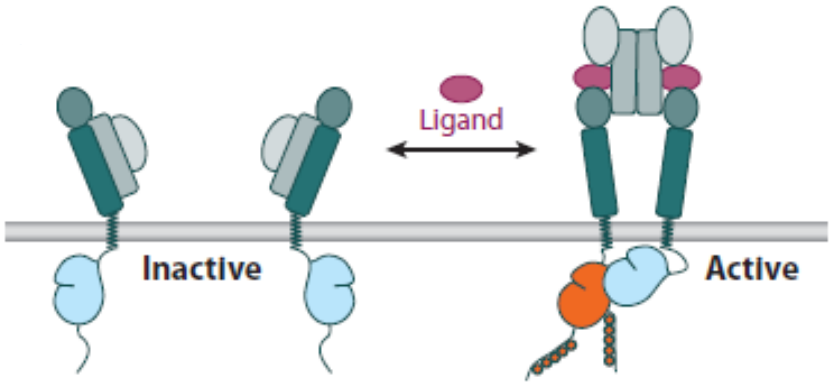


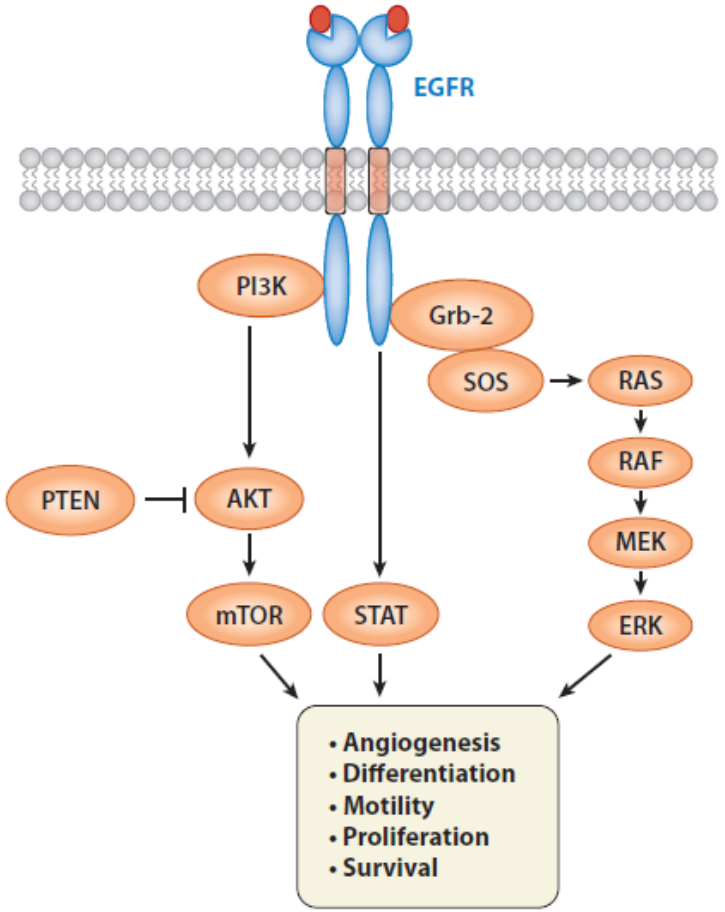


Lynch T, et al. NEJM 350: 2129-39, 2004

EGFR Activation and Signaling

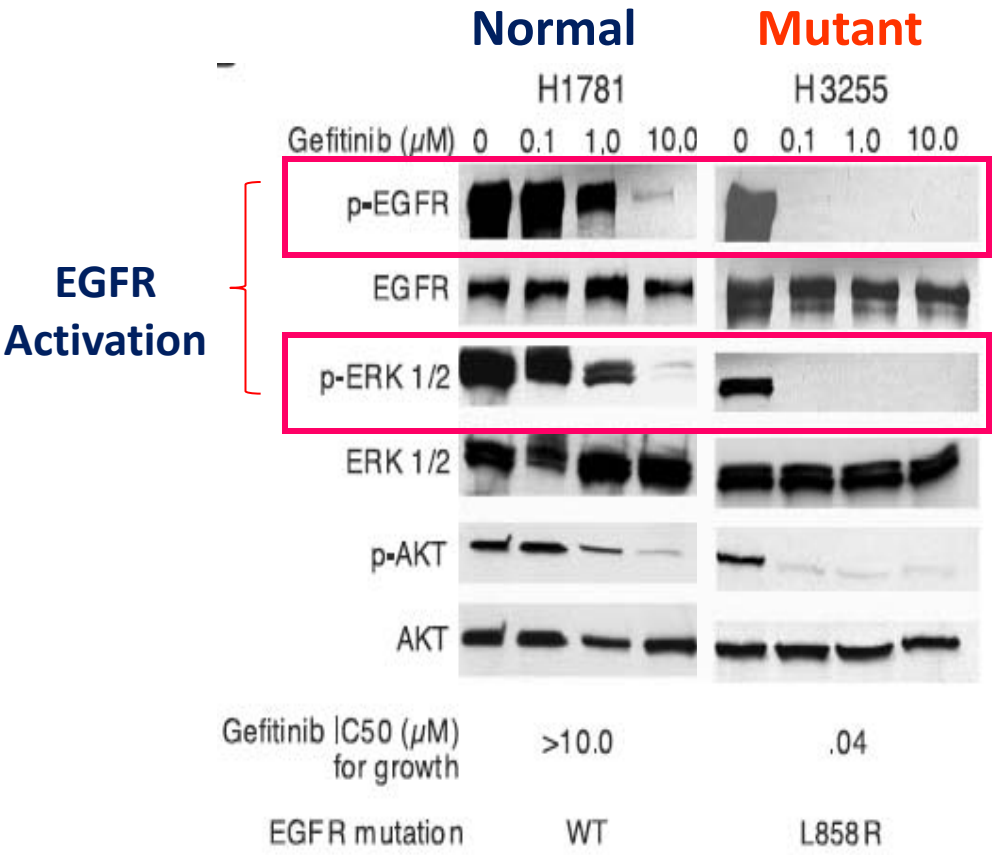


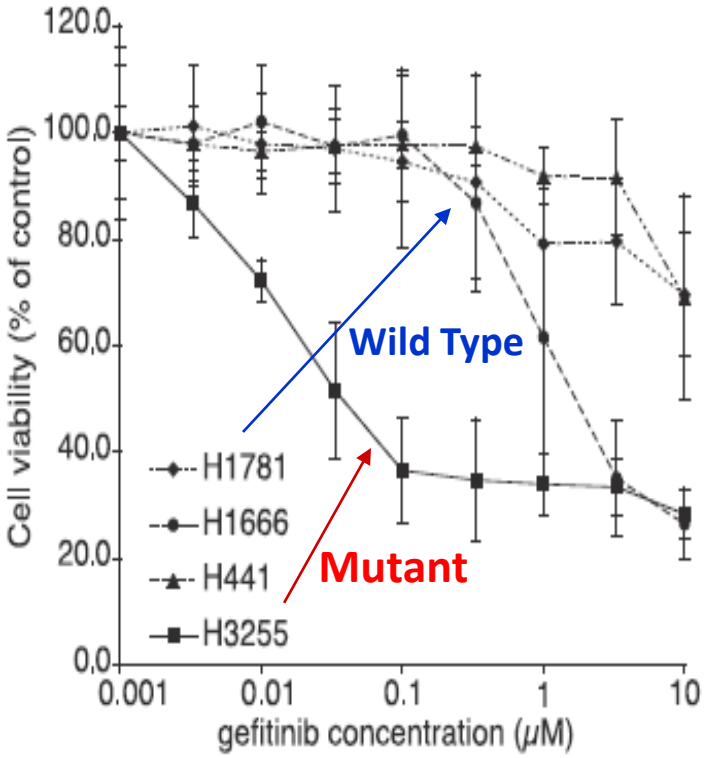




Kumar A, et al. J Clin Oncol 26:1742-1751

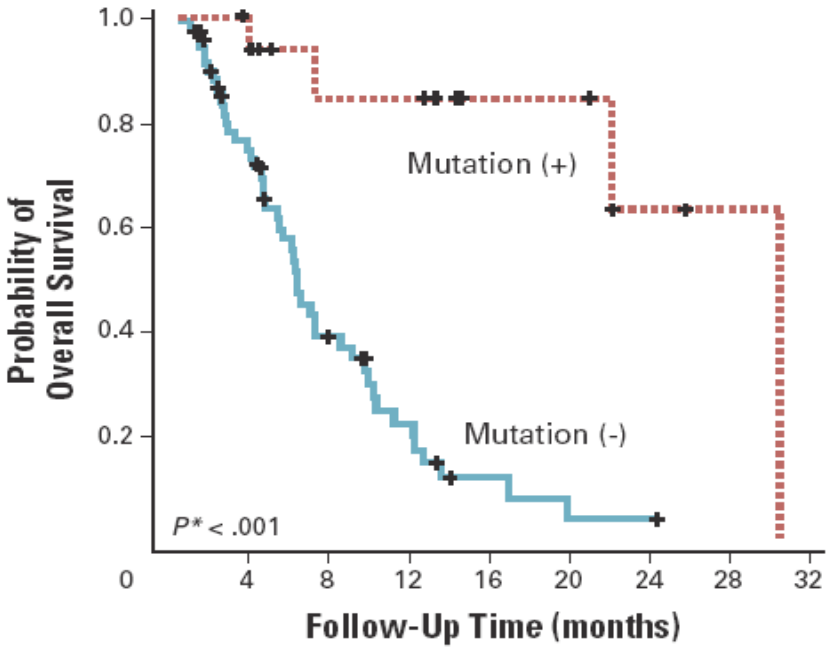
EGFR Mutant Lung Cancer Cells are more Sensitive to EGFR TK Inhibitors





Paez et al: Science 2005;304:1497-1500

EGFR TKI Improves Survival of EGFR Mutant Compared to Wild Type Patients



Han S-W et al. J Clin Oncol 2005;23: 2493-2501

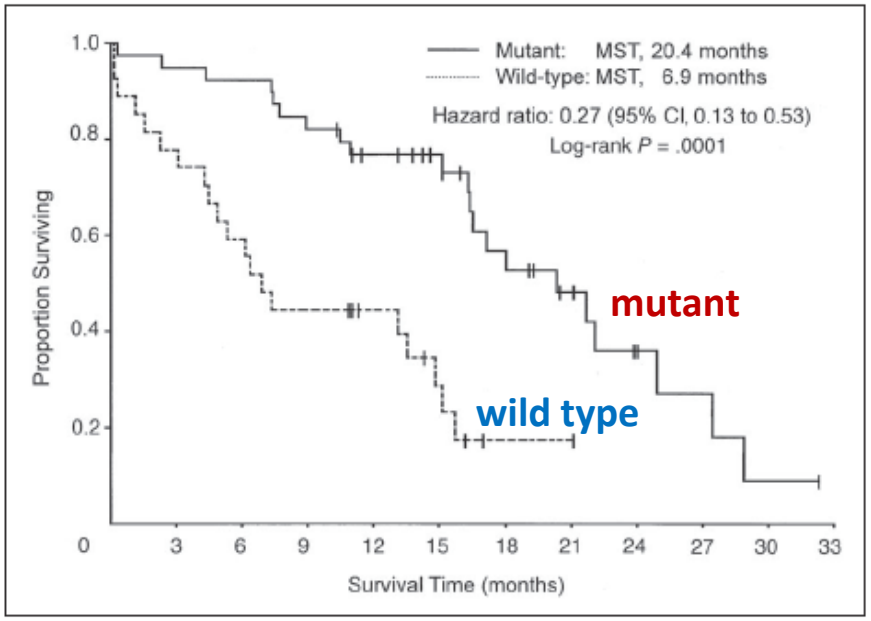


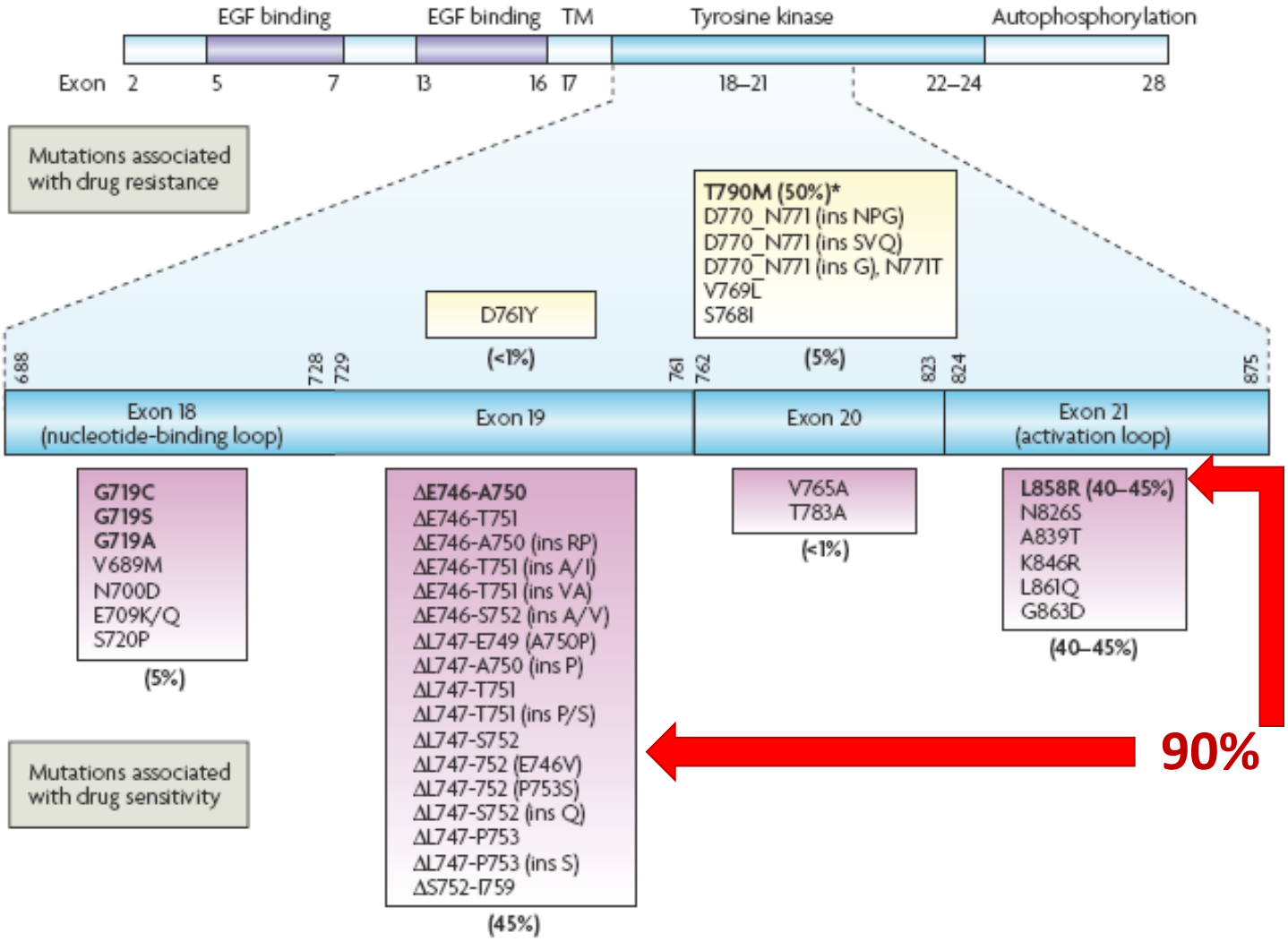
Fig 3. Kaplan-Meier plot of overall survival according to epidermal growth factor receptor (*EGFR*) mutation status. MST, median survival time.

Takano T et al. J Clin Oncol 2005;23: 6829-37

EGFR TK Domain Mutations

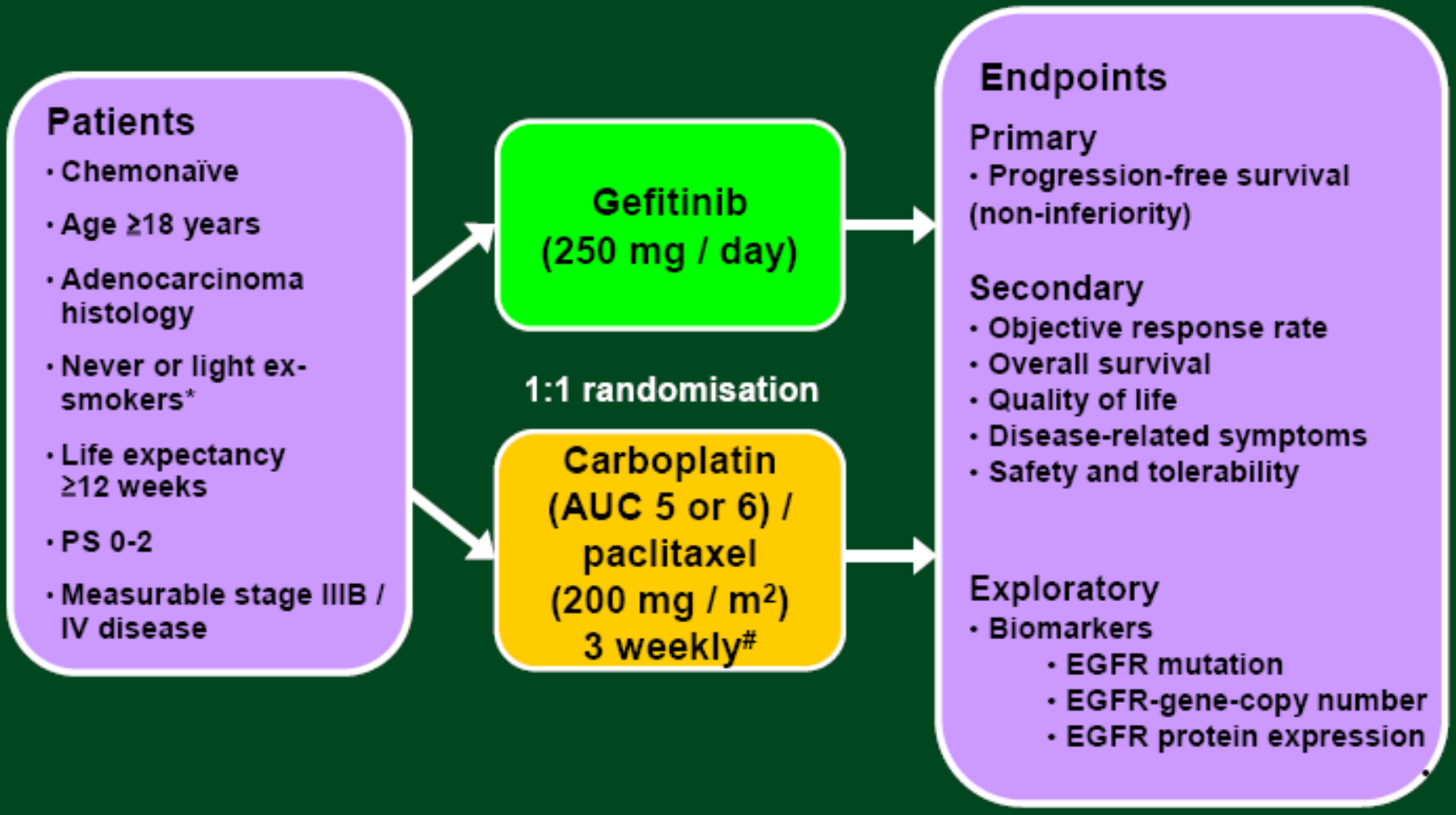
- Adeno > Squamous (<5%)
- Women > Men
- East Asian (40-50%) > Caucasian (10-20%)
- Never smokers > smokers

EGFR TK Domain Mutations



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

IPASS (Iressa Pan-ASia Study)

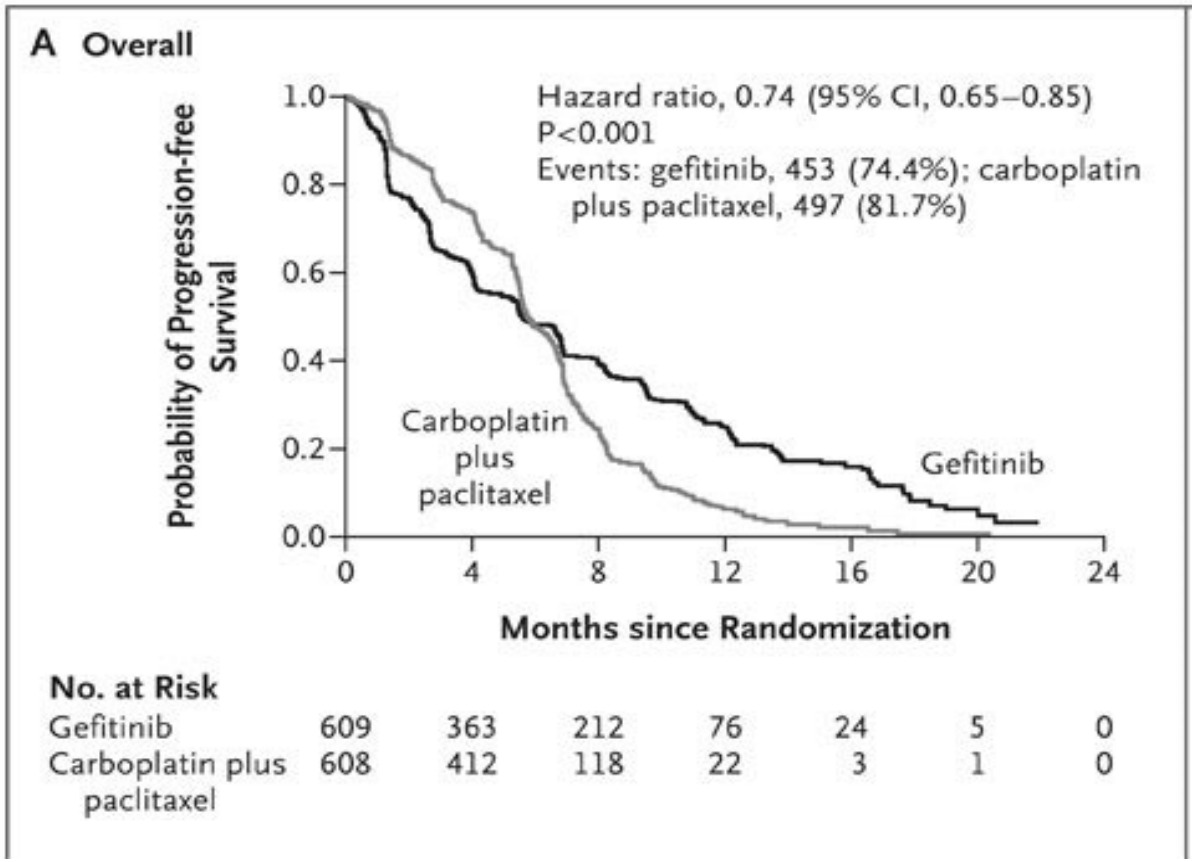


*Never smokers, <100 cigarettes in lifetime; light ex-smokers, stopped ≥15 years ago and smoked ≤10 pack years; #limited to a maximum of 6 cycles
Carboplatin / paclitaxel was offered to gefitinib patients upon progression
PS, performance status; EGFR, epidermal growth factor receptor

Mok et al, Chicago 2008

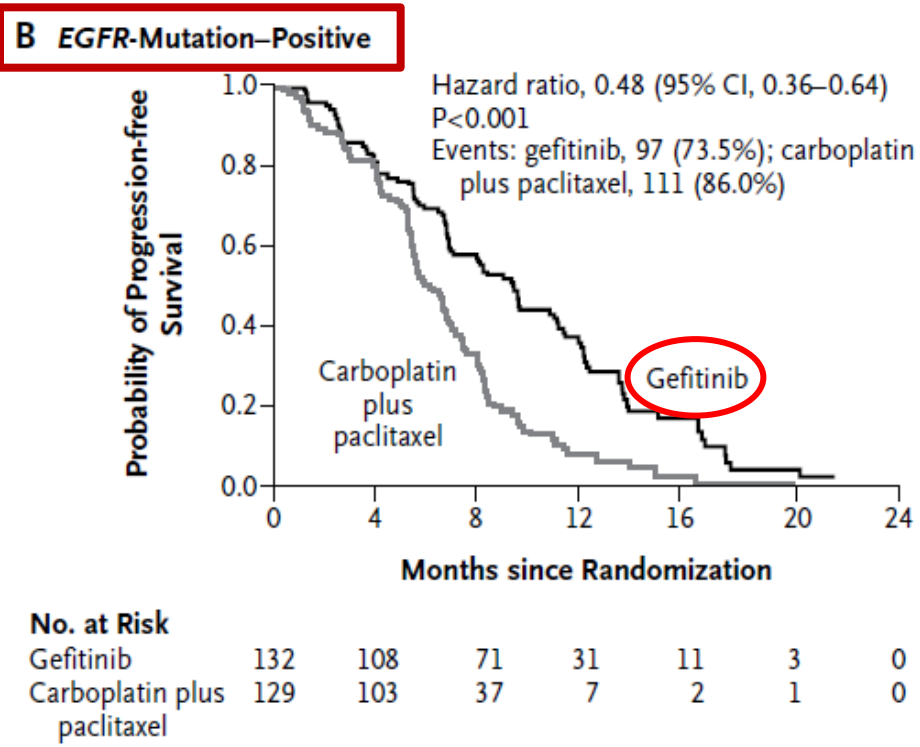
Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

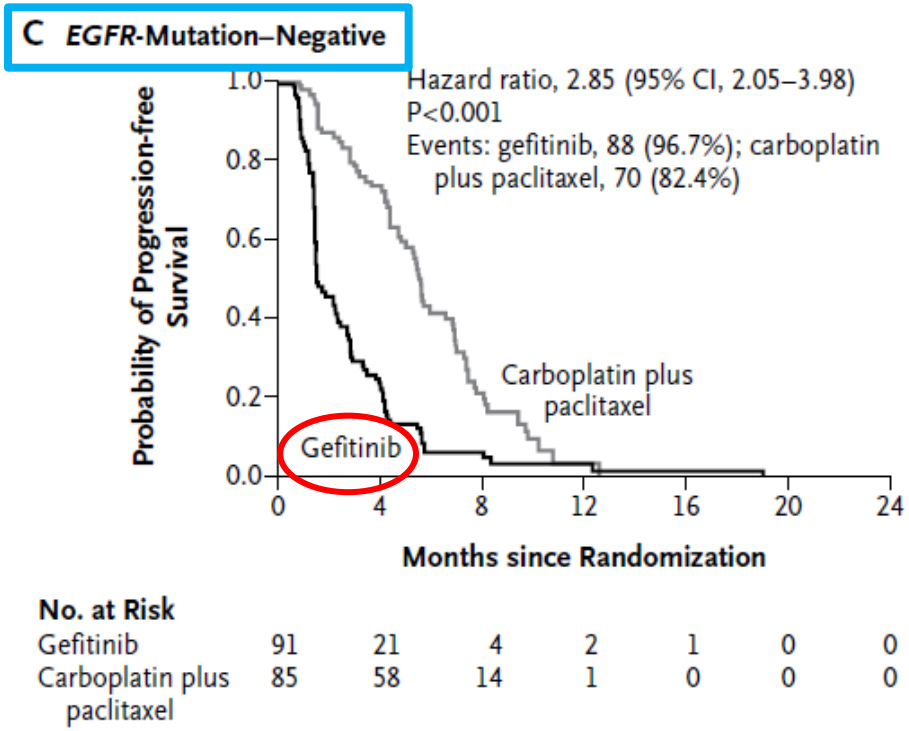
Tony S. Mok, M.D., Yi-Long Wu, M.D., F.A.C.S., Sumitra Thongprasert, M.D., Chih-Hsin Yang, M.D., Ph.D., Da-Tong Chu, M.D., Nagahiro Saijo, M.D., Ph.D., Patrapim Sunpaweravong, M.D., Baohui Han, M.D., Benjamin Margono, M.D., Ph.D., F.C.C.P., Yukito Ichinose, M.D., Yutaka Nishiwaki, M.D., Ph.D., Yuichiro Ohe, M.D., Ph.D., Jin-Ji Yang, M.D., Busyamas Chewaskulyong, M.D., Haiyi Jiang, M.D., Emma L. Duffield, M.Sc., Claire L. Watkins, M.Sc., Alison A. Armour, F.R.C.R., and Masahiro Fukuoka, M.D., Ph.D.



N Engl J Med 2009;361:947-57.

Gefitinib Treatment Should be Directed Only to EGFR Mutant Patients





N Engl J Med 2009;361:947-57.

Phase III studies of EGFR-TKI vs. Platinum doublet in EGFR Mutant Patients

Group	EGFR mutation	Primary endpoint	N (TKI vs. CT)	TKI	Control
WJOG 3405	EX19, L858R	PFS	172 (HR=0.49)	G	CDDP+DOC
NEJ 002	EX19, L858R, G719X, L861Q	PFS	320 (HR=0.69)	G	CBDCA+PAC
EURTARC	EX19, L858R	PFS	174 (HR=0.37)	E	Pt doublet
Optimal	EX19, L858R, T790M	PFS	165 (HR=0.16)	E	CBDCA+GEM

Current Guideline for EGFR Mutation Testing

- **Advanced NSCLC patients being considered for treatment with 1st line gefitinib therapy (chemotherapy naïve)**
- **Mainly for adenocarcinoma or NSCLC with adeno component – Important to use immunohistochemistry markers for more precise histological diagnosis**

EGFR Mutation Testing

- Testing performed in a certified clinical diagnostic laboratory
- Minimum test includes exon 19 deletion and exon 21 L858R mutation; rare sensitizing mutations should also be tested
- Pre-testing analysis include examination of the histology/cytology slide by a pathologist to confirm tumor type and define cellularity and areas for microdissection or coring

International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification of Lung Adenocarcinoma

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.

J Thorac Oncol. 2011;6: 244–285

Selection of Samples for Epidermal Growth Factor Receptor (EGFR) Mutation Analysis in Non-Squamous Non-Small Cell Lung Carcinoma

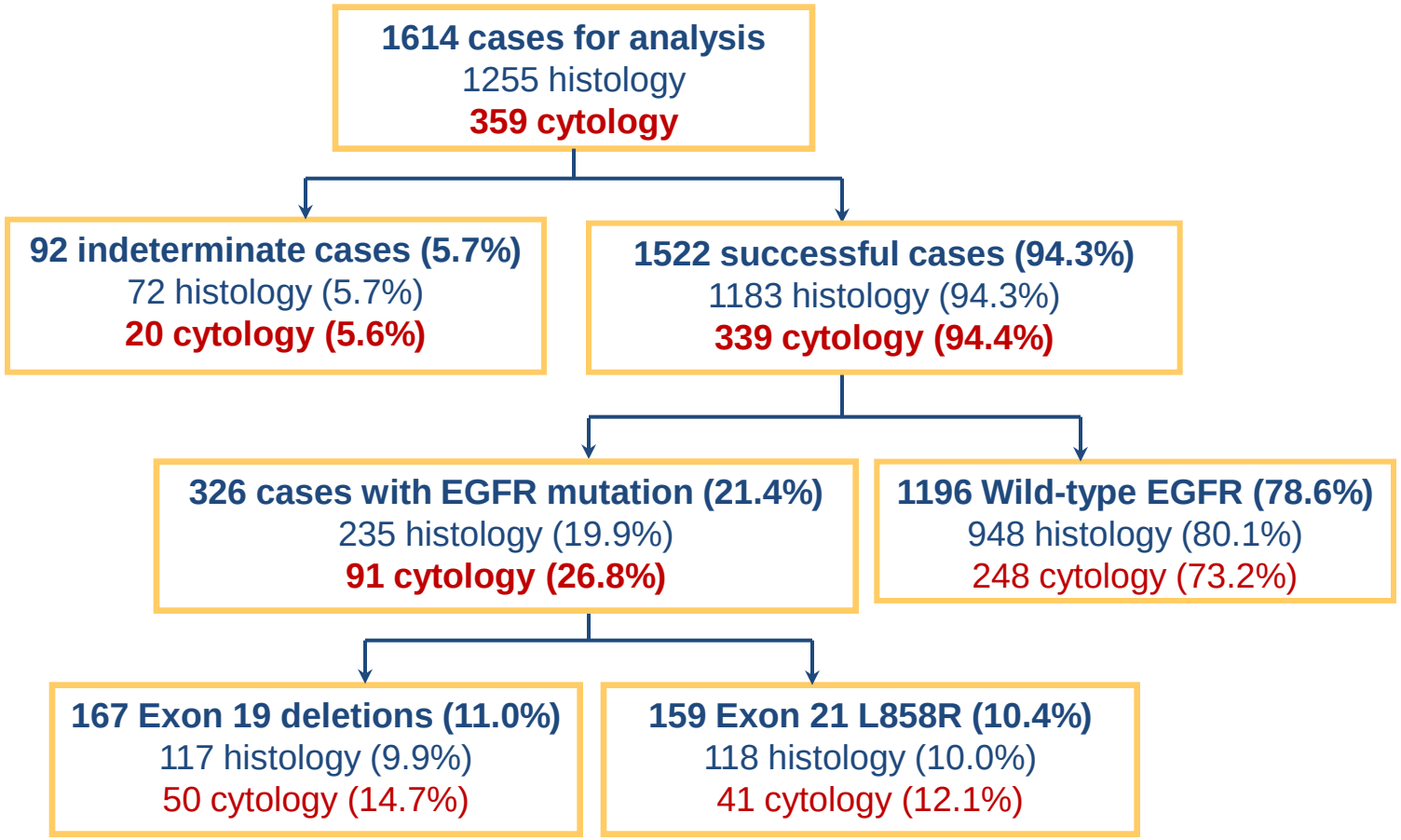
Carolyn J Shiau[1,2], Jesse Babwah [1,2], Gilda da Cunha Santos[1], Jenna Sykes[3], Melania Pintilie[3], Scott L Boerner[1], William R Geddie[1], Suzanne Kamel-Reid[1], Cuihong Wei[1], David M Hwang[1], Ming S Tsao[1]

[1] Department of Laboratory Medicine and Pathology, University Health Network; [2] Department of Laboratory Medicine and Pathobiology, University of Toronto; [3] Department of Biostatistics, University Health Network and Princess Margaret Hospital

USCAP 2012

Ontario (Canada) province-wide mutation testing program

- **1.5 year period (2010-11)**



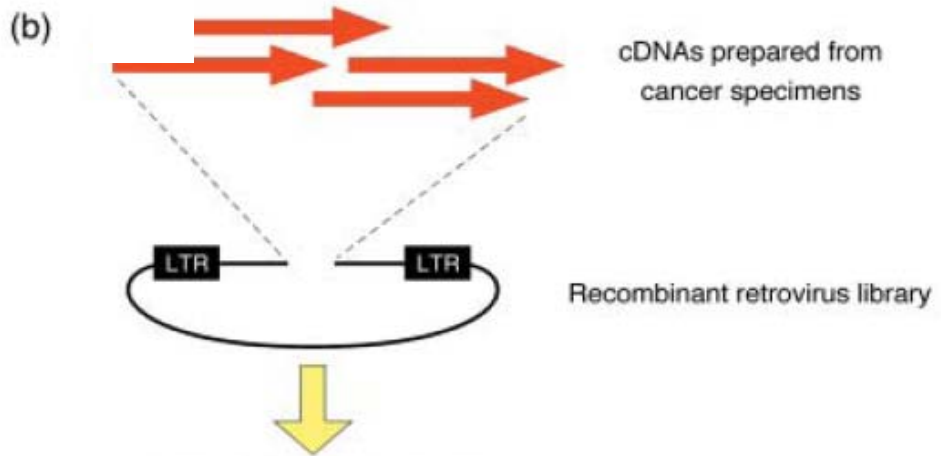
Modern Pathol 2012;25 (suppl2): 490A (Abstract 2045)

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

Nature 2007 (Aug 2);448:561-566

Manabu Soda[1,2], Young Lim Choi[1], Munehiro Enomoto[1,2], Shuji Takada[1], Yoshihiro Yamashita[1], Shunpei Ishikawa[5], Shin-ichiro Fujiwara[1], Hideki Watanabe[1], Kentaro Kurashina[1], Hisashi Hatanaka[1], Masashi Bando[2], Shoji Ohno[2], Yuichi Ishikawa[6], Hiroyuki Aburatani[5,7], Toshiro Niki[3], Yasunori Sohara[4], Yukihiko Sugiyama[2] & Hiroyuki Mano[1,7]

cDNA prepared from lung cancer specimen of a 62 year old smoker man negative for *EGFR* and *KRAS* mutation



Mouse 3T3 fibroblast

Transformed foci

EML4-ALK Gene Fusion

EML4-ALK v1

```
MDGFAGSLDDSISAASTSDVQDRLSALESRVQQQEDEITVLKAALADVLRRLAISEDHVA     60
SVKKSVSSKGQPSPRAVIPMSCITNGSGANRKPSHTSAVSIAGKETLSSAAKSGTEKKKE    120
KPQGQREKKEESHSNDQSPQIRASPSPQPSSQPLQIHRQTPESKNATPTKSIKRPSPAEK    180
SHNSWENSDDSRNKLSKIPSTPKLIPKVTKTADKHKDVIINQEGEYIKMFMRGRPITMFI    240
PSDVDNYDDIRTELPPEKLKLEWAYGYRGKDCRANVYLLPTGEIVYFIASVVVLFNYEER    300
TQRHYLGHTDCVKCLAIHPDKIRIATGQIAGVDKDGRPLQPHVRVWDSVTLSTLQIIGLG    360
TFERGVGCLDFSKADSGVHLCVIDDSNEHMLTVWDWQKKAKGAEIKTTNEVVLAVEFHPT    420
DANTIITCGKSHIFFWTWSGNSLTRKQGIFGKYEKPKFVQCLAFLGNGDVLTGDSGGVML    480
IWSKTTVEPTPGKGPKVYRRKHQELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGK    540
TSSISDLKEVPRKNITLIRGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTLPEVCSEQDE    600
LDFLMEALIISKFNHQNIVRCIGVSLQSLPRFILLELMAGGDLKSFLRETRPRPSQPSSL    660
AMLDLLHVARDIACGCQYLEENHFIHRDIAARNCLLTCPGPGRVAKIGDFGMARDIYRAS    720
YYRKGGCAMLPVKWMPPEAFMEGIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEF    780
VTSGGRMDPPKNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEYCTQDPDVINTALPIEY    840
GPLVEEEEKVPVRPKDPEGVPPLLVSQQAKREEERSPAAPPPLPTTSSGKAAKKPTAAEV    900
SVRVPRGPAVEGGHVNMAFSQSNPPSELHRVHGSRNKPTSLWNPTYGSWFTEKPTKKNNP    960
IAKKEPHERGNLGLEGSCTVPPNVATGRLPGASLLLEPSSLTANMKEVPLFRLRHFPCGN   1,020
VNYGYQQQGLPLEAATAPGAGHYEDTILKSKNSMNQPGP                        1,059
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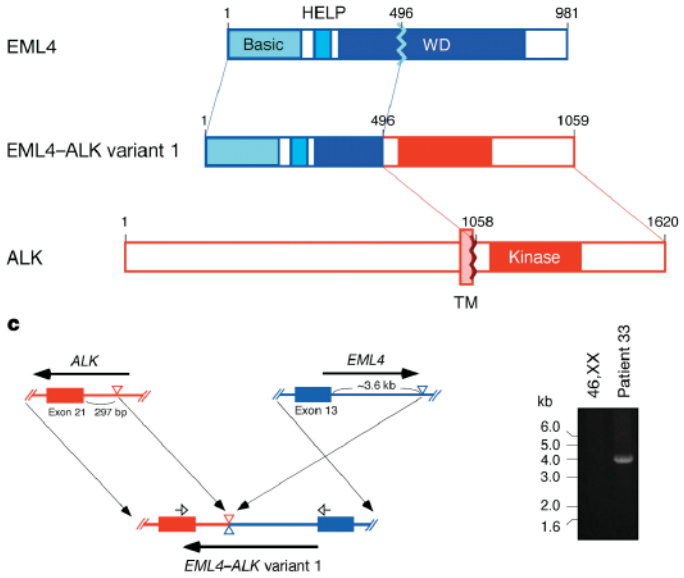
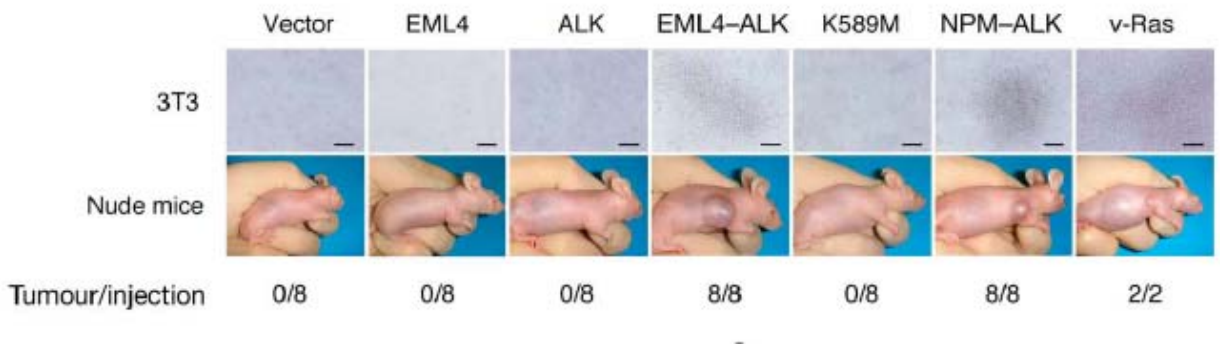
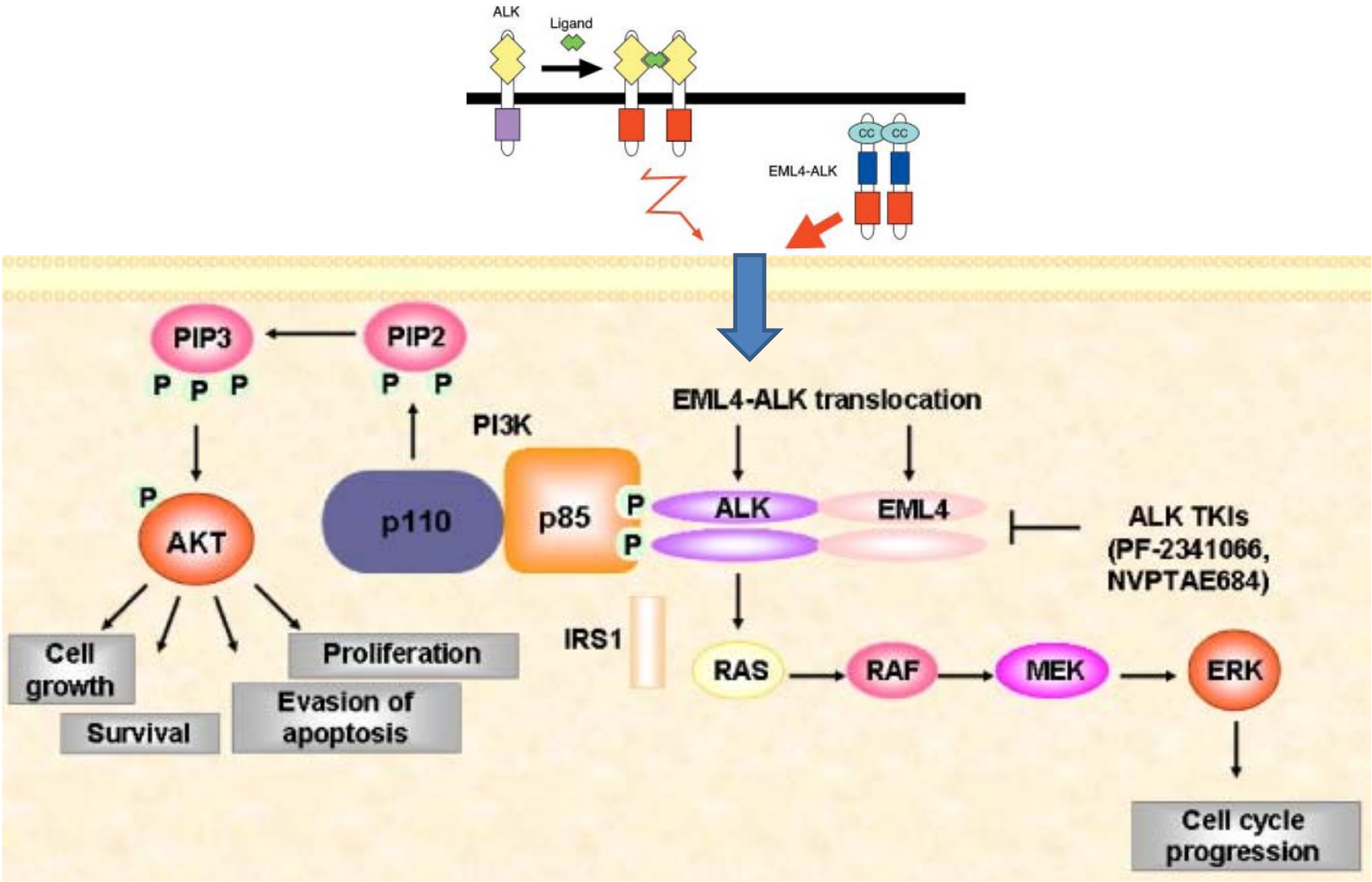
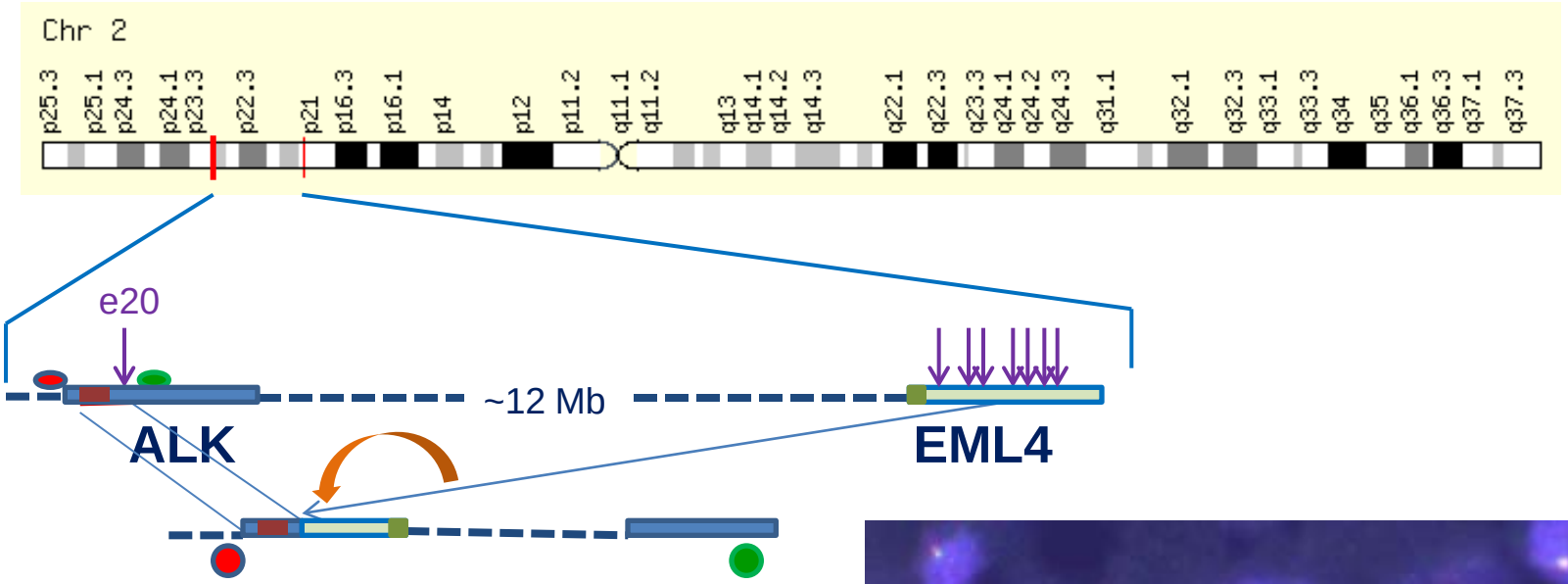


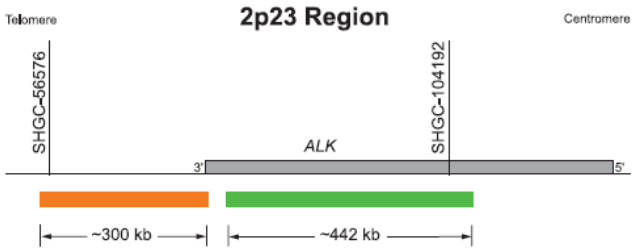


EML4-ALK Signaling Pathway

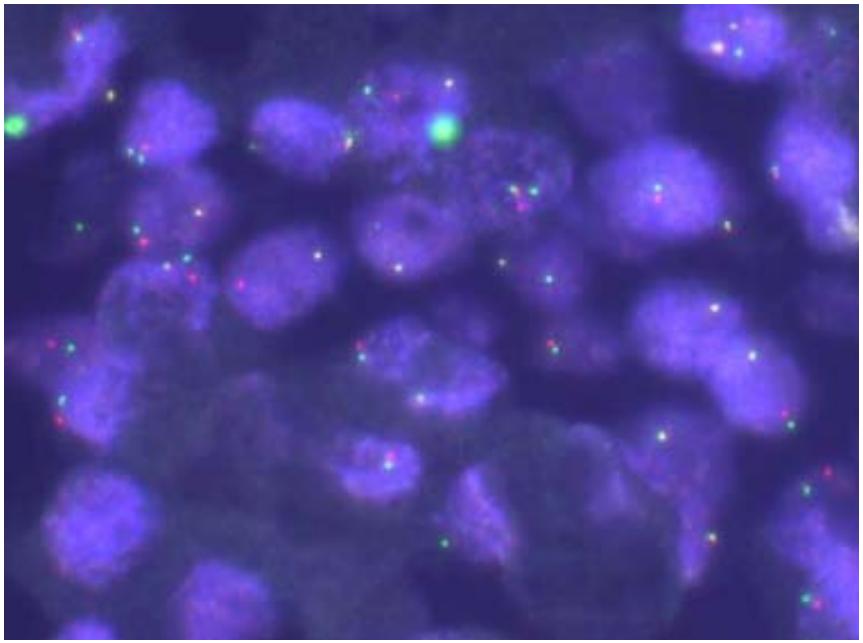


ALK Break Apart FISH

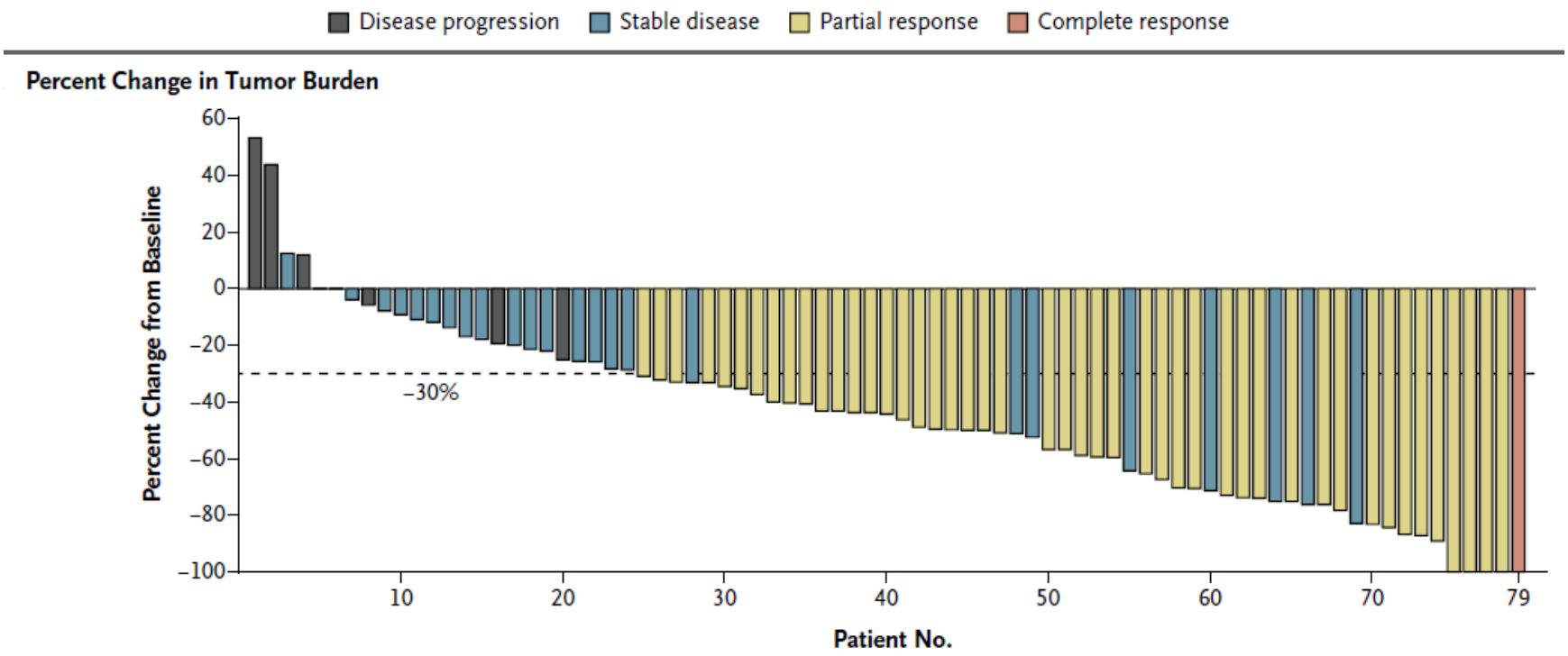




LSI ALK Dual Color, Break Apart Rearrangement Probe



EML4-ALK Positive NSCLC Patients are Highly Responsive to Crizotinib



Overall Response Rate = 57%

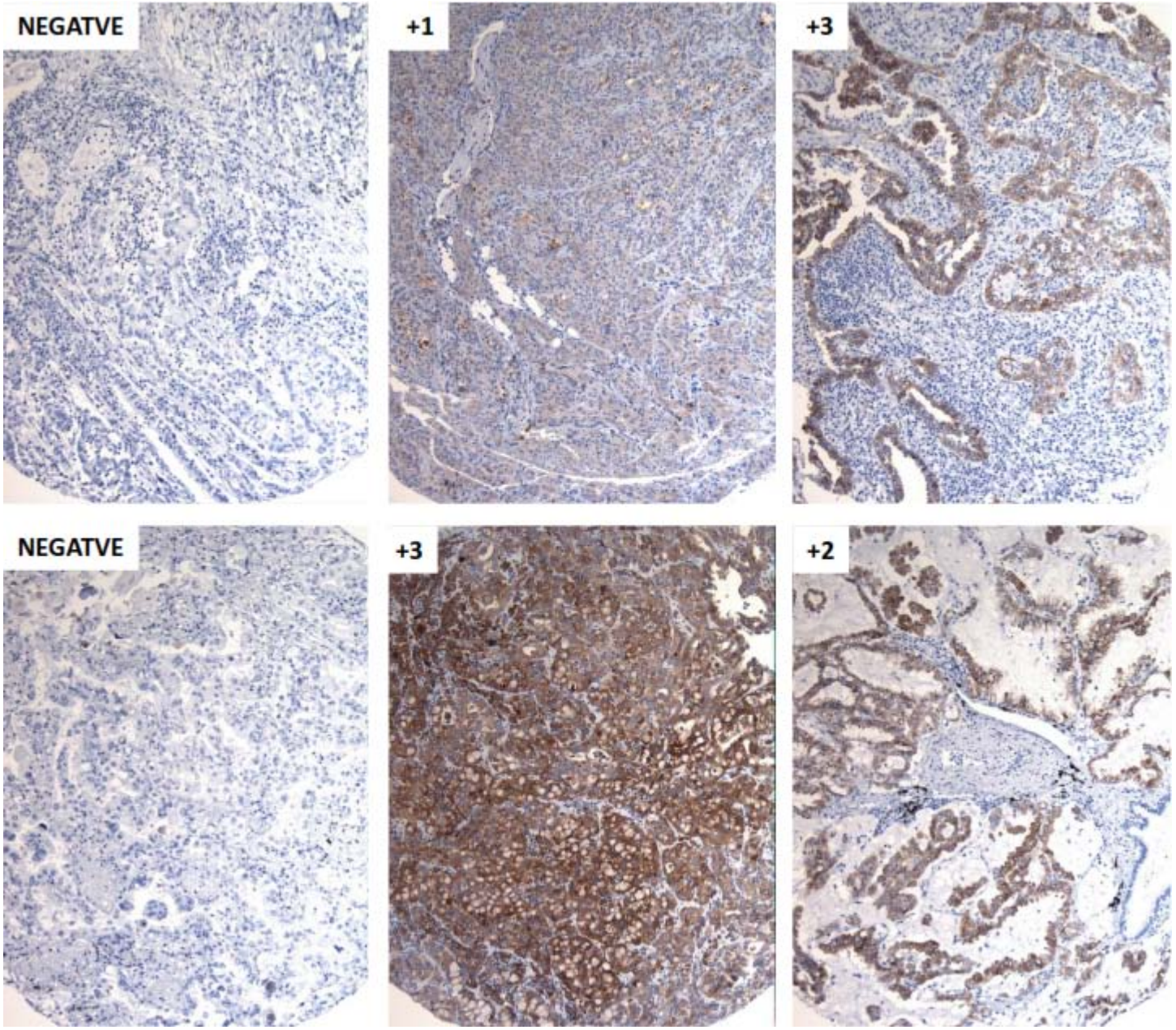
Disease Control Rate (CR+PR+SD) at 8 weeks= 87%

Kwak EL, et al. NEJM 2010;363:1693-703

(EML4)-ALK Fusion Gene Tumors Occur Mainly in Adenocarcinoma

First author	Adeno (total no.)	Squamous (total no.)	Others (total no.)
Imamura (2008)	3.4% (149)	0% (48)	0 (24)
Shinamura (2008)	4% (50)	0% (20)	0% (7)
Takeuchi (2008)	4.3% (253)	0% (71)	0% (19)
Koivunen (2008)	3.8% (208)	0% (88)	0 (100)
Rodig (2009)	5.6% (358)	-	-
Martlelli (2009)	4.8% (63)	8.3% (48)	22.2% (9)
Wong (2010)	5.3% (209)	0% (34)	8.7% (23)
Salido (2010)	4.3% (69)	0% (30)	0% (8)
Jokoji (2010)	3.1% (254)	-	-
Takahashi (2010)	2.4% (211)	0% (75)	0% (27)
Paik (2011)	6.3% (423)	0% (163)	3.7% (27)
Tumors studied	2247	577	244

ALK Immunohistochemistry



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

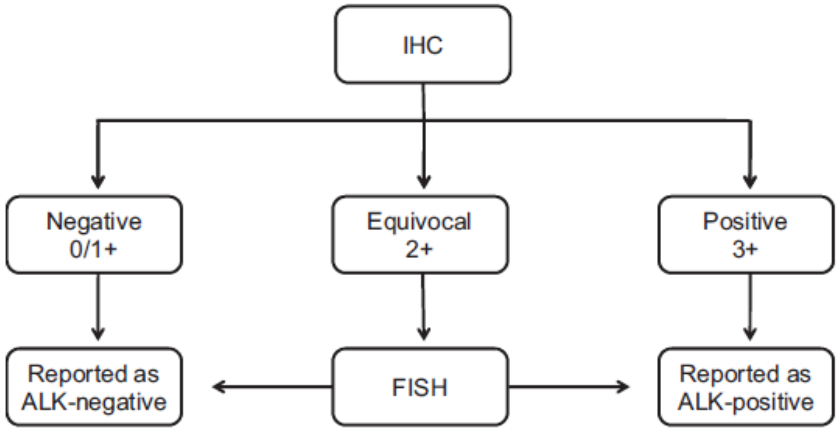
Correlation with Fluorescence In Situ Hybridization

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**

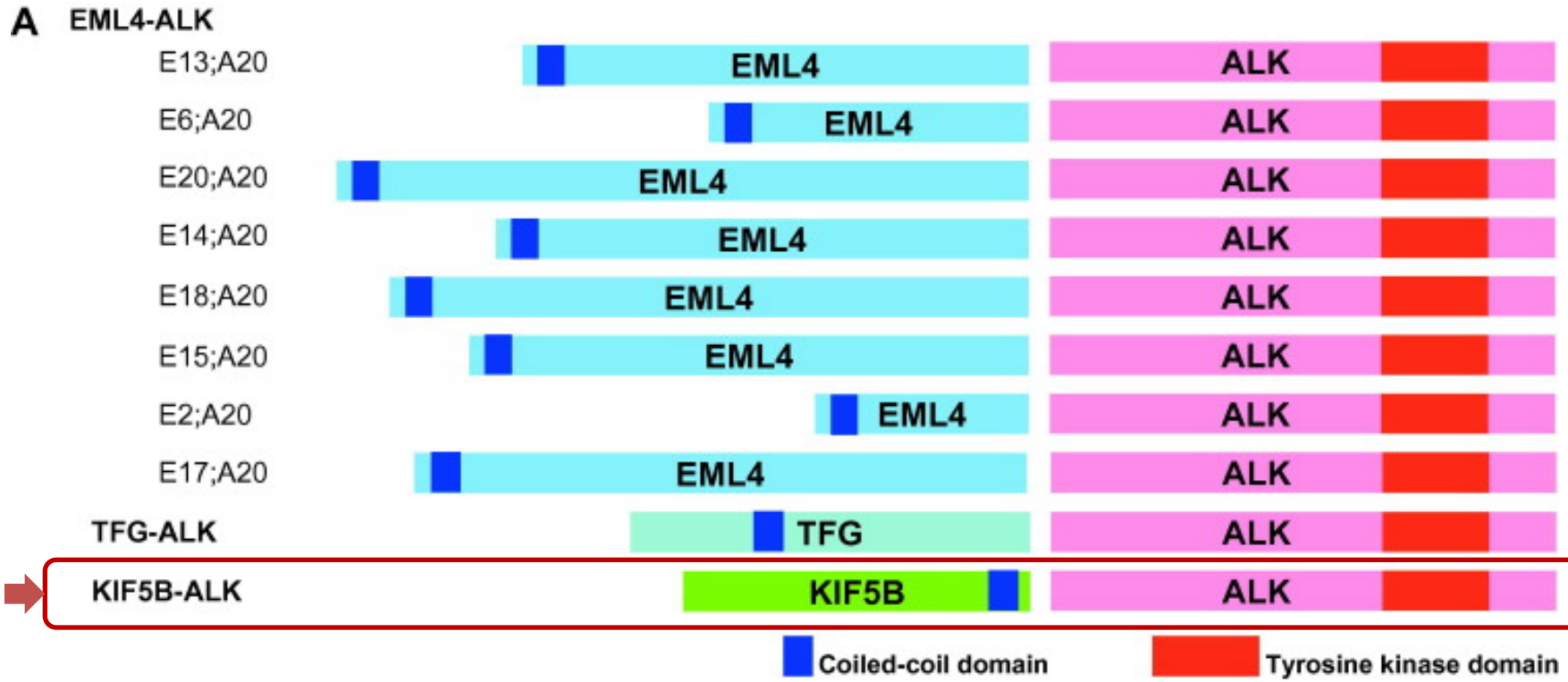
TABLE 1. The Relationship between ALK IHC and FISH in the Test Set

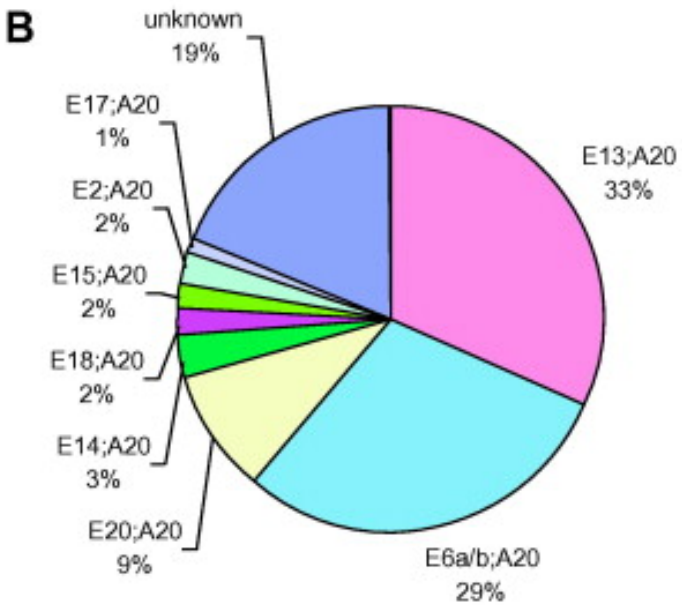
	ALK FISH		
ALK IHC	**(+)**	**(−)**	**Total (%)**
0	0	413 (91.2)	413 (91.2)
1+	0	14 (3.1)	14 (3.1)
2+	3 (0.7)	7 (1.5)	10 (2.2)
3+	16 (3.5)	0	16 (3.5)
Total	19 (4.2)	434 (95.8)	453 (100)

IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ALK, anaplastic lymphoma kinase.



J Thoracic Oncol 2011;6:466-72





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

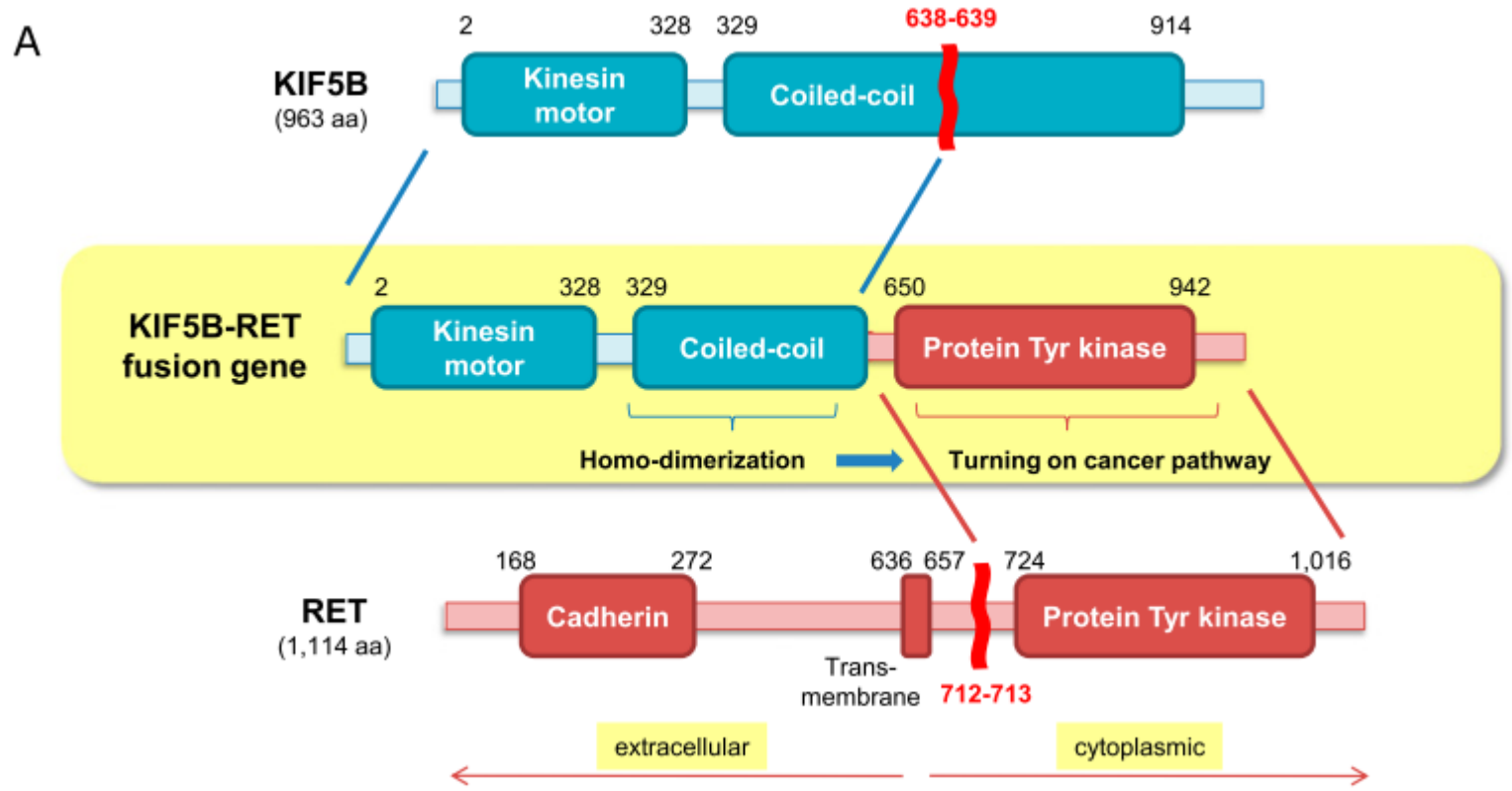
NSCLC Cell lines

H3122 and DFCI032 contain E13;A20. H2228 contain E6;A20

Sasaki T, et al, Eur J Cancer. 2010 Jul;46(10):1773-80.

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

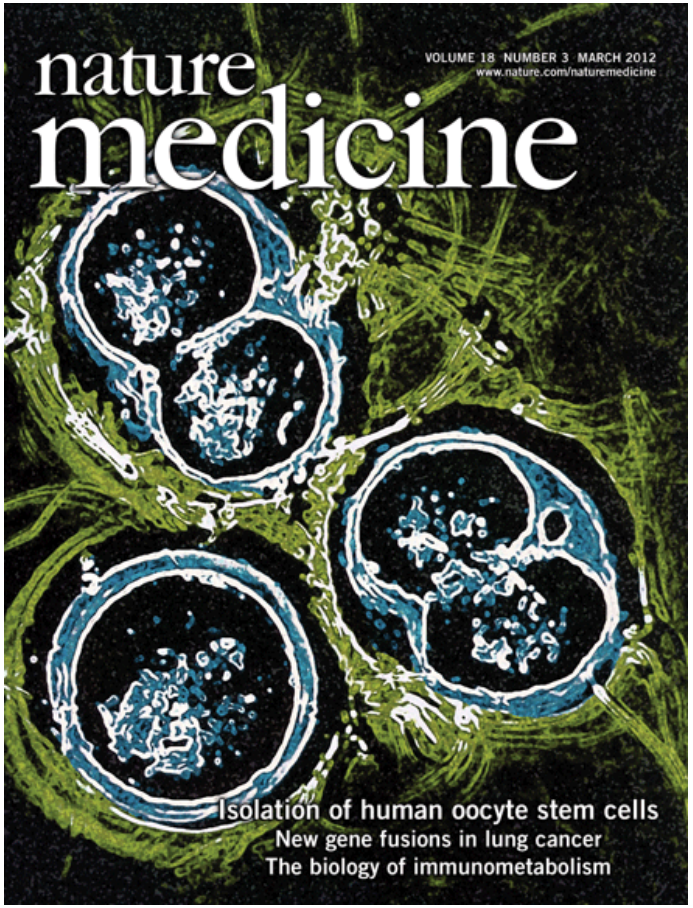
Young Seok Ju,[1,2] Won-Chul Lee,[1,3] Jong-Yeon Shin,[1,4] Seungbok Lee,[1,3] Thomas Bleazard,[1] Jae-Kyung Won,[5] Young Tae Kim,[6,7] Jong-Il Kim,[1,3,4,8] Jin-Hyoung Kang,[9] and Jeong-Sun Seo[1,2,3,4,8,10]



Genome Res 2012;22:436-445 (Epub December 22, 2011)

New Potential Target for RET inhibitors

March 2012



KIF5B-RET fusions in lung adenocarcinoma

Takashi Kohno[1,15], Hitoshi Ichikawa[2,15], Yasushi Totoki[3],

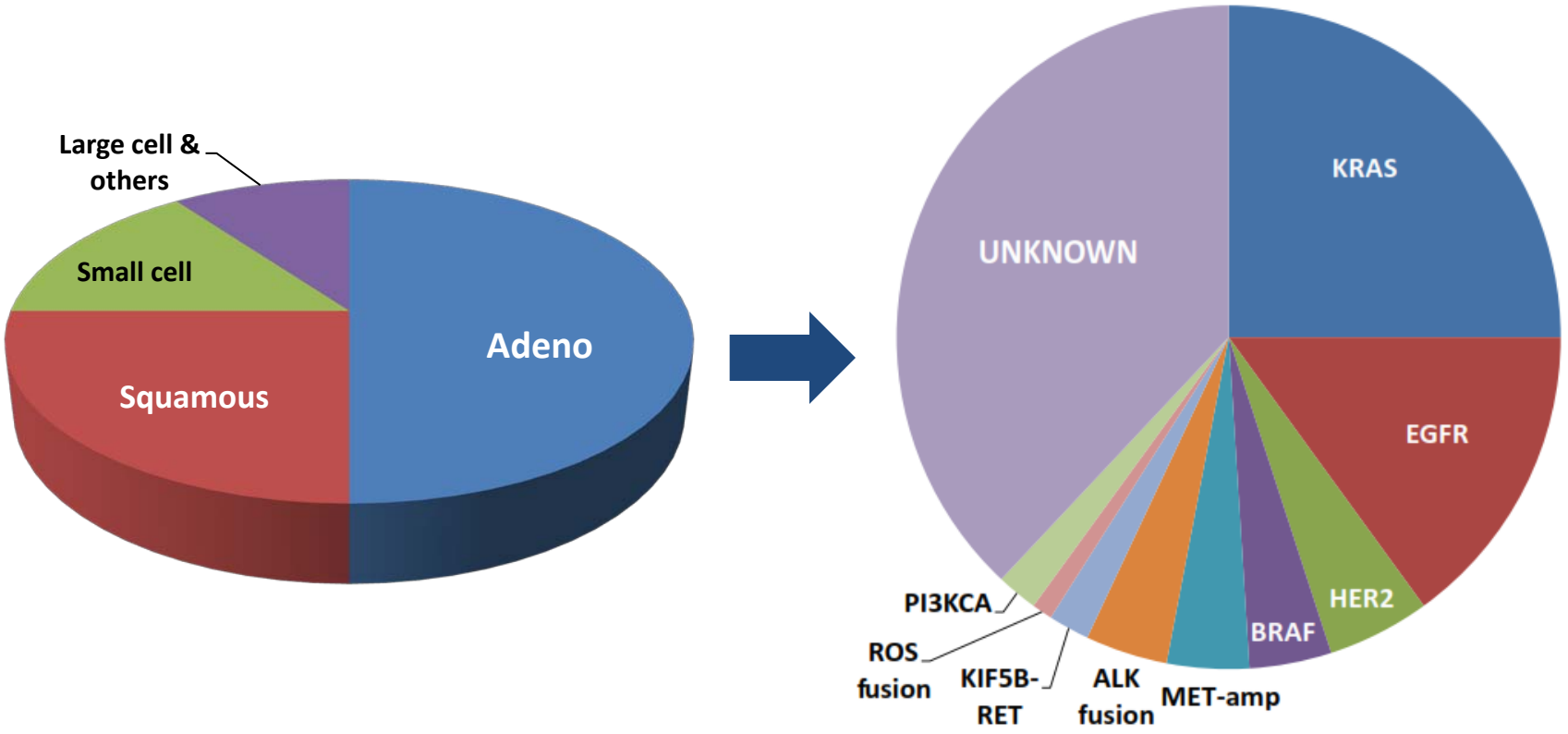
Identification of new *ALK* and *RET* gene fusions from colorectal and lung cancer biopsies

Doron Lipson[1,9], Marzia Capelletti[2,9], Roman Yelensky[1],

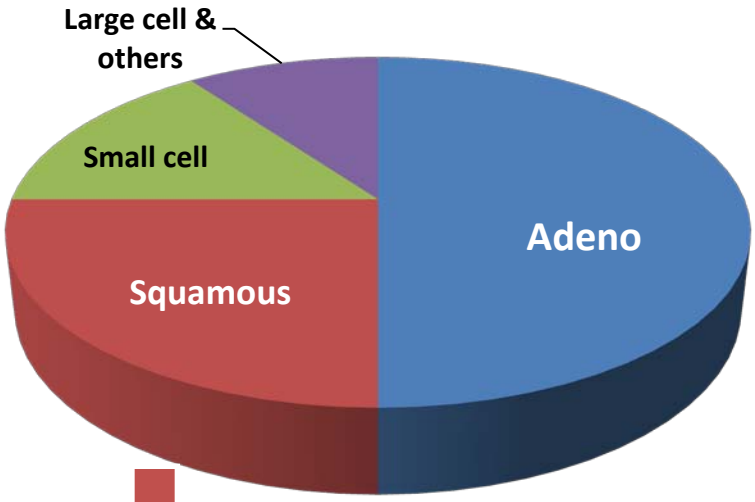
RET, ROS1 and ALK fusions in lung cancer

Kengo Takeuchi[1,2], Manabu Soda[3], Yuki Togashi[1,2],

Molecular Targets for Lung Cancer Treatment

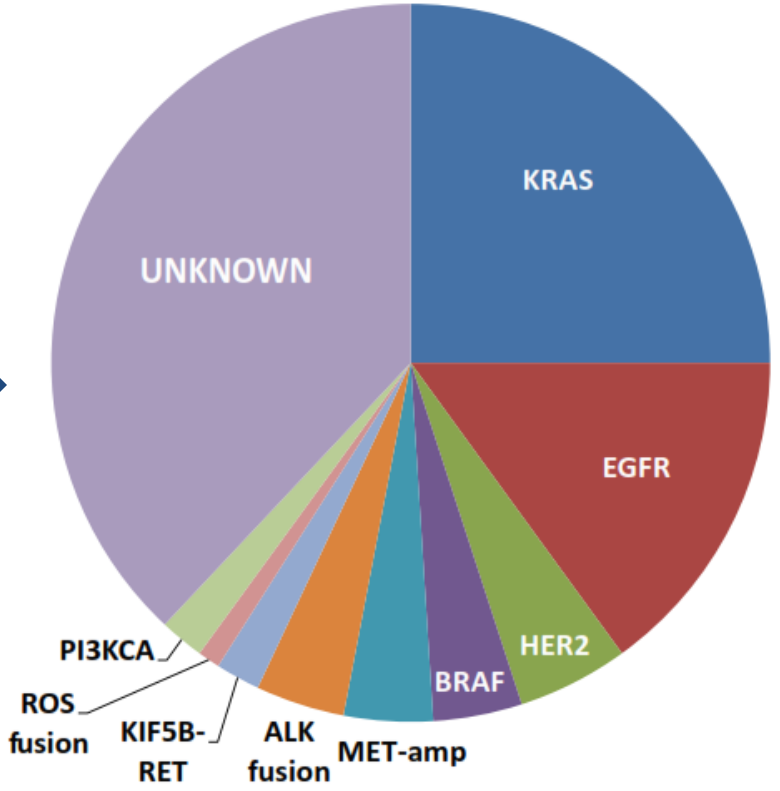


Molecular Targets for Lung Cancer Treatment



?

(30% of NSCLC)

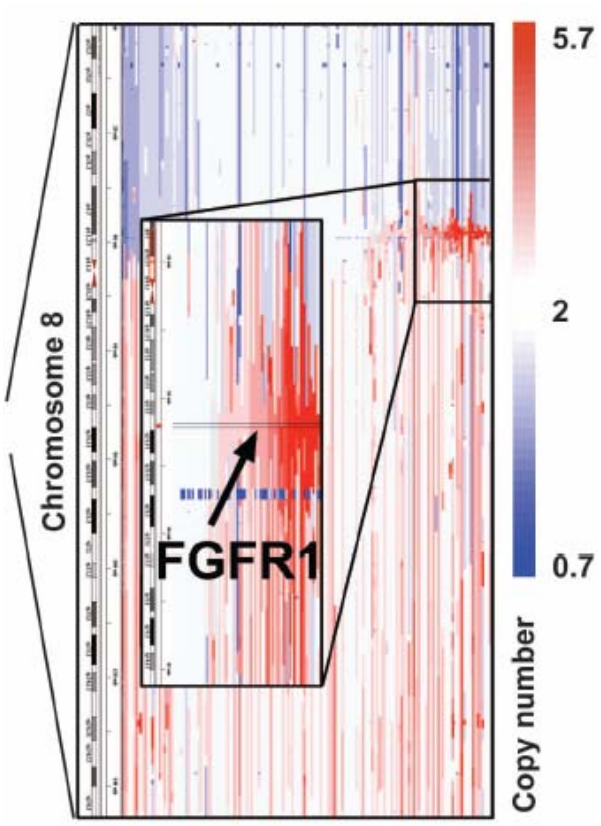


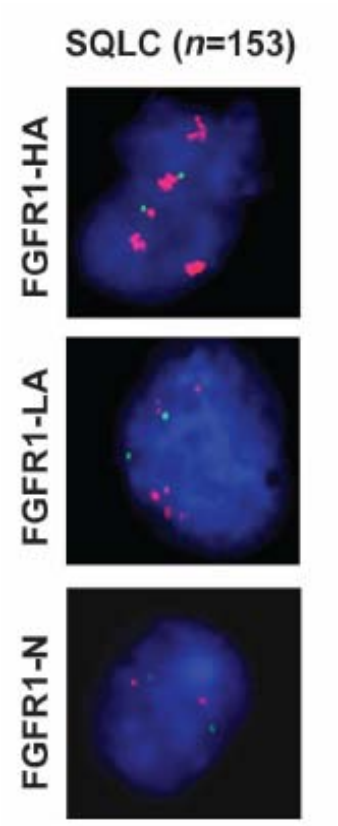
Chromosome 8p12

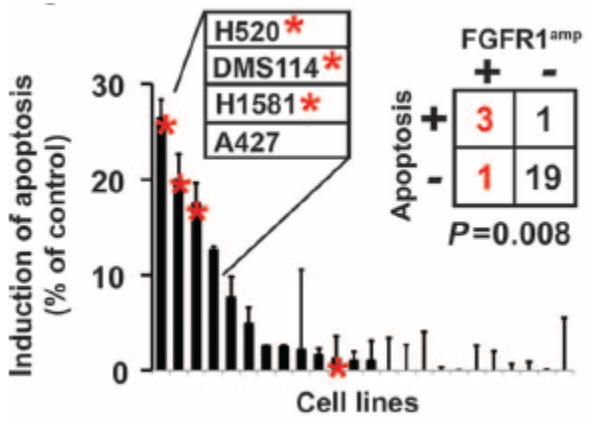
Frequent and Focal *FGFR1* Amplification Associates with Therapeutically Tractable FGFR1 Dependency in Squamous Cell Lung Cancer

Jonathan Weiss,[1]* Martin L. Sos,[1]*† Danila Seidel,[1,2]* Martin Peifer,[1] Thomas Zander,[3] *et al*

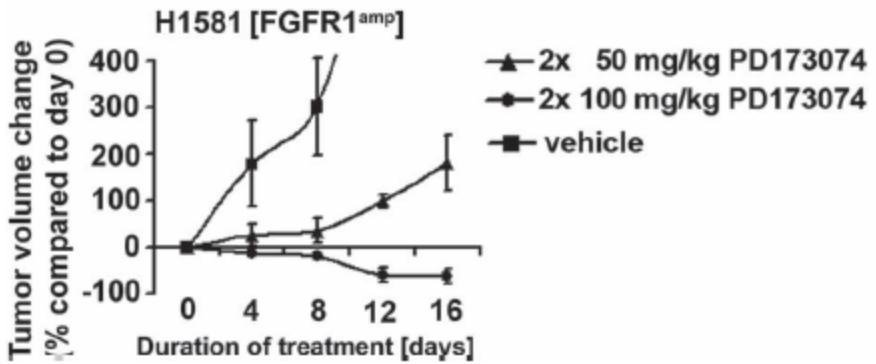
FGFR1 amplification: 15/155 (9.7%)







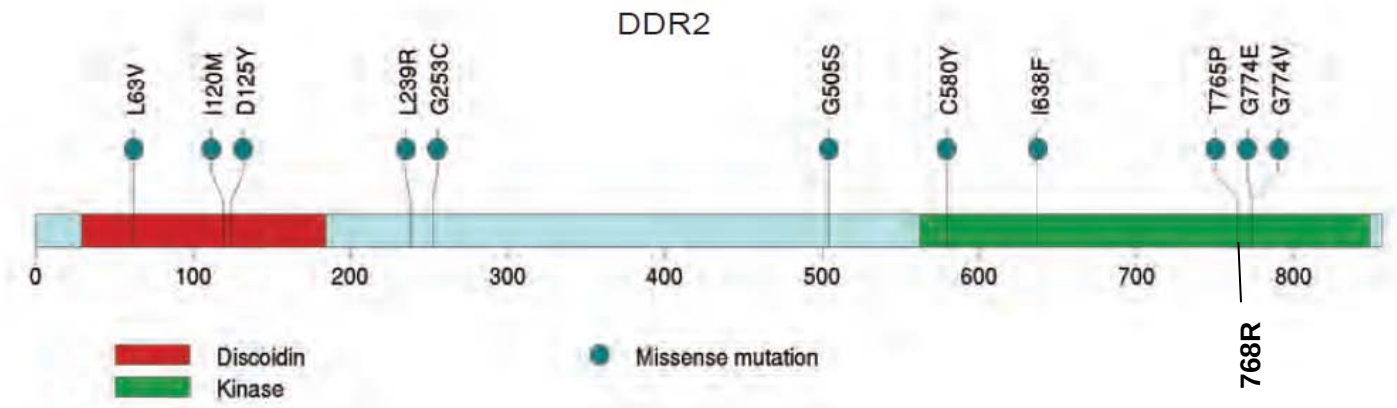
FGFR inhibitor PD173074

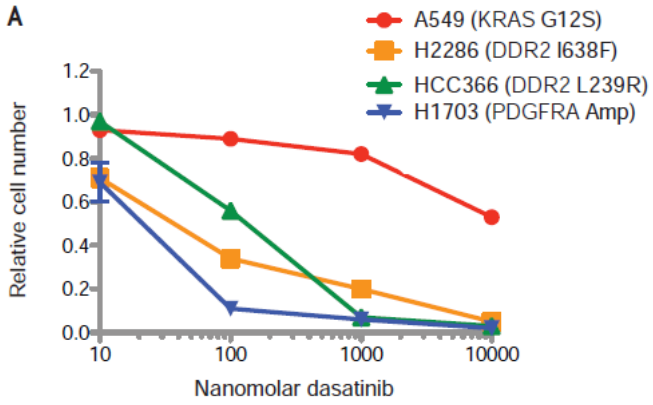


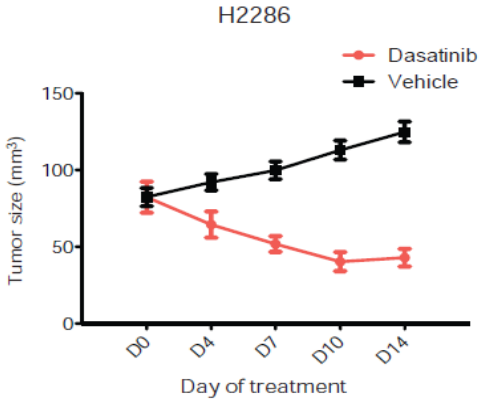
Science Trans Med 2010;2:1-7

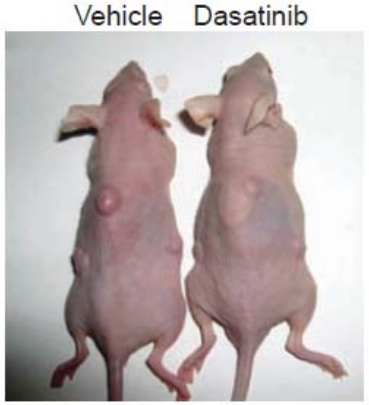
Mutations in the *DDR2* Kinase Gene Identify a Novel Therapeutic Target in Squamous Cell Lung Cancer

11/290 (3.8%) samples screened; 9/277 (3.2%) primary SqCC









Hammerman PS, et al. Cancer Discovery 2011;1:79-89.

Comprehensive genomic characterization of squamous cell lung cancers

The Cancer Genome Atlas Research Network*

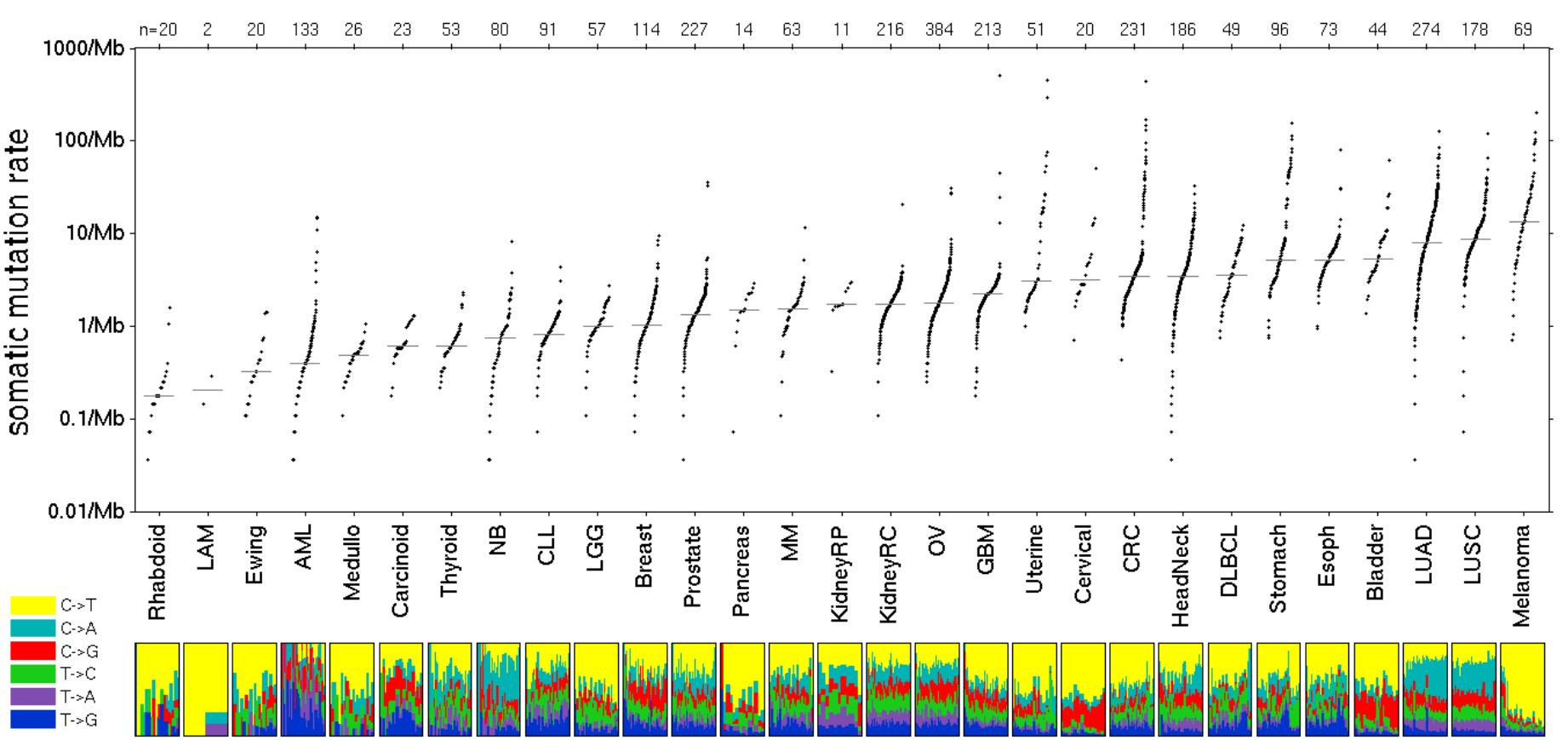
Nature September 9 (published on line)

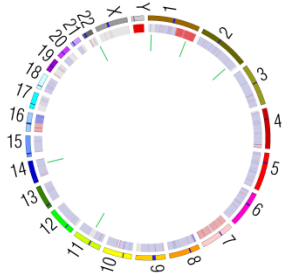
Lung squamous cell carcinoma is a common type of lung cancer, causing approximately 400,000 deaths per year worldwide. Genomic alterations in squamous cell lung cancers have not been comprehensively characterized, and no molecularly targeted agents have been specifically developed for its treatment. As part of The Cancer Genome Atlas, here we profile 178 lung squamous cell carcinomas to provide a comprehensive landscape of genomic and epigenomic alterations. We show that the tumour type is characterized by complex genomic alterations, with a mean of 360 exonic mutations, 165 genomic rearrangements, and 323 segments of copy number alteration per tumour. We find statistically recurrent mutations in 11 genes, including mutation of *TP53* in nearly all specimens. Previously unreported loss-of-function mutations are seen in the *HLA-A* class I major histocompatibility gene. Significantly altered pathways included *NFE2L2* and *KEAP1* in 34%, squamous differentiation genes in 44%, phosphatidylinositol-3-OH kinase pathway genes in 47%, and *CDKN2A* and *RB1* in 72% of tumours. We identified a potential therapeutic target in most tumours, offering new avenues of investigation for the treatment of squamous cell lung cancers.

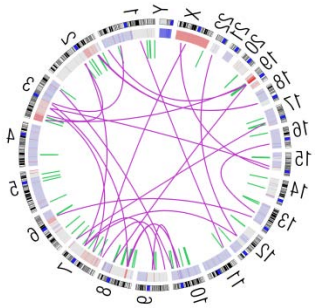
Potentially Targetable Mutated/Amplified Genes

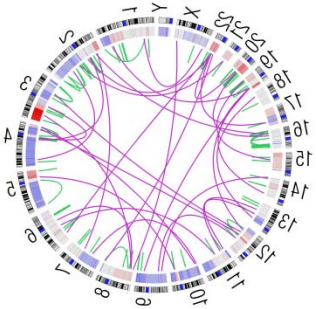
PI3KCA	PTEN	AKT 1-3	FGFR 1-3	EGFR	ERBB2	BRAF	NOTCH	RAS
16%	8%	20%	12%	9%	4%	4%	13%	6%

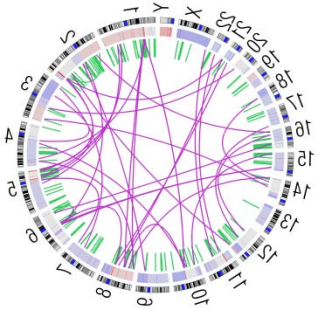
NSCLCs are among the most genomically deranged of all cancers





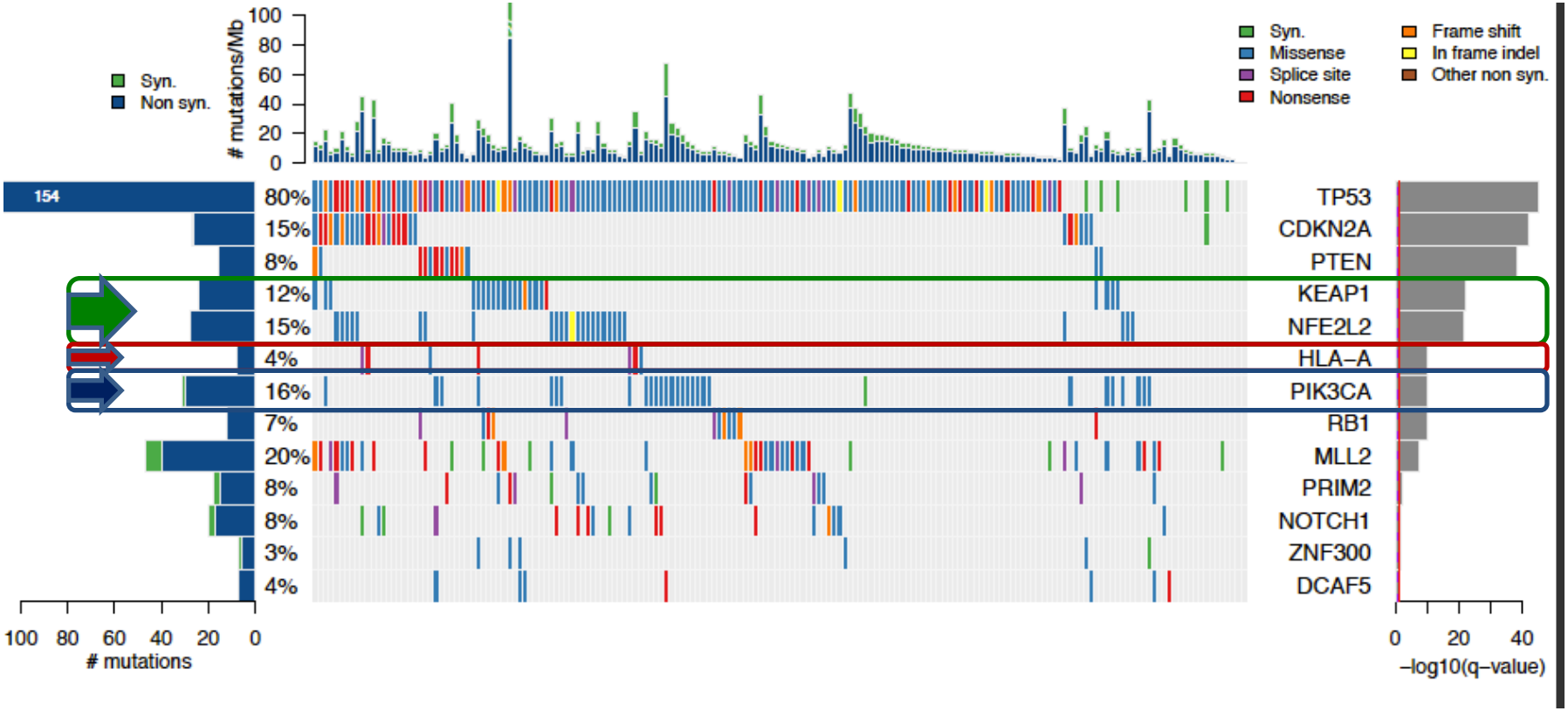






Courtesy of P. Hammerman

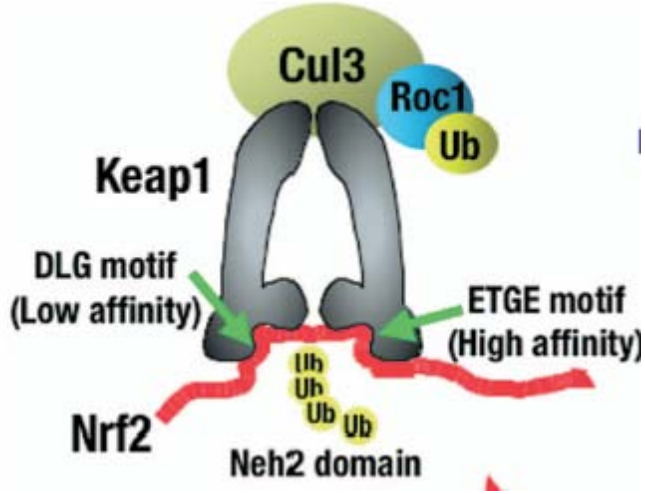
Mutations in Lung SqCCs

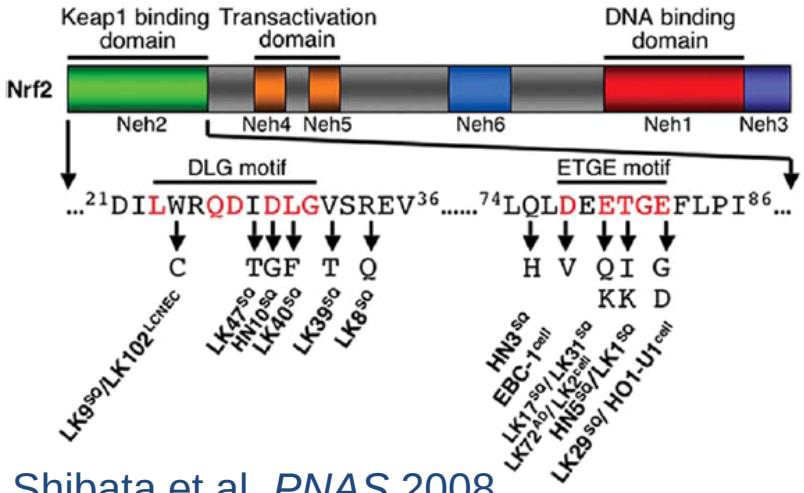


HLA-A mutations: possible mechanism for immune avoidance

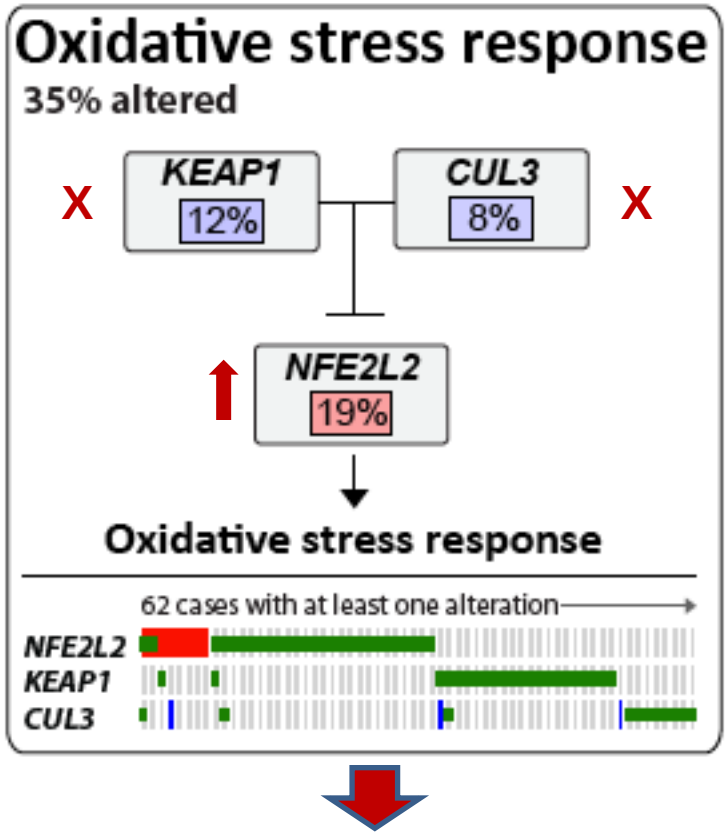
NFE2L2/KEAP1/CUL3

- **Mutations in KEAP1 are loss of function (frequent LOH of 2nd allele)**
- **Mutations in NRF2 cluster in DLG and ETGE motif -> prevent KEAP1 interaction -> results in NRF2 stabilization and nuclear entry**



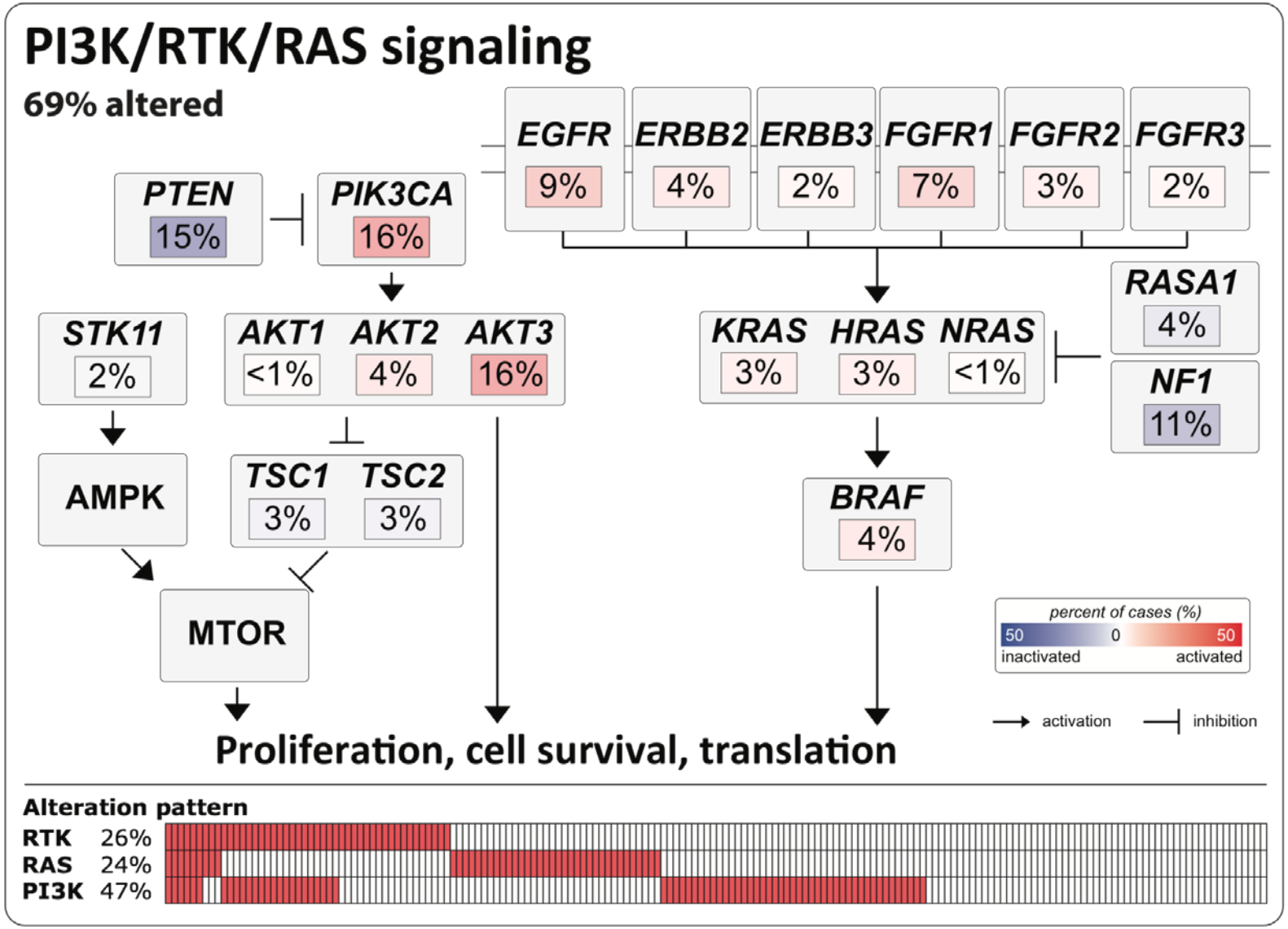


Shibata et al. *PNAS* 2008

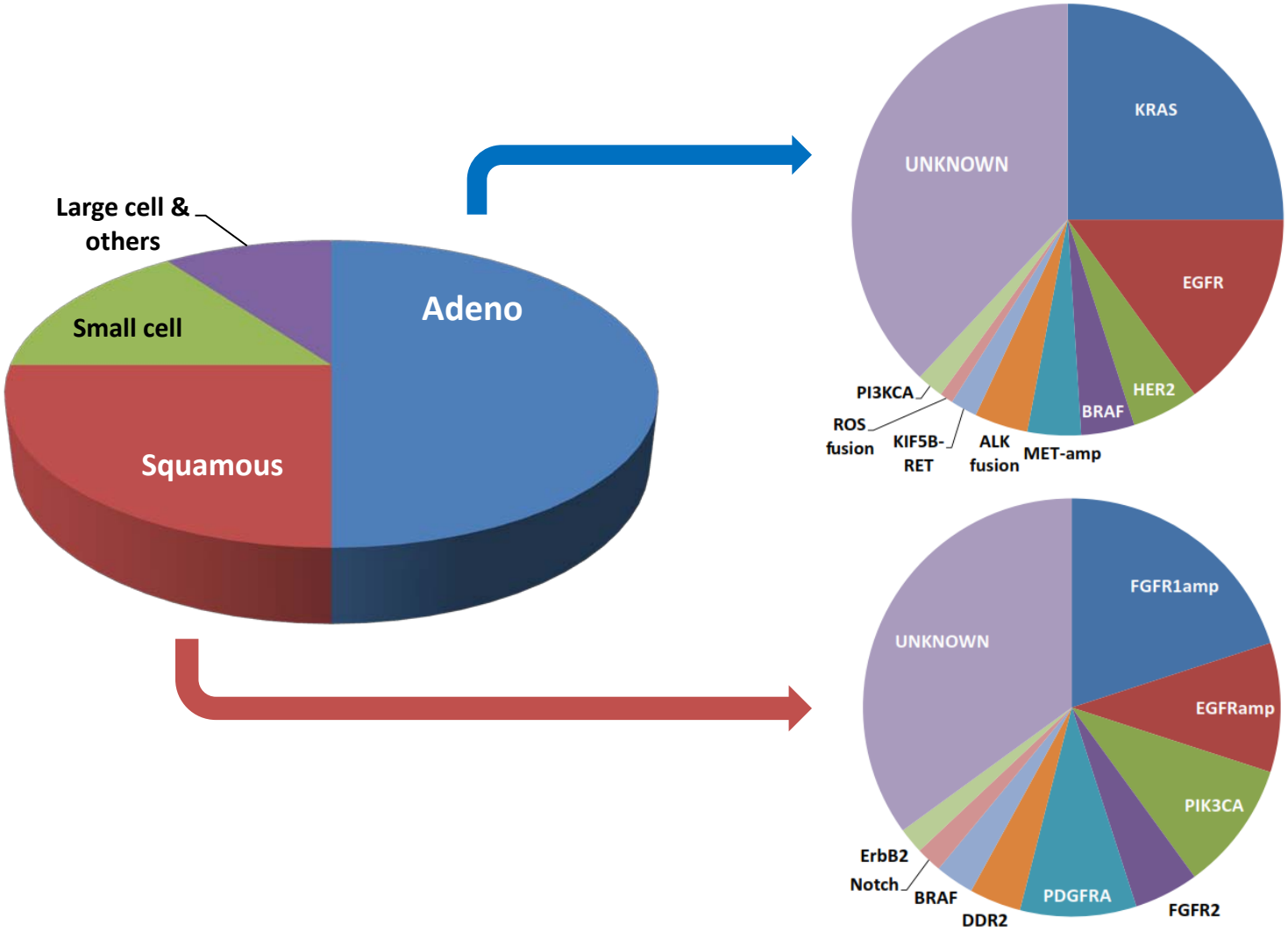


Increased resistance to chemotherapy

Altered Signaling Pathways in Lung Squamous Cell Carcinoma



Molecular Targets for Lung Cancer Treatment



Benchmarking of Mutation Diagnostics in Clinical Lung Cancer Specimens

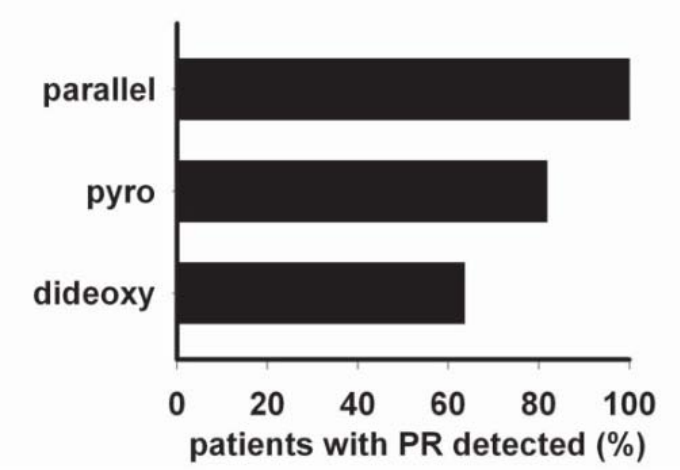
Silvia Querings[1,2☯], Janine Altmüller[3☯], Sascha Ansén[4☯], Thomas Zander[4☯], Danila Seidel[1,2], Franziska Gabler[1,2], Martin Peifer[1], Eva Markert[5], Kathryn Stemshorn[3], Bernd Timmermann[6], Beate Saal[1], Stefan Klose[7], Karen Ernestus[5], Matthias Scheffler[4], Walburga Engel-Riedel[8], Erich Stoelben[9], Elisabeth Brambilla[10], Jürgen Wolf[2,4¶], Peter Nürnberg[3¶], Roman K. Thomas[1,2,4*¶]

Illumina MiSeq (1.5 Gigabase in 1 day)

Ion Torrent PGM (1 Gigabase 6 hr)

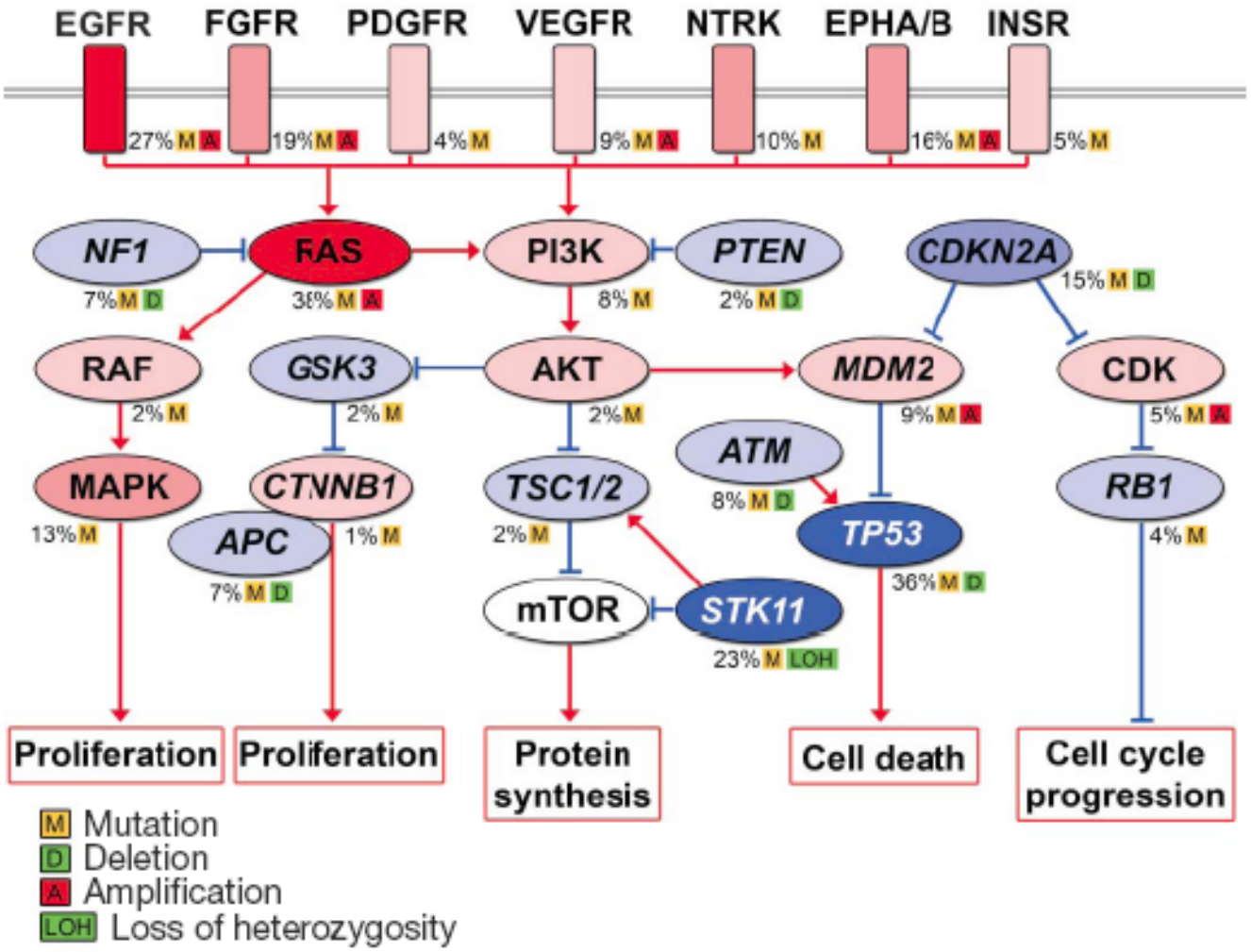
Compared 'Sanger' and pyrosequencing against massively parallel sequencing on patients who have been treated by EGFR TKI:

"…… all patients with a confirmed response to EGFR inhibition, only massively parallel sequencing detected all relevant mutations"



PLosONE 2011;6:e19601

Potential New Therapeutic Targets in Lung Adenocarcinoma



Ding L, et al. Nature vol. 455 (23 October 2008): pg. 1069

“Short” List of Targeted Agents in Non-Small Cell Lung Cancer

VEGF targeted agents

EGFR targeted agents

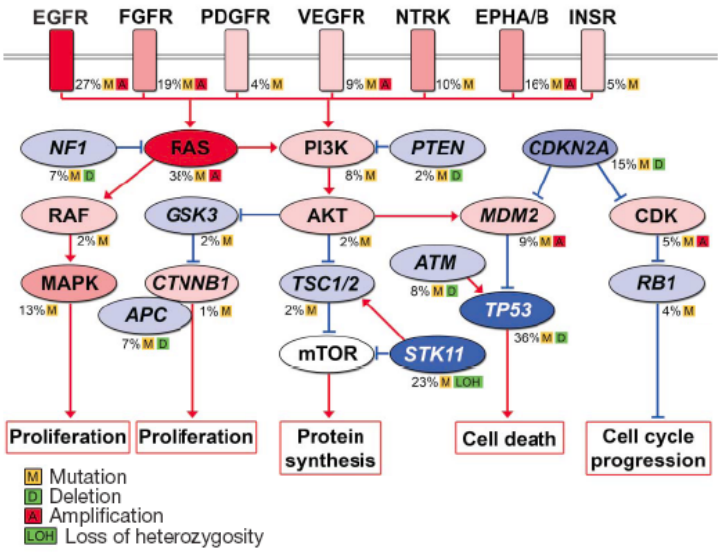
mTOR inhibitors

Proteasome inhibitors

Cell cycle targeted agent

- **PARP inhibitors**
- **CDK inhibitors**
- **Novel chemotherapy**
- **Proapoptotic agents**

Vaccine Therapy



Sundry Kinase Inhibitors:

- **PI3K**
- **AKT**
- **MAP kinase**
- **MEK (Ras, Raf)**
- **SRC**
- **Aurora kinase**
- **Polo-like kinases**
- **PKC**

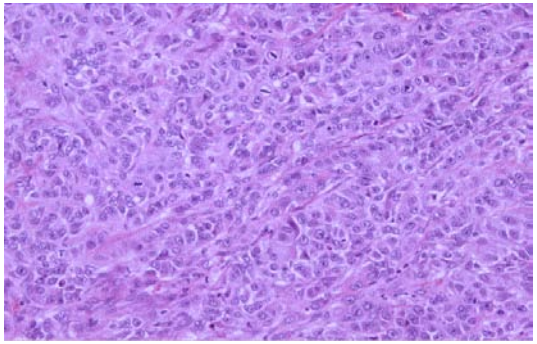
HSP 70, 90 targeted agents

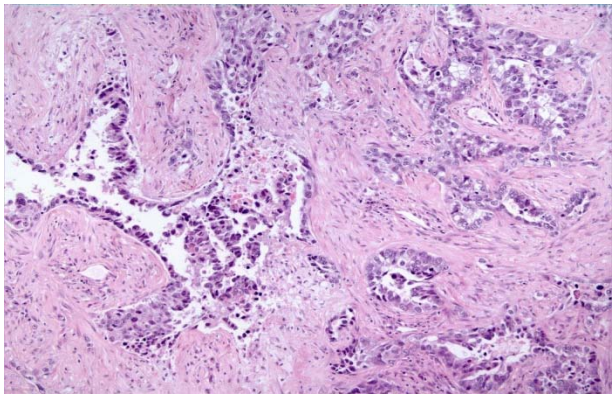
HIF1-alpha antagonists

C-met inhibitors

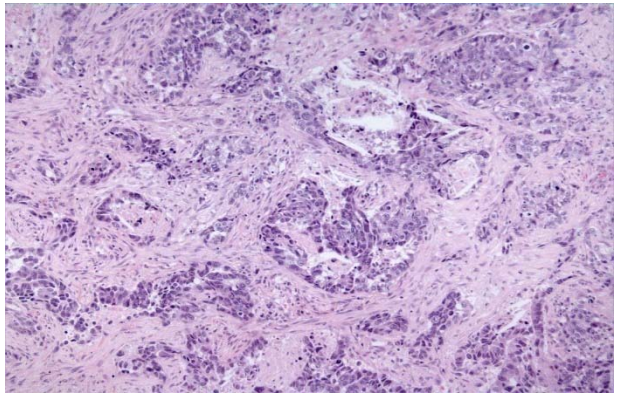
Primary Lung Cancer Xenografts Mimic Primary Tumor Histologies

A549 lung adenocarcinoma cell line

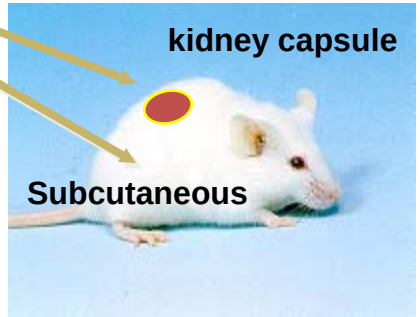




Patient Tumor

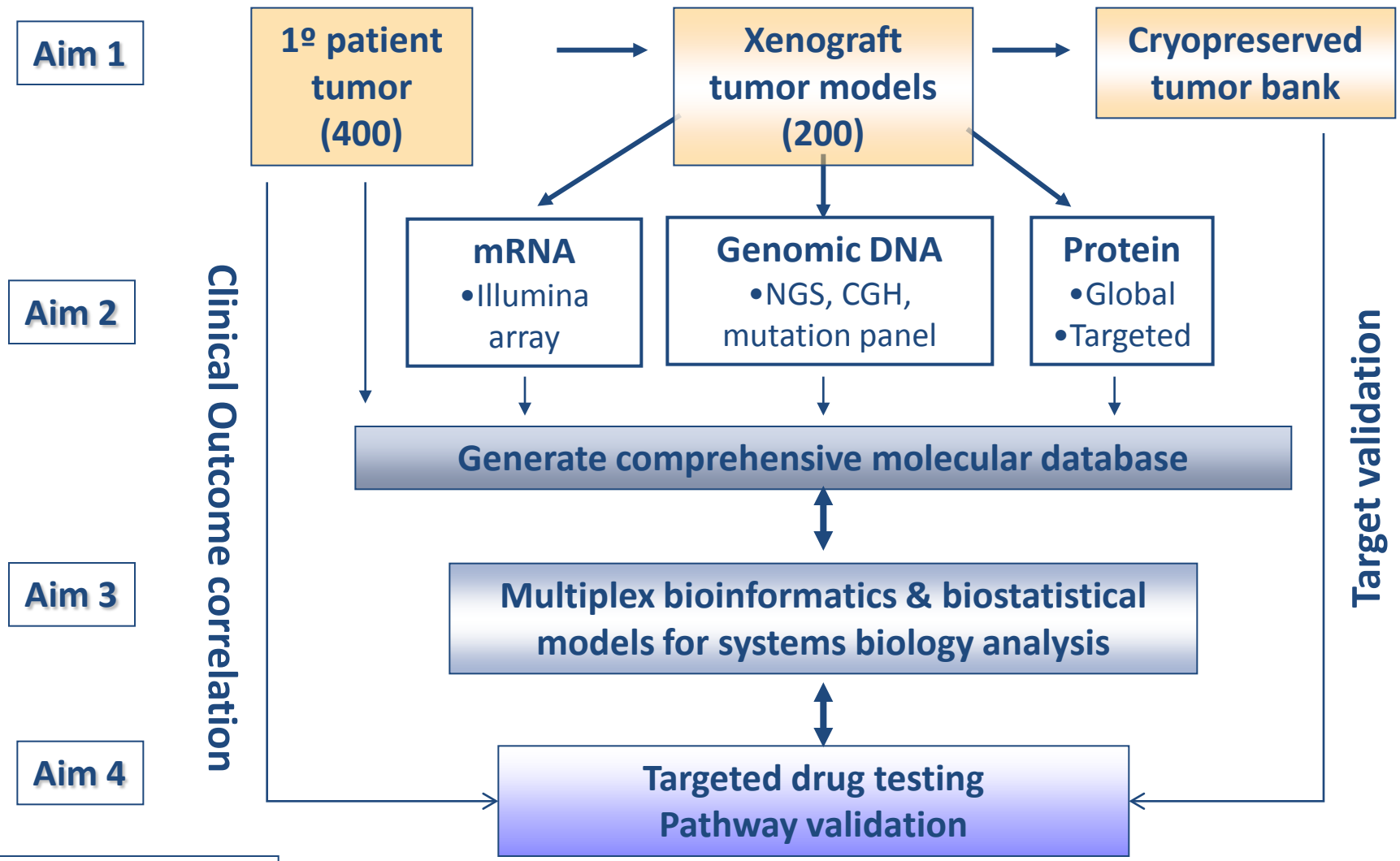


Primary Xenograft



IMP4 in Lung Cancer

(Integrated Molecular Pathology, Pharmacodynamic, Pharmacogenomic & Proteomics)



Funding: 2009-14

Primary NSCLC Xenograft Establishment

(up to End of August)

Year	Tumor Implanted	Confirmed Xenograft (>P1)	Confirmed Take Rate	Potential (still in P1)	Potential Take Rate
2005	21	5	24%	-	-
2006	24	7	29%	-	-
2007	52	21	40%	-	-
2008	53	22	42%	-	-
2009	46	19	41%	-	-
2010	85	35	41%	-	-
2011	80	31	39%	7	48%
2012	69	13	19%	14	39%
Total	**430**	**153**	**36%**	**21**	**40%**

Cancer Therapy: Preclinical

Clinical Cancer Research

The Ability to Form Primary Tumor Xenografts Is Predictive of Increased Risk of Disease Recurrence in Early-Stage Non–Small Cell Lung Cancer

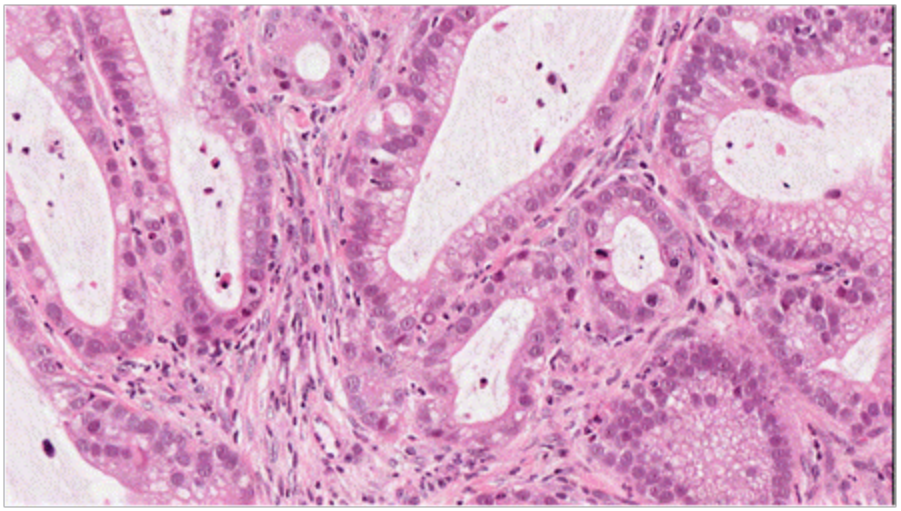
Thomas John[1,2,5], Derek Kohler[2,3], Melania Pintilie[4], Naoki Yanagawa[2,3], Nhu-An Pham[2], Ming Li[2], Devang Panchal[2], Frances Hui[2], Fannong Meng[3], Frances A. Shepherd[5,6], and Ming-Sound Tsao[2,3,7]

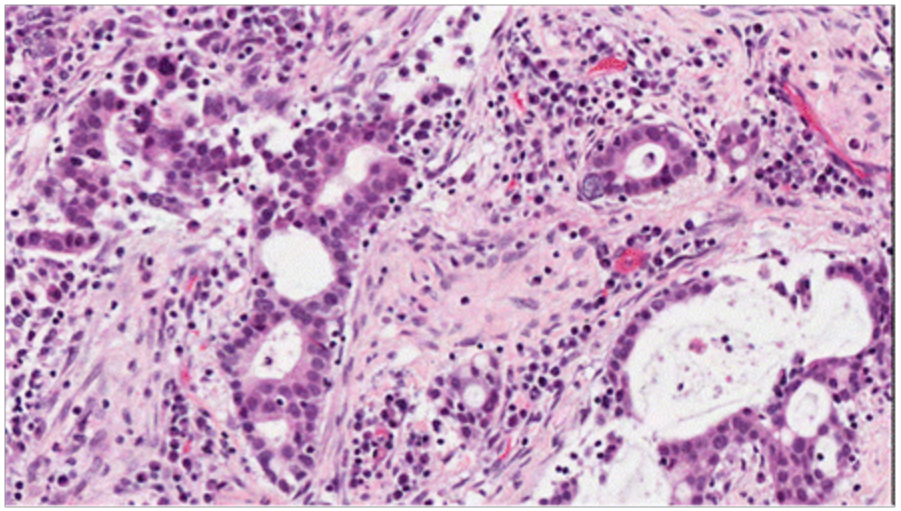
First 157 patients

Characteristic	Xenograft (%)	No Xenograft (%)	P-value (Fisher Exact Test)
Total	**63 (40%)**	**94 (60%)**	
Adenocarcinoma	29 (30%)	66 (70%)	<0.001
Squamous Cell	30 (65%)	16 (35%)	
Other	4 (25%)	12 (75%)	
Differentiation			
Well	2 (10%)	17 (90%)	0.003
Moderate	26 (38%)	41 (62%)	
Poor	35 (53%)	31 (47%)	

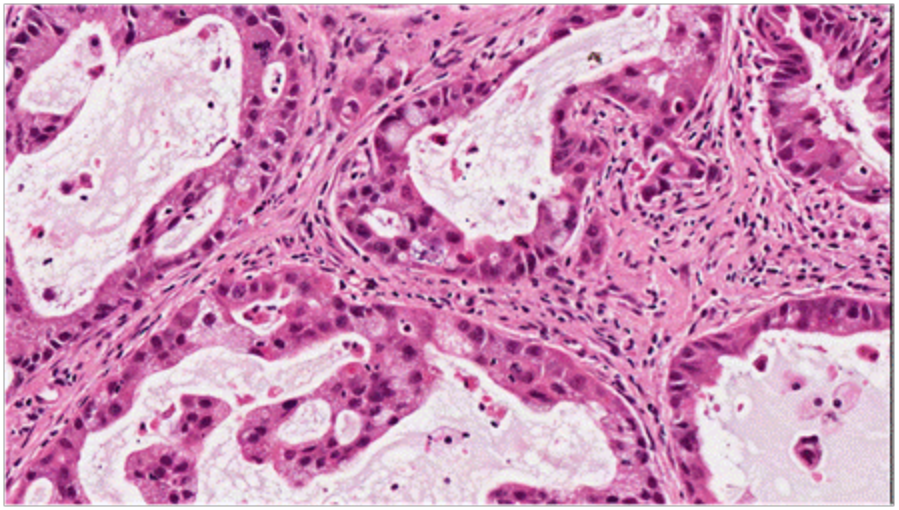
PHL-196: Similar Histologies

Primary Tumor – Mucinous adenocarcinoma

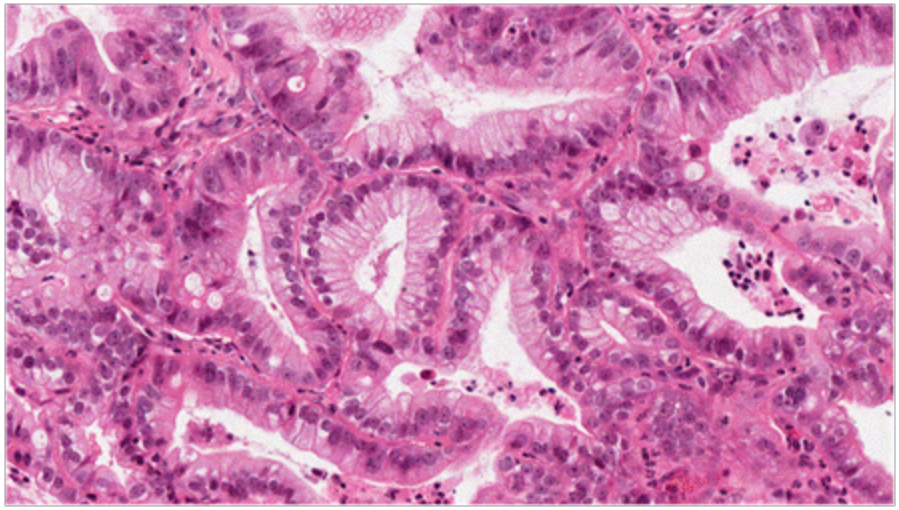




Fragment xeno

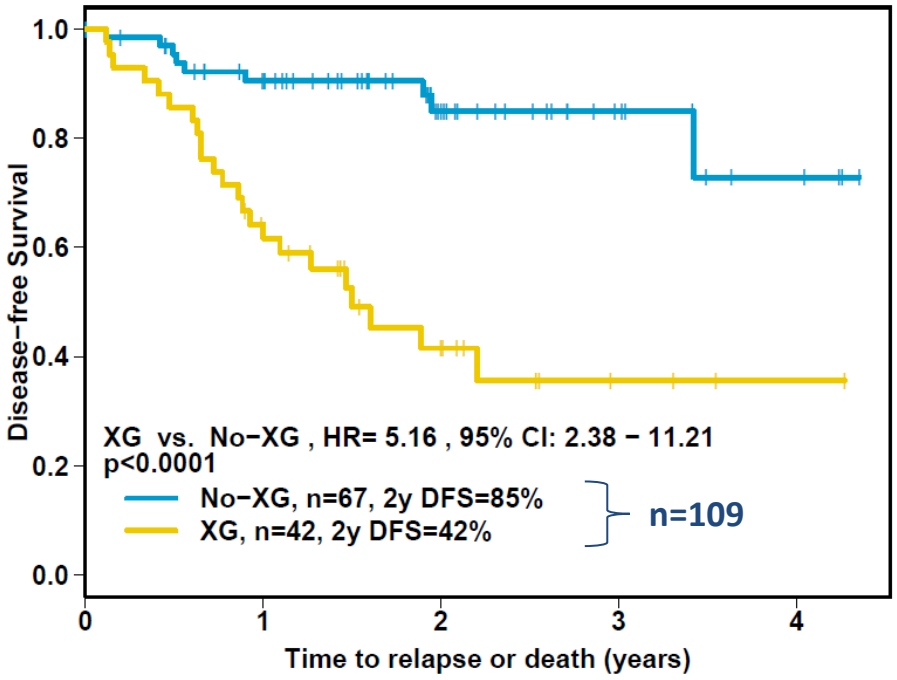


Isolated cells xeno

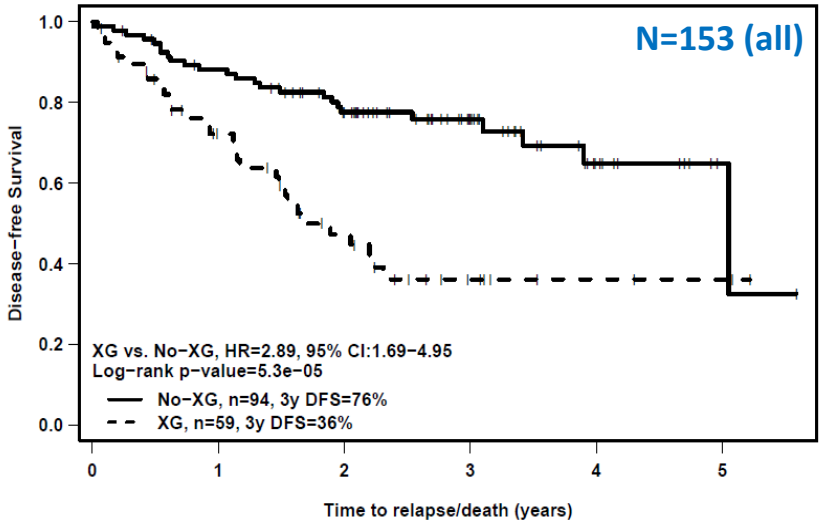


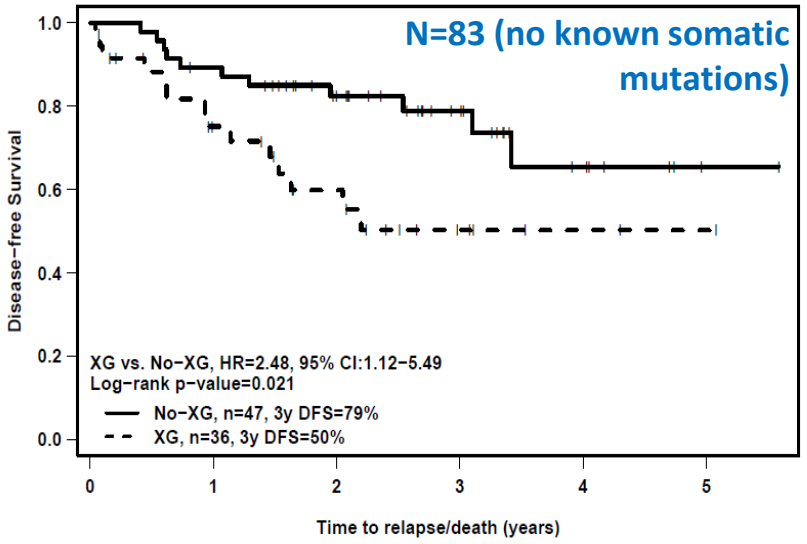
Tumor Engraftment Predictive of Higher Recurrence Rate and Poorer DFS

Clin Cancer Res 2011;17:134-141.



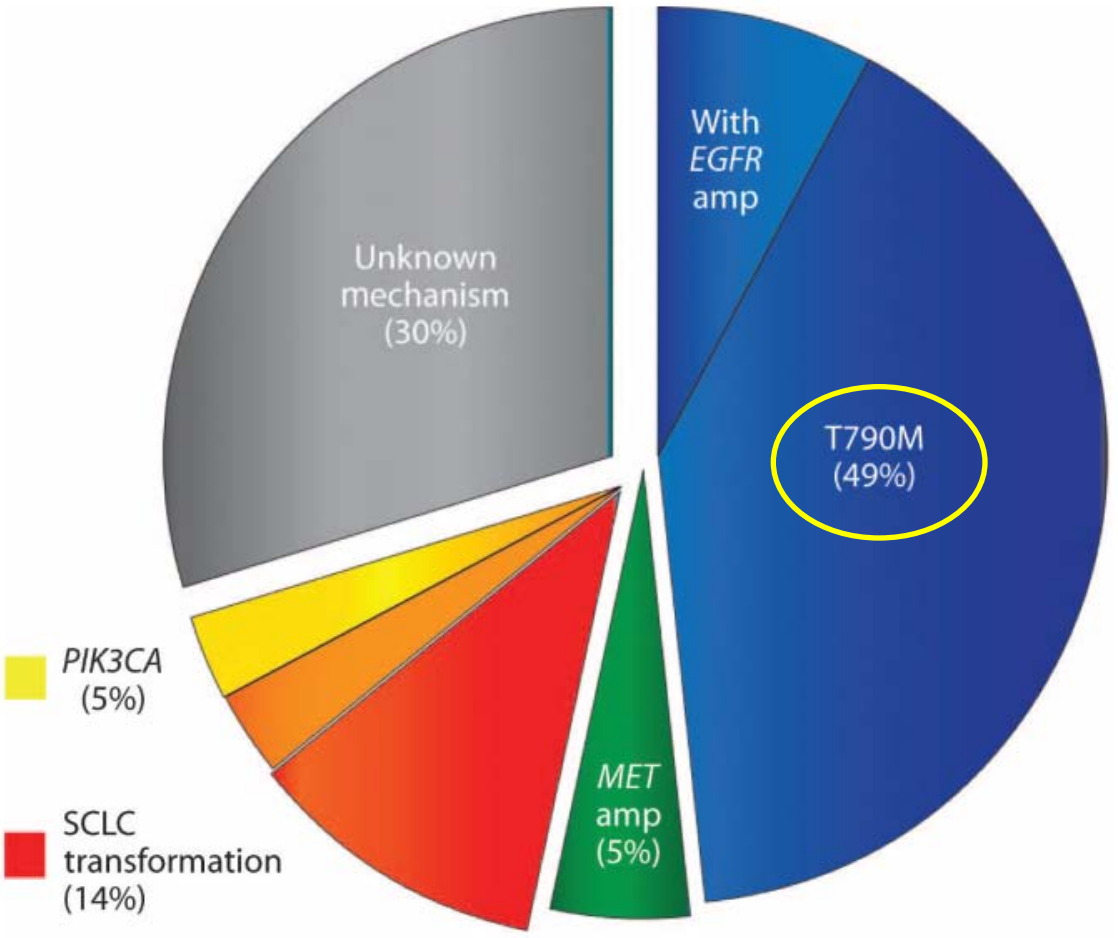
Updated Follow-up





Mechanisms of TKI Resistance

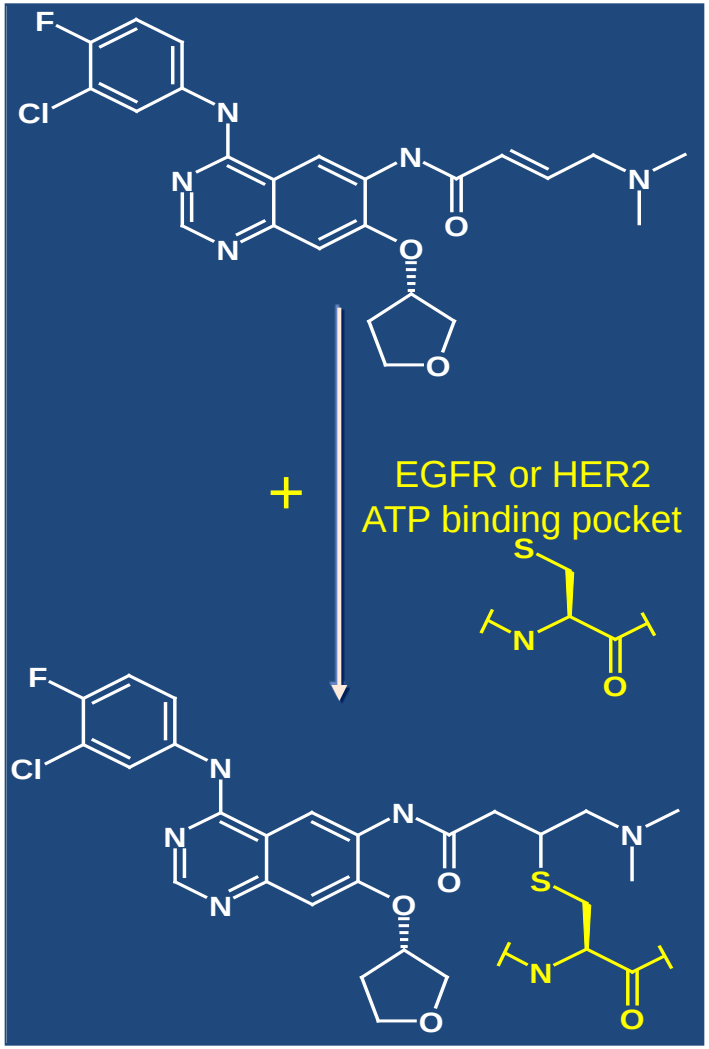
37 patients with re-biopsy of tumor that progress during EGFR TKI therapy



Sequist LV, et al. Sci Transl Med. 2011 March 23; 3(75): 75ra26.

Afatinib (BIBW 2992)

- Afatinib is an irreversible EGFR and HER2 inhibitor with preclinical activity against H1975 (L858R/T790M) (EC_{50}: 99 nM)
- Designed to irreversibly bind to the ATP binding pocket of EGFR and HER2
- Highly specific for EGFR and HER2
  - EGFR IC_{50}: 0.50 nM
  - HER2 IC_{50}: 14 nM

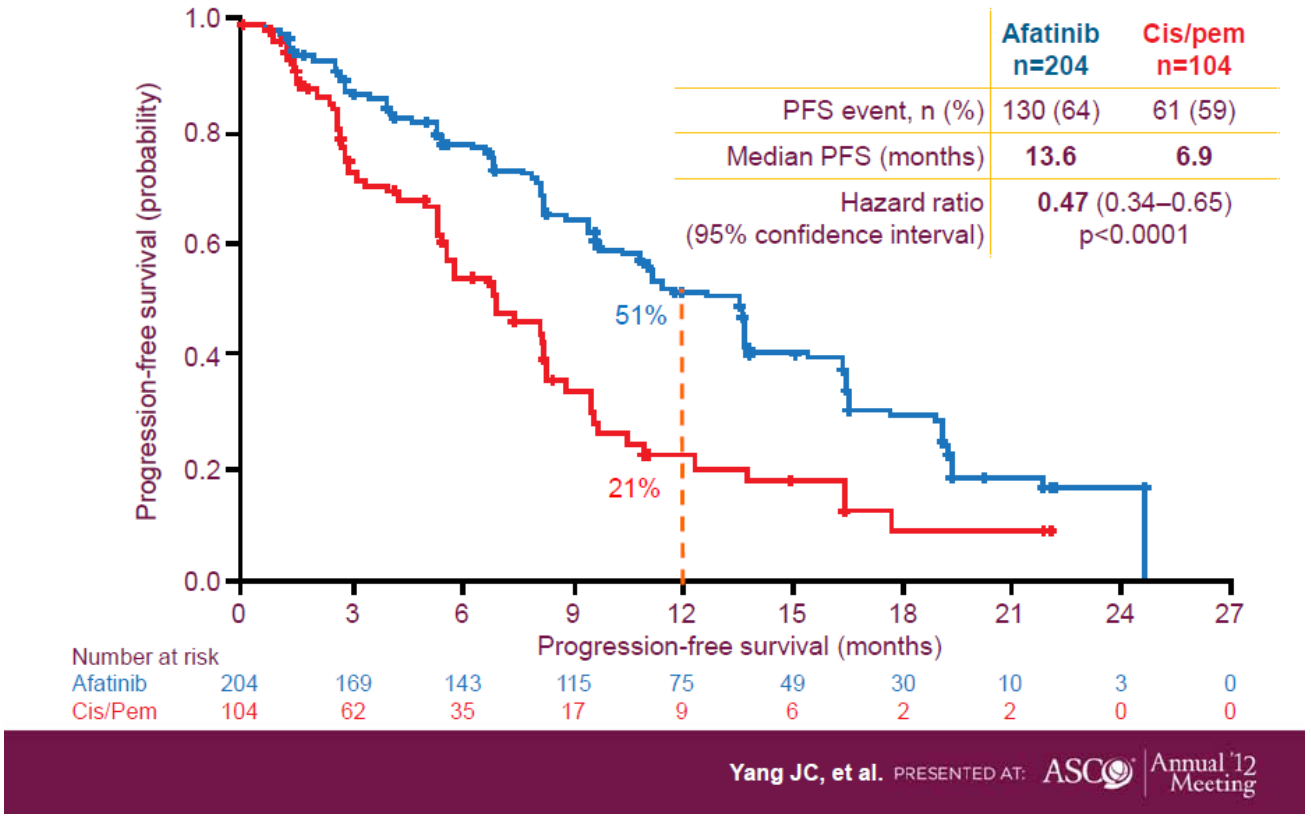


LUX-Lung 3

Phase 3 trial comparing Afatinib vs Chemotherapy in EGFR mutant Patients

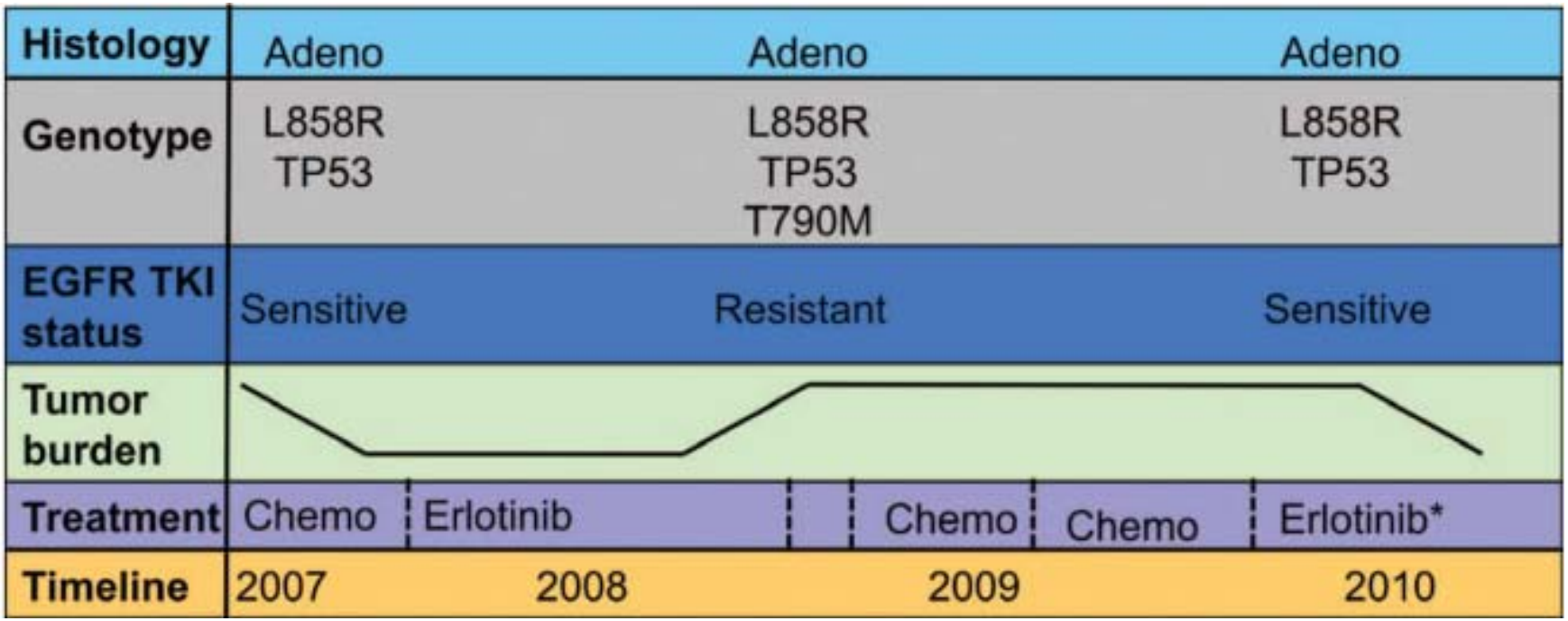
PFS: Common mutations (Del19/L858R)

Independent review – patients with Del19/L858R (n=308)



Yang JC, et al. PRESENTED AT: ASCO Annual '12 Meeting

Interruption of TKI Therapy May Lead to Loss of Resistant Mutant Tumor Clones and Restore Sensitivity



Sequist LV, et al. Sci Transl Med. 2011 March 23; 3(75): 75ra26.

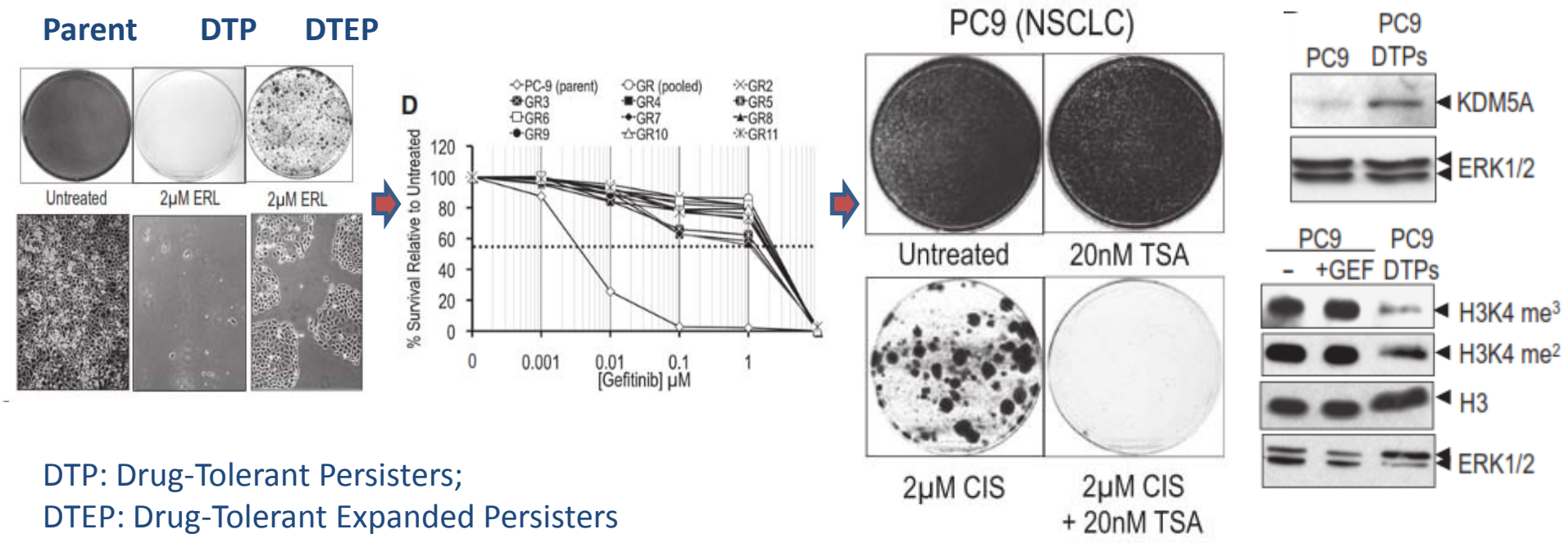
Mechanism of Resistance

More complicated than resistant mutant clones or amplification

A Chromatin-Mediated Reversible Drug-Tolerant State in Cancer Cell Subpopulations

Sreenath V. Sharma,[1] Diana Y. Lee,[1] Bihua Li,[1] Margaret P. Quinlan,[1] Fumiyuki Takahashi,[1] Shyamala Maheswaran,[1] Ultan McDermott,[1] Nancy Azizian,[1] Lee Zou,[1] Michael A. Fischbach,[1] Kwok-Kin Wong,[2] Kathleyn Brandstetter,[2] Ben Wittner,[1] Sridhar Ramaswamy,[1] Marie Classon,[1,3,*] and Jeff Settleman[1,3,*]

Induction of DTP and DTEP appears linked to chromatin alteration



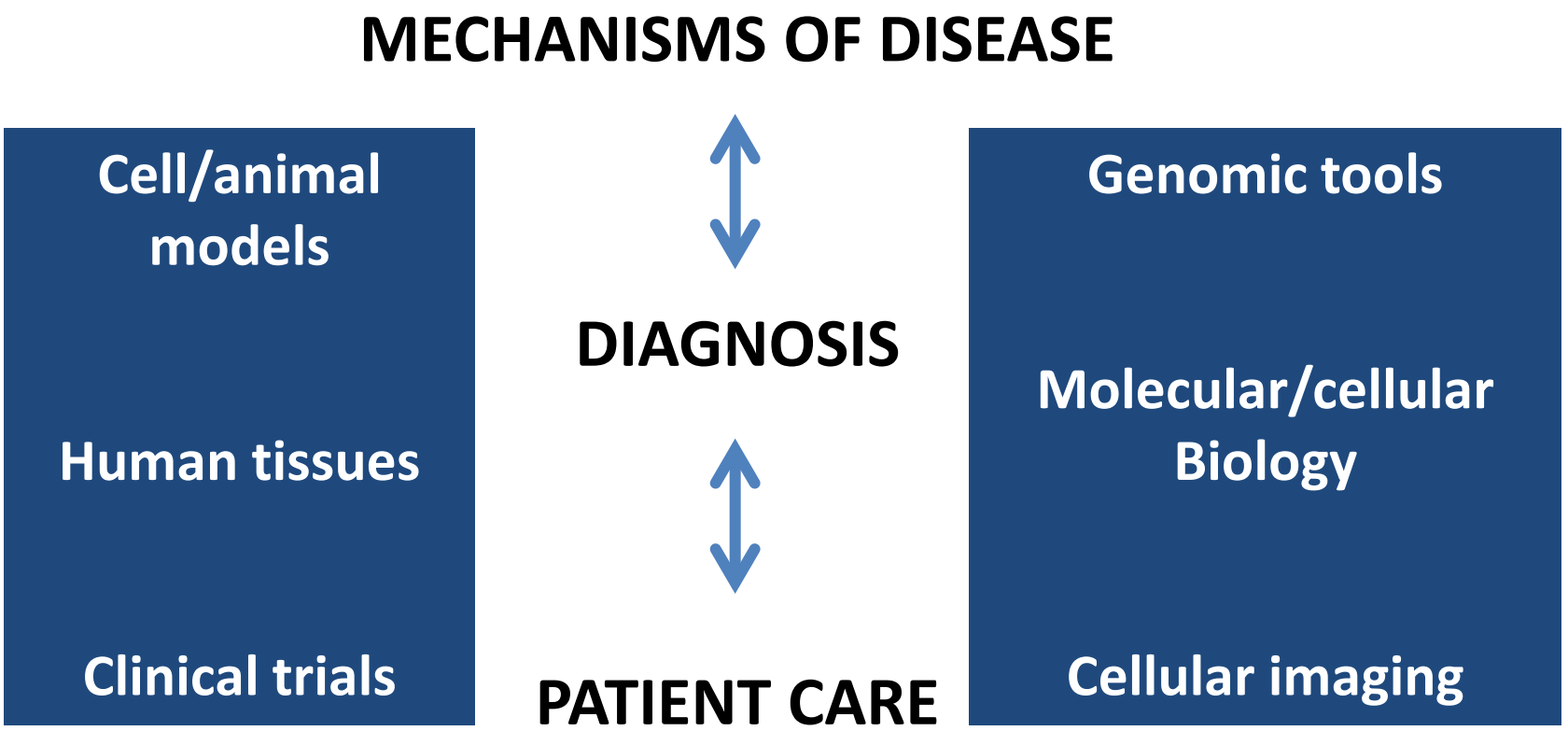
DTP: Drug-Tolerant Persisters;
DTEP: Drug-Tolerant Expanded Persisters

Cell 2010;141:1-12

Conclusions

1. Future pathology practice must be both diagnostic and predictive (of therapeutic outcome)
2. Predictive pathology will mostly be based on molecular markers
3. Molecular profiling will soon become routine in oncologic pathology reporting
4. Future cancer classification system must integrate molecular features of tumors
5. The FUTURE IS ALREADY HERE!

Opportunities in Pathology



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