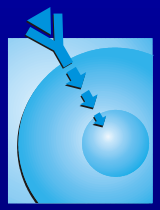


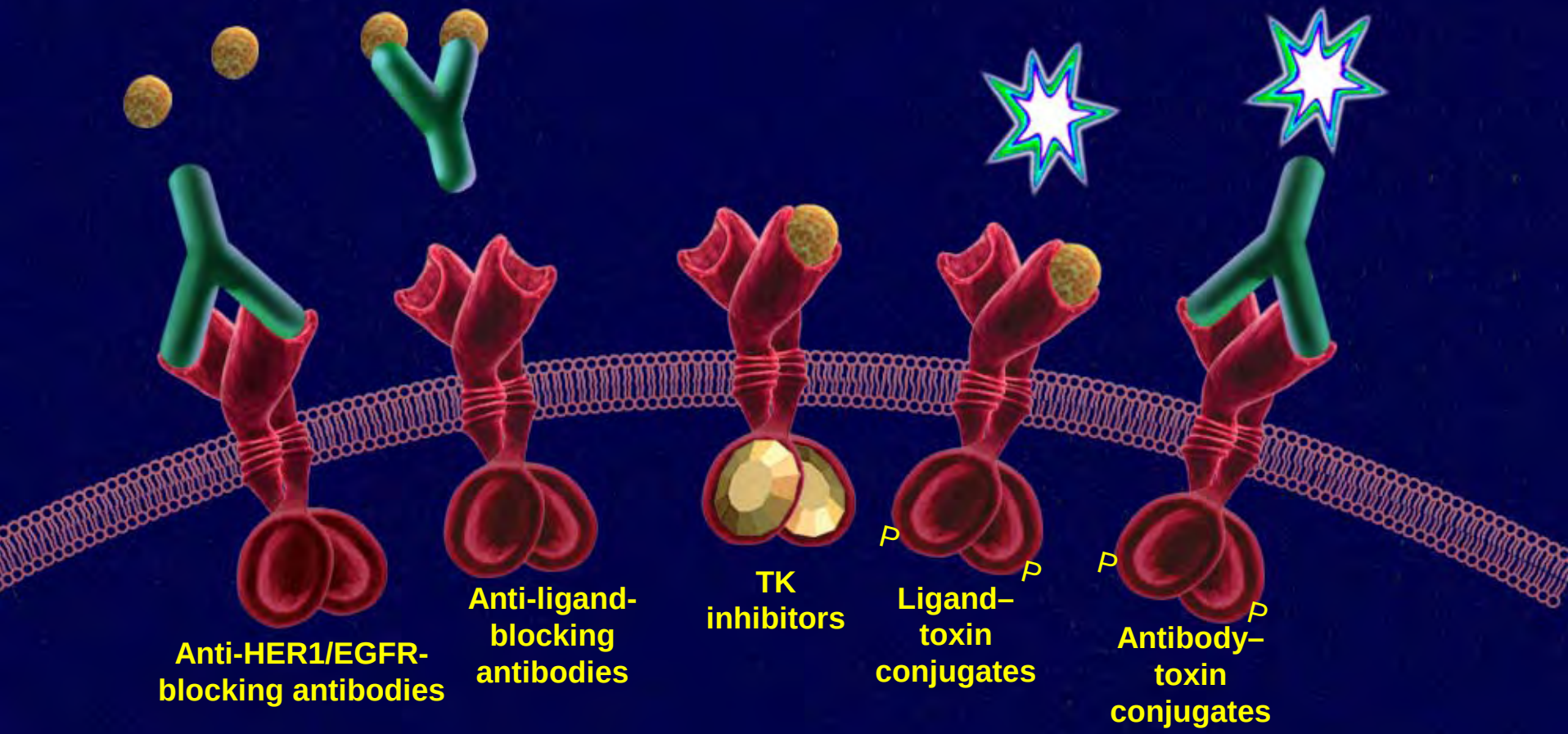
Laboratoř experimentální medicíny, DK LF UP a FN, Olomouc

Možnosti predikce účinnosti nízkomolekulárních EGFR1 inhibitorů

[1,4]Hajdúch M., Berkovcová J., [1]Trojanec R.,
[1]Janošťáková A., [2]Kolek V, [2]Grygárková I, Witta S.

[1]Laboratoř experimentální medicíny při Dětské klinice LF UP a FN Olomouc,
[2]Klinika TRN, LF UP a FN Olomouc,
[3]Colorado University, Aurora, Colorado,
[4]Onkologická klinika LF UP a FN Olomouc,

Common Approaches to Targeting HER1/EGFR



Slamon DJ, Leyland-Jones B, Shak S, et al. *N Engl J Med.* 2001;344:783-792; Mendelsohn J, Baselga J. *Oncogene.* 2000;19:6550-6565; Noonberg SB, Benz CC. *Drugs.* 2000;59:753-767; Raymond E, Faivre S, Armann JP. *Drugs.* 2000;60(Suppl 1):15-23; Arteaga C. *J Clin Oncol.* 2001;19:32s-40s; Pedersen MW, Meltom M, Damstrup L, et al. *Ann Oncol.* 2001;12:745-760.

Somatic Mutations in the Tyrosine Kinase Domain of EGFR

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

Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M.

SCIENCE April 29, 2004

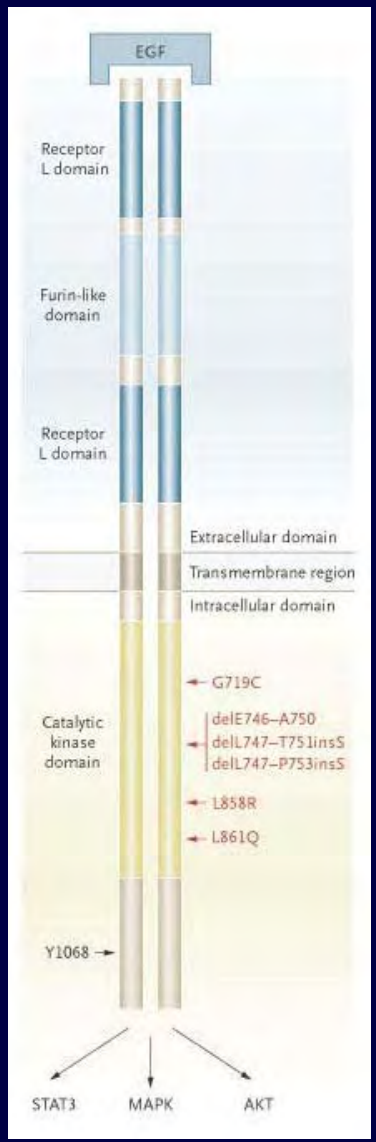
Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non –Small-Cell Lung Cancer to Gefitinib

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.

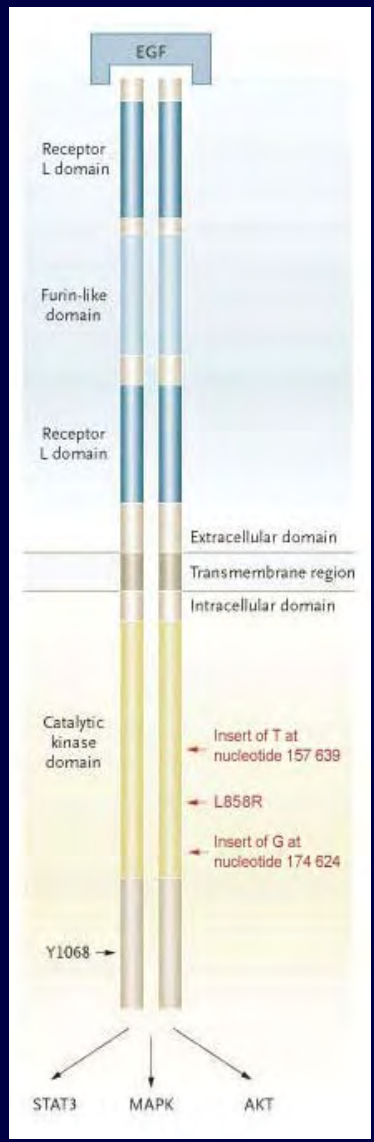
NEW ENGLAND JOURNAL OF MEDICINE, MAY 20, 2004

LABORATORY OF EXPERIMENTAL MEDICINE

Mutations in gefitinib NSCLC responders

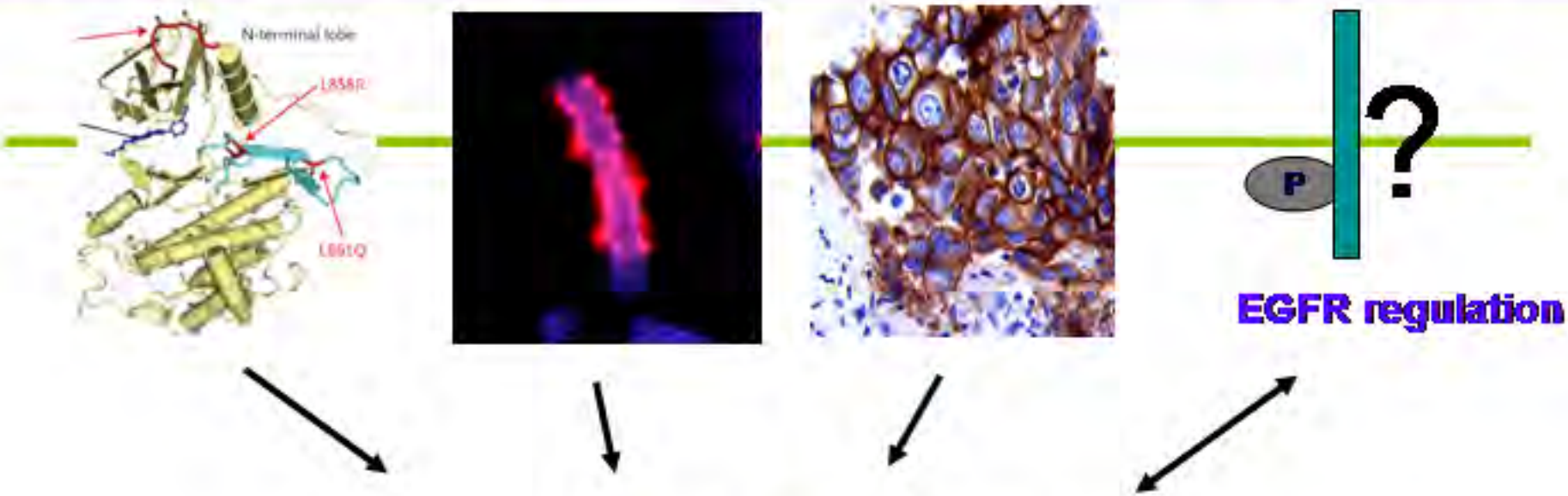


NEJM, 2004



LEM, 2004

Gene Expression and Predicting Response the EGFR TK-Inhibitors



Δ Gene Expression

Develop Predictive Assays
Novel Targets
Pathway alterations to improve sensitivity

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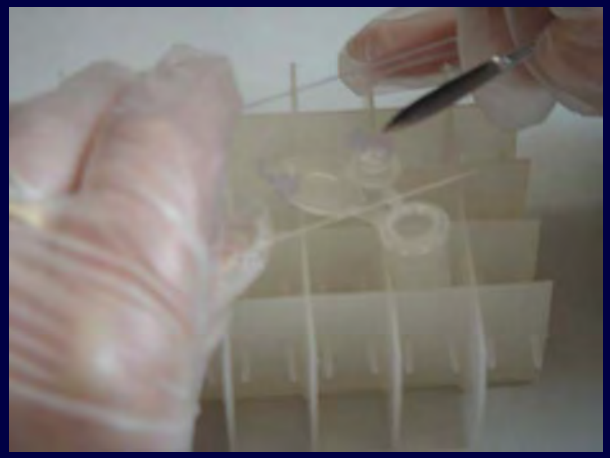
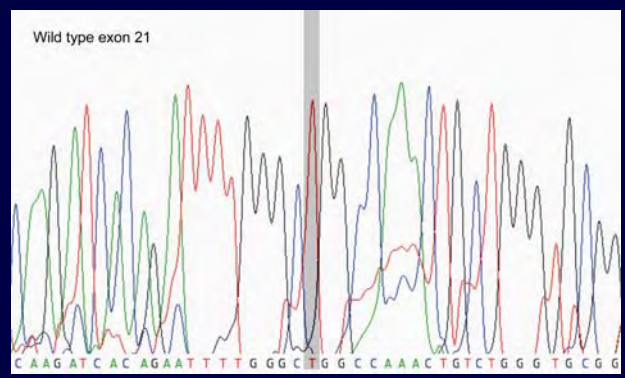
Tissue processing: direct DNA extraction



DNA extraction:

2. Tissue elution
3. Phenol-chloroform extraction
4. DNA easy tissue kit (Qiagen)

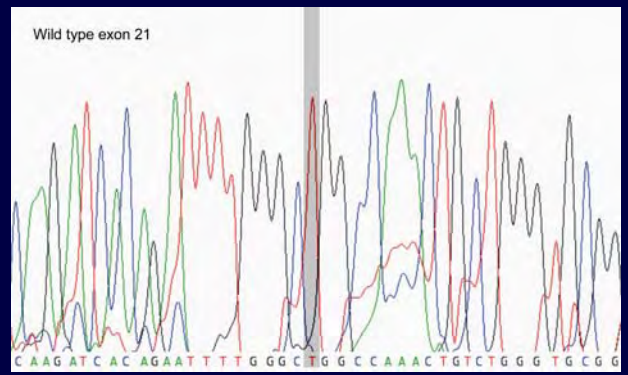
Specific DNA amplification & sequencing

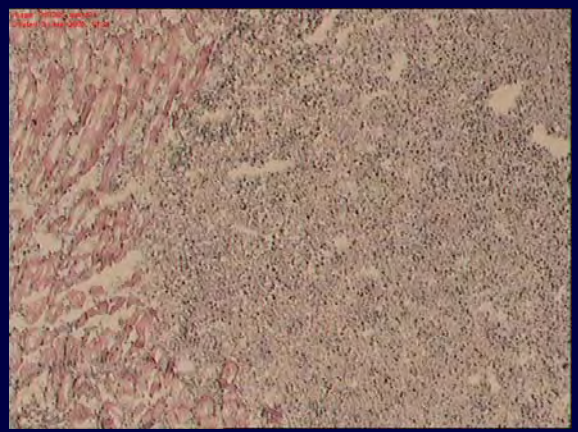


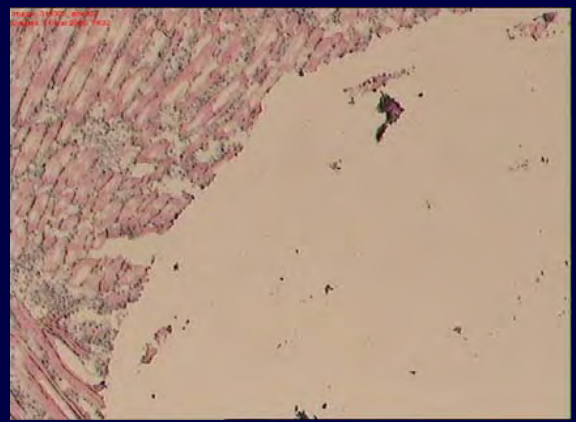
LABORATORY OF EXPERIMENTAL MEDICINE

Tissue processing: macrodissection

Specific DNA amplification & sequencing





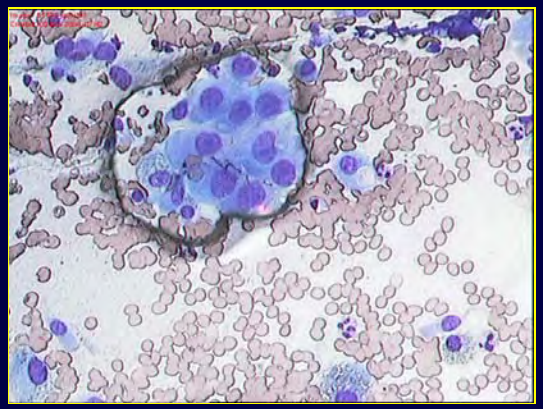


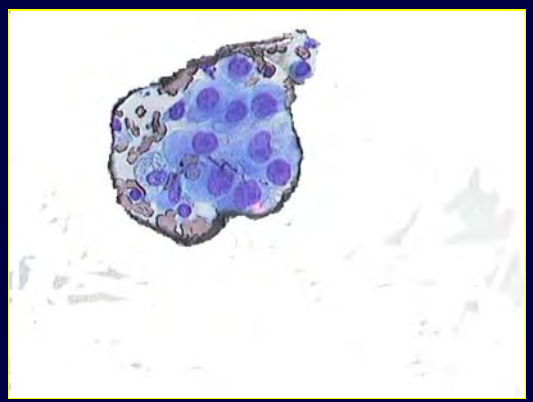
DNA extraction:

2. Tissue elution
3. Phenol-chloroform extraction
4. DNA easy tissue kit (Qiagen)

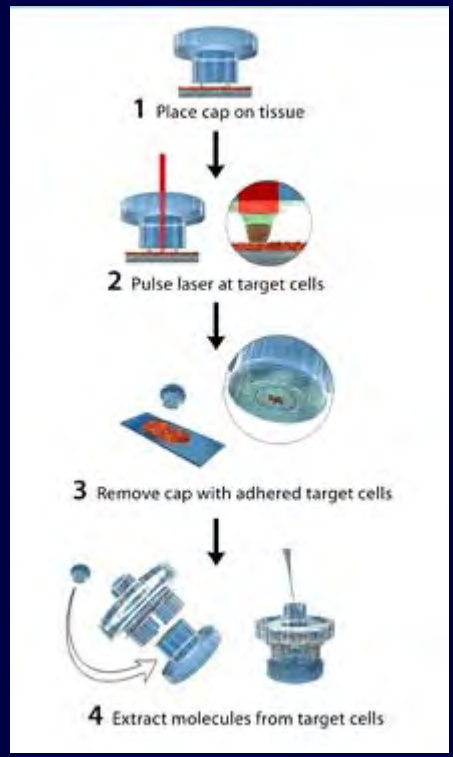
LABORATORY OF EXPERIMENTAL MEDICINE

Tissue processing: Laser capture microdissection (LCM)

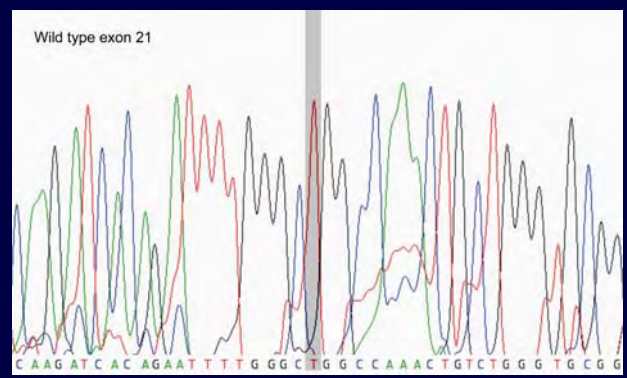




DNA extraction and linear amplification



Specific DNA amplification & sequencing



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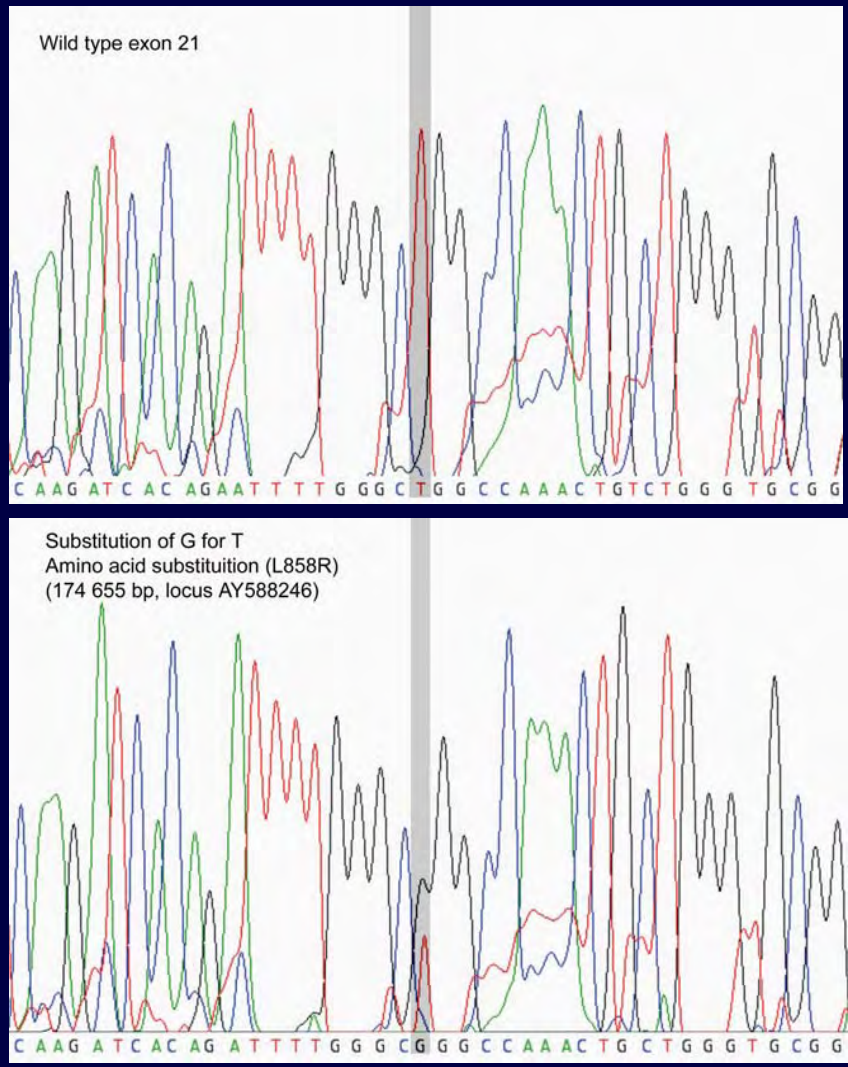
EGFR alterations in gefitinib NSCLC partial responder

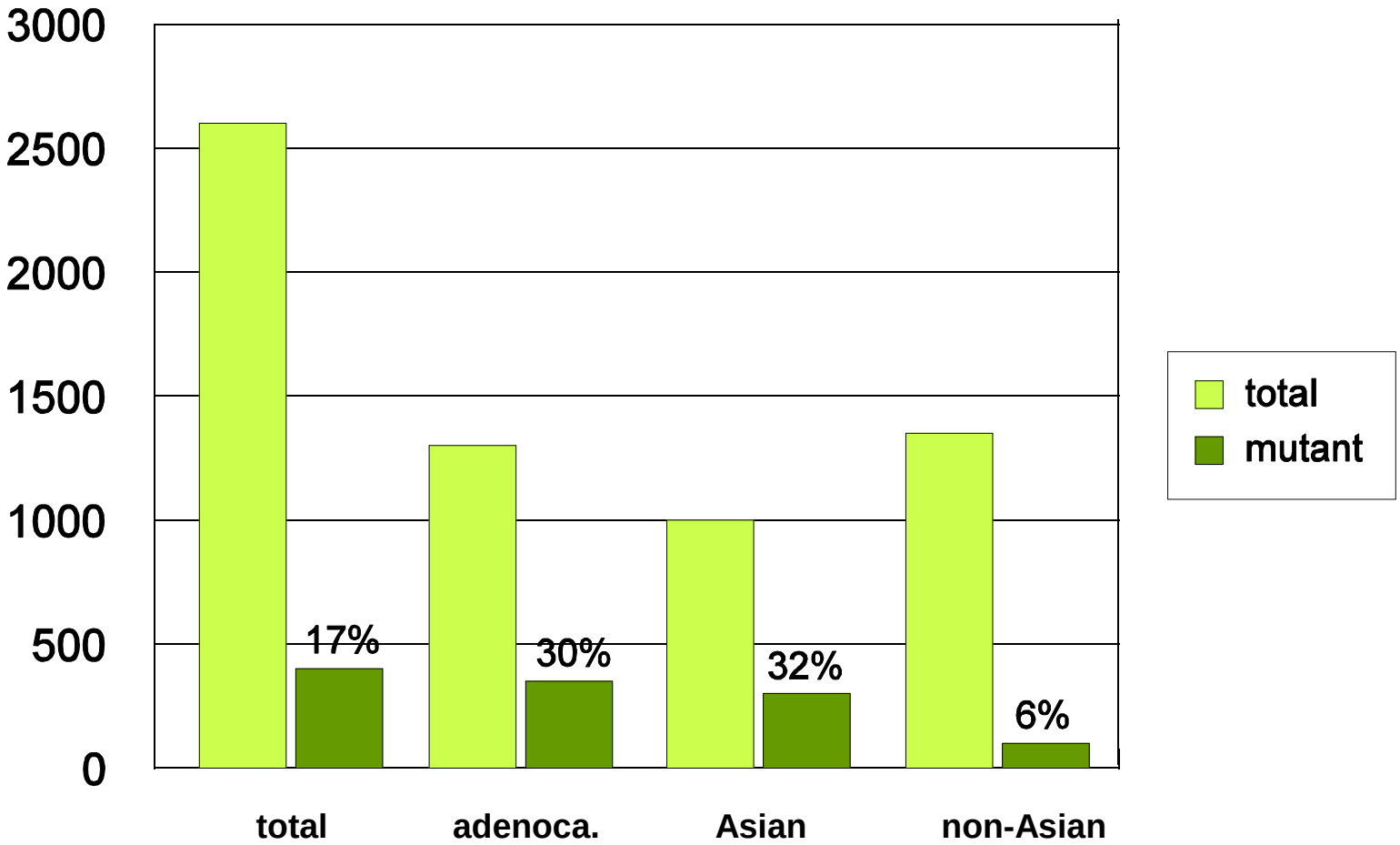
Exon 21



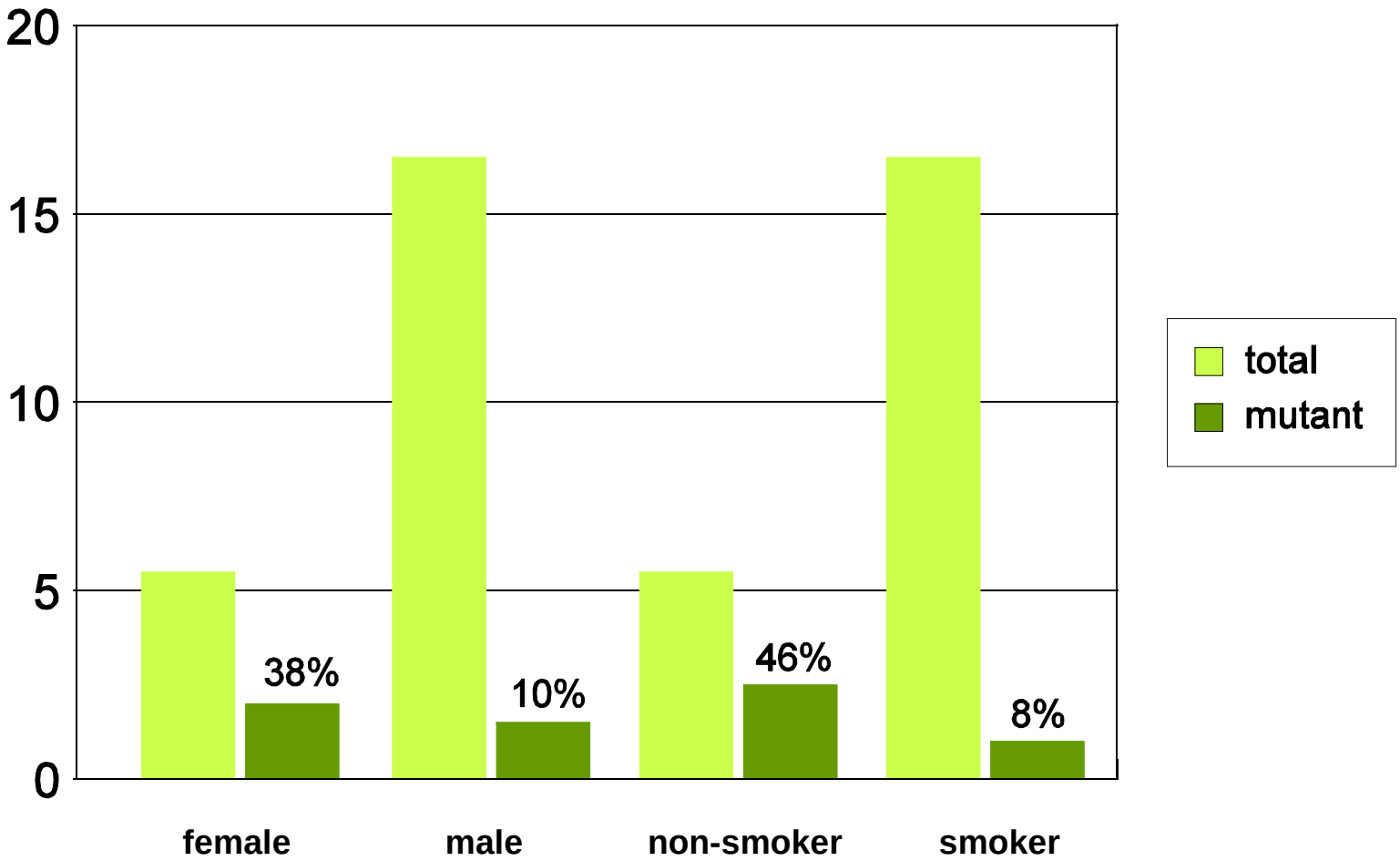
EGFR mutations in resected NSCLC patients

Author	# patients	Mutations		Race	Comments
		Total	adenoca		
Huang 04	101	39 (39%)	38 / 69 (55%)	Chinese	No difference in smoking history
Kosaka 04	277	111 (40%)	110 / 224 (49%)	Japanese	More mutations in female, adenoca, never-smokers; reciprocal exclusion with K-ras
Tokumo 05	120	38 (32%)	37 / 82 (45%)	Japanese	More mutations in female, adenoca, never-smokers
Qin 05	41	10 (24%)	7 / 17 (41%)	Chinese	More mutations in adenoca
Soung 05	153	30 (20%)	25 / 69 (38%)	Korean	More in adenoca and never smokers; reciprocal exclusion with K-ras
Marchetti 05	860	39 (4.5%)	39 / 375 (10%)	Italian	No mutations in squamous and large cell carcinomas; reciprocal exclusion with K-ras
Stephens 04	120	2 (2%)	2 / 51 (4%)	English	
Weber 05	60	2 (3%)	NR	Caucasians	
Shigematsu 05	617	130 (21%)	114 / 289 (40%)	Mixed, including East Asians	More mutations in female, adenoca, never-smokers and East Asians; reciprocal exclusion with K-ras
Yan 05	219	26 (12%)	25 / 16 (5%)	Caucasians and African Americans	More mutations in female, adenoca, never-smokers and Caucasians

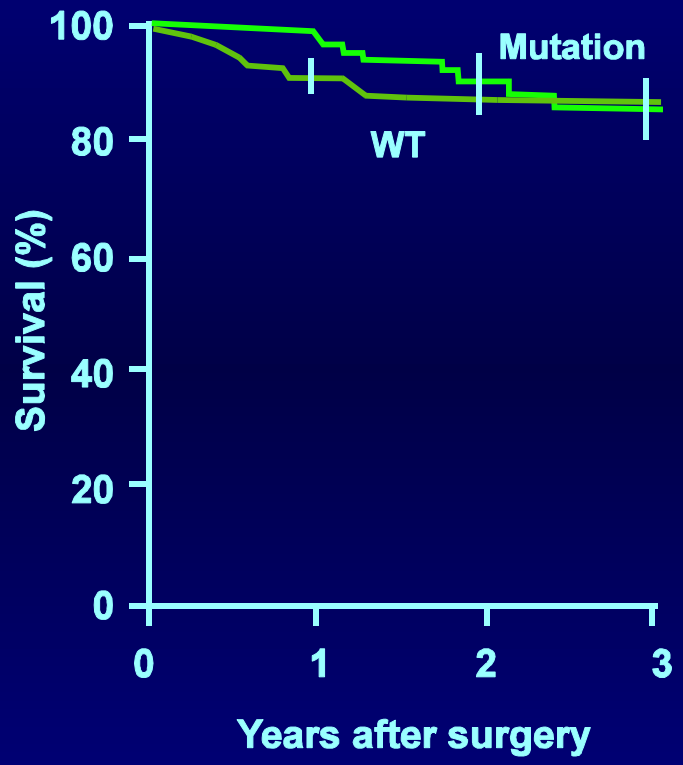
10 studies, 2568 resected NSCLC

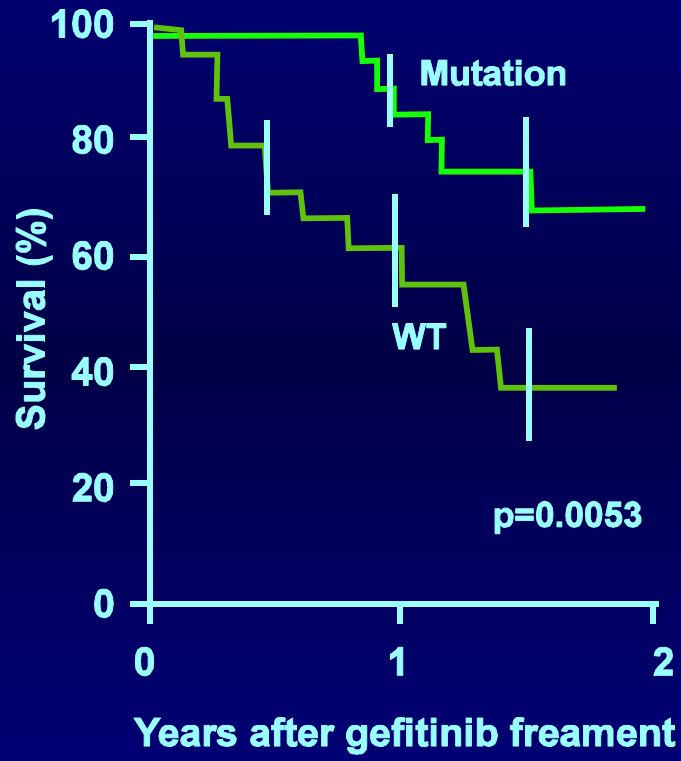


8 studies, 2295 resected NSCLC



Effect of HER1/EGFR mutations on survival from surgery and from gefitinib



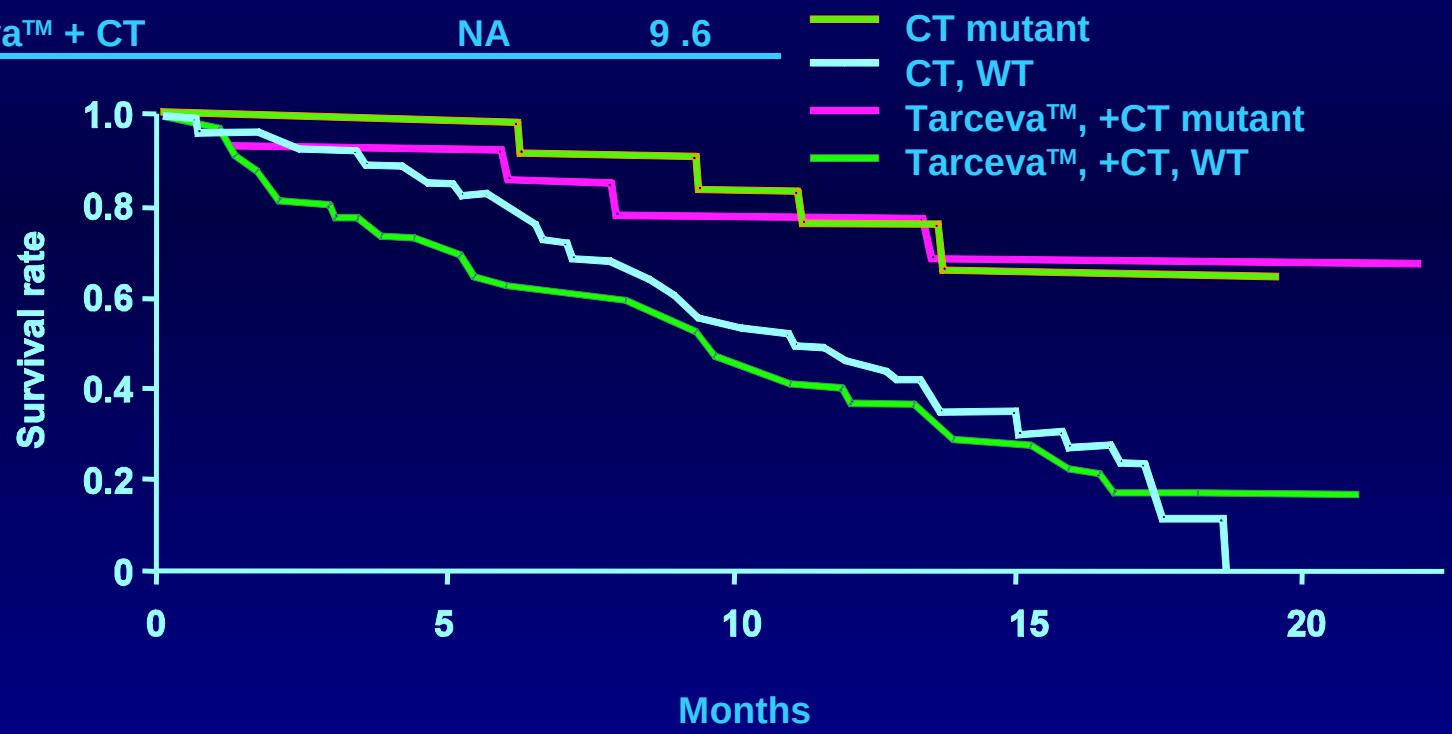


Number of patients at risk				
Mutation	91	87	47	4
WT	145	129	71	9

Number of patients at risk			
Mutation	33	25	16
WT	26	15	14

TRIBUTE: overall survival by treatment and HER1/EGFR TK mutations status

	Median overall survival	
	TK mutation	WT
Chemotherapy (CT) alone	NA	11.7
Tarceva™ + CT	NA	9 .6

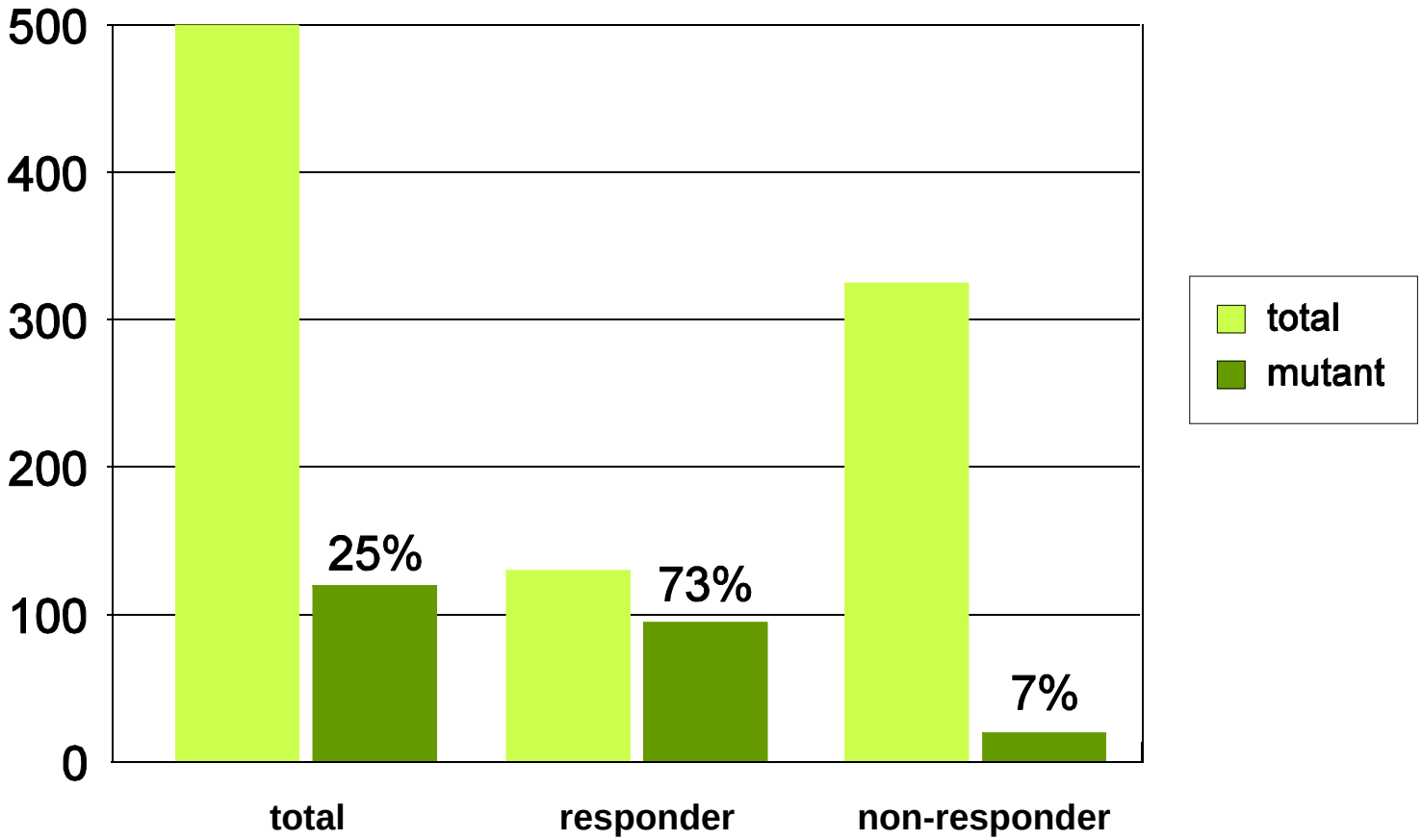


Eberhart et al., EORTC-NCI-AACR, 2005

Patients treated with EGFR TKIs and EGFR mutations

Author	EGFR inhibitor	# patient	Series	Mutants / Responders	Mutants / Non-responders	Race
Lynch 04	gefitinib	16	selected	8 / 9	0 / 7	Caucasians
Paez 04	gefitinib	9	selected	5 / 5	0 / 4	Caucasians
Pao 05	gefitinib	24	selected	9 / 12	0 / 12	Caucasians
Pao 05	gefitinib	36	selected	8 / 10	0 / 26	Caucasians
Amann 05	gefitinib	3	selected	3 / 3	0	Caucasians
Huang 04	gefitinib	16	consecutive	7 / 9	1 / 7	Chinese
Han 05	gefitinib	90	consecutive	11 / 21	6 / 69	Korean
Mitsudomi 05	gefitinib	59	consecutive	24 / 26	5 / 24	Japanese
Kim 05	gefitinib	27	consecutive	6 / 8	0 / 19	Korean
Tokumo 05	gefitinib	21	consecutive	8 / 10	1 / 11	Japanese
Cortes-Funes 05	gefitinib	83	consecutive	6 /10	4 / 66	Caucasians
Cappuzzo 05	gefitinib	89	consecutive	8 / 15	7 / 74	Caucasians
Giaccone 05	erlotinib	26	consecutive	1 / 5	0 / 22	Caucasians

EGFR mutations and response to EGFR TKIs; 13 reports, 499 patients



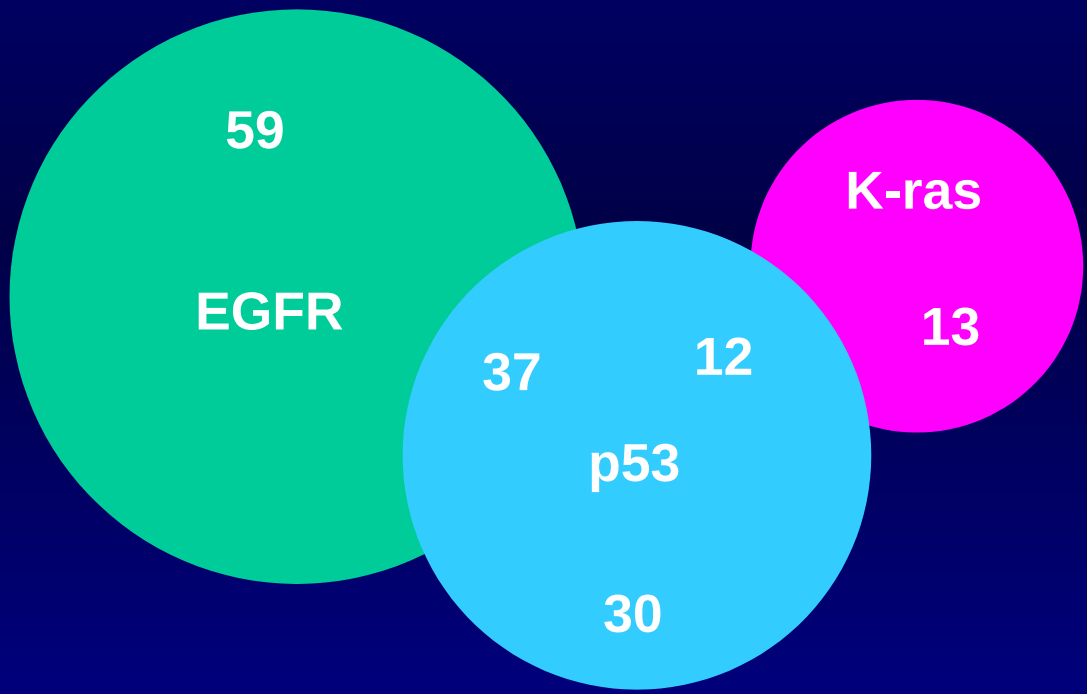
Response to EGFR TKIs and K-ras mutations

Author	EGFR TKI	K-ras mutants / responders	K-ras mutants / non-responders
Pao et al. 2005	Gefitinib or Erlotinib	0 /21	9 / 38
Mitsudomi et al. 2005	Gefitinib	0 / 2	5 / 18
Giaccone et al. 2005	Erlotinib	0 / 4	8 / 22

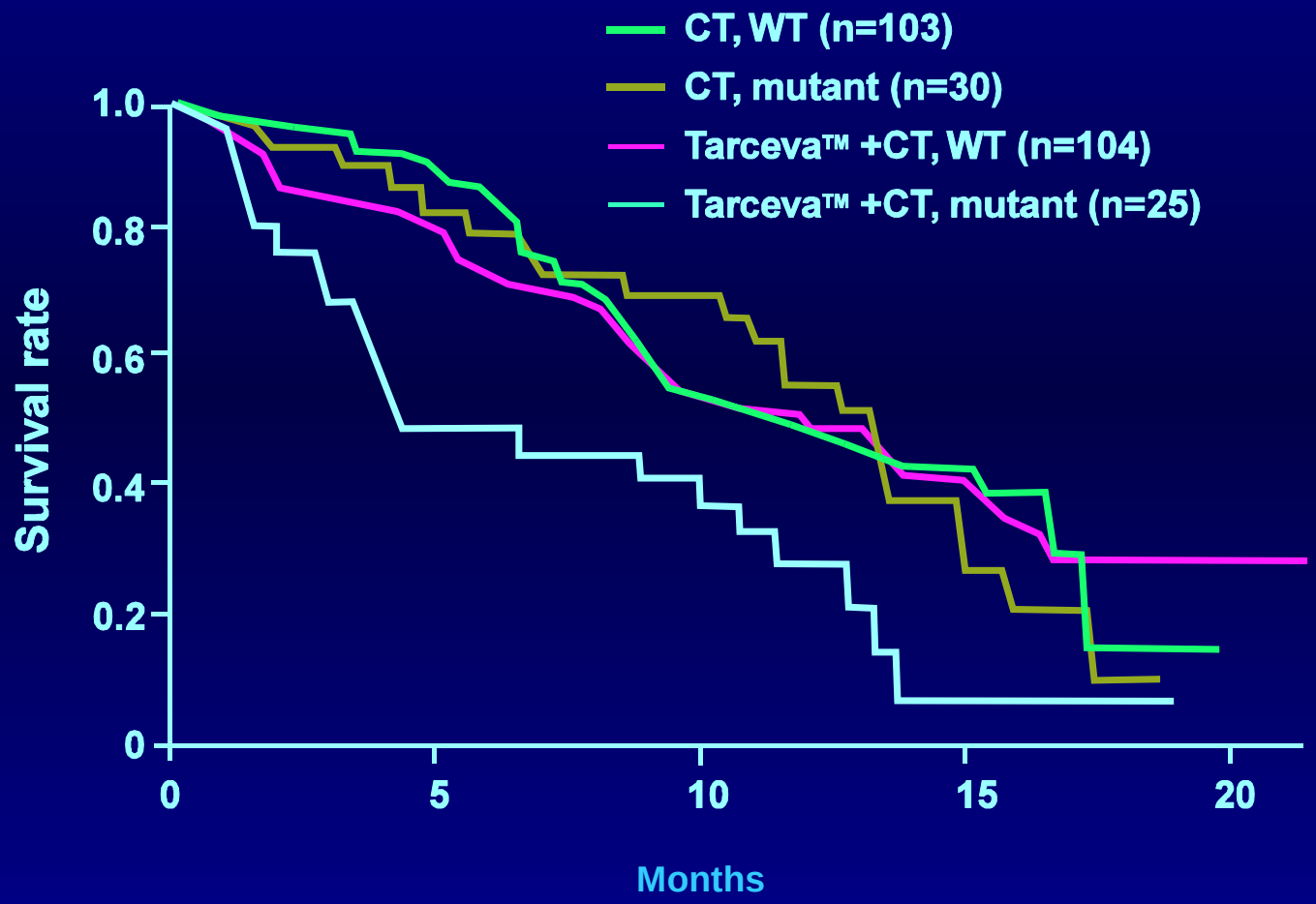
HER1/EGFR gene mutations in resected NSCLC

n=192

41 no mutations



Overall survival by treatment versus K-ras mutation status (TRIBUTE)



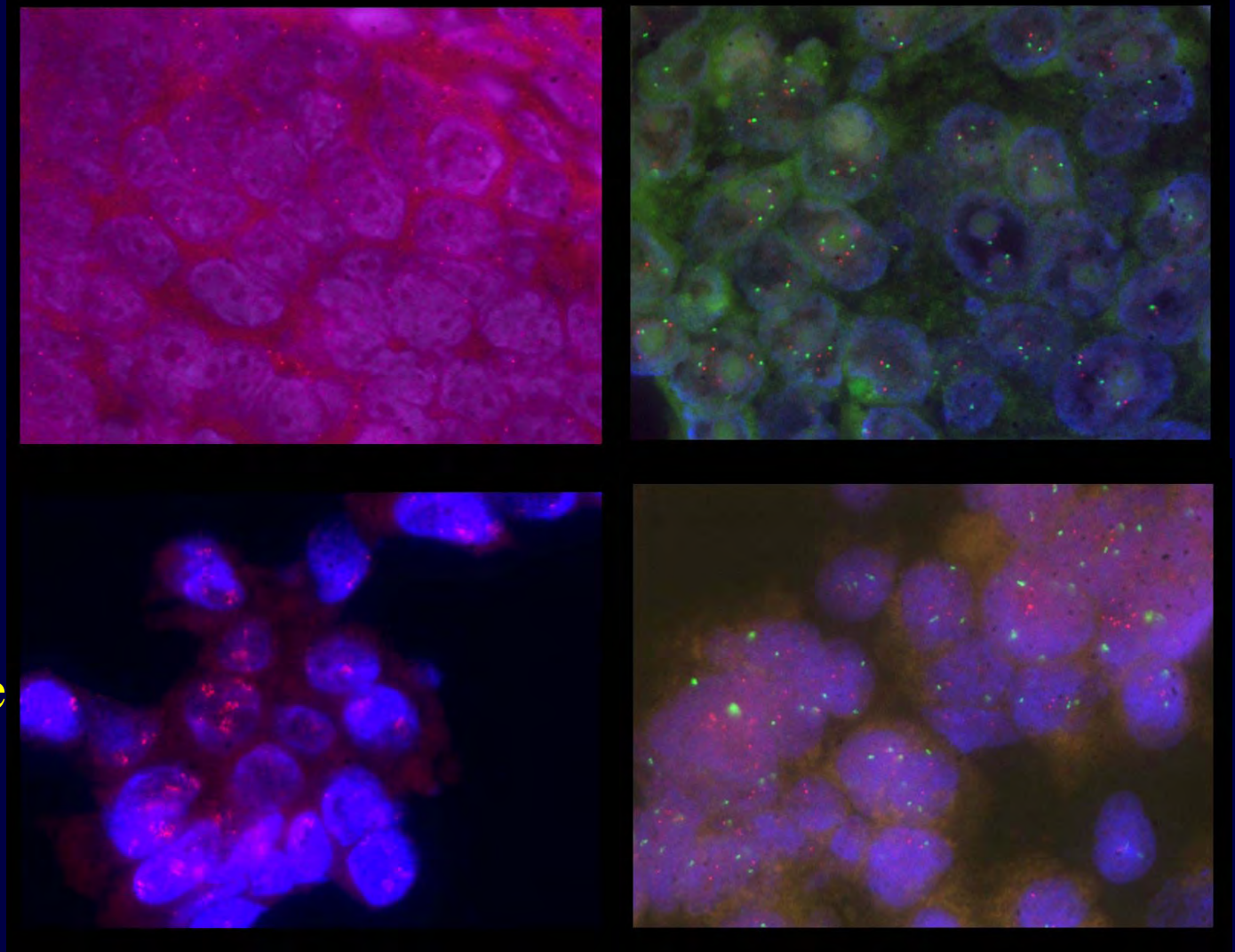
LABORATORY OF EXPERIMENTAL MEDICINE

HER1/2 status in gefitinib NSCLC (non)responders

EGFR1 (HER1) HER2/neu

diploidní

amplifikace



Combination of gene copy number (FISH), protein expression (IHC) and mutation analyses and outcome to gefitinib therapy

Combinations	Number of Patients	Response Rate (N/%)	Disease Control Rate (N/%)	Median Time to Progression (months)	Median Survival (months)
FISH+/IHC+	22	10/45.5	17/77.3	9.1	20.8
FISH+/IHC-	11	2/18.2	5/45.5	2.9	8.3
FISH-/IHC+	36	2/5.6	15/41.7	3.2	10.9
FISH-/IHC-	29	0	3/10.3	2.3	4.5
P (any positive versus negative/negative)		*0.009*	*<0.001*	*<0.001*	*0.015*
FISH+/Mutation +	9	7/77.7	7/77.7	15.6	20.8
FISH+/Mutation-	18	4/22.2	10/55.5	5.3	9.3
FISH-/Mutation+	6	1/16.6	2/33.3	2.1	3.2
FISH-/Mutation-	56	0	14/25.0	2.4	7.3
P (any positive versus negative/negative)		*<0.001*	*0.002*	*0.001*	*0.1*
IHC+/Mutation+	11	7/63.6	8/72.7	13.5	20.8
IHC+/Mutation-	37	3/8.1	17/45.9	3.6	11.4
IHC-/Mutation+	4	1/25.0	1/25.0	2.1	2.9
IHC-/Mutation-	33	1/3.0	7/21.2	2.3	5.3
P (any positive versus negative/negative)		*0.02*	*0.008*	*0.003*	*0.02*

Cappuzzo F., et al. JNCI, 2005

Molecular Analysis of the Epidermal Growth Factor Receptor (EGFR) Gene and protein expression in Patients Treated with Erlotinib in National Cancer Institute of Canada Clinical Trials Group (NCIC CTG) BR.21

M. S. Tsao, A. Sakurada, I. Lorimer, J. Cutz, S. Kamel-Reid, J. Squire, K. Ding, F. Richardson, L. Seymour, F. Shepherd

Ontario Cancer Institute/Princess Margaret Hospital

Ottawa Health Research Institute

OSI Pharmaceuticals

University of Toronto & Queen's University

BR.21 Summary of Significant Clinical Predictors of Response

		Erlotinib (N = 427)	P value*
Gender	Female (146)	14.4 %	0.006
	Male (281)	6.1 %	
Histology	Adeno (209)	13.9 %	<0.001
	Other (218)	4.1 %	
Ethnicity	Asian (53)	18.9 %	0.02
	Other (374)	7.5 %	
Ever smoked	Yes (311)	3.8 %	<0.001
	No (93)	24.7 %	
	UNK (23)	13 %	

*** Significance between subgroup**

BR.21 Summary of Treatment Effect: Survival

		Hazard ratio	CI	P*
Gender	Male (475)	0.8	0.6-0.9	0.76
	Female (256)	0.8	0.6-1.1	
Histology	Adeno (365)	0.7	0.6-0.9	0.37
	Other	0.8	0.6-1.0	
Ethnicity	Asian (91)	0.6	0.4-1.0	0.44
	Other (640)	0.8	0.7-0.9	
Smoking History	Ever (545)	0.9	0.7-1.0	0.02
	Never (146)	0.4	0.3-0.6	
	Unknown (40)	1.1	0.5-2.6	

***P value for interaction between erlotinib and clinical variables**

EGFR Mutational Analysis of the TK Domain

	Number of Patients
In the entire trial	731
Suitable for analysis	197

Mutations Found

Ex19 746-752 deletions	13 (29%)
Ex21- L858R mutation	8 (18%)
Other mutations	24 (53%)

Patient Characteristics Relating to Frequency of Mutations

	No. of pts	Percent with mutation	P value*
Adeno	95	28 %	0.05
Non-adeno	82	16 %	
Female	66	24 %	NS
Male	111	22 %	
Never smoked**	42	31 %	NS
Ever smoked**	131	21 %	
Asian	12	50 %	0.03
Other	165	21 %	

***Significance between subgroup**
****Smoking status unknown in 4**

Response* to Erlotinib Therapy for Patients with Mutation Results

	No. of pts	No. evaluable for response	Overall response rate (95% C.I.)	P value
Wild type	137	81	7.4% (3.5%, 15.4%)	0.37
Mutation	40	19	15.8% (6.1%, 39.6%)	

*** Assessed by RECIST**

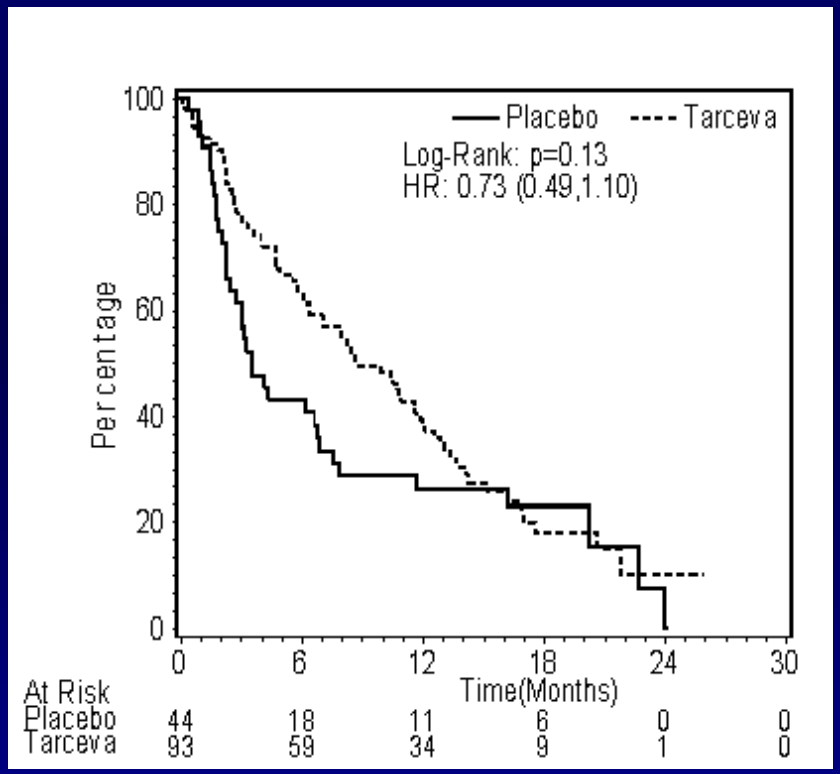
Survival Benefit from Erlotinib in Patients with Mutation Results

	No. of pts	HR	CI	P*	P**
Wild type	137	0.73	0.49-1.10	0.13	0.97
Mutant	40	0.77	0.40-1.50	0.45	

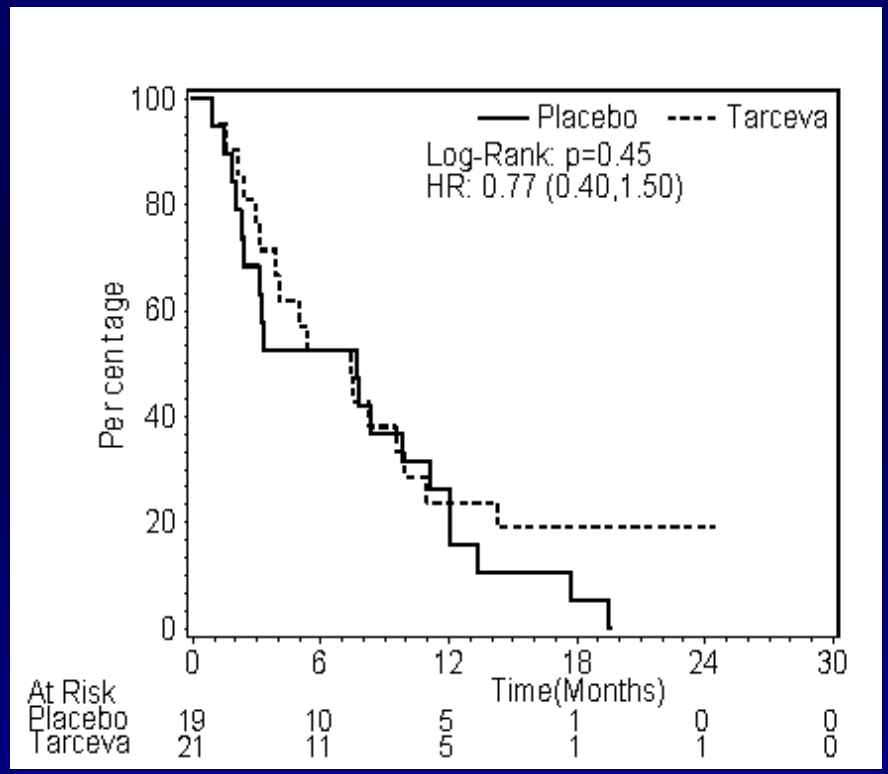
***P value for subgroup compared to placebo**
****P value for interaction**

Survivals According to EGFR Genotype

NO MUTATION



+ MUTATION



P value for interaction= 0.97

Are Other EGFR Parameters Prognostic?

- EGFR protein expression assessed by immunohistochemistry (IHC)
- EGFR gene copy number assessed by fluorescence in situ hybridization (FISH)

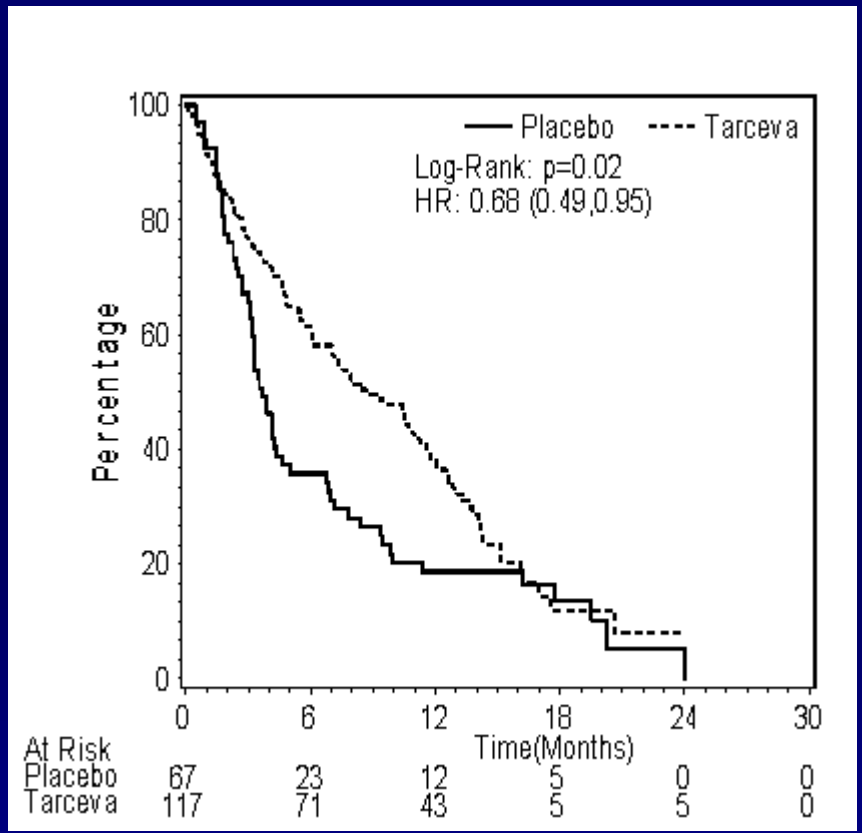
Response* to Erlotinib Therapy for Patients with EGFR IHC

	No. of pts	No. evaluable for response	Overall response rate(95% C.I.)	P value
All pts	731	427	8.9% (6.6%, 12.0%)	
IHC +	184	106	11.3% (6.7, 18.9%)	0.1
IHC -	141	80	3.8% (1.4%, 10.6%)	

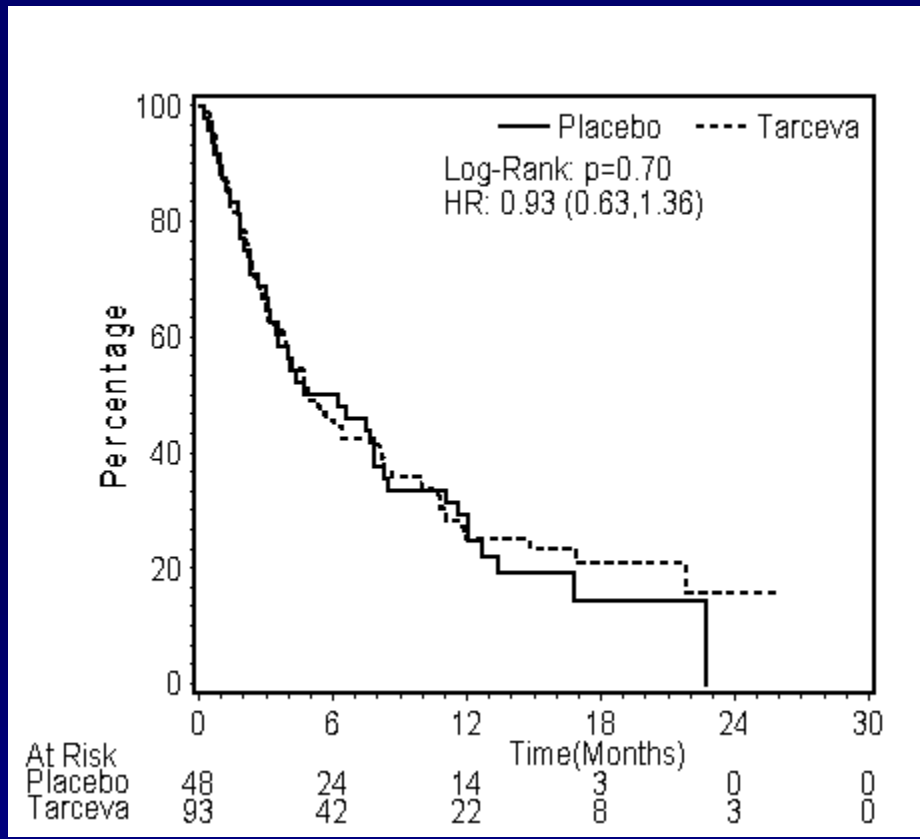
* Assessed by RECIST

Survival According to EGFR Protein Expression

EGFR +



EGFR -



P value for interaction = 0.25

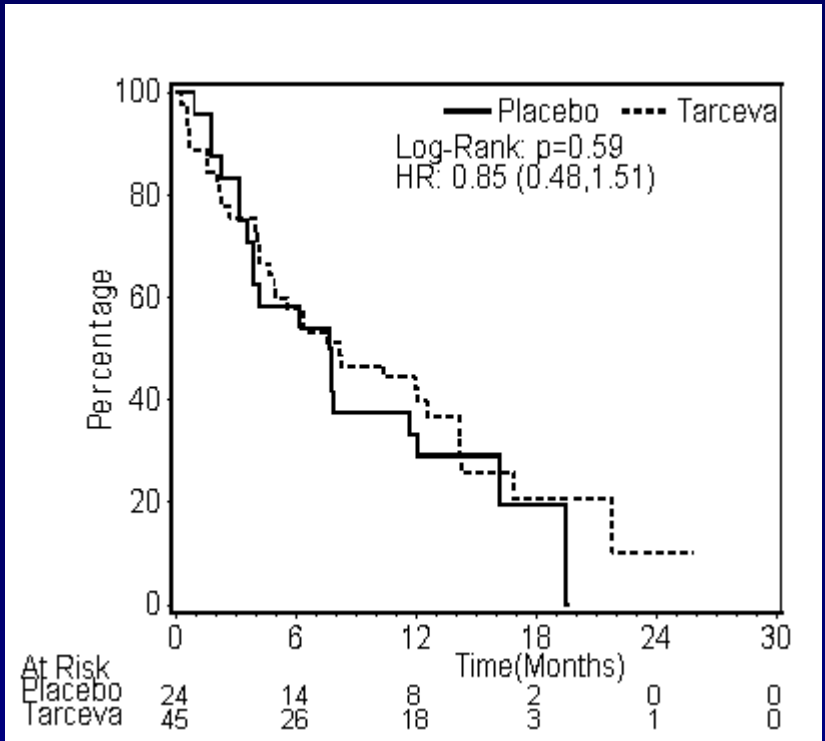
Response* to Erlotinib Therapy for Patients with EGFR FISH Results

	No. of pts	No. evaluable for response	Overall response rate(95% C.I.)	P value
Low copy	69	41	2.4% (0.6%, 12.9%)	0.03
High copy	56	25	20% (9.4%, 40.7%)	

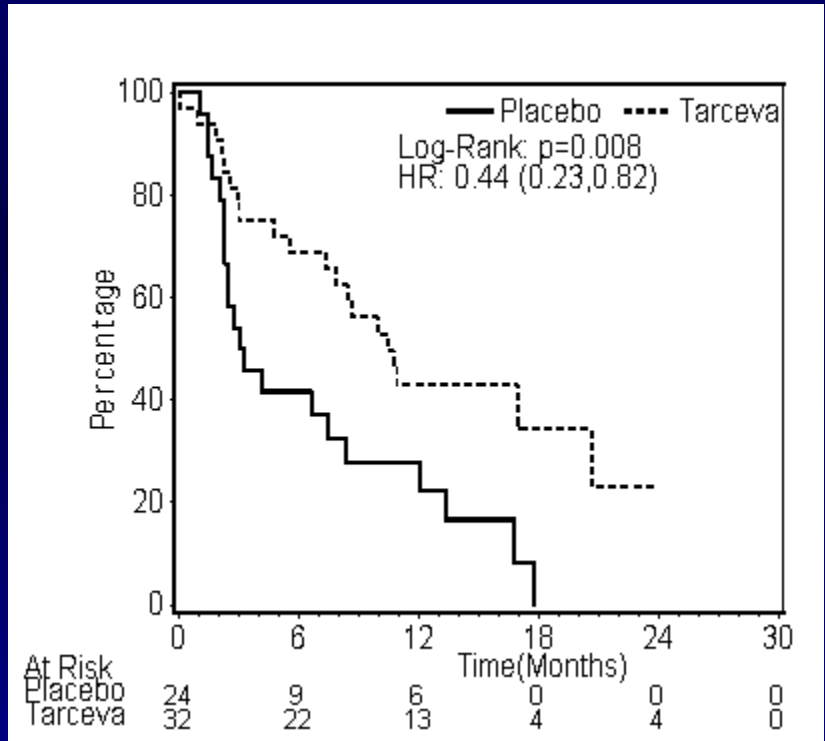
*** Assessed by RECIST**

Survival according to EGFR gene copy number

Low copy



High copy



P value for interaction = 0.10

Conclusions

- Response rate to EGFR1 inhibitors was higher in patients with mutations, IHC+ tumors & high gene copy number.
- Mutations better predict response than survival benefit (10-fold higher sensitivity of EGFR1 mutated tumors to EGFR1 inhibitors).
- Mutations better predict response to gefitinib (dose?).
- EGFR1 copy numbers with/without IHC predict response and possible survival benefit for both gefitinib and erlotinib.
- K-ras mutations are negative predictors for response (exon 12).
- EGFR1 mutations are unique for NSCLC.
- Prediction of therapeutic benefit for EGFR1 inhibitors in NSCLC is comparable to trastuzumab in metastatis breast cancer.
- EGFR1 amplification predicts also response to cetuximab (ASCO2005).