

EGFR Mutation in Never-Smoker's Lung Cancer: Impact of Environmental Tobacco Smoke

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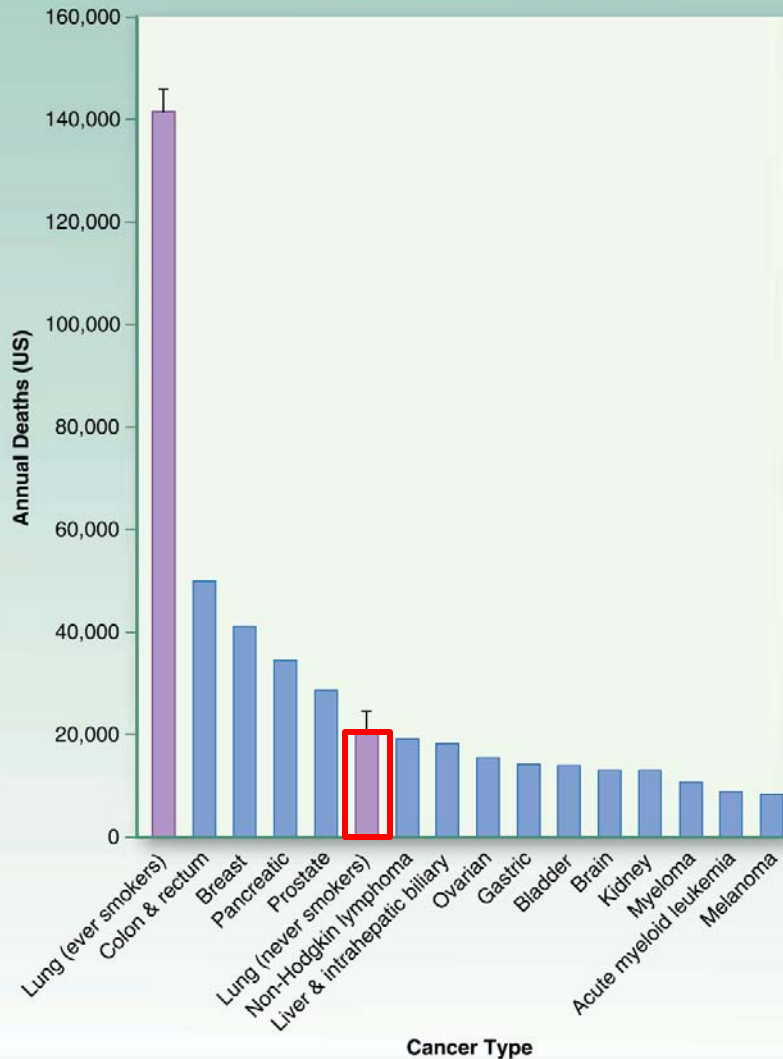
Agenda

- Lung cancer in never smokers (LCINS)
 - Epidemiology
 - Risk factors
 - Molecular biology, esp *EGFR* mutation
 - Prognosis and response to therapy
- Environmental Tobacco Smoke (ETS) and *EGFR* mutation in LCINS
- Conclusion

Lung Cancer in Never Smokers (LCINS)

- Cigarette smoking causes the majority of new cases of lung cancer worldwide
- At a surprisingly high rate, lung cancer also occurs in lifelong never smokers
 - In the US, >30,000 patients diagnosed with NSCLC each year are never smokers
 - Despite very low rate of women who smoke (3.9%), ~30% of newly diagnosed NSCLC patients were never smoked women in Korea 2006
- LCINS has a distinct biology, pathogenesis, prognosis, and response to therapy

Common Cause of Cancer Death (USA, 2008)



- **LCINS is worldwide public health concern, with mortality similar to other major cancer type**
- **In the US, LCINS is almost as common as a cause of death as atherosclerosis, liver cancer, and esophageal cancer**

Risk factors of LCINS

- Exposure to environmental tobacco smoke (ETS)
- Radon
- Ionizing radiation
- Asbestos
- Cooking fumes
- Underlying COPD
- Genetic susceptibility (13q31.3 GPC5)
- Oncogenic viruses

Is Most Lung Cancer really preventable?

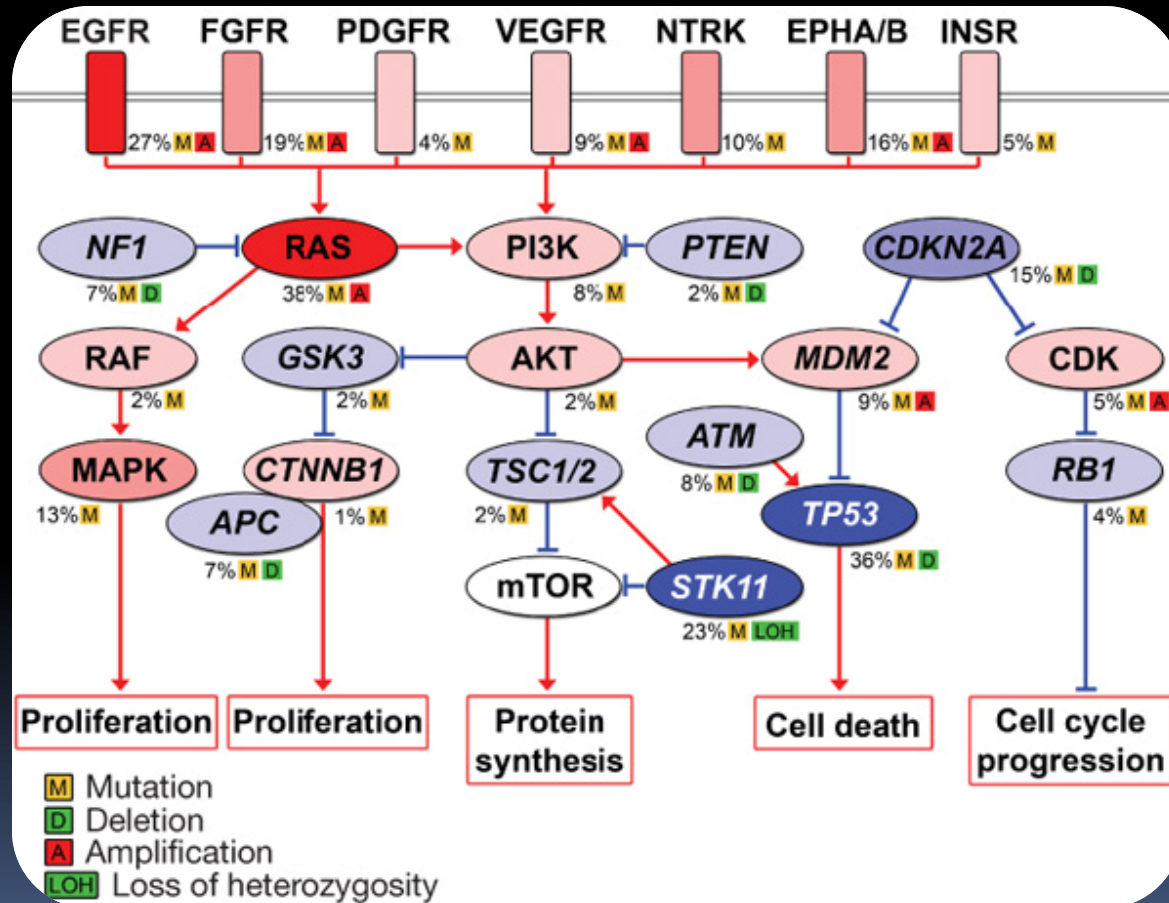
Environmental Tobacco Smoke (ETS)



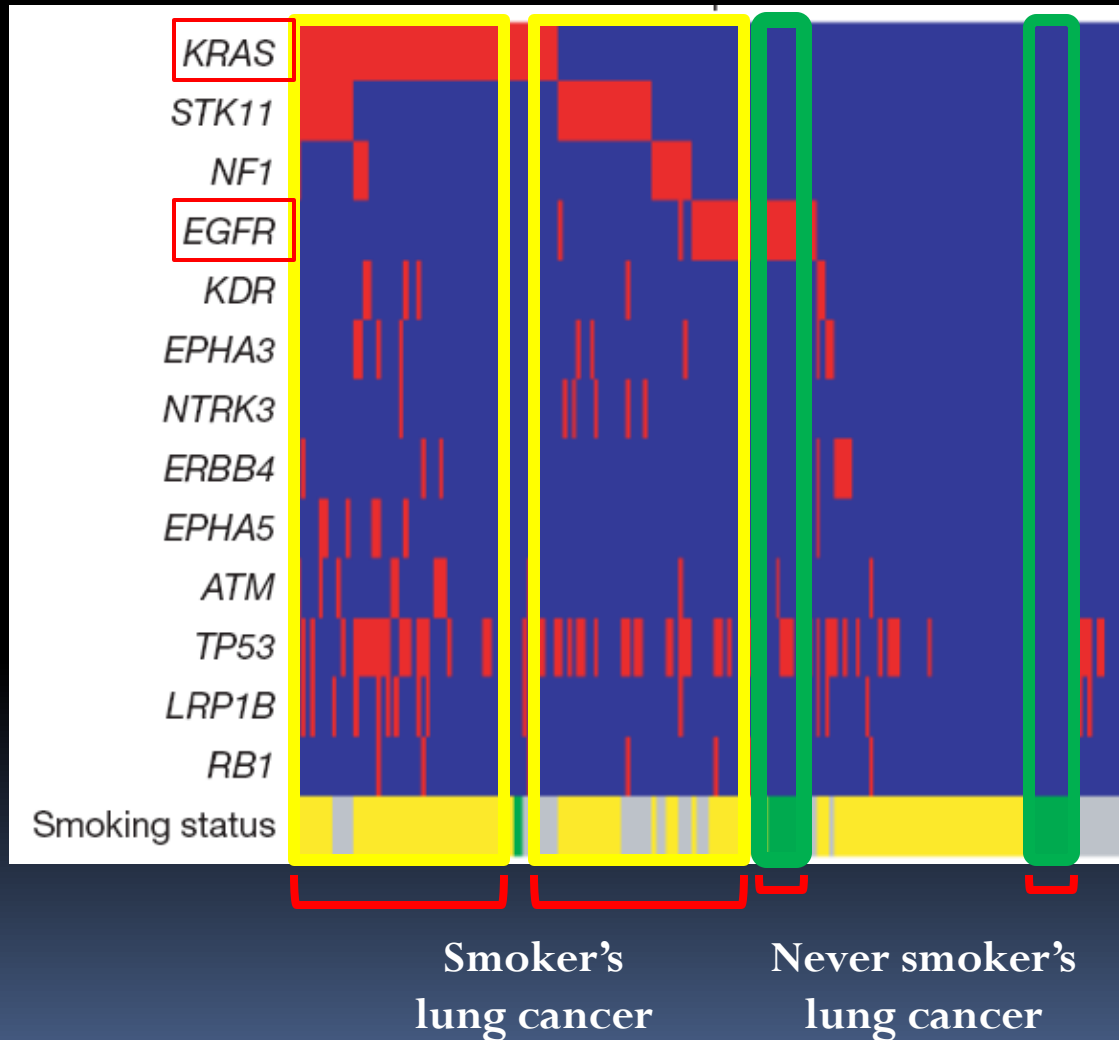
- ETS is a mixture of sidestream smoke from the burning tip of cigarette and the mainstream smoke exhaled by smoker
- ETS increased risk of lung cancer in never smokers by 20-30%*
- In the EU, 19,000 passive smokers die annually from smoking-related diseases
- 540 million passive smokers and 100,000 annual deaths from ETS in China
- **In contrast to other risk factors, ETS is easily identifiable and thus avoidable**

*US Surgeon General report, 2006

Significantly Mutated Pathways in Lung Adenocarcinomas



LCINS has Much Fewer Somatic Mutation



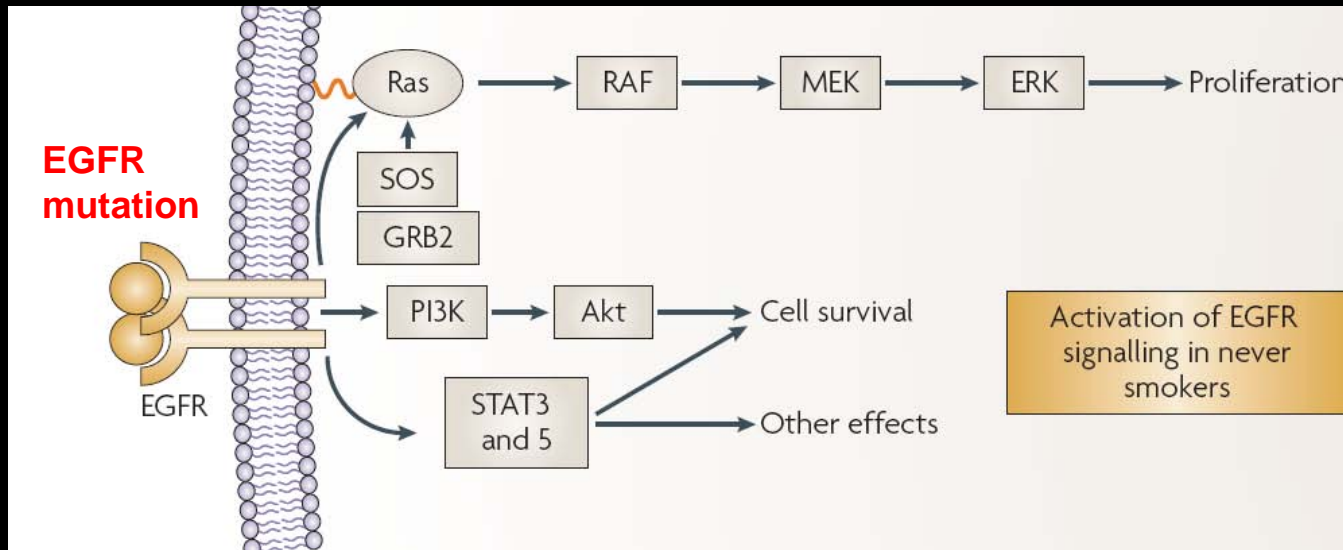
Molecular Characteristics of LCINS vs Tobacco-related Lung cancer

Molecular Markers	LCINS	Tobacco-related Lung Cancer
Chromosomal aberrations		
Chromosome 16p gain	Common, 59%	Very rare, <5%
Gene mutations		
<i>p53</i> G→T, to G→A transversion	Low, ratio= 0.23	High, ratio= 1.5
<i>p53</i> transition	Very common, 83%	Rare, 20%
<i>EGFR</i>	Common	Rare
<i>K-ras</i>	Very rare, 0-7%	Common, 30-43%
<i>EML-ALK</i> fusion gene	4.3-8.5%	0.8-2.3%
Epigenetic changes		
Methylation Index	Low	High
<i>p16</i> and <i>APC</i> methylation	Low	High
Hypermethylation of <i>hMLH1</i> , <i>hMLH2</i>	Common	Rare

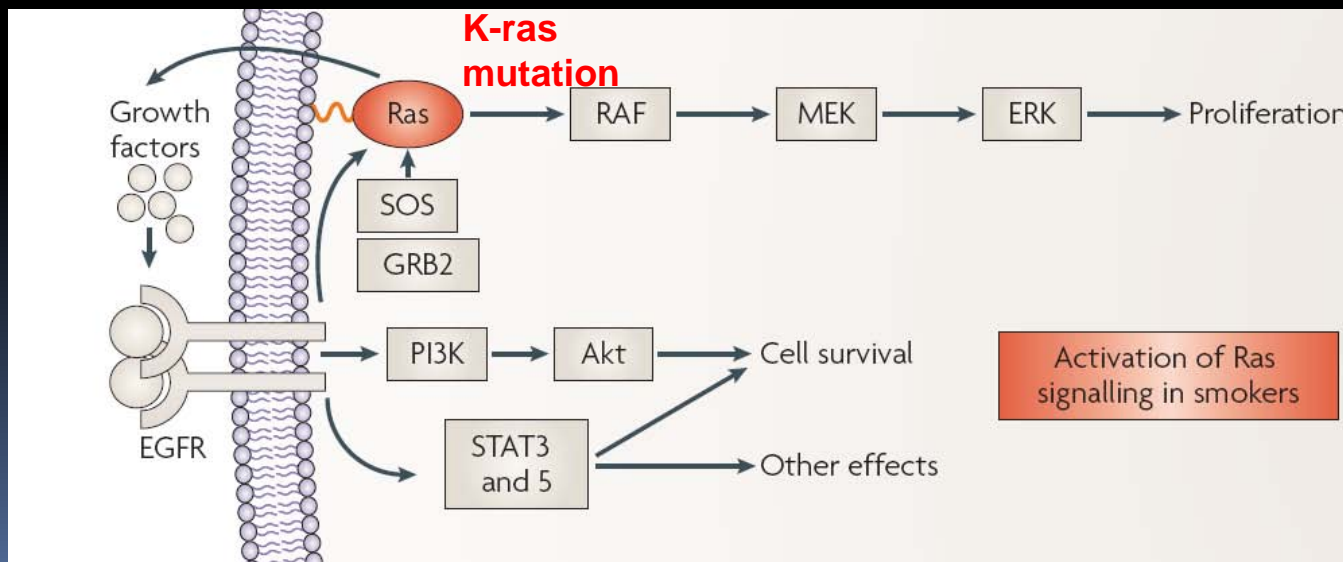
Incidence of EGFR TK Domain Mutation in Various Subgroups

Characteristics of NSCLC Tumors	No. of Tumors Evaluated	No. of Tumors With EGFR Mutation	Positive for EGFR Mutation (%)
Smoking history			
Never-smokers	181	92	50.8
Smokers	434	39	9.0
Sex			
Female	216	81	37.5
Male	422	55	13
Histology			
Adenocarcinomas	453	142	31.3
Non-adenocarcinomas	306	7	2.3
Ethnicity			
East Asian	419	122	29.1
Non-East Asian	340	27	7.9
American (United States)	262	25	9.5
Total	759	149	19.6

Pathogenesis of LCINS: Two Separate Carcinogenesis

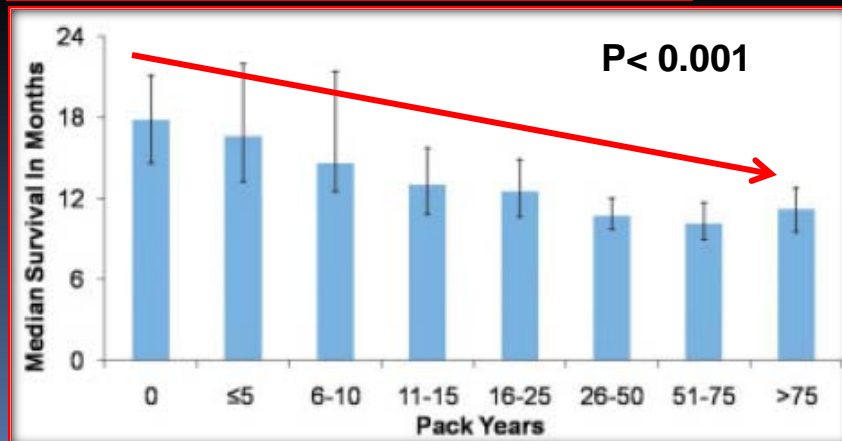
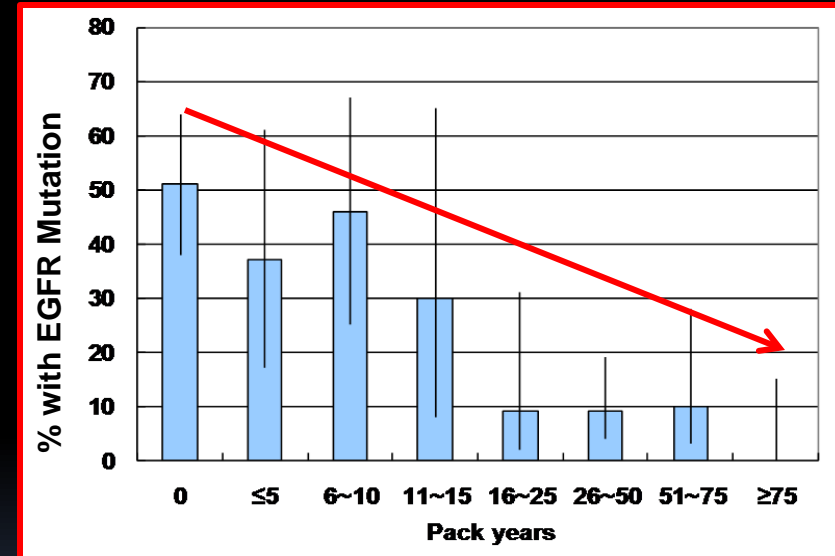
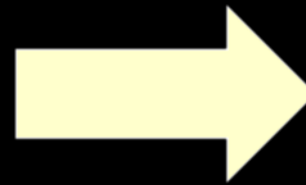
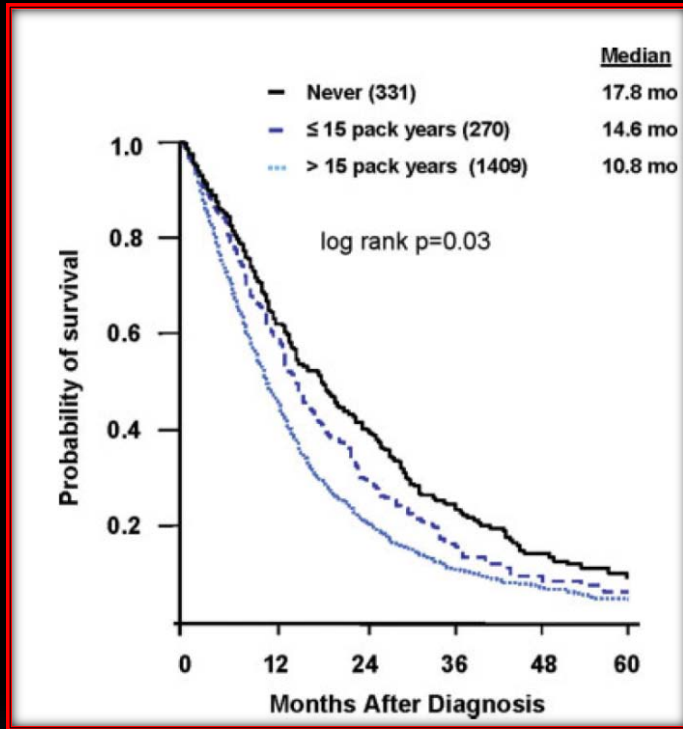


Never smoker's lung cancer
~50% EGFR Mut+

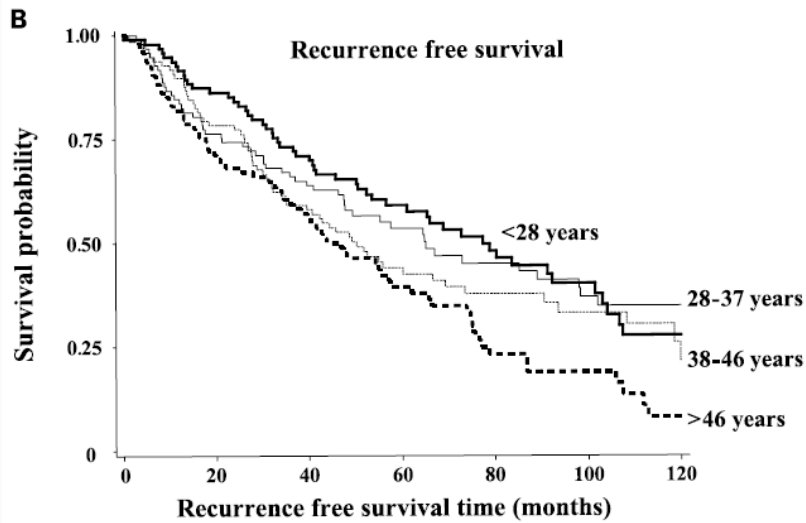
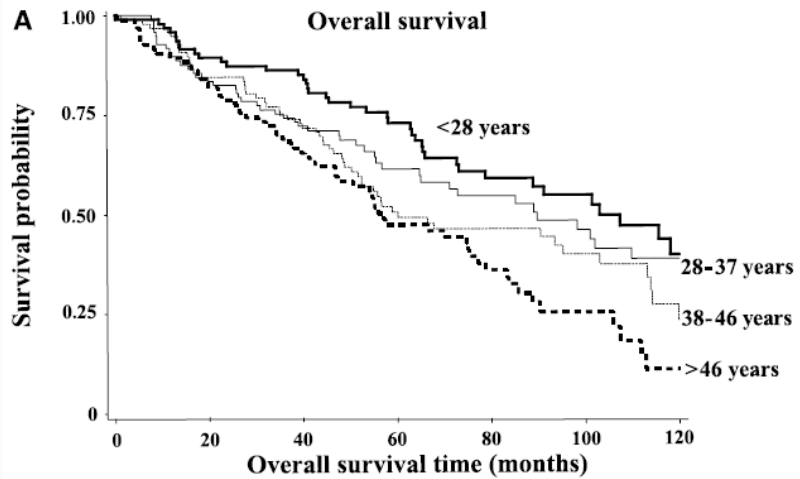


Smoker's lung cancer
~30% K-ras Mut+

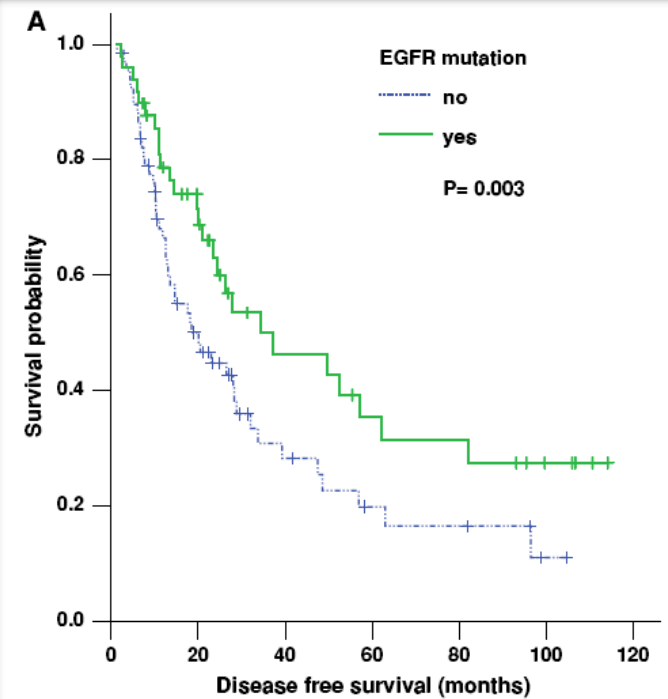
Prognostic Impact of Never Smoking History in Stage IIIB/IV NSCLC



ETS Exposure and Survival in Early-Stage NSCLC Patients



**EGFR mutation:
Molecular predictor of RFS
in resected NSCLC**



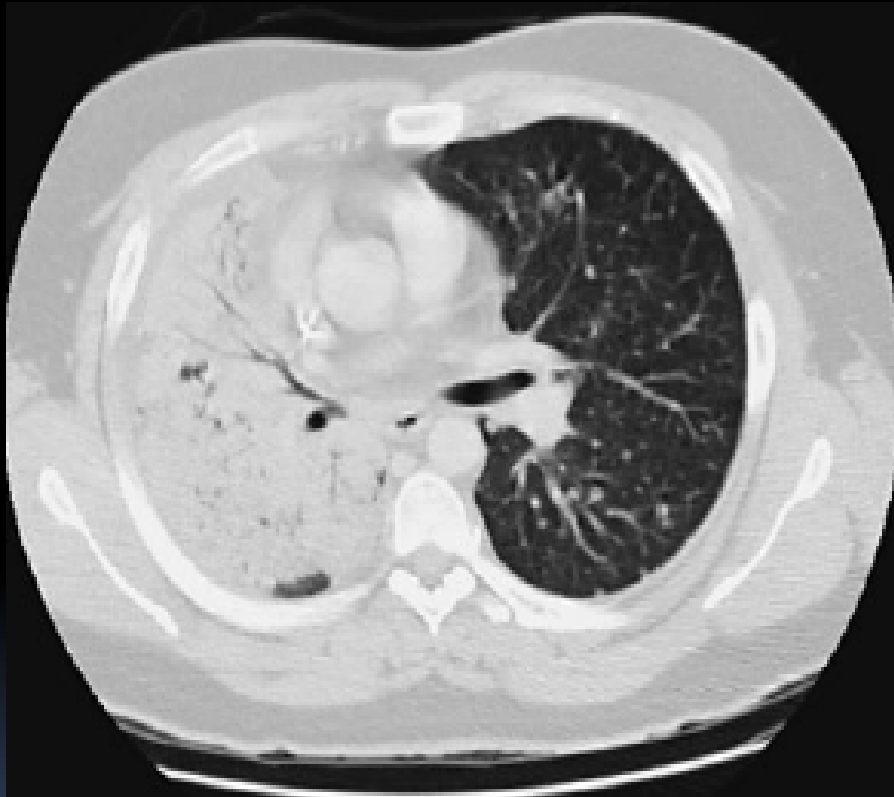
BR21 predictors of response

- Higher response seen with

- Adenocarcinoma 14% v 4%
- **Never smokers** 25% v 4%
- EGFR expression > 10% 11.3% v 3.8%
- Women 14.4% v 6%
- Asian ethnicity 19% v 7.5%

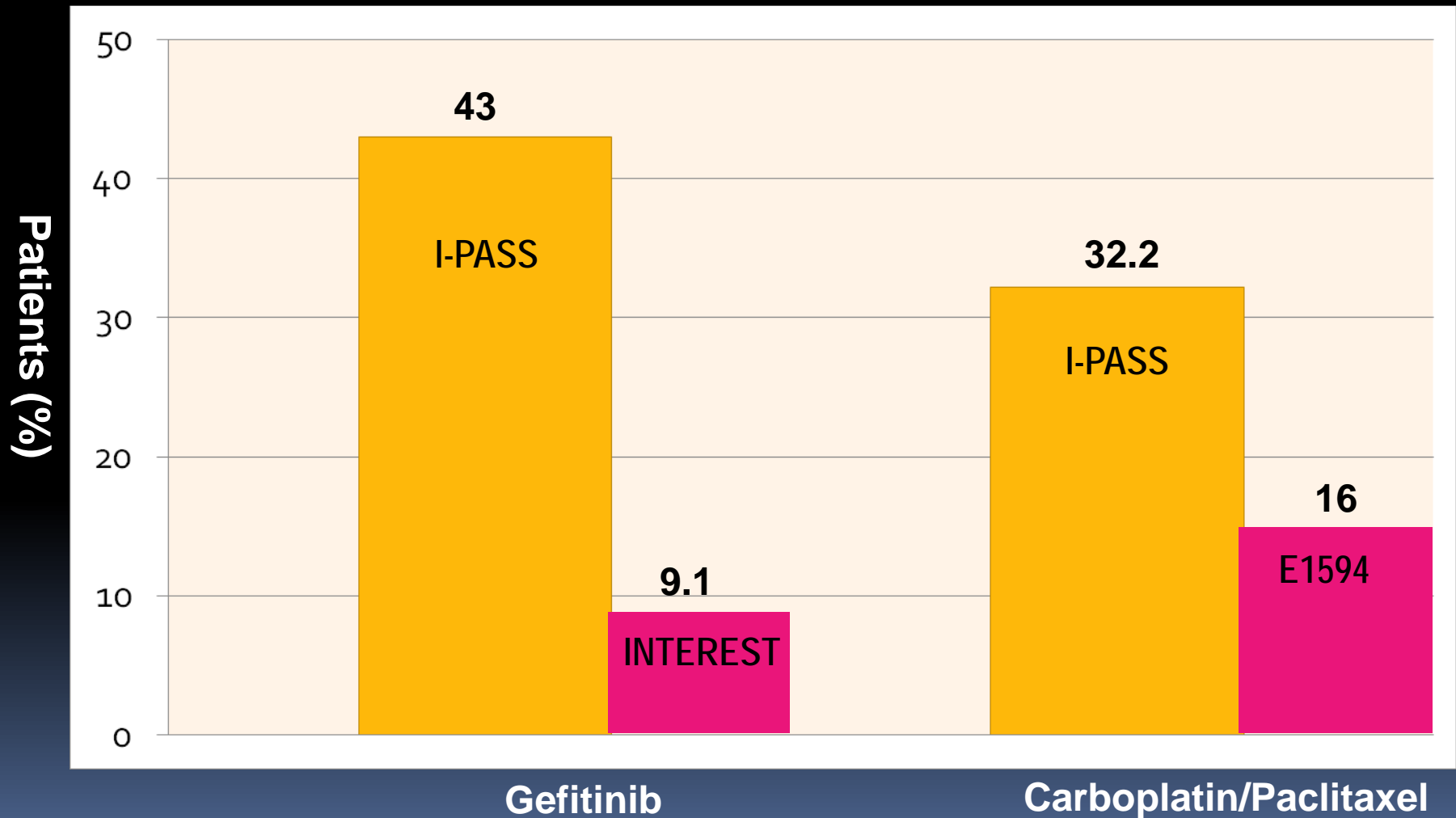
'Lazarus Response'

71 year-old female, adenocarcinoma, never smoker

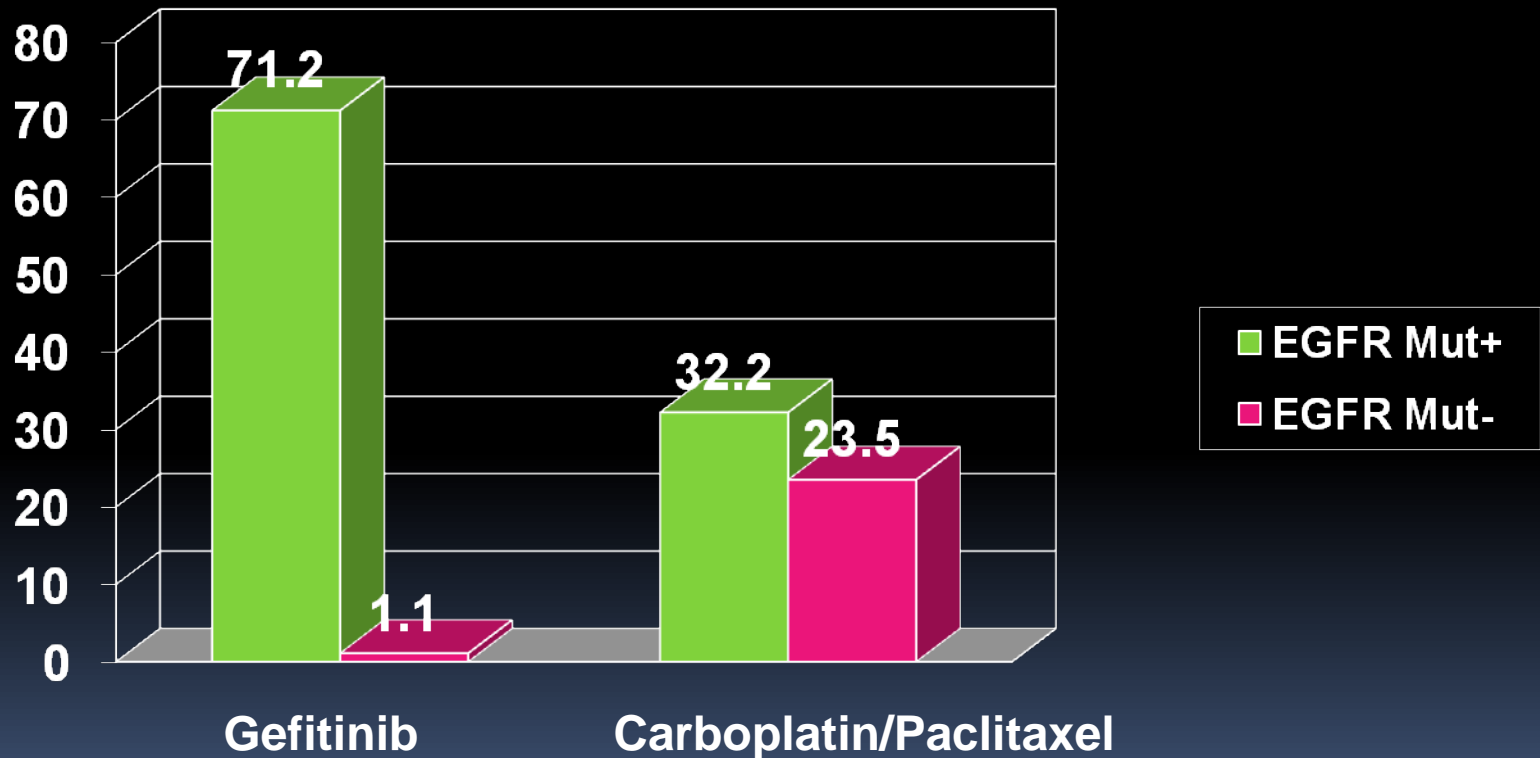


Six weeks of erlotinib

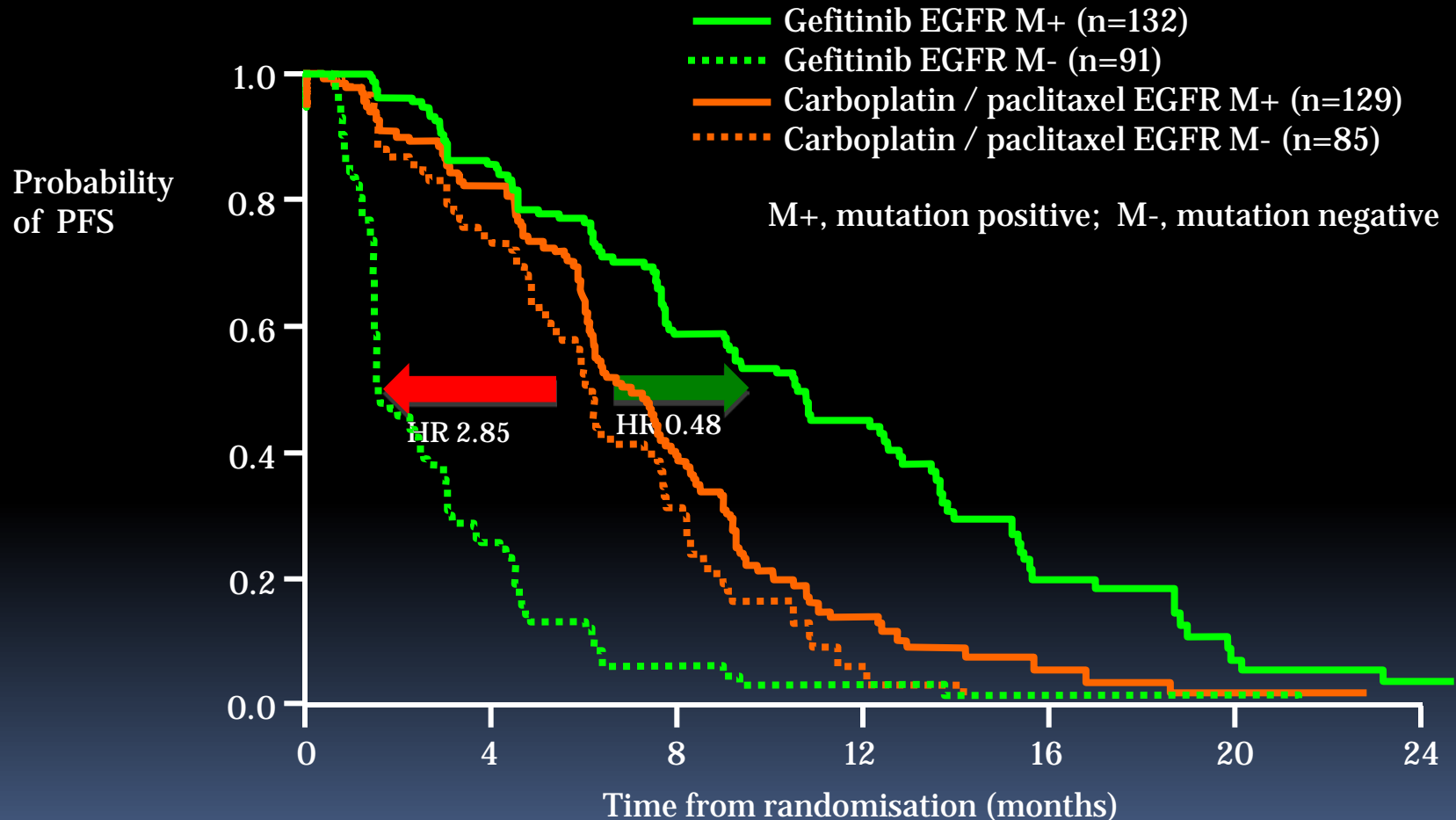
I-PASS (>93% never smoker): Response to Therapy



I-PASS: Comparison of ORR by Mutation Status within Treatment arm



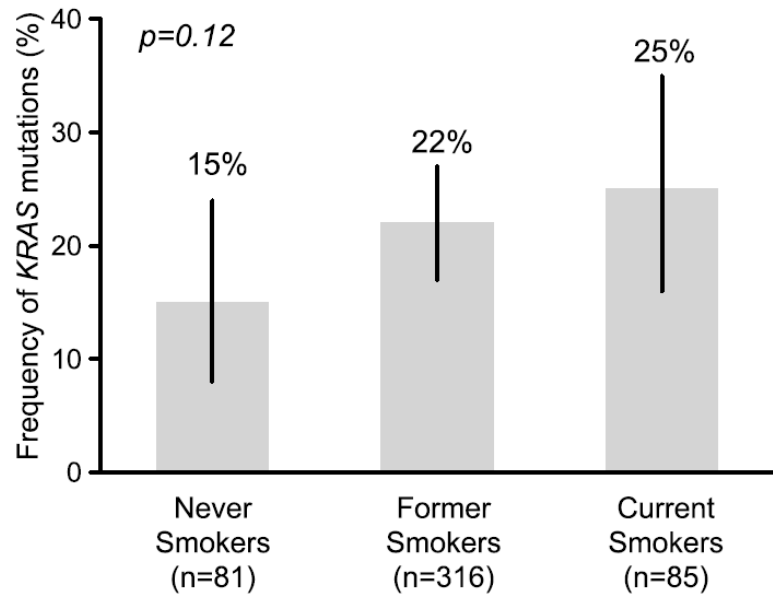
I-PASS: *EGFR* Mutation, not demographics, Determines Outcome following Treatment



Hazard ratio <1 implies a lower risk of progression in the M+ group than in the M- group

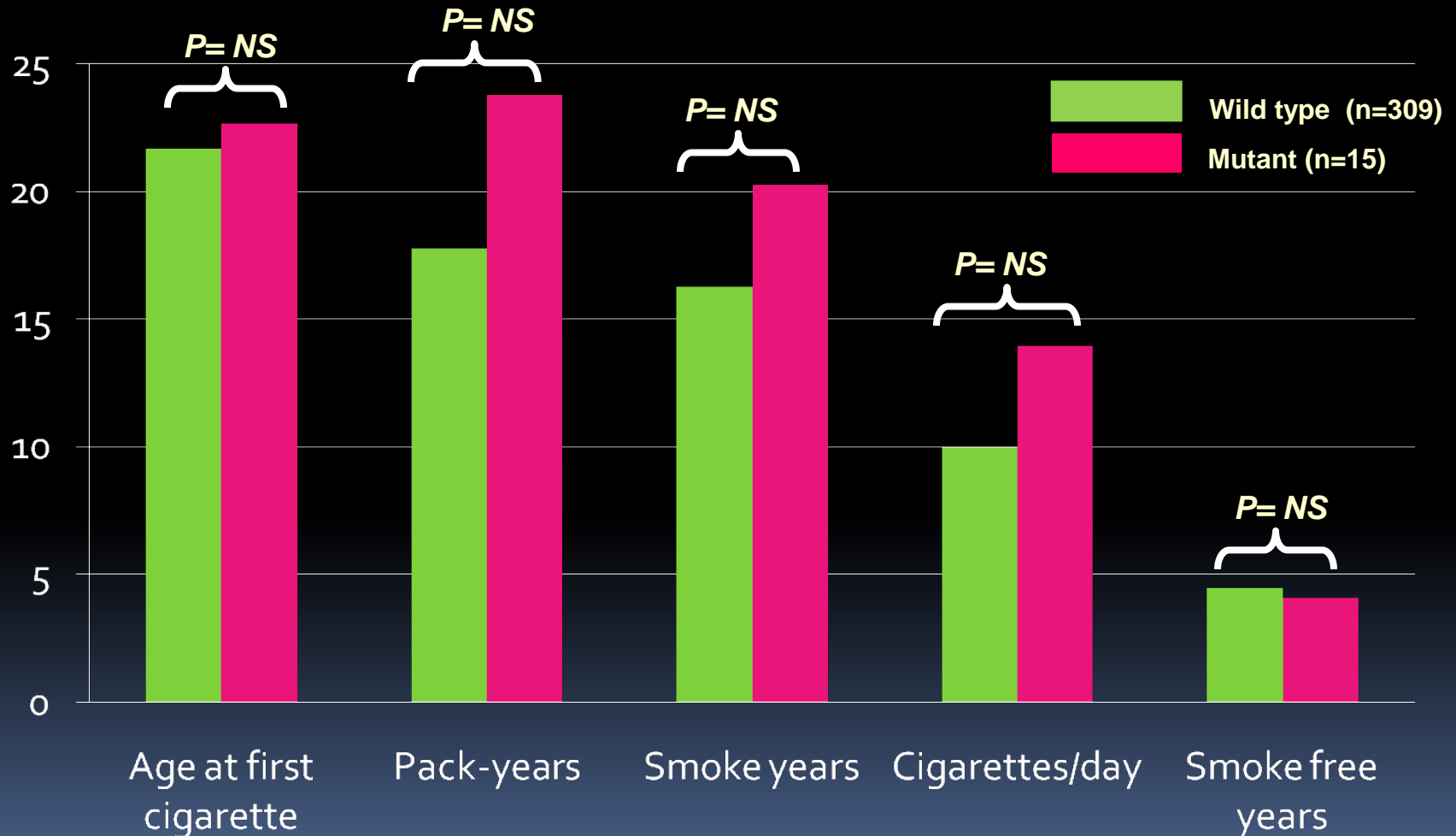
M+, mutation positive; M-, mutation negative

Frequency and Type of K-RAS mutations According to Smoking

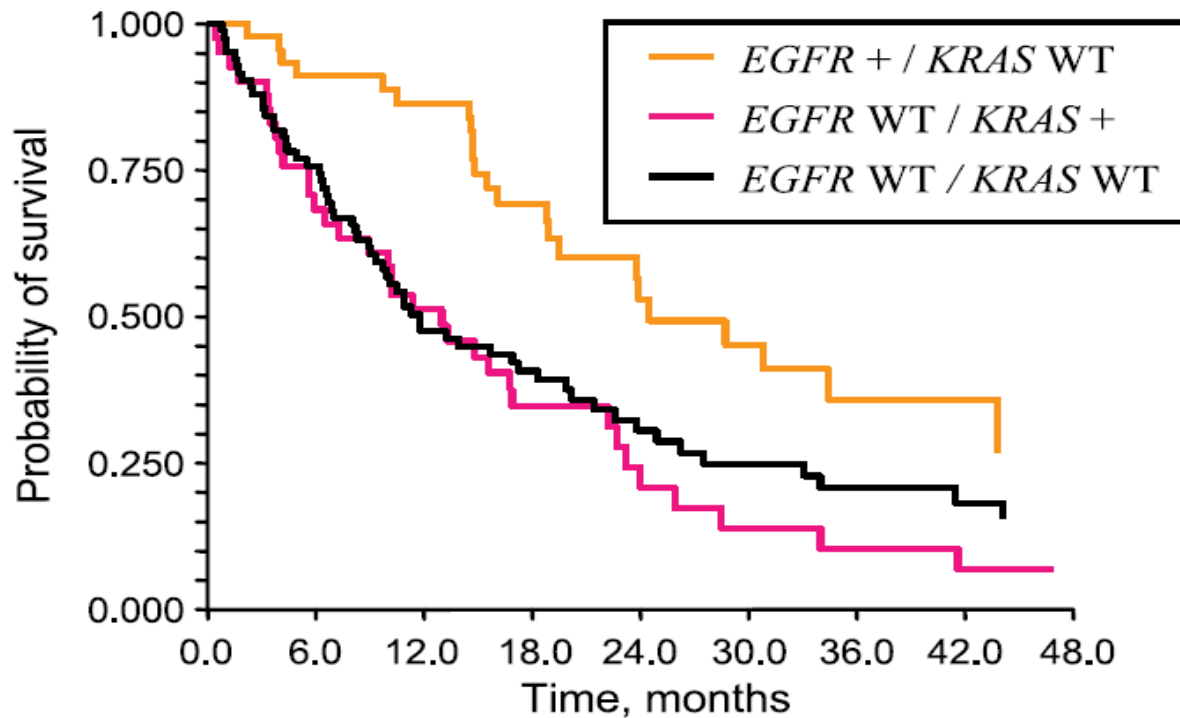


<i>KRAS</i>		Former/current	Never	Total
Mutation	Nucleotide			
G12A	GGT→GCT	13	0	13
G12C	GGT→TGT	38	0	38
G12V	GGT→GTT	20	1	21
G13C	GGC→TGC	2	0	2
G13D	GGC→GAC	1	0	1
G12D	GGT→GAT	15	10	25
G12S	GGT→AGT	1	1	2
Total		90	12	

K-ras Mutation According to Smoking History ($n=324$)



Survival based on *EGFR* and *KRAS* Mutation

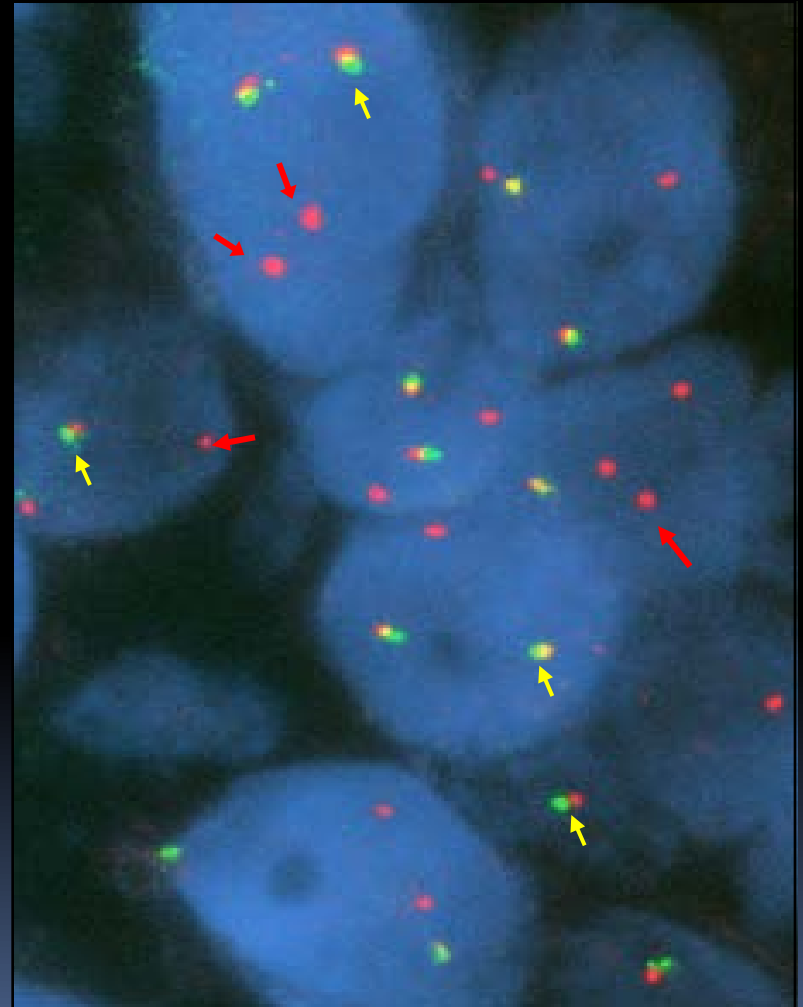
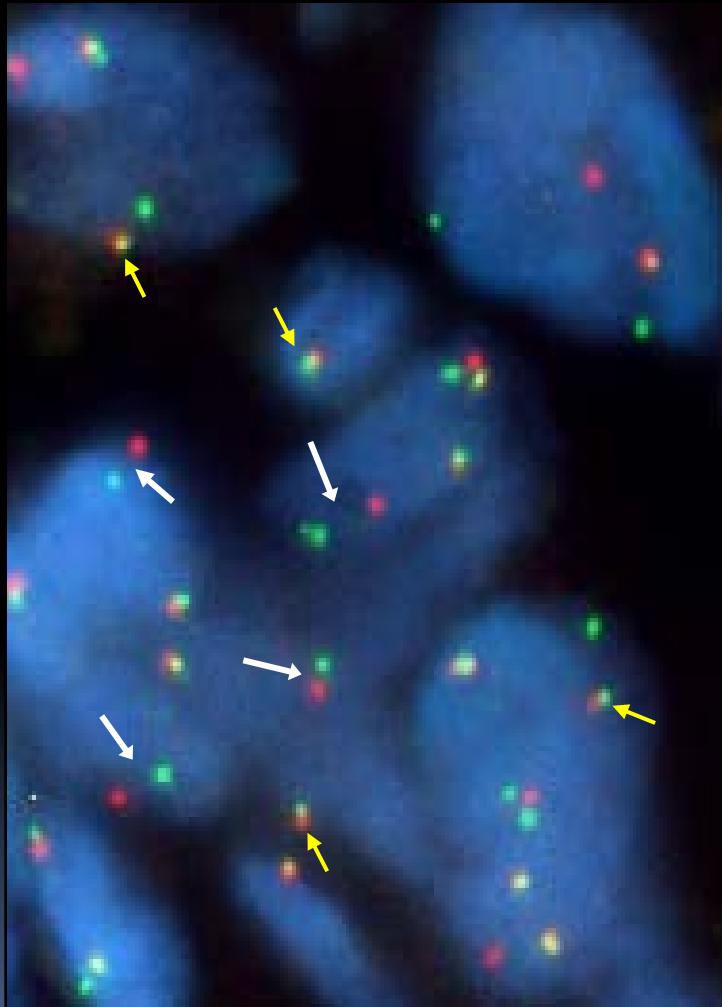


	<i>EGFR</i> + / <i>KRAS</i> WT	<i>EGFR</i> WT / <i>KRAS</i> +	<i>EGFR</i> WT / <i>KRAS</i> WT	P
N	47	41	83	
RR	68%	0	5%	< .001
Median TTP (months)	13.1	3.3	3.1	< ,0001
Median OS	24.5	13.0	11.8	.002

Testing NSCLC by ALK Break-Apart FISH



2p23



ALK Rearrangements in NSCLC

References	Method	N	ALK rearrangements	%
Soda et al., 2007	RT-PCR	75	5	6.7
Rikova et al., 2007	RT-PCR	103	4	3.9
Shinmura et al., 2008	RT-PCR	77	2	2.6
Inamura et al., 2008	RT-PCR	200	5	2.5
Takeuchi et al., 2008	RT-PCR	253	11	4.3
Wong et al, 2009	RT-PCR	266	13	4.9
Takeuchi 2009 CCR	RT-PCR	130	8	6.2
Martelli et al., 2009	RT-PCR	120	9	7.5
	IHC	662	0	0.0
Boland et al., 2009	IHC, FISH	335	6	1.9
Rodig et al, 2009	IHC, FISH	227	1	0.4
Koivunen et al., 2008	FISH	305	8	2.6
Perner et al., 2008	FISH	603	16	2.7
Shaw et al., 2009	FISH	141	19	13.5*
Total		3456	88	2.6%

Clinical Features of EML4-ALK NSCLC

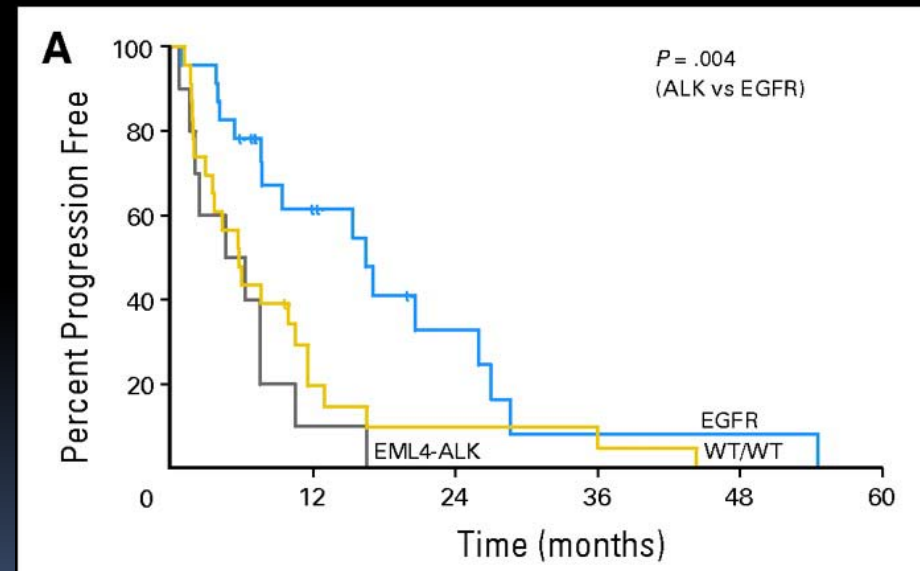
Characteristic	ALK (N=19)	EGFR (N=31)	WT/WT (N=91)
Age, yrs			
Median	52	66	64
Range	29-76	36-90	29-87
Sex			
Male	58%	26%	32%
Female	42%	74%	68%
Smoking history			
Never smoker	74%	68%	26%
Light smoker	26%	19%	16%
Smoker	0%	13%	57%
Stage			
I-III	11%	16%	31%
IV	89%	84%	58%

TTP & OS of EML4-ALK-positive patients compared with EGFR mutant or WT/WT tumors

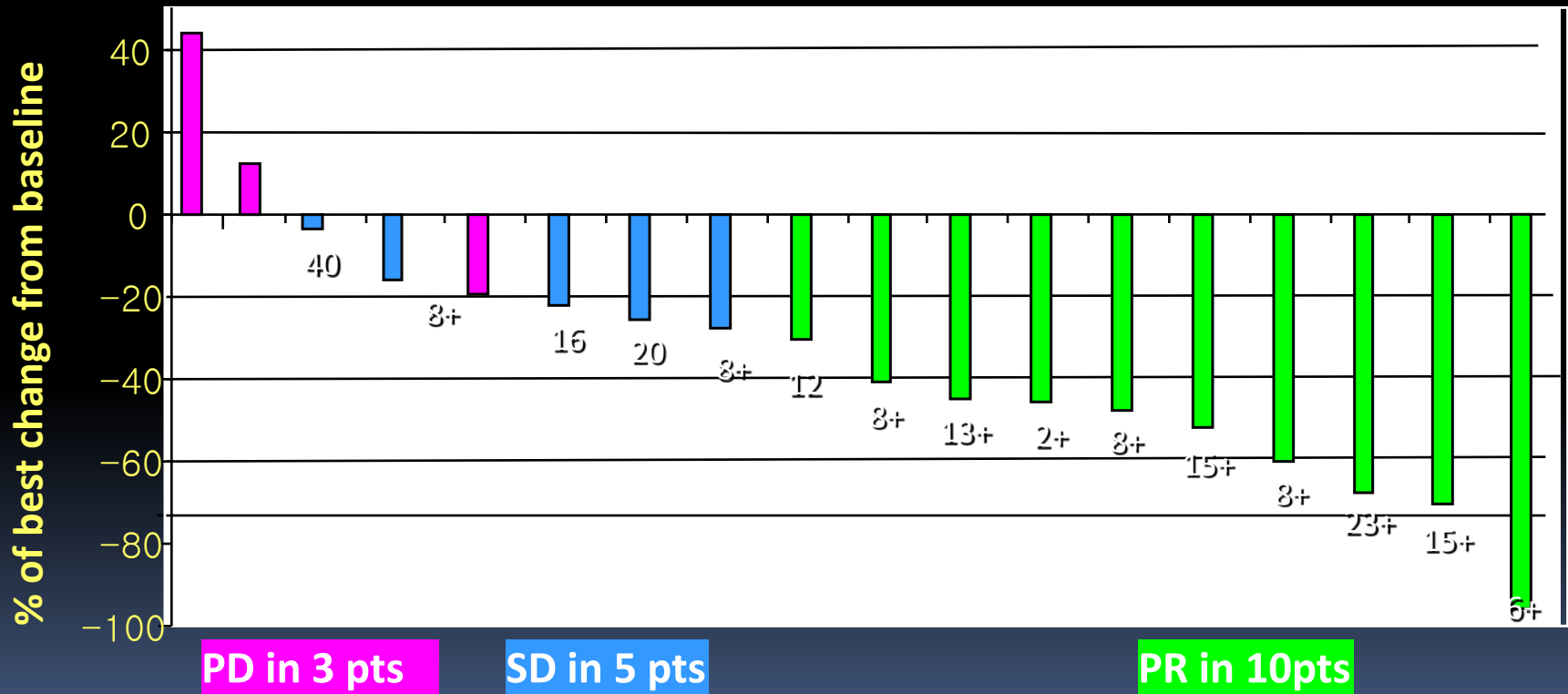
ORR to EGFR TKI

<i>ALK</i>	<i>EGFR</i>	<i>WT/WT</i>	
0%	69%	13%	$P < 0.001$

TTP for EGFR TKI

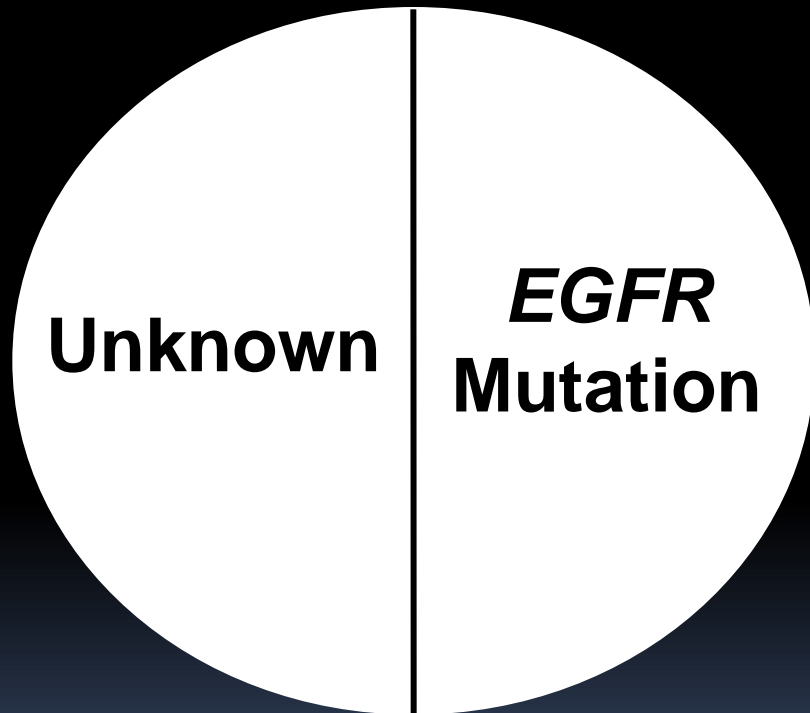


Tumor Responses to PF02341066 for NSCLC Patients with ALK Fusions

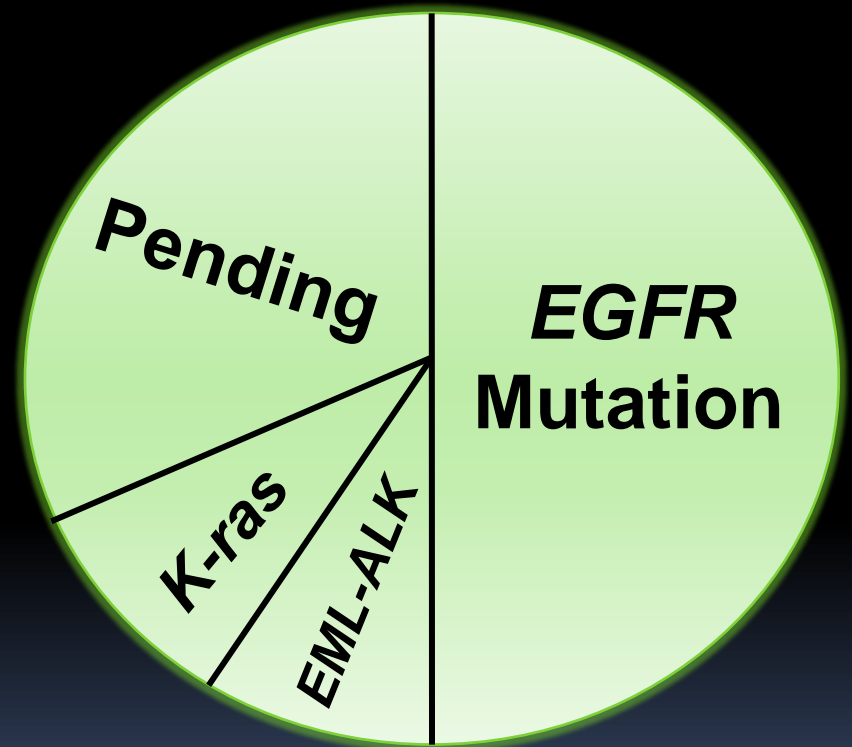


An Evolving View of Molecular Targets in LCINS

LCINS in 2004



LCINS in 2010

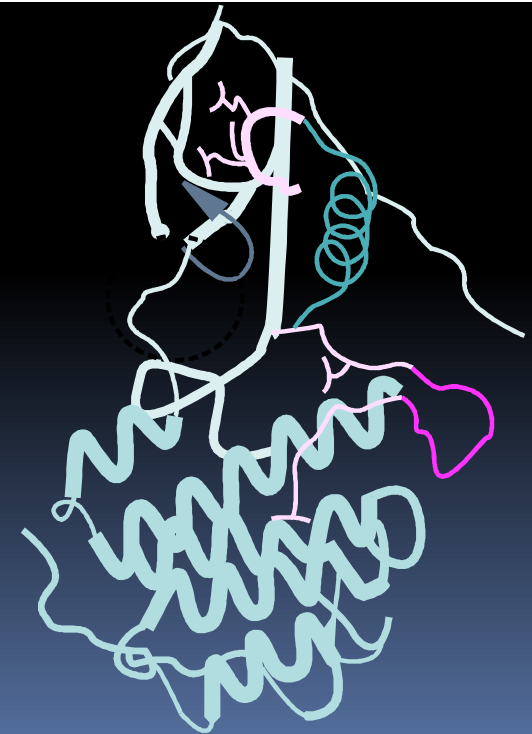
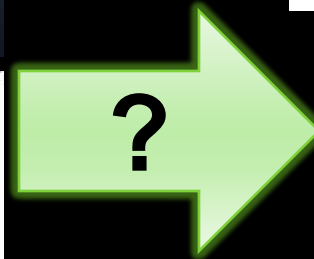
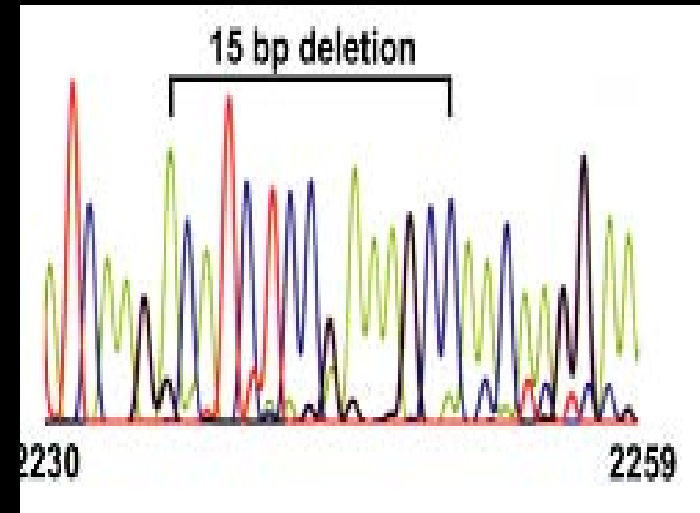


Need to identify genotype-specific subsets and treat them with appropriate kinase inhibitors

ETS is an alarming public health hazard



EGFR Mutation



Impact of Environmental Tobacco Smoke on the Incidence of Mutations in Epidermal Growth Factor Receptor Gene in Never-Smoker Patients With Non–Small-Cell Lung Cancer

Young Joo Lee, Byoung Chul Cho, Sun Ha Jee, Jin Wook Moon, Se Kyu Kim, Joon Chang, Kyung Young Chung, In Kyu Park, Sung Ho Choi, and Joo-Hang Kim

Objective:

To investigate an association between ETS exposure and *EGFR* mutations in never smokers with NSCLC

Patients and methods (1)

- **Histologically proven newly diagnosed NSCLC between June 2006 and December 2008 at Yonsei Cancer Center**
- **Lifetime never smoker**
 - **patients whose lifetime smoking dose was less than 100 cigarettes**
- **Tumor tissue samples for *EGFR* mutational analysis**

Patients and methods (2)

- **The use of an interviewer-administered questionnaire**
 - **A detailed history of ETS exposure by using prospectively administered questionnaire**
 - **Past Hx of lung disease**
 - **Family Hx of upper respiratory tract cancer**
 - **Employment**
 - **Urban residency**

Patients and methods (3)

- **The history of ETS exposure**

A regular exposure to ETS produced by an active smoker within a confined space for at least 1 year

- **Age at first exposure**
- **Exposure period: Childhood or Adulthood (≥ 19 yr)**
- **Exposure place: Home or Workplace**
- **Exposure source: Parents, Spouse, or Coworkers**
- **Exposure years per source**

Patients and methods (4)

- ***Mutational analysis***

- To determine the presence of activating *EGFR* mutations in exon 19 or exon 21
- By nested PCR amplification and direct DNA sequencing on exon 18-21 of *EGFR* gene

Patients characteristics (N=179)

Characteristics	Environmental Tobacco Smoke		P*
	Never	Ever	
No. of patients	44 (24.6)	135 (75.4)	
Age, median (range, years)	57 (37-76)	59 (33-80)	0.641 [†]
Gender	Male	7 (15.9)	0.485
	Female	37 (84.1)	
Histology	ADC	39 (88.6)	0.647
	Non-ADC	5 (11.4)	
Past history of lung disease [‡]	Never	3 (6.8)	0.827
	Ever	41 (93.2)	
Family history of URT cancer	Yes	4 (9.1)	1.000
	No	40 (90.9)	
Employment	Yes	14 (31.8)	0.776
	No	30 (68.2)	
Urban residency	Ever	38 (86.4)	0.389
	Never	6 (13.6)	

Notes: *tested by χ^2 test; [†]tested by t-test; [‡]past history of lung disease includes previous physician-diagnosed pneumonia, chronic bronchitis, emphysema, asthma, tuberculosis, silicosis, asbestosis, and idiopathic pulmonary fibrosis. Abbreviations: ADC, adenocarcinoma; URT, upper respiratory tract.

Summary of ETS History

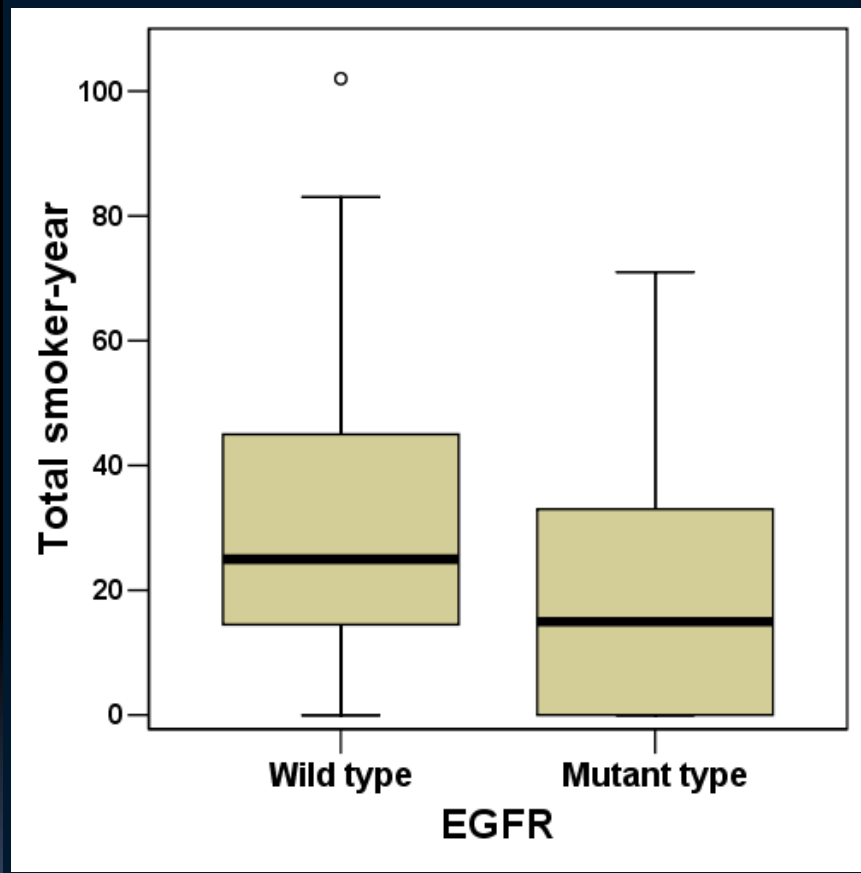
Characteristics		N= 135
Age at first exposure, median (range, years)		1 (1–45)
Total number of active smokers, median		2 (1–7)
Total smoker year, median (range, years)		30 (2–102)
ETS period	Childhood	6.7%
	Adulthood	42.2%
	Both	51.1%
ETS place	Home	62.2%
	Workplace	17.1%
	Both	20.7%

The incidence of *EGFR* mutations

Characteristics		Mutation/N (%)	<i>P</i> *
Total		79/179 (44.1)	
Age	<58 y	38/84 (45.2)	0.780
	≥ 58y	41/95 (43.2)	
Gender	Male	12/23 (52.2)	0.406
	Female	67/156 (42.9)	
Histology	ADC	72/155 (46.5)	0.113
	Non-ADC	7/24 (29.2)	
ETS exposure	Ever	52/135 (38.5)	0.008
	Never	27/44 (61.4)	

* tested by χ^2 test; ADC, adenocarcinoma; ETS, environmental tobacco smoke

Total smoker-year according to *EGFR* mutation



EGFR	Total smoker-year median (range)
<i>Wt</i>	25 (0-102)
<i>Mutant</i>	15 (0-71)

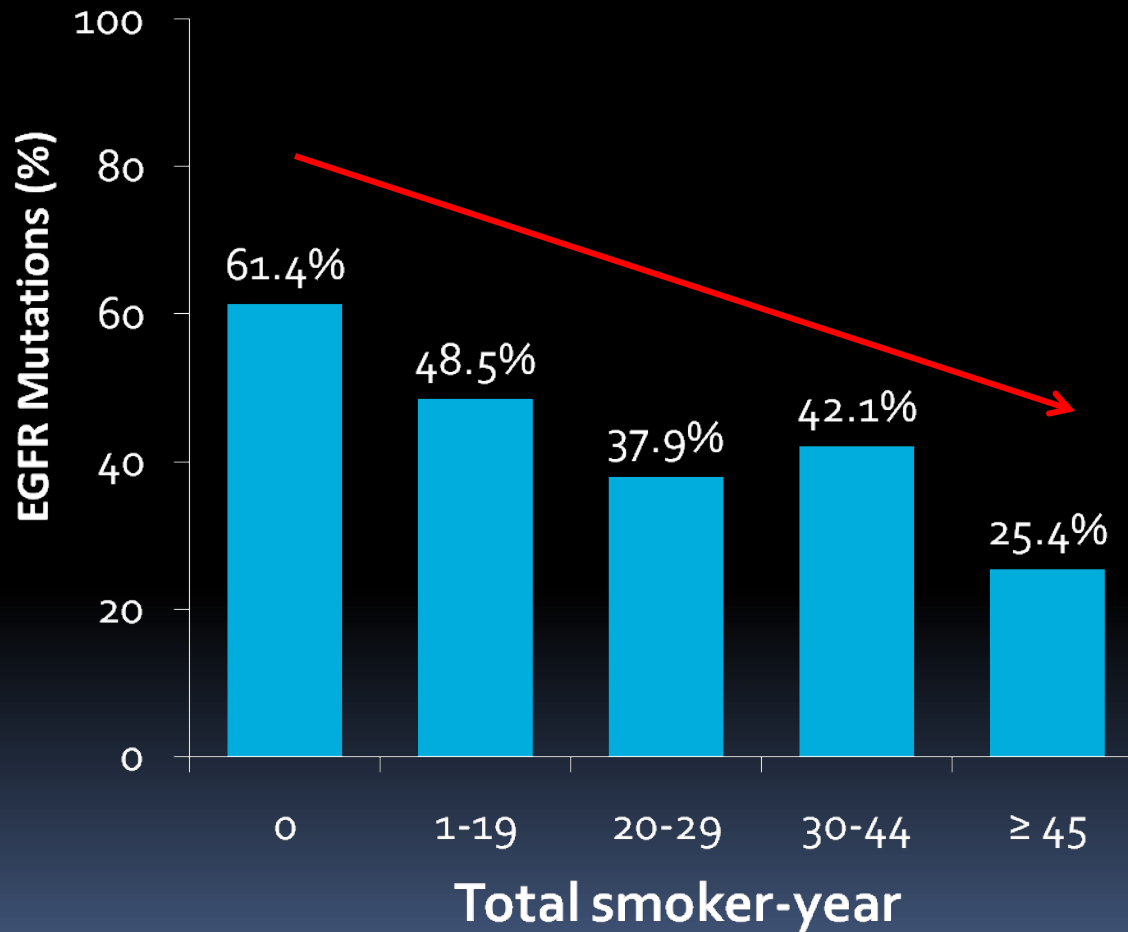
Mann-Whitney U test, $P= 0.002$

Incidence of *EGFR* Mutations by ETS Exposure in Subgroups

Subgroups	Never ETS Exposure			Ever ETS Exposure				P*
	Mutation		AOR	Mutation		AOR	95% CI	
	No. of Mutations/ Total No.	%		No. of Mutations/ Total No.	%			
Total	27/44	61.4	1.00	52/135	38.5	0.40	0.20 to 0.81	.011
Age, year [†]								
< 58	14/23	60.9	1.00	24/61	39.3	0.42	0.16 to 1.14	.091
≥ 58	13/21	61.9	1.00	28/74	37.8	0.41	0.15 to 1.12	.081
Sex								
Male	6/7	85.7	1.00	6/16	37.5	0.14	0.01 to 1.55	.109
Female	21/37	56.8	1.00	46/119	38.7	0.48	0.23 to 1.01	.054
Adenocarcinoma	25/39	64.1	1.00	47/116	40.5	0.39	0.18 to 0.84	.015
Nonadenocarcinoma	2/5	40.0	1.00	5/19	26.3	0.68	0.08 to 5.45	.718

Abbreviations: *EGFR*, epidermal growth factor receptor; *ETS*, environmental tobacco smoke; *AOR*, adjusted odds ratio. *Tested by logistic regression with sex, histology, and exposure to *ETS*. Never *ETS* exposure group was treated as the reference group. [†]Dichotomized by median value.

Incidence of *EGFR* mutations by total smoker-year of ETS (1)



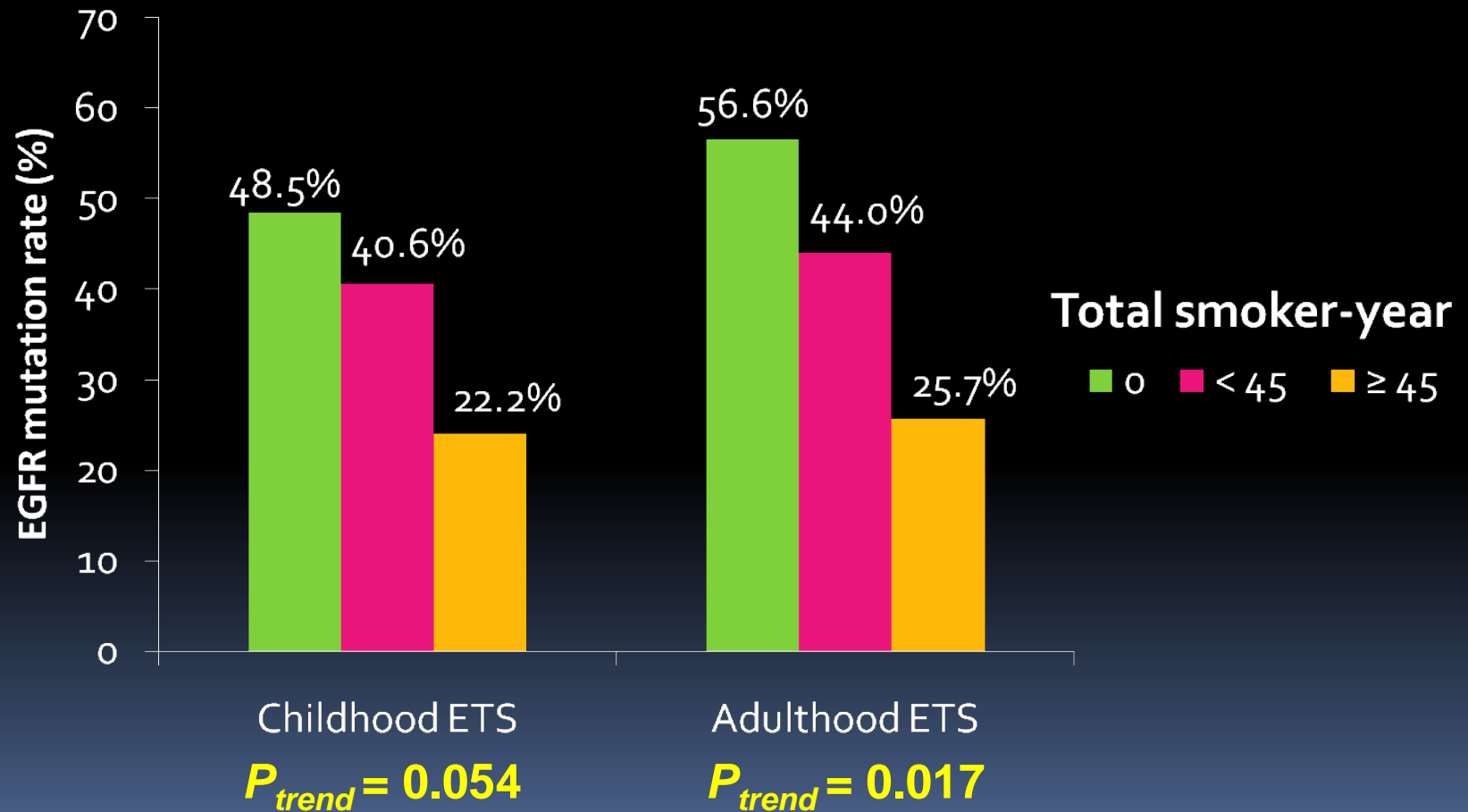
$P_{trend} = 0.028$ (by χ^2 test for linear trend)

Incidence of *EGFR* Mutations by Total Smoker-Year of ETS (2)

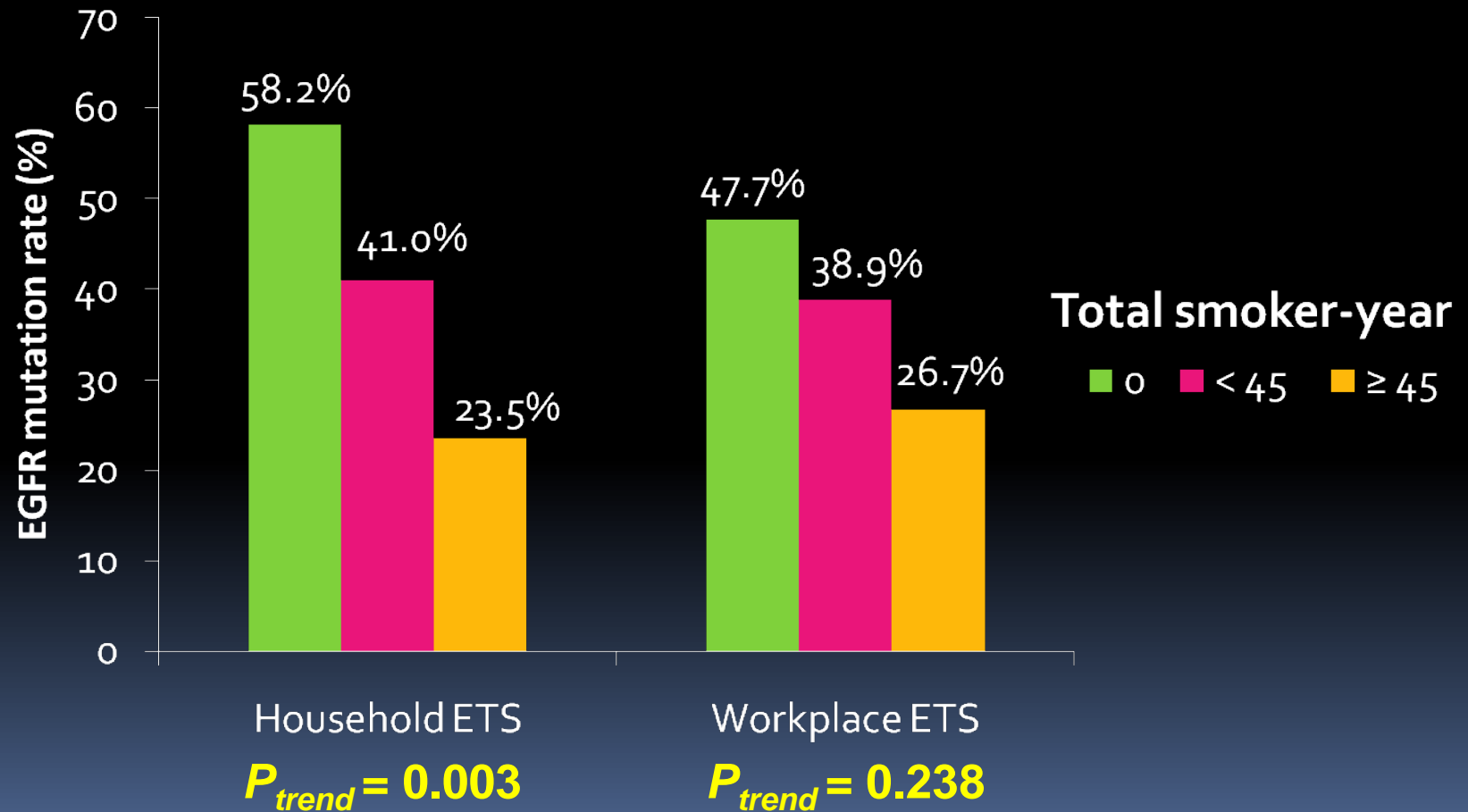
Total smoker-year		Mutation/ N (%)	AOR*	95% CI	P
Never	0	27/44 (61.4)	1.00	-	-
Q1	1-19	16/33 (48.5)	0.59	0.23-1.49	0.264
Q2	20-29	11/29 (37.9)	0.50	0.17-1.50	0.184
Q3	30-44	16/38 (42.1)	0.48	0.20-1.18	0.112
Q4	≥ 45	9/35 (25.7)	0.23	0.08-0.62	0.004

Abbreviations: EGFR, epidermal growth factor receptor; ETS, environmental tobacco smoke; OR, odd ratio. *Adjusted with sex and histology. In all of analyses to estimate odds ratio, never-smoker group was treated as the reference group.

Incidence of *EGFR* mutations by ETS exposure period



Incidence of *EGFR* mutations by ETS exposure place



Response to EGFR-TKIs

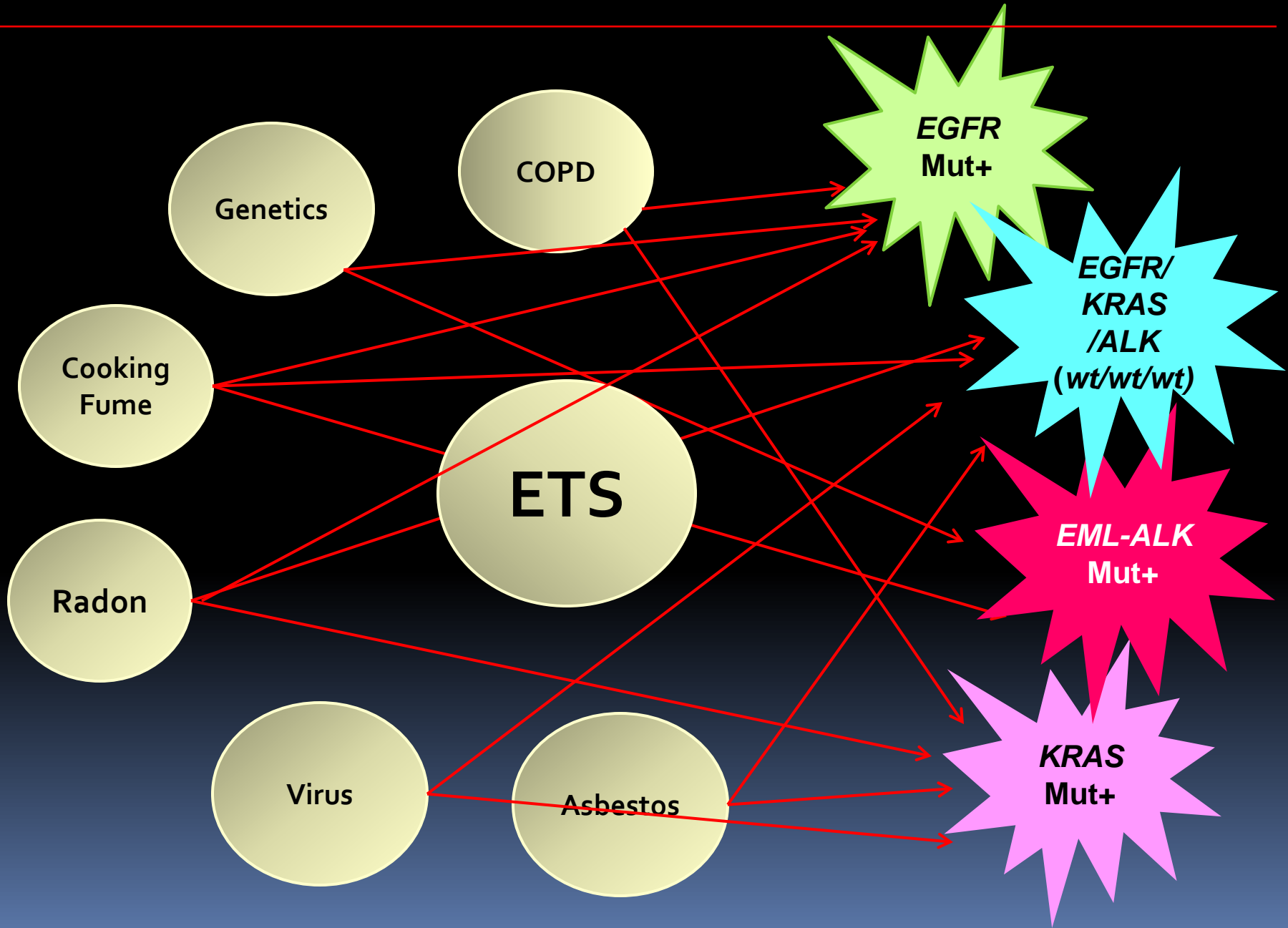
Variables		Responder/N	%	P*
Total		28/90	31.1	
Gender	Male	2/9	22.2	0.715
	Female	26/81	32.1	
Histology	ADC	25/76	32.9	0.536
	Non-ADC	3/14	21.4	
EGFR mutation	Yes	23/36	63.9	< 0.001
	No	5/54	9.3	
ETS exposure	Ever	15/61	24.6	0.053
	Never	13/29	44.8	
Previous chemotherapy	Never	3/8	37.5	0.253
	Ever	25/82	30.1	

Notes: *tested by χ^2 test ; Abbreviations: ADC, adenocarcinoma; EST, environmental tobacco smoke

Conclusions

- **LCINS, compared with smoker's lung cancer, showed better prognosis and higher response to therapy, underlying significance of *EGFR* mutation**
- ***EGFR* mutations are the first specific genetic mutation associated with LCINS, and have strong negative correlation with active smoking**
- **Appropriate selection of targeted therapy should be guided by identifying the molecular features of patients, no matter what they are never smokers or not**
- **Exposure to ETS is negatively associated with the incidence of *EGFR* mutations in LCINS**
- **Exposure to ETS might greatly influence tumor biology, natural history, and responsiveness to therapy by reducing the incidence of *EGFR* mutation**

LCINS: At least Four Different Diseases



Urgent Asian Task: Tracing the Cause of *EGFR* Mutation

- Contrast to extensive genetic and epigenetic change in smoker's bronchial epithelium, there is little field cancerization in never smoker's (*EGFR*-mutated) tumor
 - *Small particle carcinogen?*
- Based on ours and other works,
 - *Tobacco carcinogen is the least to be the suspect for *EGFR* mutation*
- Much higher incidence of *EGFR* mutation in Asians
 - *One of the prevalent environmental factors in Asia?*
 - *Asian-specific genetic factor, such as tobacco-carcinogen metabolizing enzymes?*
- Striking contrast to *P53* or *K-ras* mutations prevalent in various types of cancers, *EGFR* mutation is virtually found only in NSCLC
 - *Aerosol type carcinogens?*



Thank You!