Ruolo del K-ras come fattore predittivo di Risposta ad anticorpi anti-EGFR nel carcinoma del colon retto

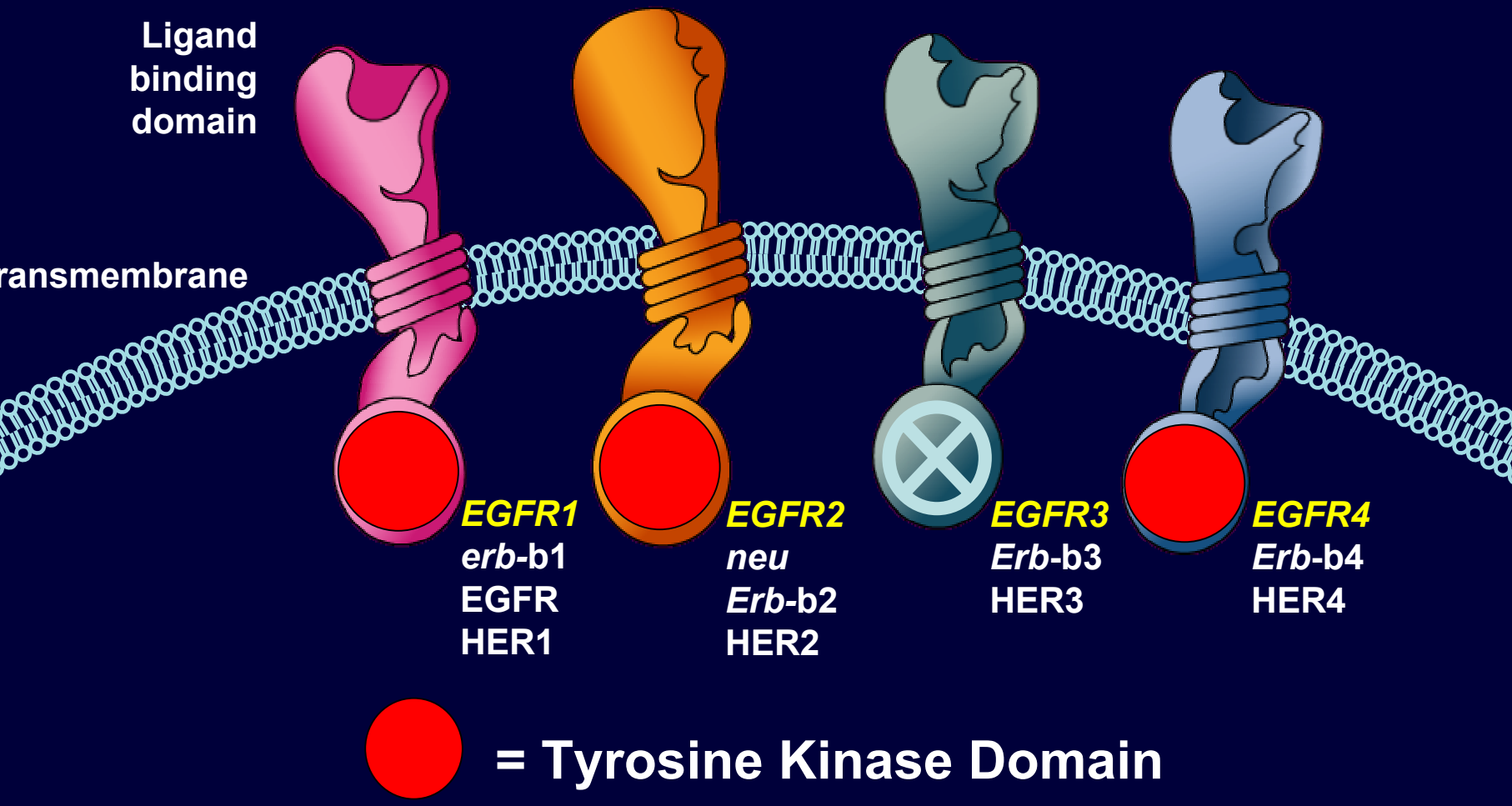
Antonio Marchetti

Pathology and Oncogenetic Unit,

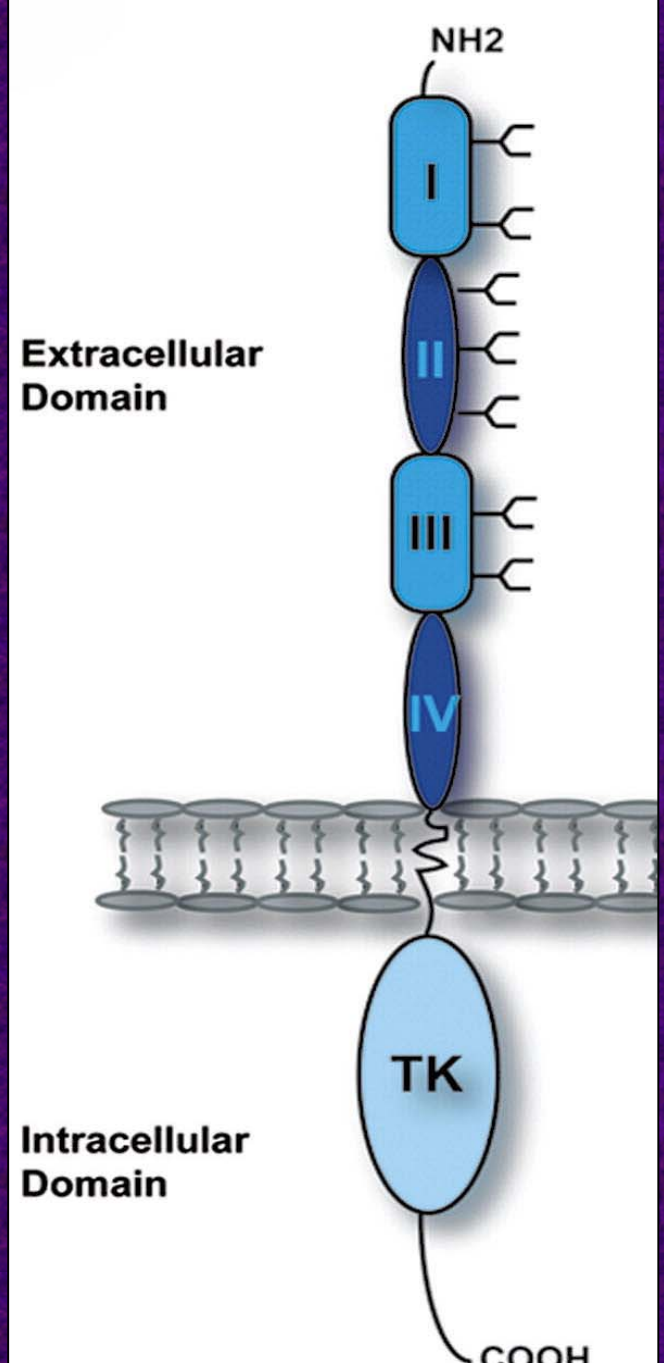
Center of Excellence on Aging

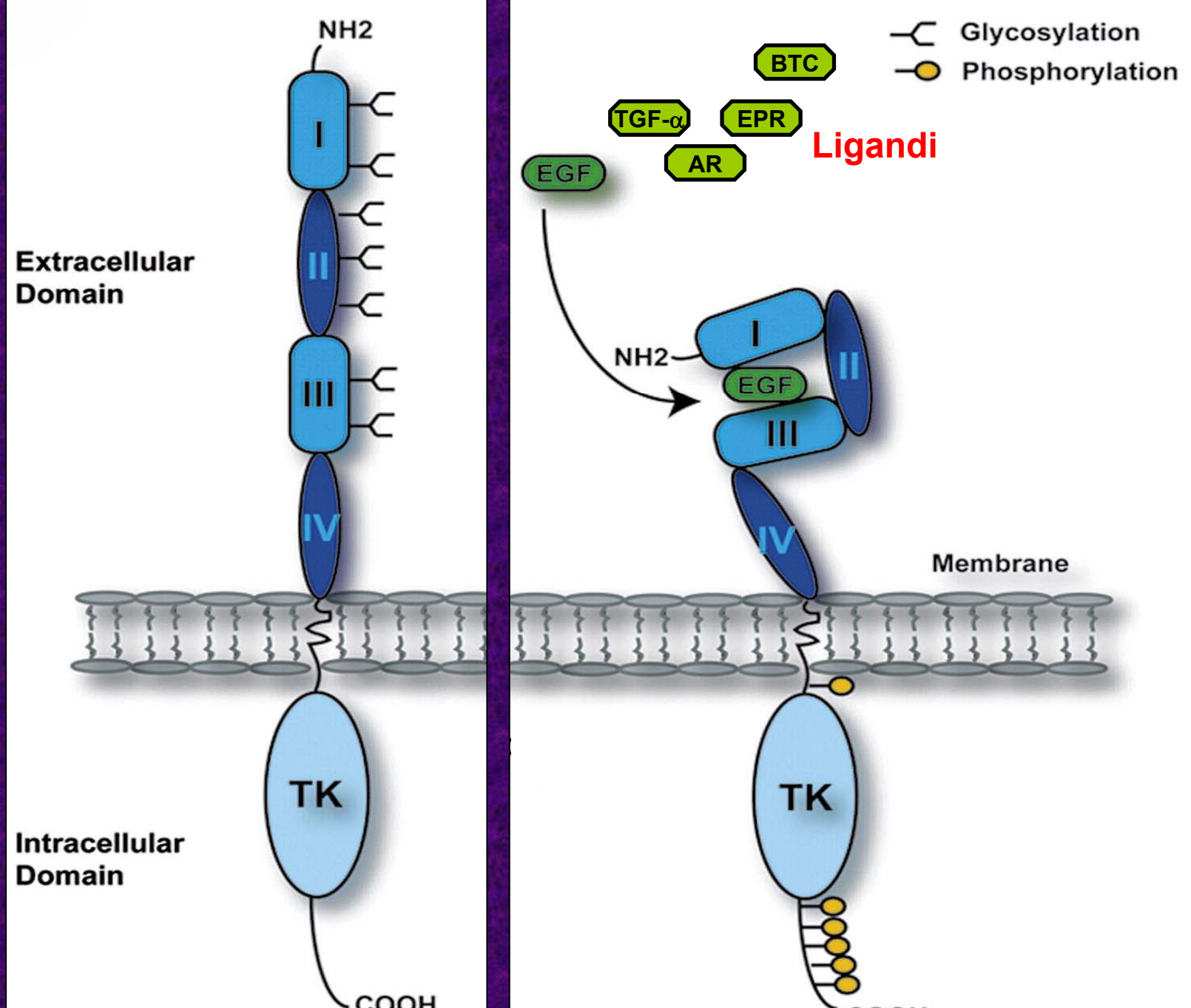
University-Foundation "G D'Annunzio", Chieti

The EGFR/HER Family

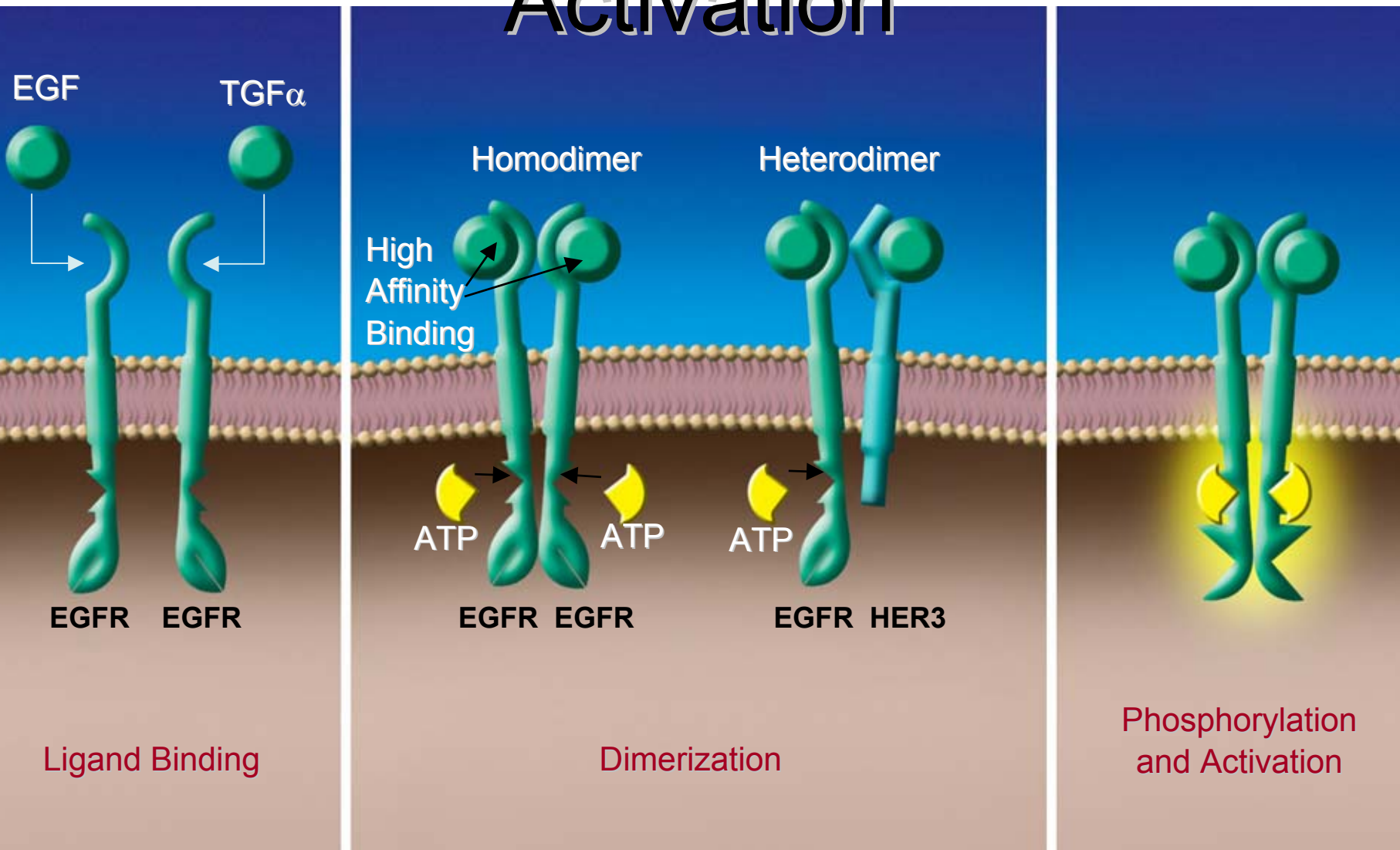


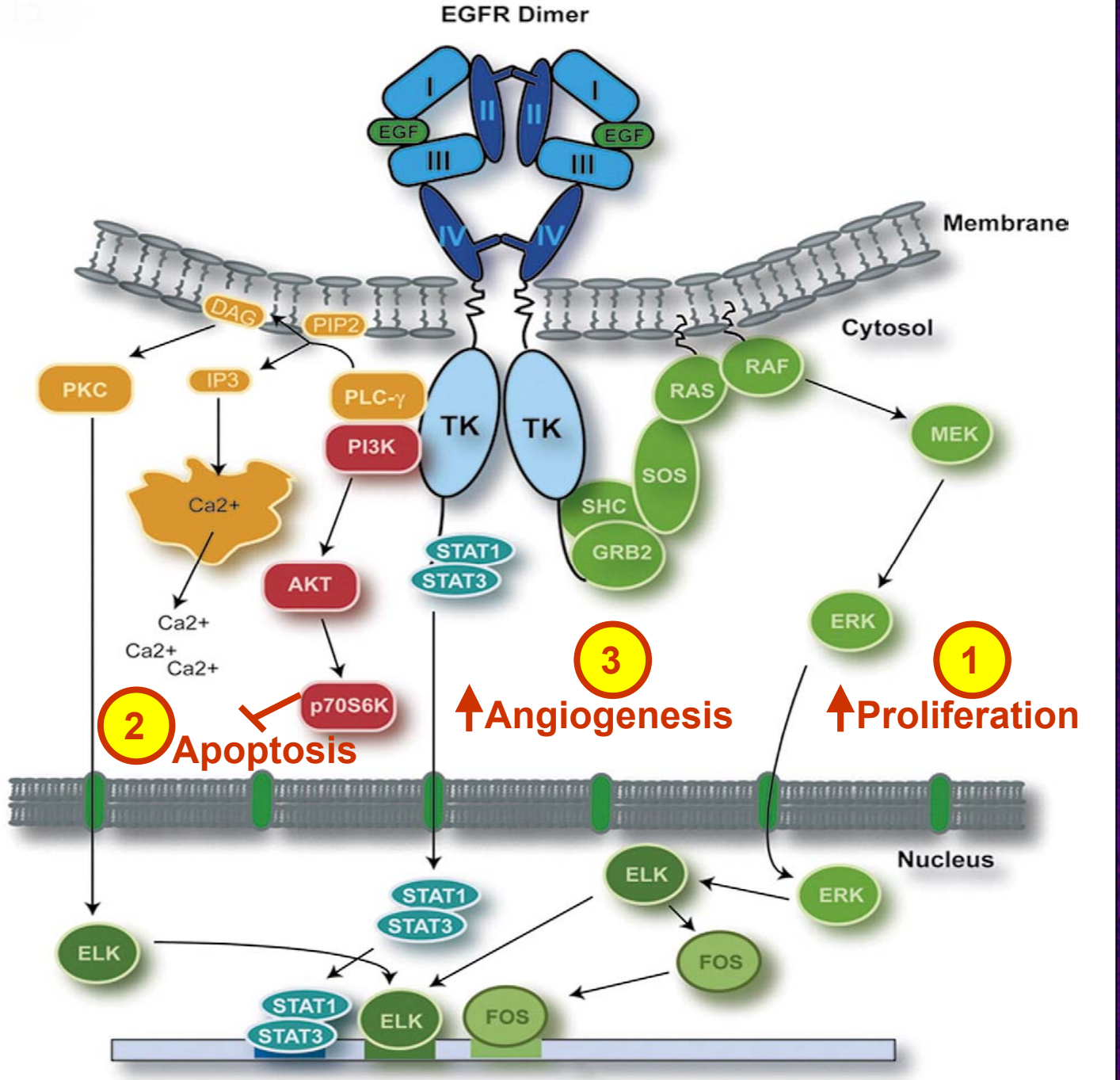
EGFR-1

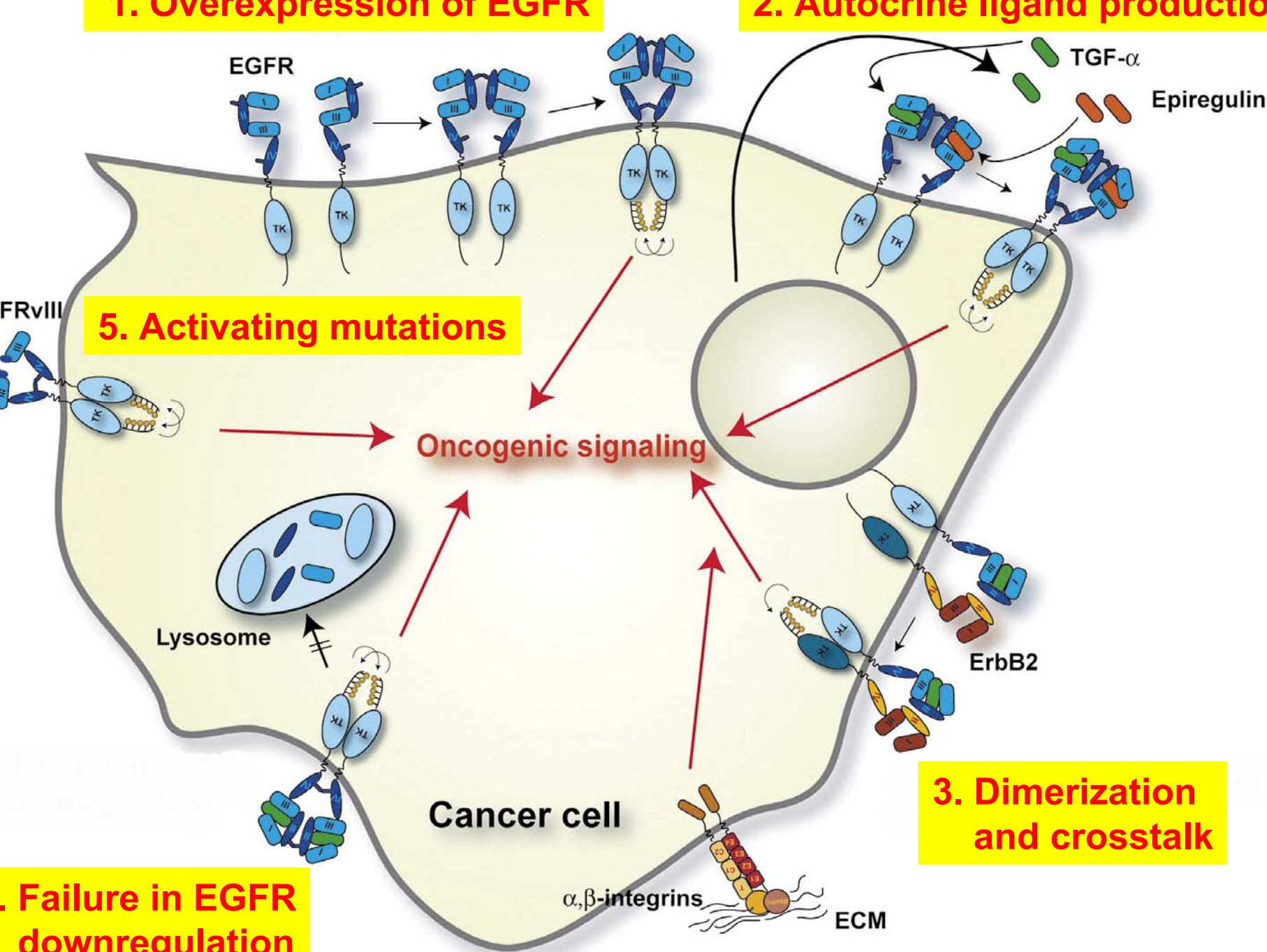

NH2
I
II
III
IV
Extracellular
Domain
TK
Intracellular
Domain
COOH


NH2
I
II
III
IV
Extracellular Domain
TK
Intracellular Domain
COOH
Glycosylation
Phosphorylation
BTC
TGF-α
EPR
AR
Ligandi
EGF
NH2
I
II
EGF
III
IV
Membrane
TK

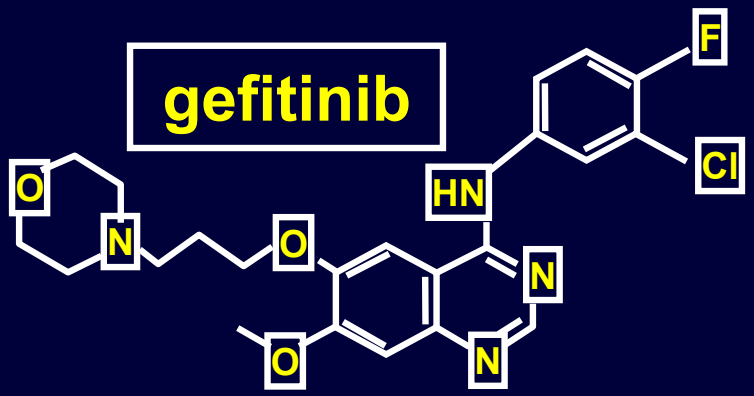
Ligand Binding and Dimerization Result in TK Activation

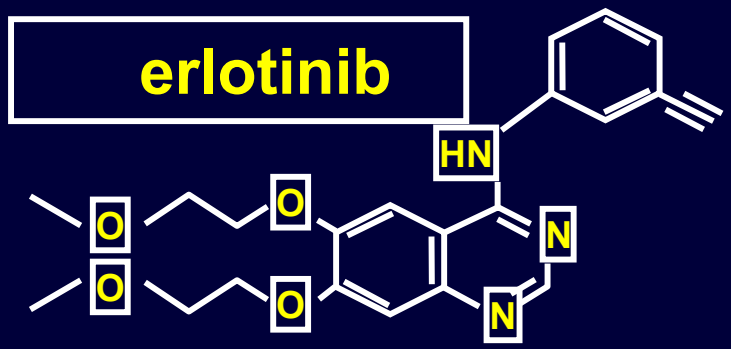



EGFR Dimer
I
II
III
IV
EGF
Membrane
Cytosol
DAG
PIP2
PKC
IP3
PLC-γ
PI3K
TK
RAS
RAF
MEK
SOS
SHC
GRB2
Ca2+
AKT
STAT1
STAT3
ERK
p70S6K
1
Proliferation
2
Apoptosis
3
Angiogenesis
Nucleus
ELK
FOS

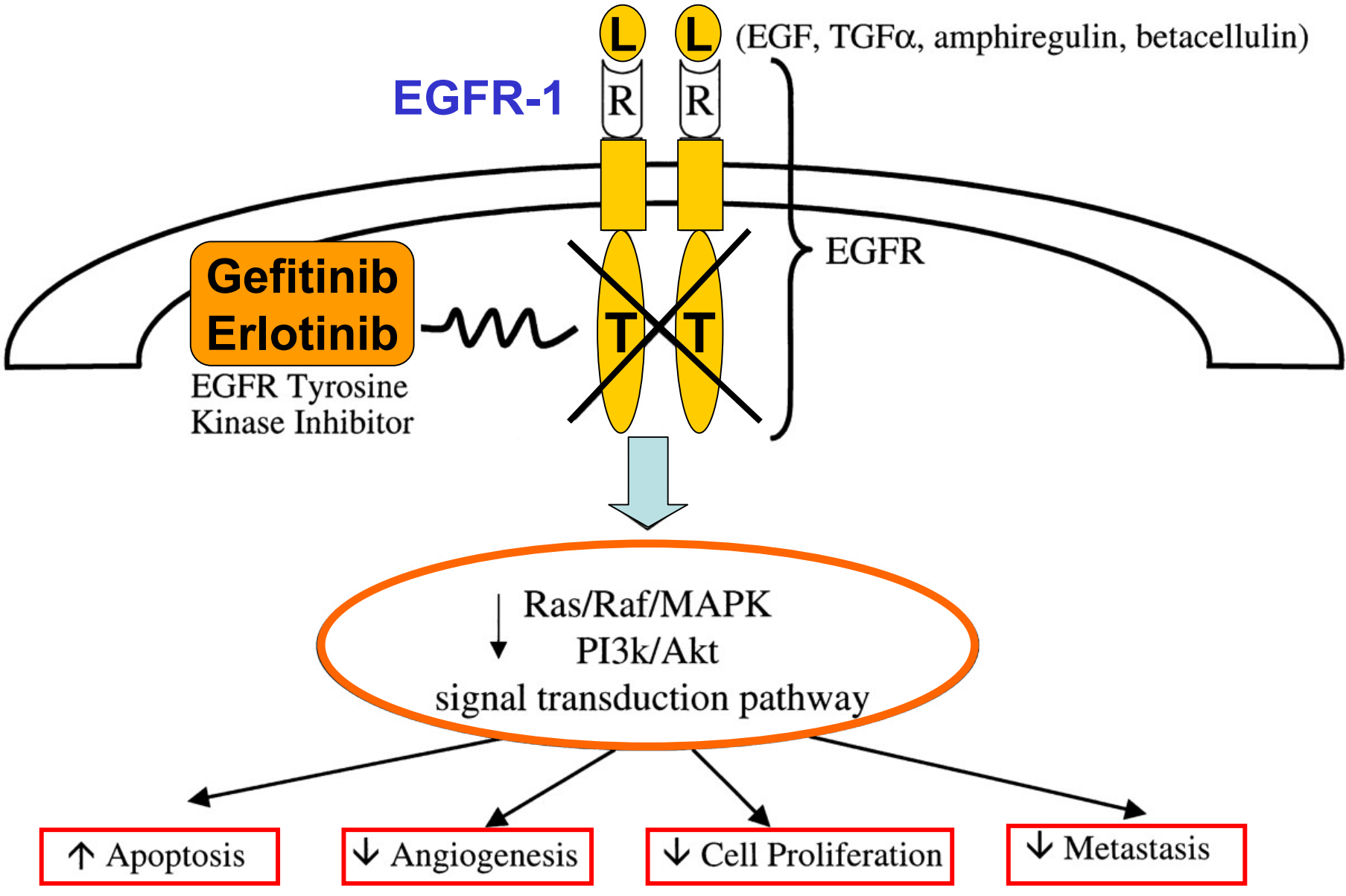

1. Overexpression of EGFR
2. Autocrine ligand productio
EGFR
TGF-α
Epiregulin
TK
FRvIII
5. Activating mutations
Oncogenic signaling
Lysosome
ErbB2
3. Dimerization and crosstalk
Cancer cell
α,β-integrins
ECM
. Failure in EGFR downregulation

EGFR Selective Small Molecule Tyrosine Kinase Inhibitors



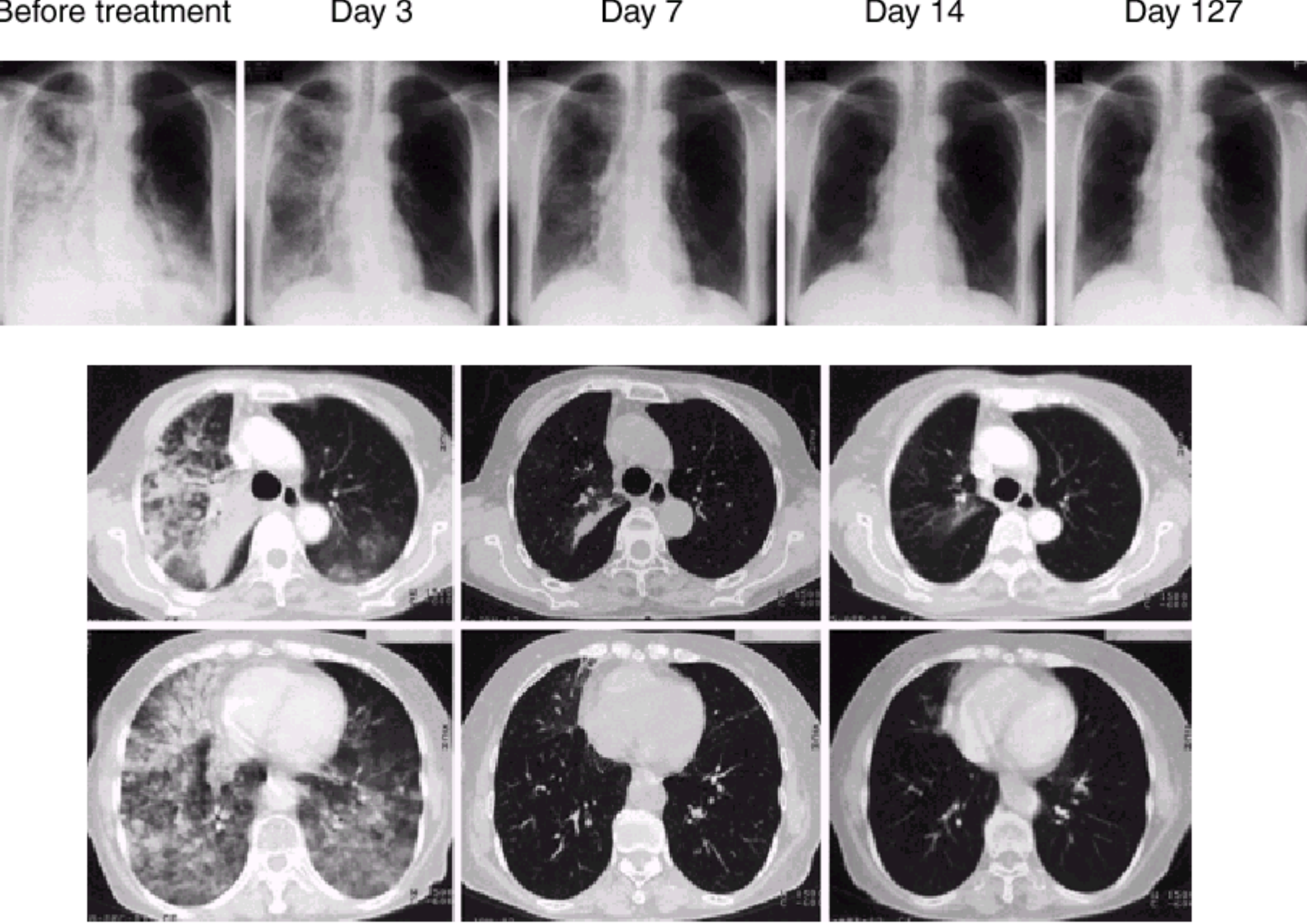


- The tyrosine kinase activity requires ATP
- gefitinib and erlotinib compete for ATP binding
- Orally bioavailable small molecules


L
L
(EGF, TGFα, amphiregulin, betacellulin)
EGFR-1
R
R
EGFR
Gefitinib
Erlotinib
EGFR Tyrosine
Kinase Inhibitor
T
T
Ras/Raf/MAPK
PI3k/Akt
signal transduction pathway
↑ Apoptosis
↓ Angiogenesis
↓ Cell Proliferation
↓ Metastasis

… questi inibitori del TKD, a differenza della chemioterapia classica, sono **farmaci mirati** ed hanno pertanto **minimi effetti collaterali** …

… si è cominciato ad utilizzare questi farmaci, **a scopo compassionevole** in varie forme neoplastiche in fase avanzata, incluso il ca. polmonare …


Before treatment
Day 3
Day 7
Day 14
Day 127
Before treatment
Day 28
Day 127

Phase III studies

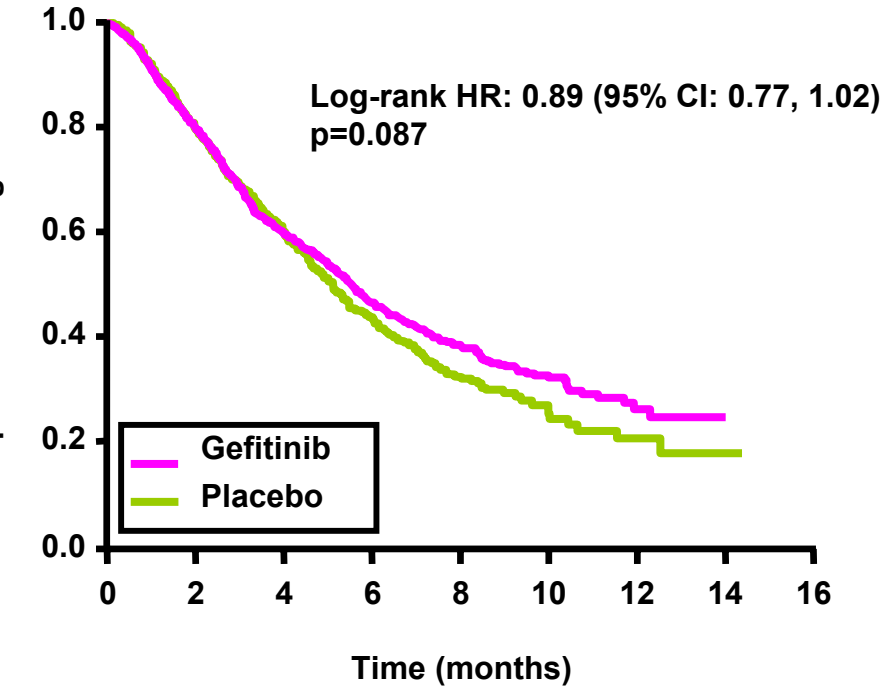
ISEL (gefitinib)

Previous treatment: platinum
Cases: 1129 gefitinib, 563 placebo
Response rate: Gefitinib: 8%, Placebo 1%.
Ov.survival: Gefitinib: 27 months, Placebo 21.

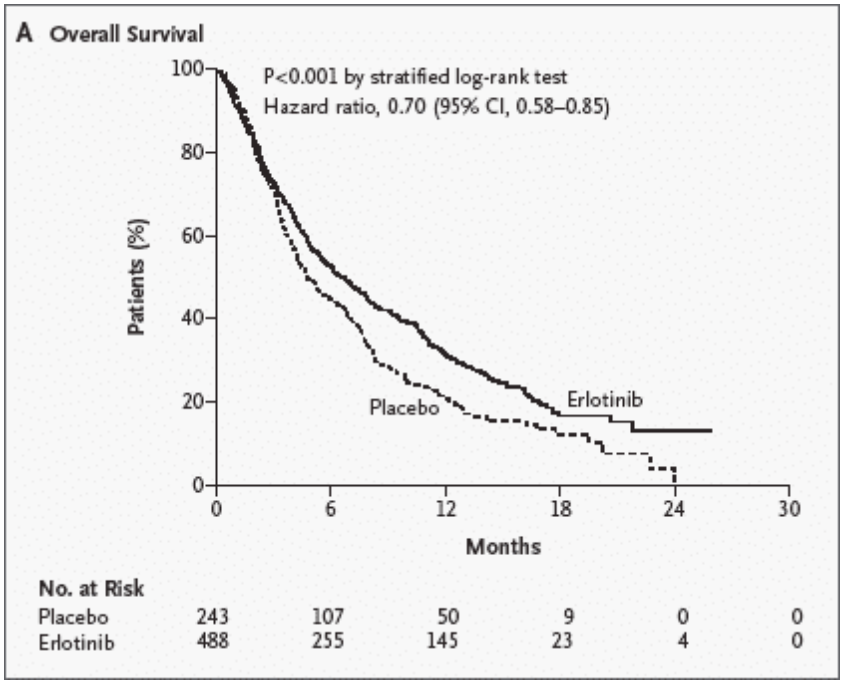
BR-21 (erlotinib)

Previous treatment: platinum
Cases: 488 Erlotinib, 243 placebo
Response rate: Gefitinib: 9%, Placebo 1%.
Ov.survival: Erlotinib: 31 months, Placebo 22.

ISEL



BR.21



Response in subgroup of patients

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 MAY 20, 2004 VOL. 350 NO. 21

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

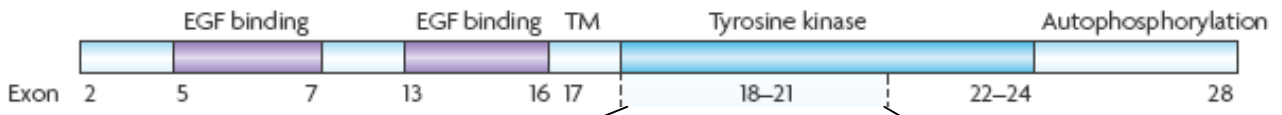
Thomas J. Lynch, M.D., Daphne W. Bell, Ph.D., Raffaella Sordella, Ph.D., Sarada Gurubhagavatula, M.D., Ross A. Okimoto, B.S., Brian W. Brannigan, B.A., Patricia L. Harris, M.S., Sara M. Haserlat, B.A., Jeffrey G. Supko, Ph.D., Frank G. Haluska, M.D., Ph.D., David N. Louis, M.D., David C. Christiani,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

SCIENCE VOL 304, 4 June 2004

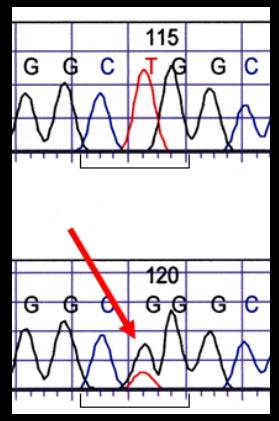
EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy

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†]

Pathology of EGFR Mutants

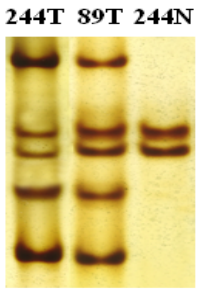


Mutations associated with drug sensitivity

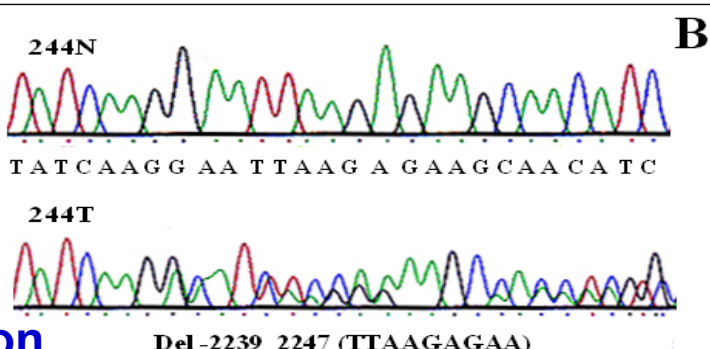


L858R

Exon 18 (nucleotide-binding loop)	Exon 19	Exon 20	Exon 21 (activation loop)
G719C **G719S** **G719A** V689M N700D E709K/Q S720P	**ΔE746-A750** ΔE746-T751 ΔE746-A750 (ins RP) ΔE746-T751 (ins A/I) ΔE746-T751 (ins VA) ΔE746-S752 (ins A/V) ΔL747-E749 (A750P) ΔL747-A750 (ins P) ΔL747-T751 ΔL747-T751 (ins P/S) ΔL747-S752 ΔL747-752 (E746V) ΔL747-752 (P753S) ΔL747-S752 (ins Q) ΔL747-P753 ΔL747-P753 (ins S) ΔS752-I759	V765A T783A	**L858R (40–45%)** N826S A839T K846R L861Q G863D
(5%)	**48%**	(<1%)	**43%**

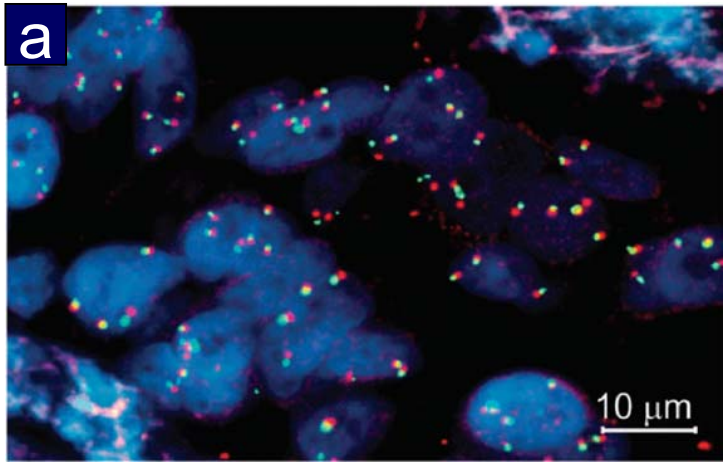


Ex 19 Deletion

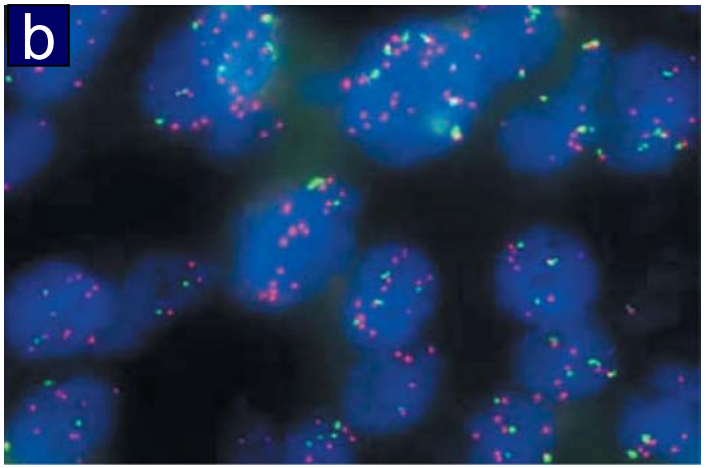


EGFR
gene copy number

FISH



High polysomy



EGFR1 gene amplification

Patient with lung ADK

Mutational analysis

Gene copy number analysis (FISH)

KRAS Mutations and Primary Resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib

William Pao[1,2*], Theresa Y. Wang[1], Gregory J. Riely[2], Vincent A. Miller[2], Qiulu Pan[3], Marc Ladanyi[3], Maureen F. Zakowski[3], Robert T. Heelan[4], Mark G. Kris[2], Harold E. Varmus[1]

Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, **2** Department of Medicine, Memo an-Kettering Cancer Center, New York, New York, United States of America, **3** Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New Yo ited States of America, **4** Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America

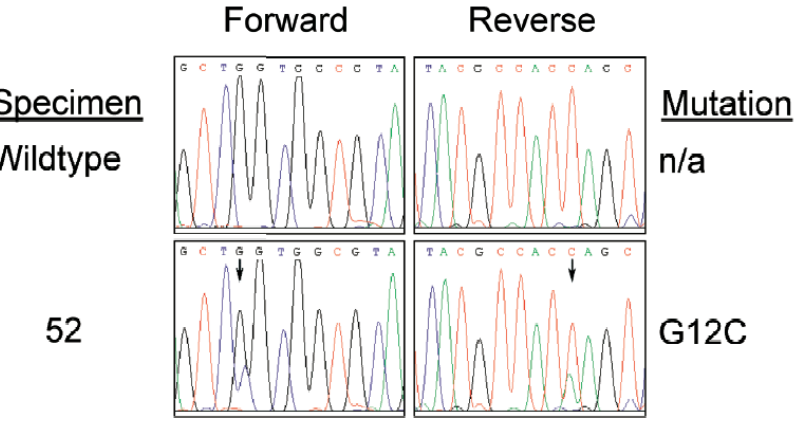


Table 1. *EGFR* and *KRAS* Mutation Status in Lung Adenocarcinomas Sensitive or Refractory to Gefitinib or Erlotinib

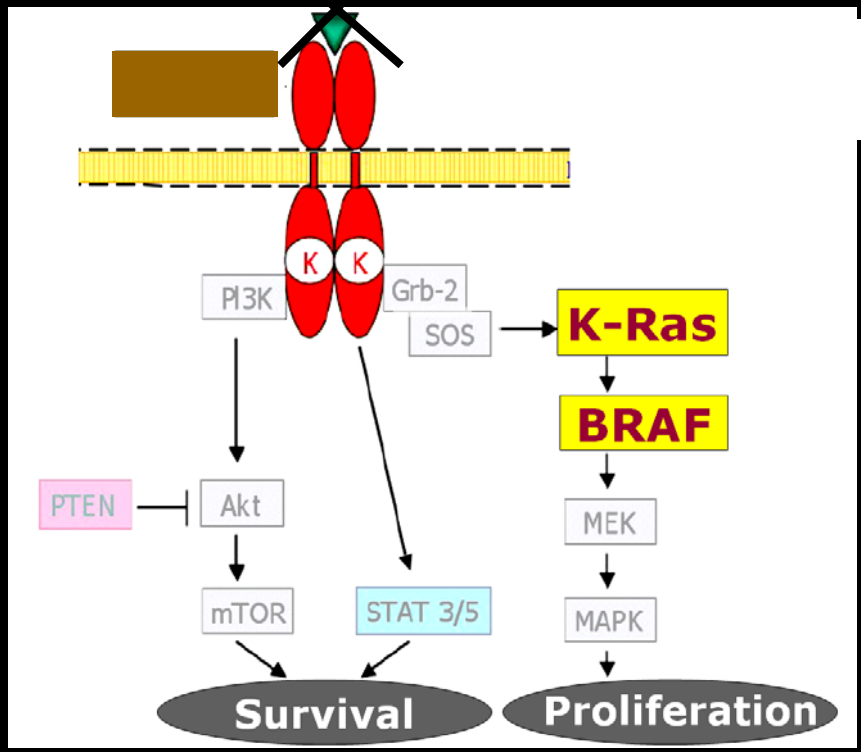
Drug	Gene Mutated	Proportion Sensitive	Proportion Refractory	p-Value
Gefitinib	*EGFR*	9/12	0/12	0.0034
	KRAS	0/12	5/12	0.0373
Erlotinib	*EGFR*	8/10	0/26	1.487×10^{-6}
	KRAS	0/9[a]	4/26	0.5531[b]
Gefitinib or Erlotinib	*EGFR*	17/22	0/38	6.801×10^{-11}
	KRAS	0/21[a]	9/38	0.0201

Intrinsic resistance to TKI

K-ras mutations

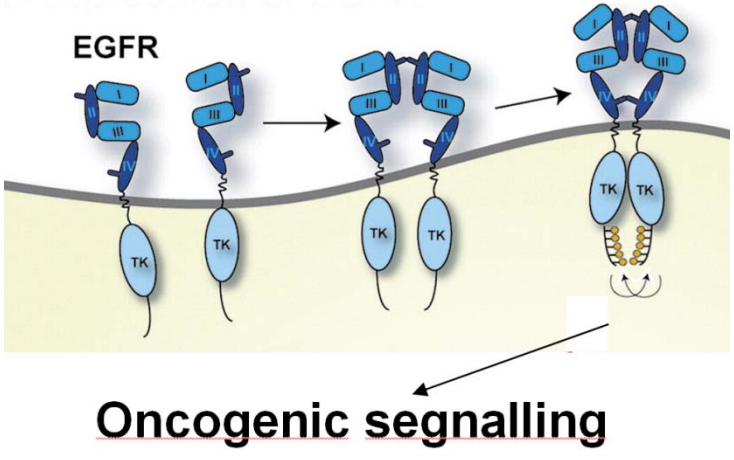
↓

Resistance to TKI



Epidermal Growth Factor Receptor (EGFR) in Colorectal Cancer

Overexpression of EGFR

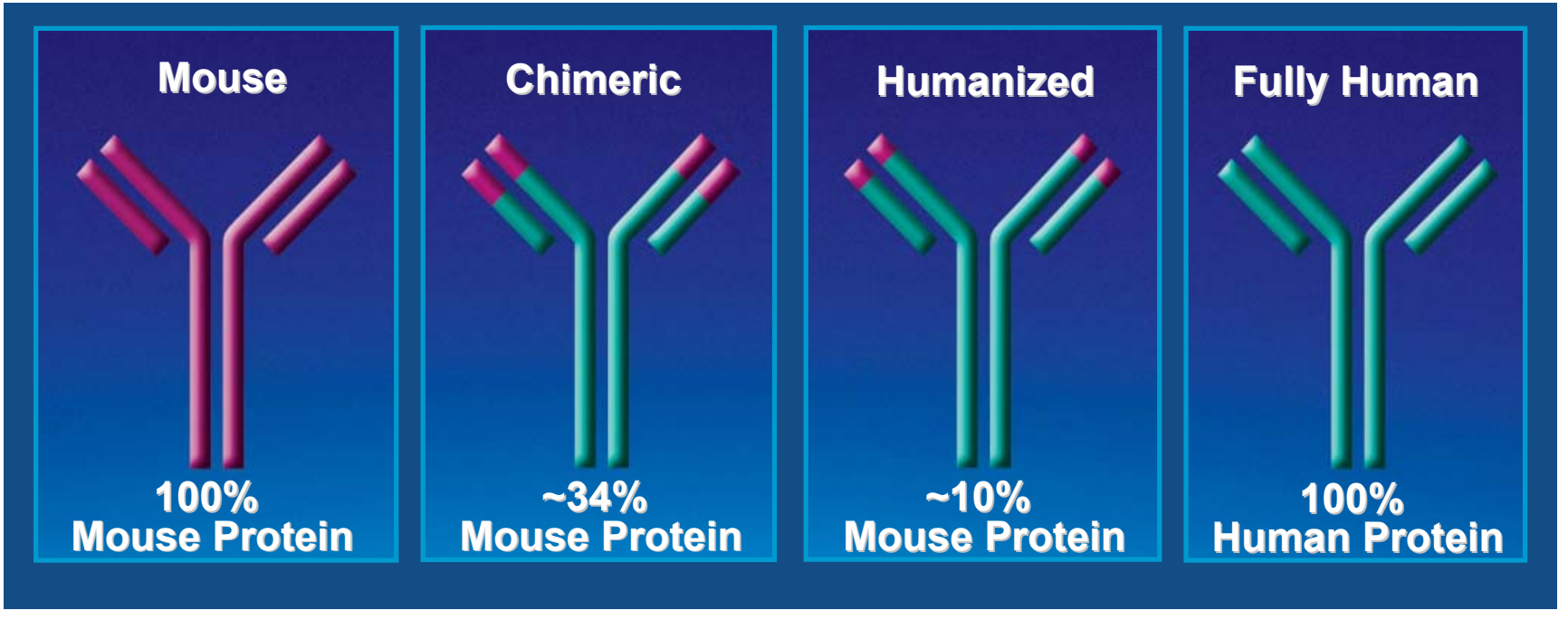


In vitro and in vivo data indicate that:

- preventing the binding of ligands to EGFR results in inhibition of tumor cell growth

- monoclonal antibodies can inhibit the binding of ligands

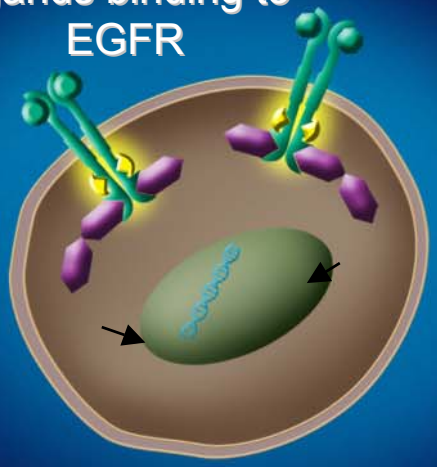
The Development of Human Monoclonal Antibodies



panitumumab

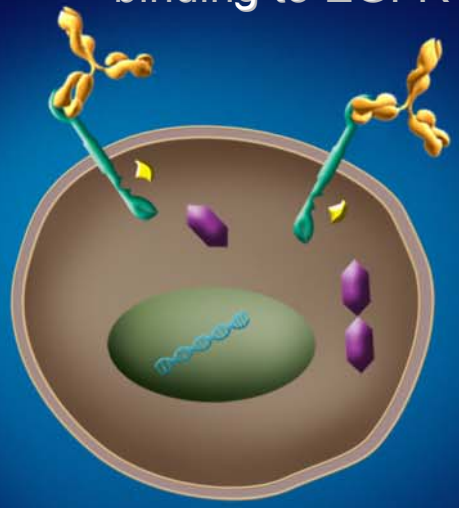
Panitumumab Inhibits Ligand Binding to EGFR and Dimerization

EGF, TGFα or other ligands binding to EGFR



Panitumumab

Inhibition of EGF and TGFα binding to EGFR



- A fully human* IgG2 monoclonal antibody to EGFR
- High affinity, $K_D = 5 \times 10^{-11}$ M
- Inhibits ligand-induced EGFR tyrosine phosphorylation

This may lead to:
↓ Cell proliferation
↓ Cell survival
↓ Angiogenesis
↓ Metastatic spread

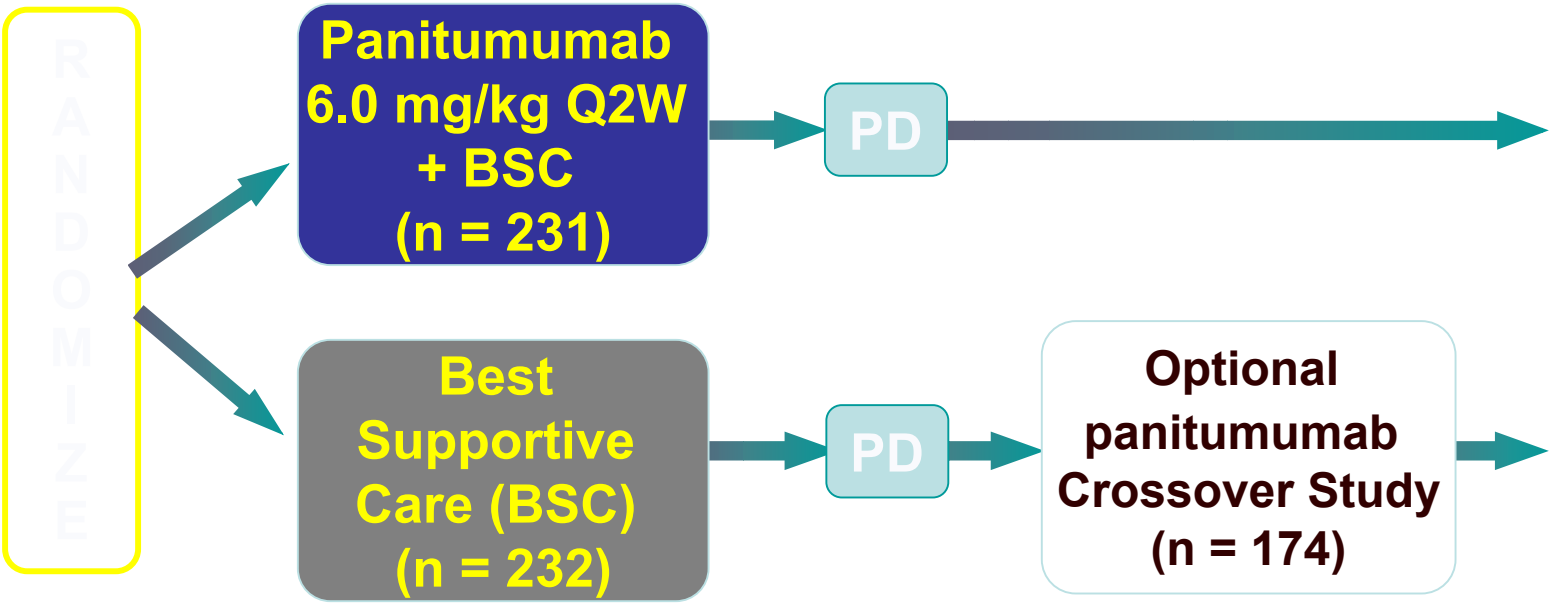
Panitumumab pharmacokinetics

- Panitumumab administered as a single agent or in combination with chemotherapy exhibits nonlinear pharmacokinetics
- Steady-state is obtained after 3 doses at 6 mg/kg given once every 2 weeks without the need of a loading dose
- The mean half-life value during the dosing interval is 7.5 days (range: 3.6 -10.9 days) for the 6 mg/kg dose

Based on preliminary data on outcomes in colorectal cancer, a large, randomized, phase 3 trial in EGFR-expressing tumors of patients with metastatic colorectal cancer resistant to 2 lines of chemotherapy was designed.

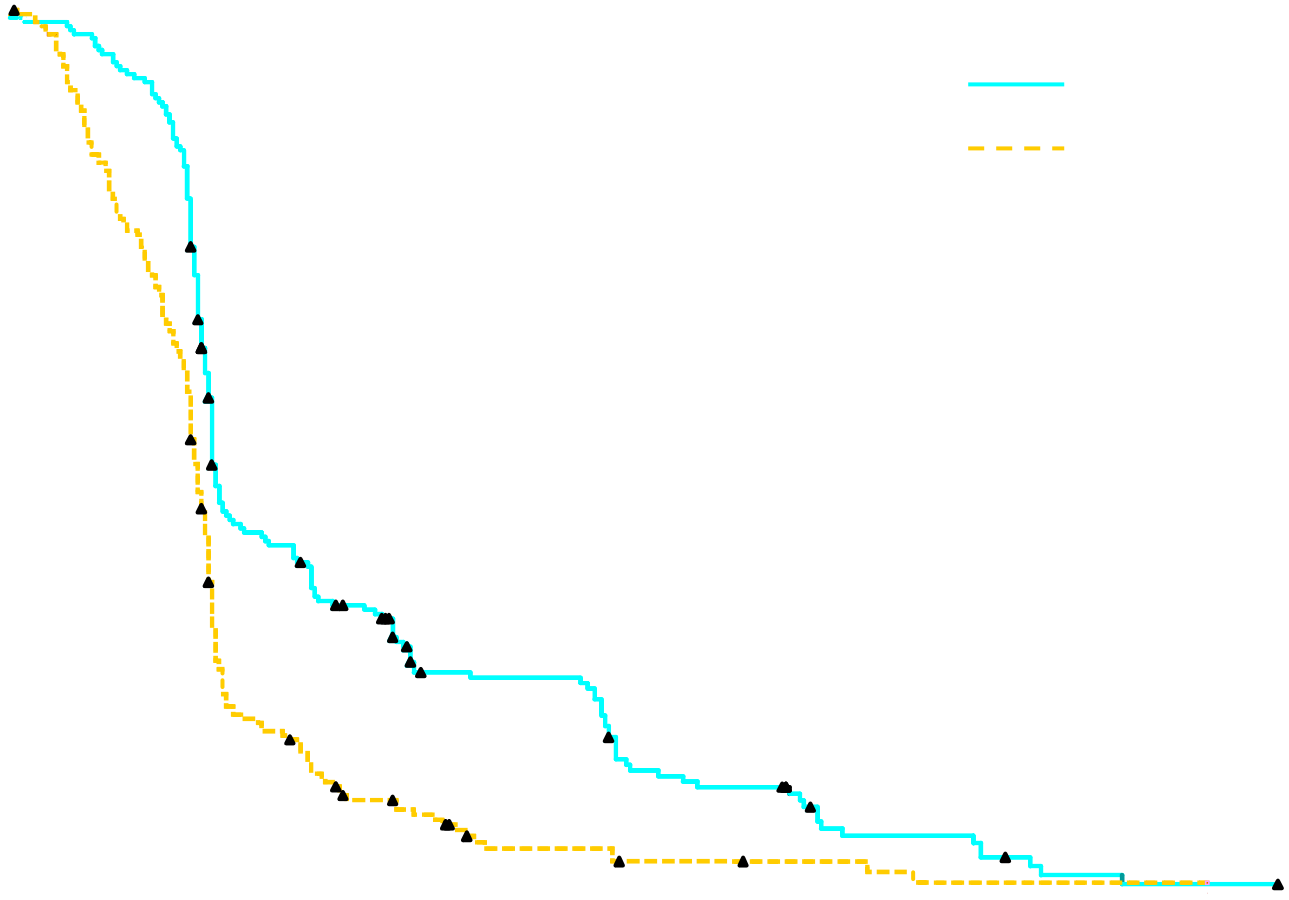
Study Design

Van Cutsem E et al JCO 2007; 25(13):1658-1664

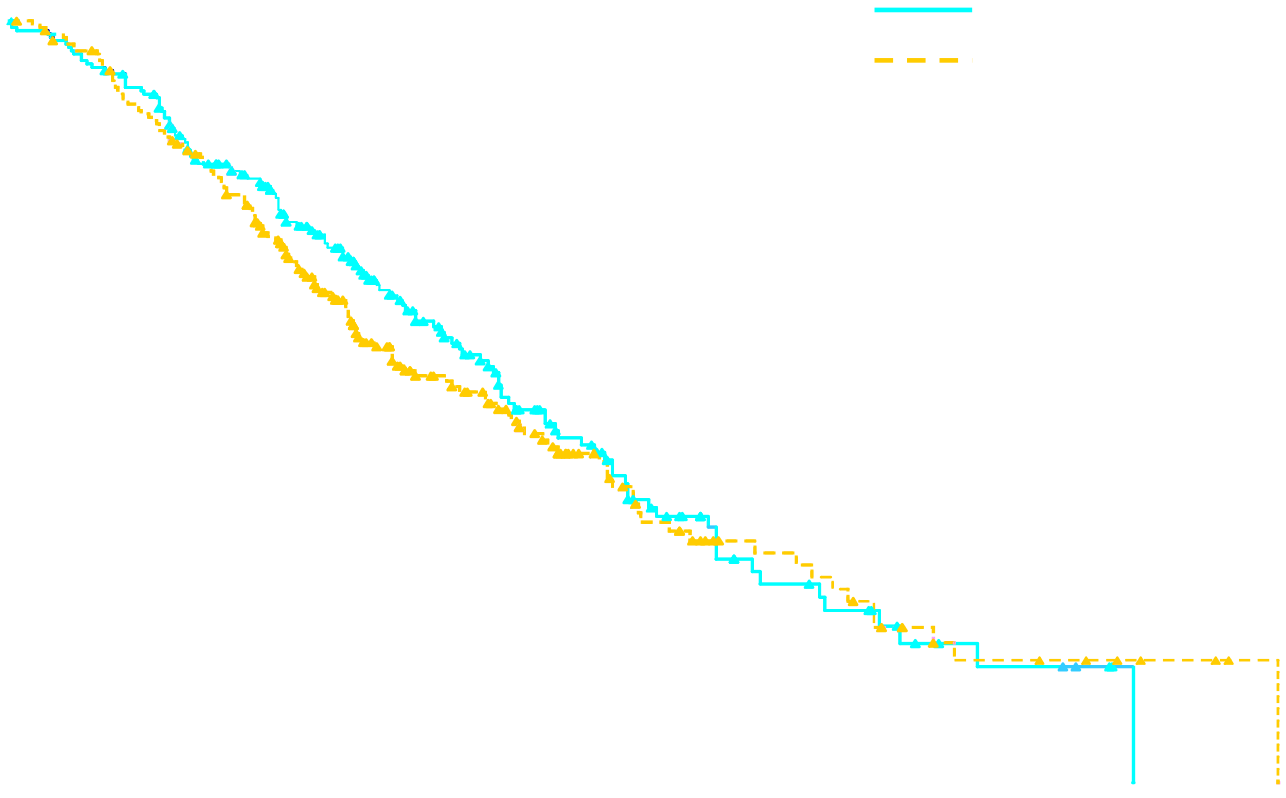


75% of BSC alone patients entered crossover study

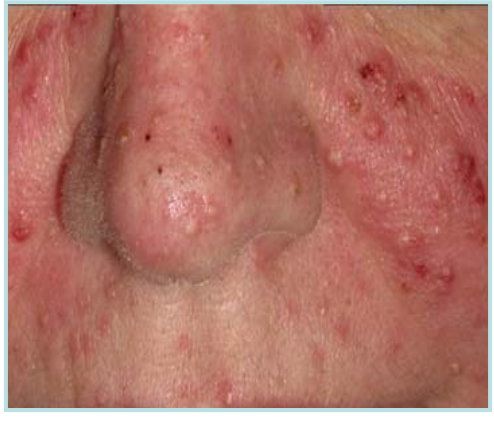
Progression-Free Survival

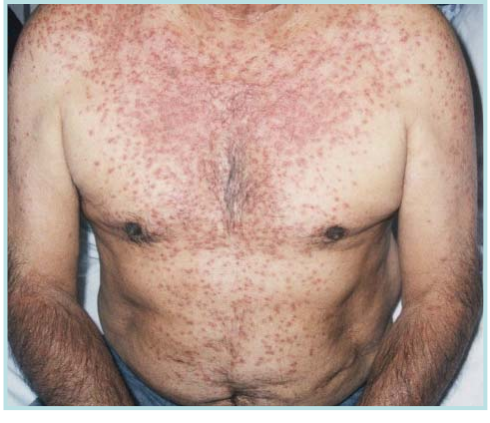


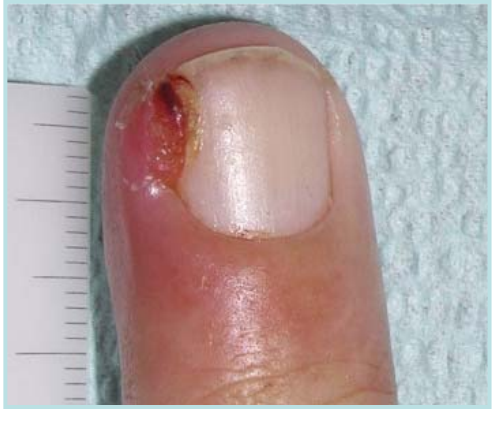
Overall Survival
(All Randomized Analysis Set)



Skin Toxicities Are the Most Common Adverse Events With Panitumumab





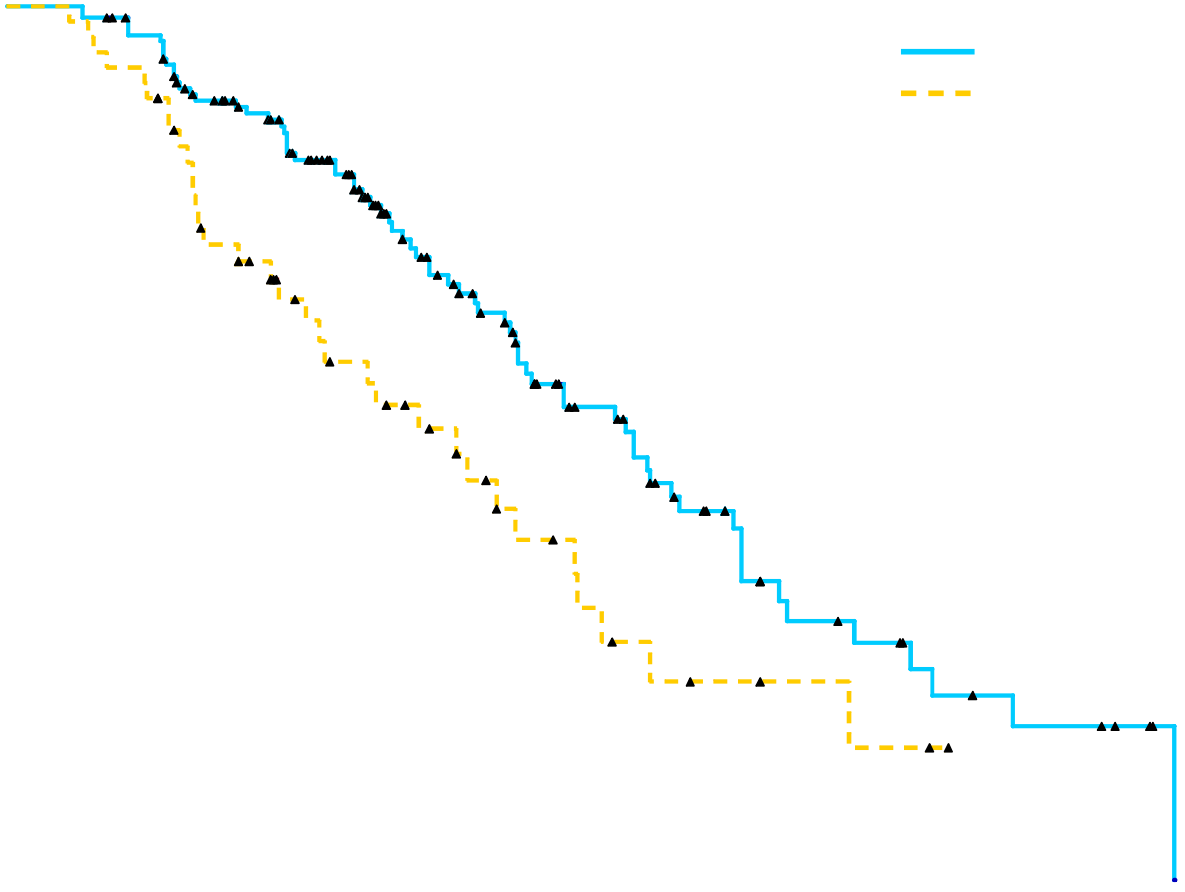


- **Dermatologic toxicities** **89% of patients**
- **12%** **grade 3**

Skin Toxicity was the Most Commonly Reported Adverse Event

Adverse Event	Panitumumab + BSC (n = 229)		BSC alone (n = 234)	
	All grades	Grade 3-4	All grades	Grade 3-4
All Skin/Integument toxicity	90%	16%	9%	0%
Skin	90%	14%	6%	0%
Erythema	65%	5%	1%	0%
Acneiform dermatitis	57%	7%	1%	0%
Pruritus	57%	2%	2%	0%
Skin exfoliation	25%	2%	0%	0%
Rash	22%	1%	1%	0%
Skin fissures	20%	1%	<1%	0%
Dry skin	10%	0%	0%	0%
Acne	13%	1%	0%	0%
Nail				
Paronychia	25%	2%	0%	0%
Other nail disorder	9%	0%	0%	0%
Hair	9%	0%	1%	0%
Growth of eyelashes	6%	0%	0%	0%
Eye	15%	<1%	2%	0%

Skin Toxicity in the Panitumumab Patients



US FDA Label

'Panitumumab has been approved for treatment of EGFR-expressing metastatic colorectal carcinoma in patients with disease progression or following chemotherapy containing fluoropyrimidine, oxaliplatin, and irinotecan'

www.fda.gov

In search for a predictive marker

- **CLINICAL MARKER:** Skin toxicity

- **BIOLOGICAL MARKERS:**
- EGFR mutations
- Immunohistochemical expression
- EGFR copy number
- K-ras mutations

EGFR mutations in CRC

N Engl J Med 2004;351:2883.

EGFR mutations: 1/293 (0,34%)

Clin Cancer Res 2005;11:1368–1371.

EGFR mutations: 4/33 (12%)

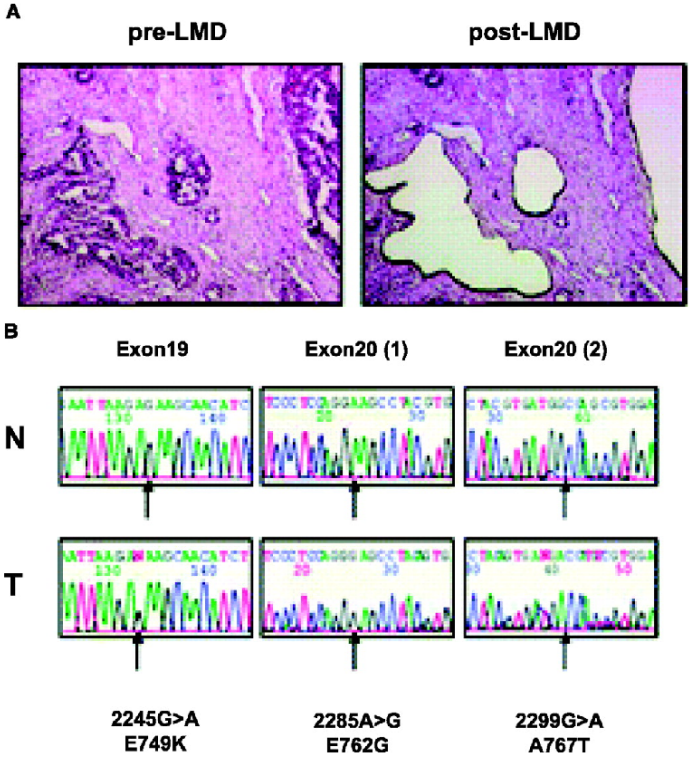
Vol. 11, 1368–1371, February 15, 2005 Clinical Cancer Research

Somatic Mutations of Epidermal Growth Factor Receptor in Colorectal Carcinoma

Hisashi Nagahara,1,2 Koshi Mimori,1 Mitsuhiko Ohta,1 Tohru Utsunomiya,1 Hiroshi Inoue,1 Graham F. Barnard,3 Masaichi Ohira,2 Kosei Hirakawa,2 and Masaki Mori1

1Department of Surgery, Medical Institute of Bioregulation, Kyushu University, Beppu, Japan; 2Department of Surgical Oncology, Osaka City University Graduate School of Medicine, Osaka, Japan; and 3Department of Medicine, University of Massachusetts, Worcester, Massachusetts

Laser microdissection



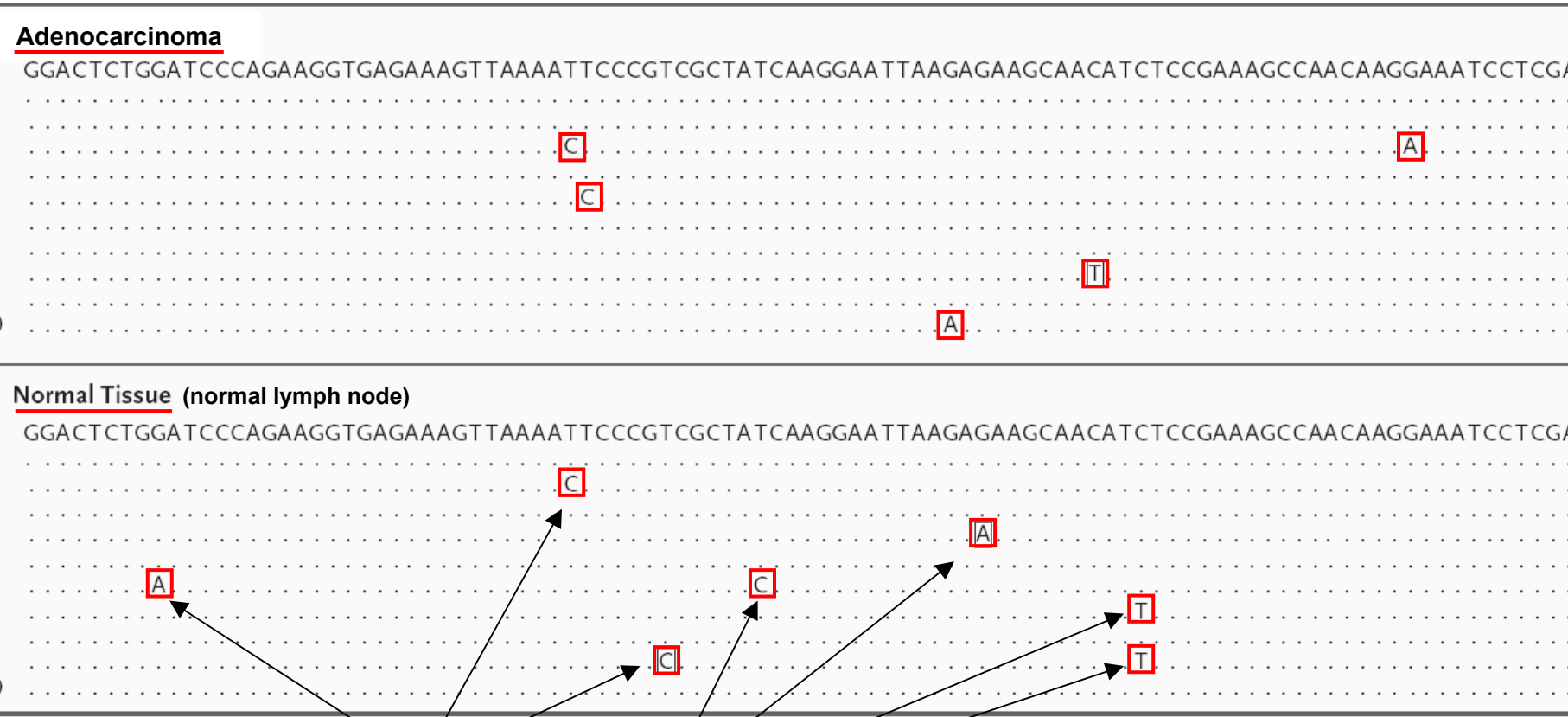
EGFR mutations

4/33 (12%)

G-A transitions

The NEW ENGLAND JOURNAL of MEDICINE

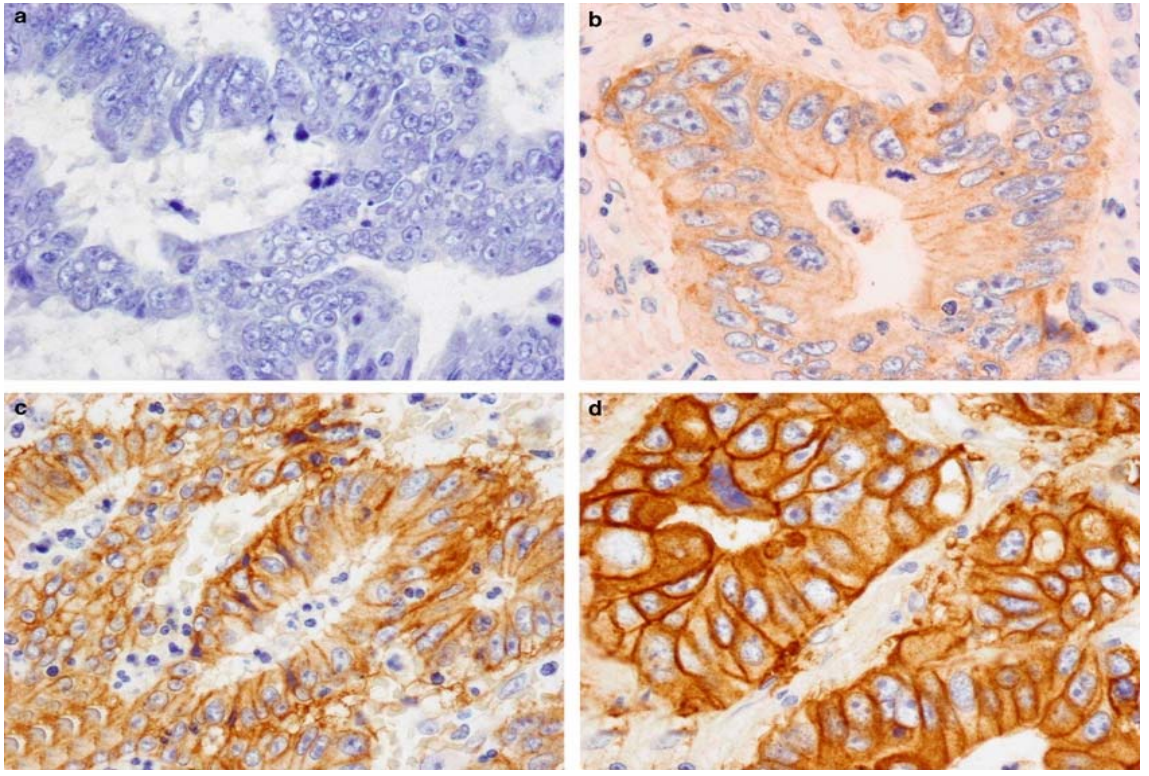
Assessing *EGFR* Mutations



Artifactual mutations in normal tissues

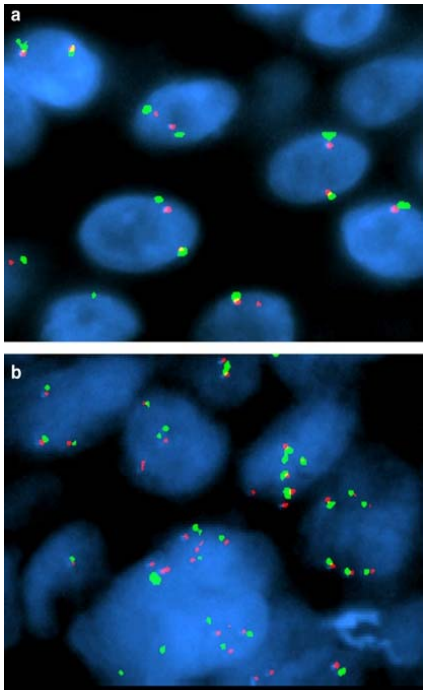
Antonio Marchetti, M.
Lara Felicioni, M.D.

EGFR IHC expression



Cunningham D et al. N Engl J Med 351:337–345, 2004
Chung et al. J Clin Oncol 23:1803–1810, 2005

EGFR copy number



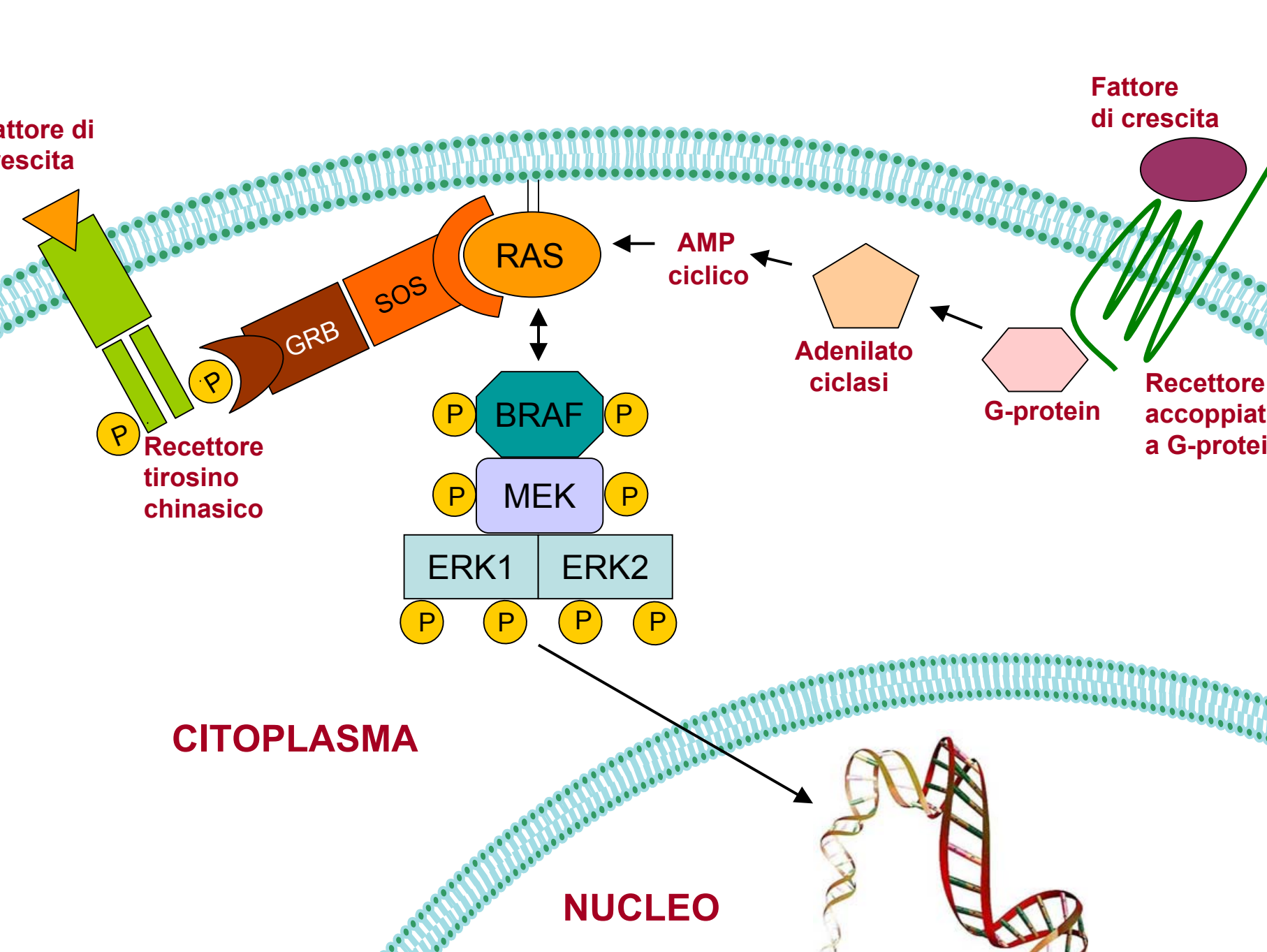
Moroni M et al. Lancet Oncol 6:279–286, 2005.
Italiano A et al. Ann Surg Oncol 15:649–654, 2008.

The Role of K-Ras in Patient Selection for Therapeutic EGFR Inhibitors

Geni della famiglia *RAS*

H-RAS ***K-RAS*** ***N-RAS***

- **Codificano proteine di 21 kd ad attività GTPasica**


ttore di
escita
Fattore
di crescita
RAS
SOS
GRB
P
P
Recettore
tirosino
chinasico
AMP
ciclico
Adenilato
ciclasi
G-protein
Recettore
accoppiat
a G-protei
P
BRAF
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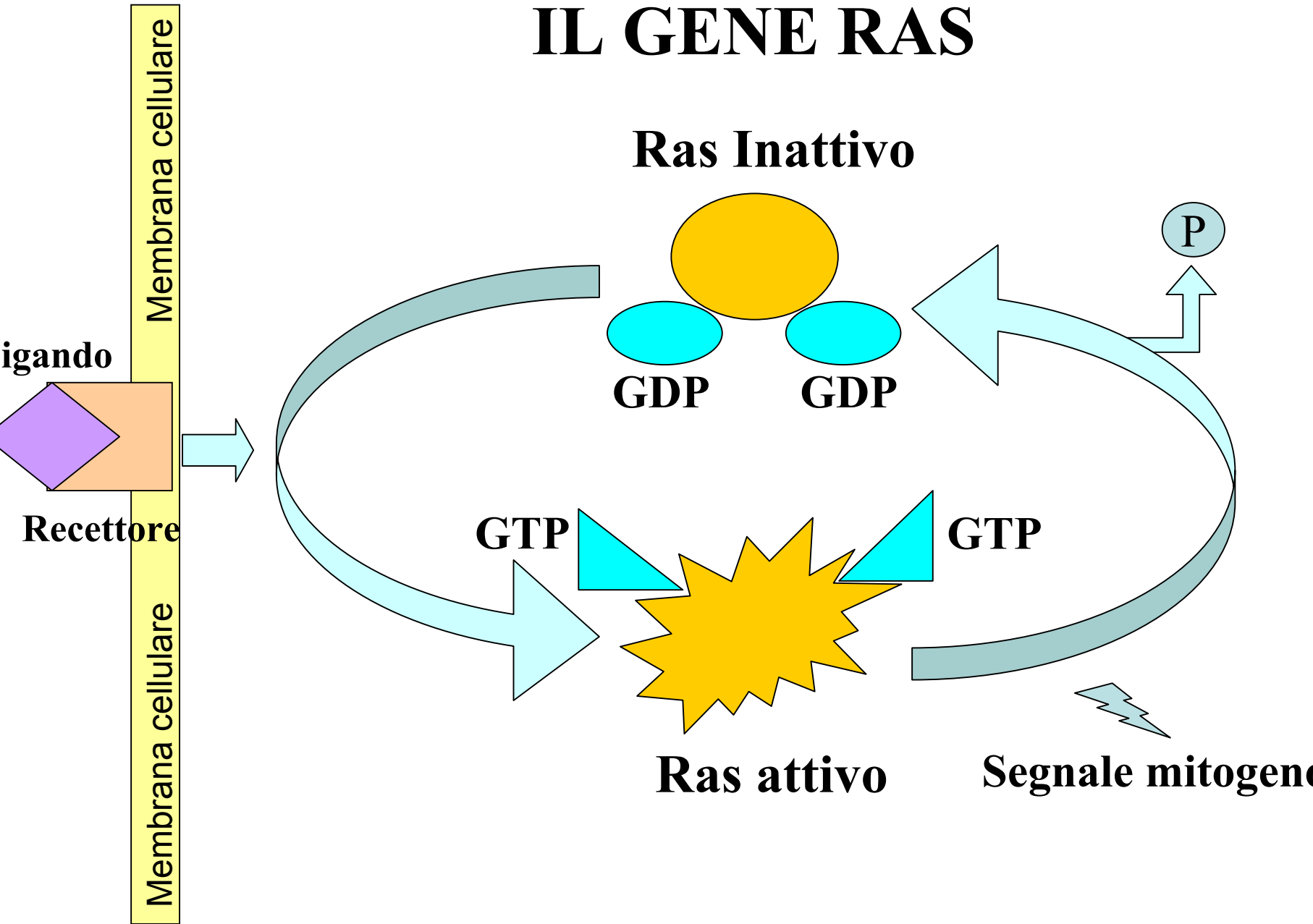
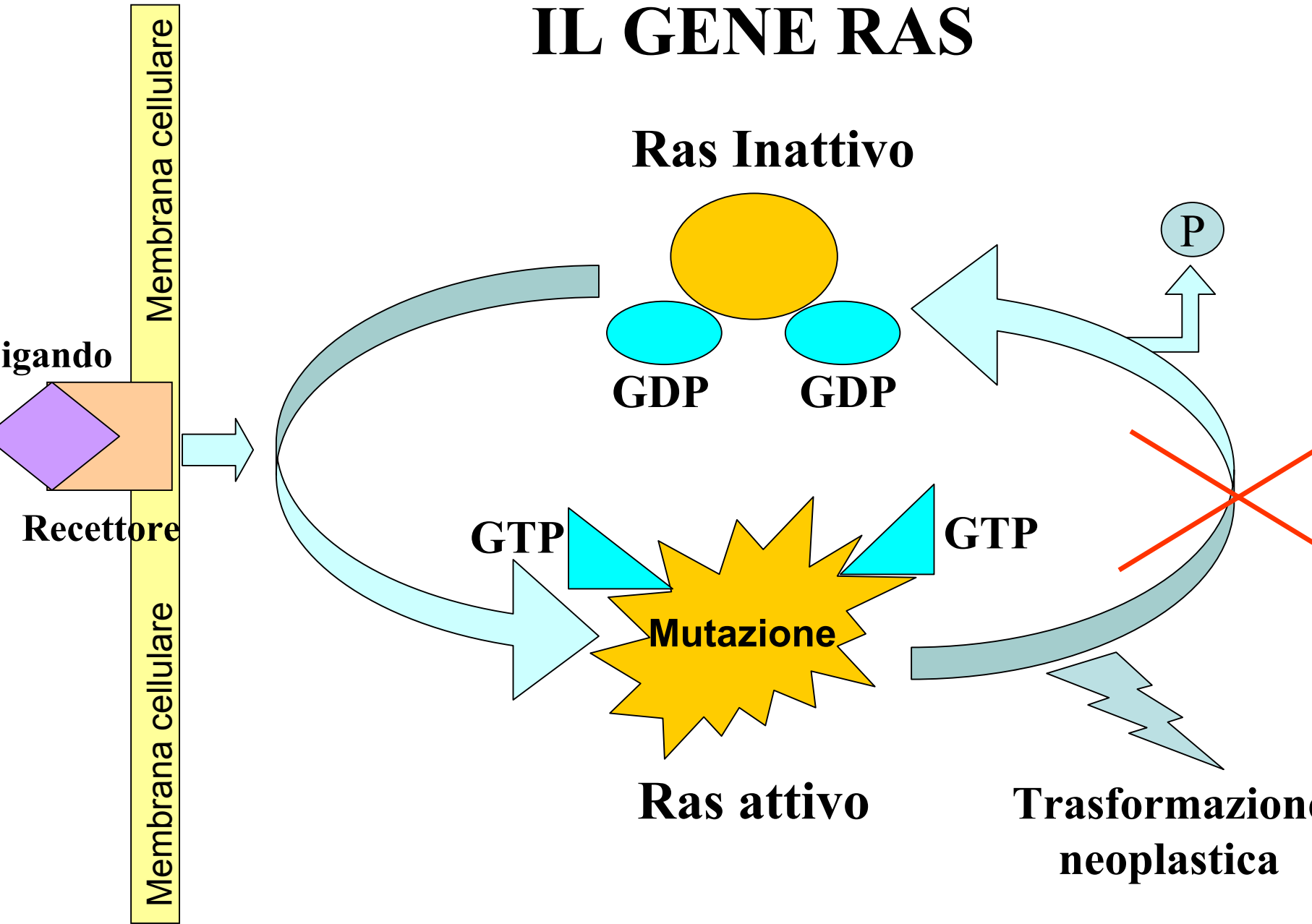
MEK
P
ERK1
ERK2
P
P
P
P
CITOPLASMA
NUCLEO

IL GENE RAS


Membrana cellulare
igando
Recettore
Membrana cellulare
Ras Inattivo
P
GDP
GDP
GTP
GTP
Ras attivo
Segnale mitogeno

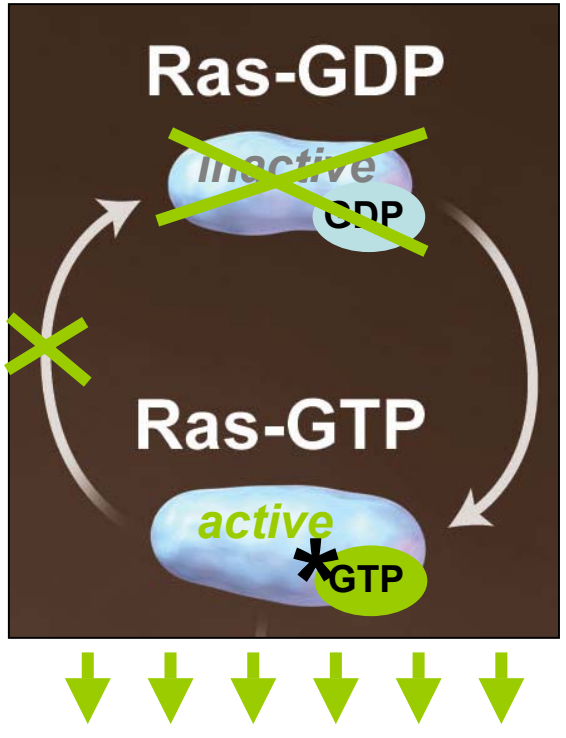
IL GENE RAS


Membrana cellulare
igando
Recettore
Membrana cellulare
Ras Inattivo
GDP
GDP
P
GTP
GTP
Mutazione
Ras attivo
Trasformazione
neoplastica

Mutated Ras May Lead to Uncontrolled Cell Signaling and Cancer

– K-ras mutations

- Mutations at codon 12, 13 (exon 2) and 61 (exon 3) near the GTP binding site.
- Mutatated proteins lose their GTPase activity.
- This type of mutation is often called “dominant activating”
- There is no way for the cell to “turn off” a protein that has a dominant activating mutation



ABNORMAL
- Growth
- Proliferation
- Differentiation

1. Esteller M, et al. *J Clin Oncol.* 2001; 19:299-304.
2. Schubbert S, et al. *Nature Rev Cancer*. 2007; 7:295-308.

Mutated *KRAS* is Prevalent in Many Different Tumor Types

Cancer Type	Reported Incidence of Mutated *KRAS*
Pancreatic	72 – 90%
Colon	**32 – 51% (40%)**
Lung	15 – 33%
Ovarian	5 – 50%
Gall bladder	14 – 38%
Multiple myeloma	16 – 33%

Adapted from: Friday BB and Adjei AA. *Biochim. Biophys. Acta.* 2005; 1756:127-144

K-ras mutations in CRC revealed by direct sequencing

Studio	n° casi studiati	n° mutazioni	%	Codone 12 (%)	Codone 13 (%)
Urosevic et al. [1993]	37	17	46		
Breivik et al. [1994]	251	99	39		
Andreyev et al. [1993]	679	225	33		
Rajagopalan et al. [2002]	330	169	51		
Brink et al. [2003]	737	271	37	72	22
Moroni et al. [2005]	31	10	32	60	40
Ogino et al. [2005]	30	10	33	60	40
Lièvre et al. [2006]	30	13	43		
Benvenuti et al. [2007]	48	16	33	63	37
Wojcik et al. [2008]	163	57	35	66	22
Benvenuti et al. [2008]	175	70	44		
Freeman et al. [2008]	62	24	39		
Totale	2573	981	38	64	32

Single-Arm Studies Support the Hypothesis for *KRAS* as a Biomarker for EGFR Inhibitors

	Treatment (panitumumab or [illegible])	No of patients (WT:MT)	Objective Response N (%)	
			MT	WT
A. Lièvre, et al. *(AACR Proceedings, 2007)*	cmab ± CT	76 (49:27)	0 (0)	24 (49)
S. Benvenuti, et al. *(Cancer Res, 2007)*	pmab or cmab or cmab + CT	48 (32:16)	1 (6)	10 (31)
W. De Roock, et al. *(ASCO Proceedings, 2007)*	cmab or cmab + irinotecan	113 (67:46)	0 (0)	27 (40)
D. Finocchiaro, et al. *(ASCO Proceedings, 2007)*	cmab ± CT	81 (49:32)	2 (6)	13 (26)
F. Di Fiore, et al. *(Br J Cancer, 2007)*	cmab + CT	59 (43:16)	0 (0)	12 (28)
S. Khambata-Ford, et al. *(J Clin Oncol, 2007)*	cmab	80 (50:30)	0 (0)	5 (10)
	TOTAL	**457 (290:167)**	**3 (1.7)**	**91 (20)**

KRAS Analysis of a Phase 3, Randomized Trial Comparing Panitumumab vs Best Supportive Care (BSC) in Colorectal Cancer

Amado R et al. *JCO:26 (10) April* 2008.

Hypothesis:

RANDOMIZE

Panitumumab 6.0 mg/kg Q2W + BSC → PD → Follow-up

BSC → PD → Follow-up

Optional Panitumumab Crossover Study

Method Used to Detect *KRAS* Mutational Status

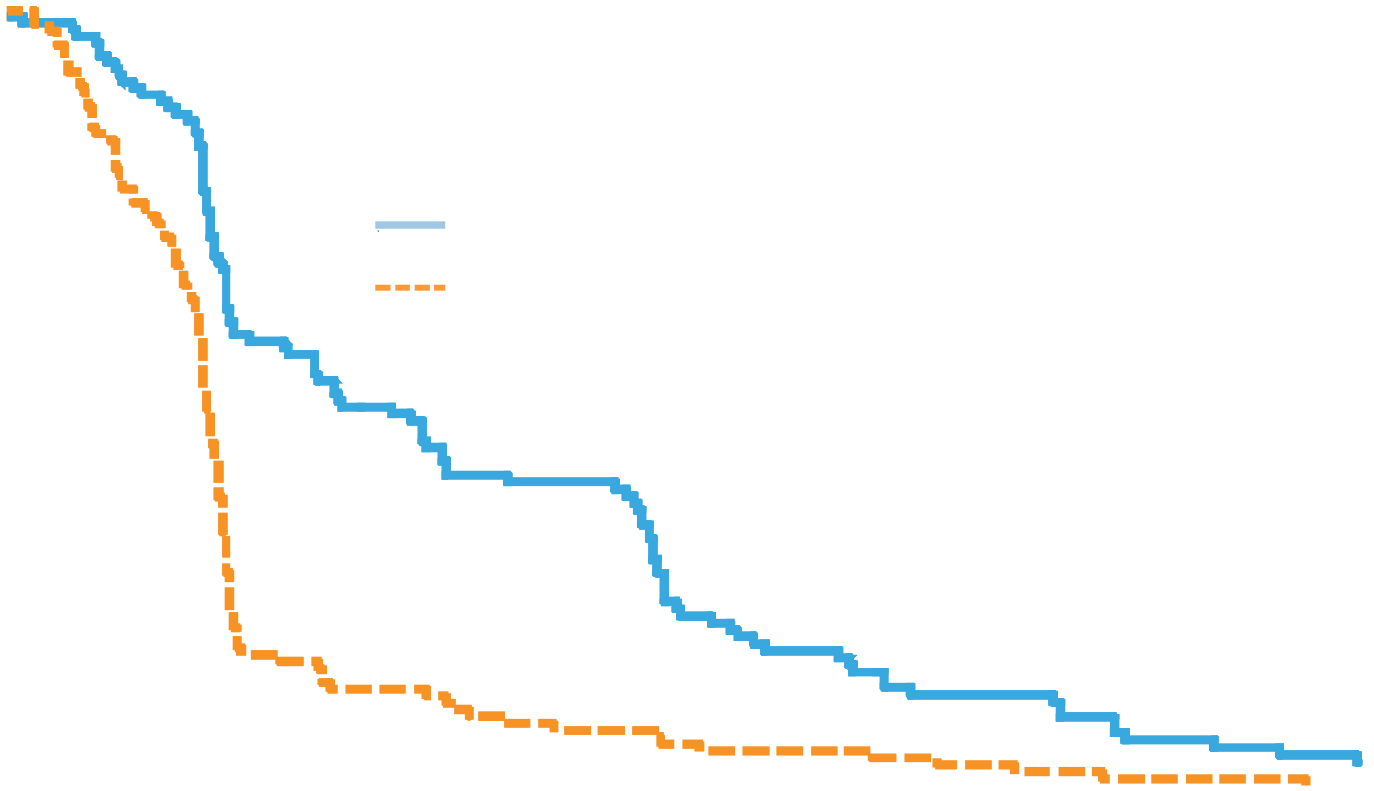
- DNA was isolated from fixed tumor samples
- Mutant *KRAS* was detected using a *KRAS* mutation kit (DxS Ltd, Manchester, UK) that used allele-specific, real-time PCR

RESULTS

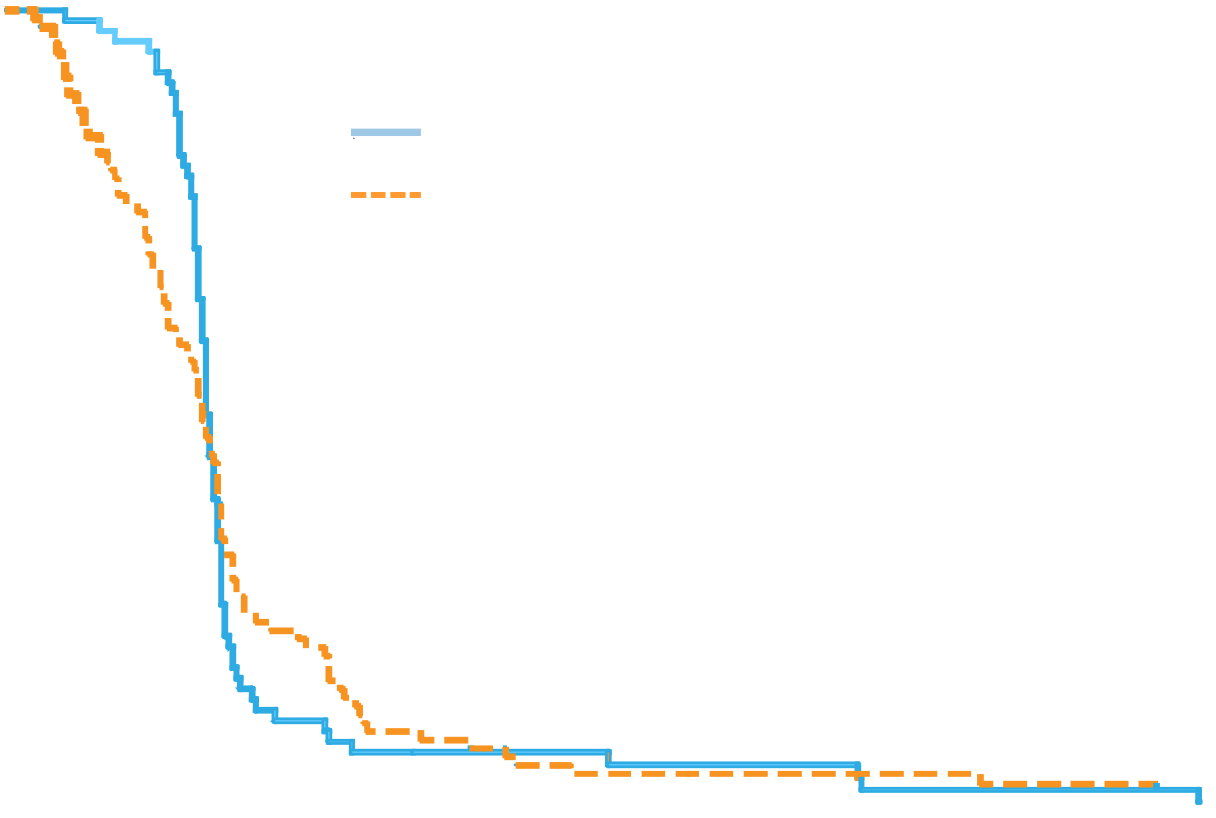
Prevalence of Mutant *KRAS*

	Total
Patients included in *KRAS* analysis, n (%)	427 (92)
Wild-type *KRAS*, n (%)	243 (57)
Mutant *KRAS*, n (%)	184 (43)

Treatment



Mutant *KRAS* Subgroup PFS by Treatment



Objective Tumor Response (Central Radiology)

	KRAS					
	All Evaluable n (%)		Mutant n (%)		Wild-type n (%)	
Response	Pmab (N = 208)	BSC (N = 219)	Pmab (N = 84)	BSC (N = 100)	Pmab (N = 124)	BSC (N = 119)
CR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PR	21 (10)	0 (0)	0 (0)	0 (0)	21 (17)	0 (0)
SD	52 (25)	22 (10)	10 (12)	8 (8)	42 (34)	14 (12)
PD	104 (50)	149 (68)	59 (70)	60 (60)	45 (36)	89 (75)
CR, PR, SD	73 (35)	22 (10)	10 (12)	8 (8)	63 (51)	14 (12)

PR partial response;

Objective Tumor Response (Central Radiology)

	KRAS					
	All Evaluable n (%)		Mutant n (%)		Wild-type n (%)	
Response	Pmab (N = 208)	BSC (N = 219)	Pmab (N = 84)	BSC (N = 100)	Pmab (N = 124)	BSC (N = 119)
CR	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PR	21 (10)	0 (0)	0 (0)	0 (0)	21 (17)	0 (0)
SD	52 (25)	22 (10)	10 (12)	8 (8)	42 (34)	14 (12)
PD	104 (50)	149 (68)	59 (70)	60 (60)	45 (36)	89 (75)
CR, PR, SD	73 (35)	22 (10)	10 (12)	8 (8)	63 (51)	14 (12)

CR, complete response; PR partial response;

SD, stable disease;

Panitumumab (Vectibix)
Anticorpo monoclonale anti-EGFr

- **Parere positivo del Committee for Medicinal Products for Human Use (CHMP): 20 settembre 2007**

- **Decisione della Commissione Europea : 5 dicembre 2007**
- **Vectibix è indicato** *« come monoterapia per il trattamento di pazienti con carcinoma colorettale metastatico esprimenti il recettore per il fattore di crescita epidermico (EGFR) dopo fallimento di regimi chemioterapici contenenti fluoropirimidine,*

Meta-Analysis

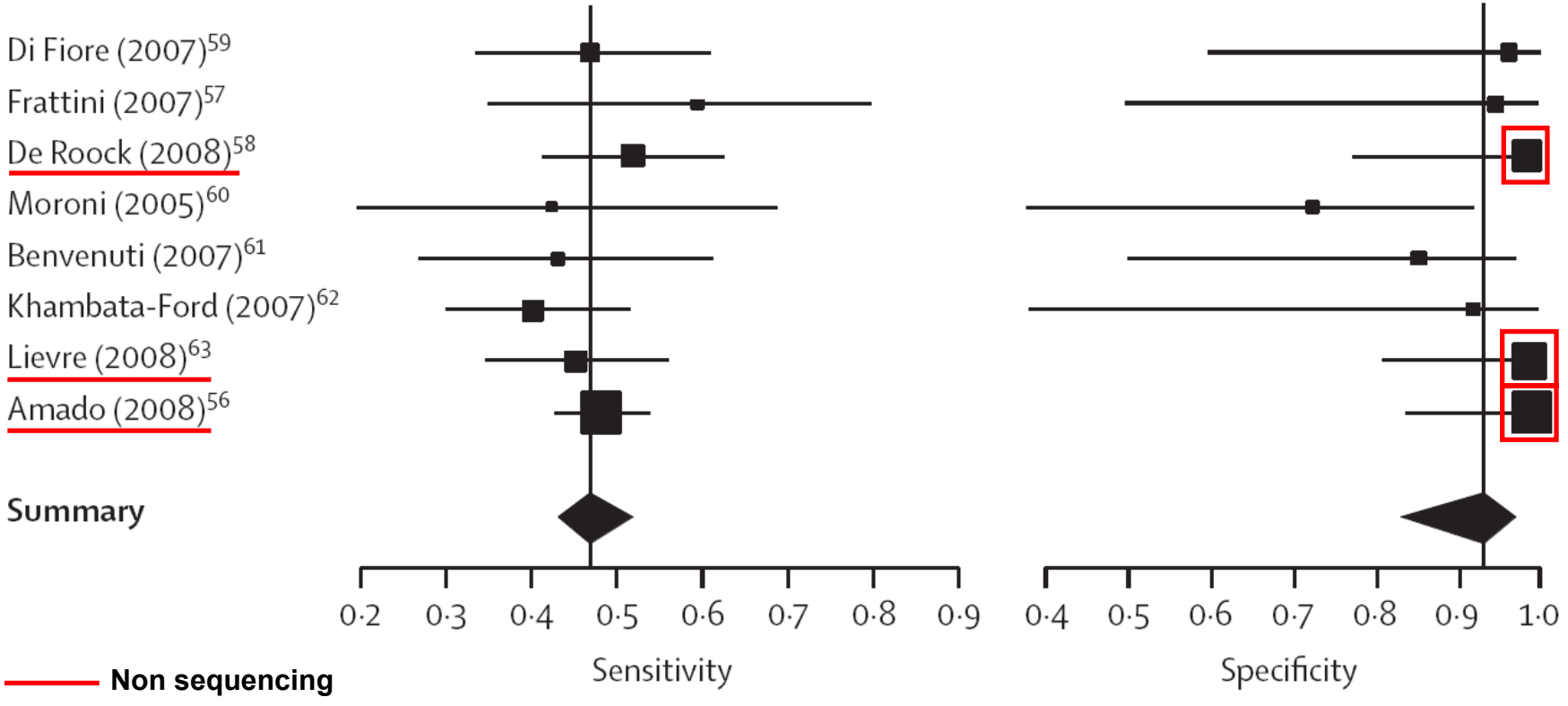
Linardeou H. Lancet Oncol, October 2008; 9: 962–72

	Patients included in analysis, n	*k-RAS*-mutation positive, n (%)	Study design	Mutation analysis	Previous treatment*	Study treatment
mado (2008)[56]†‡	376	147 (39)	Prospective	DxS	I,O,F	P§±BSC
attini (2007)[57]¶	23	9 (39)	Retrospective	Ex2 bi-ds	≥1 chemotherapy	C+I-based; or C+CAP
e Roock (2008)[58]	108	42 (39)	Retrospective‖	AD+sequencing	I	C+I; or C alone**
i Fiore (2007)[59]	59	22 (37)	Retrospective	Ex2 bi-ds	≥1 chemotherapy	C+I; or C+O
oroni (2005)[60]¶	20	7 (35)	Retrospective††	Ex2 bi-ds	≥1 chemotherapy	C+I based; or P alone C alone
envenuti (2007)[61]¶	37	13 (37)	Retrospective	Ex2 bi-ds	I	C+I based; or P alone C alone
hambata-Ford (2007)[62]	80	30 (38)	Prospective	NR	≥1 chemotherapy	C‡‡
evre (2008)[63]§§¶¶	114	36 (32)	Retrospective	AD+sequencing	≥1 chemotherapy	C+I; or C+FOLFIRI

Comparison of all studies and subgroups for metastatic colorectal cancer

	Sensitivity (95% CI)	p value	Specificity (95% CI)	p value	+LR	–LR	Predictive odds ratio
Overall (n=8)	0·47 (0·43–0·52)	..	0·93 (0·83–0·97)	..	6·82	0·57	12·01
MoAb							
Panitumumab (n=4)	0·48 (0·41–0·55)	0·25	0·84 (0·54–0·96)	0·86	2·95	0·62	4·74
Cetuximab (n=4)	0·41 (0·30–0·54)	..	0·86 (0·48–0·98)	..	2·94	0·68	4·30
MoAb combination							
Panitumumab (n=4)	0·48 (0·42–0·54)	0·72	0·84 (0·58–0·95)	0·21	2·95	0·62	4·74
Cetuximab±chemotherapy (n=10)	0·46 (0·40–0·53)	..	0·93 (0·83–0·97)	..	6·75	0·57	11·74
Chemotherapy							
MoAb (n=8)	0·46 (0·41–0·52)	0·54	0·87 (0·69–0·95)	0·14	3·59	0·62	5·82
MoAb+chemotherapy (n=6)	0·49 (0·41–0·57)	..	0·96 (0·85–0·99)	..	11·05	0·53	20·67
Detection method*							
Sequencing (n=4)	0·47 (0·35–0·60)	0·82	0·87 (0·62–0·96)	0·02	3·65	0·61	6·01
Other (n=3)	0·48 (0·42–0·54)	..	0·99 (0·89–1·00)	..	33·36	0·52	63·58

Forest plots representing all studies for *k-RAS*-mutation sensitivity and specificity in colorectal carcinoma

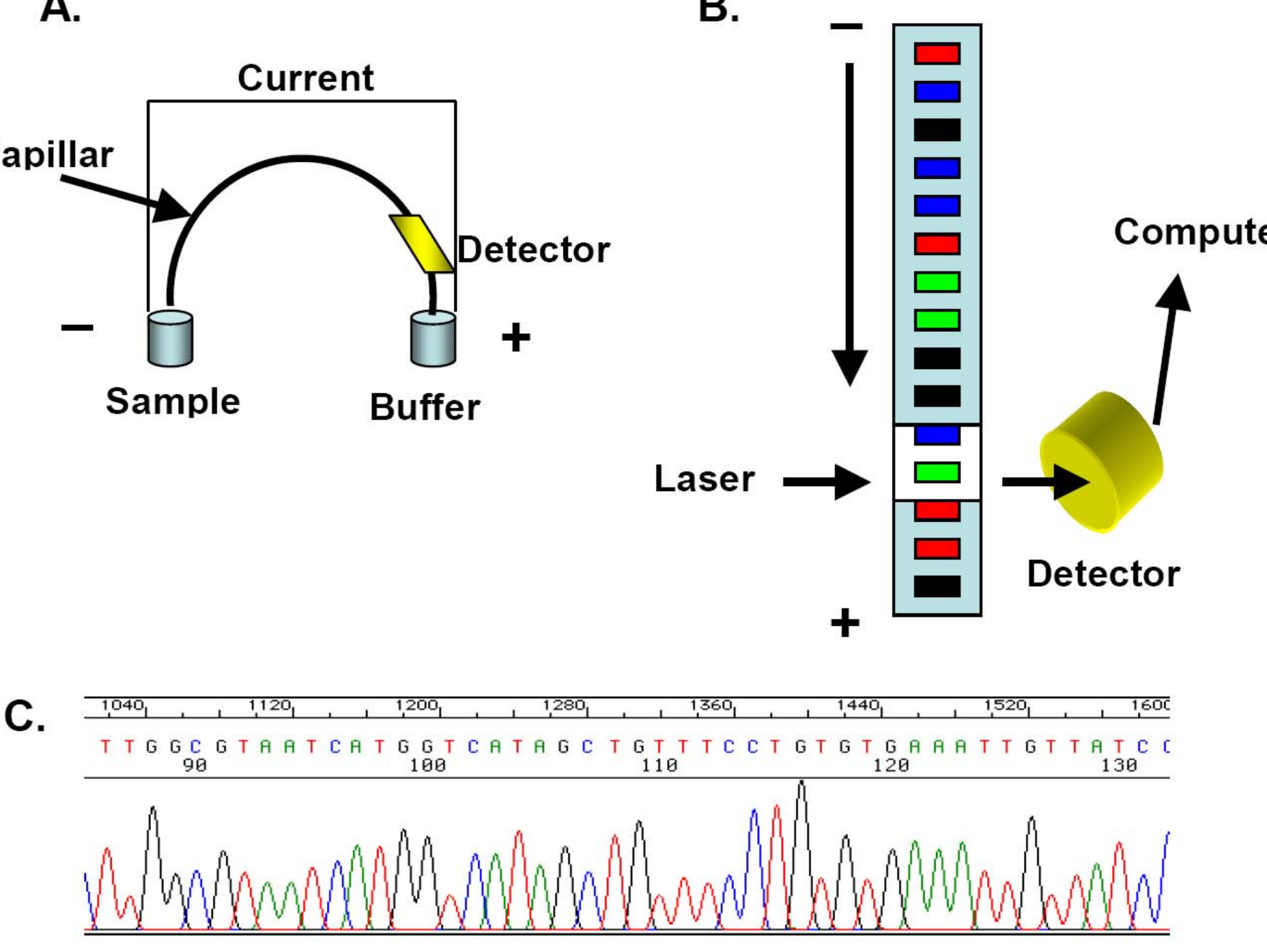


The main problem is the low sensitivity of the K-ras marker

Resistenza farmacologica su base mutazionale.

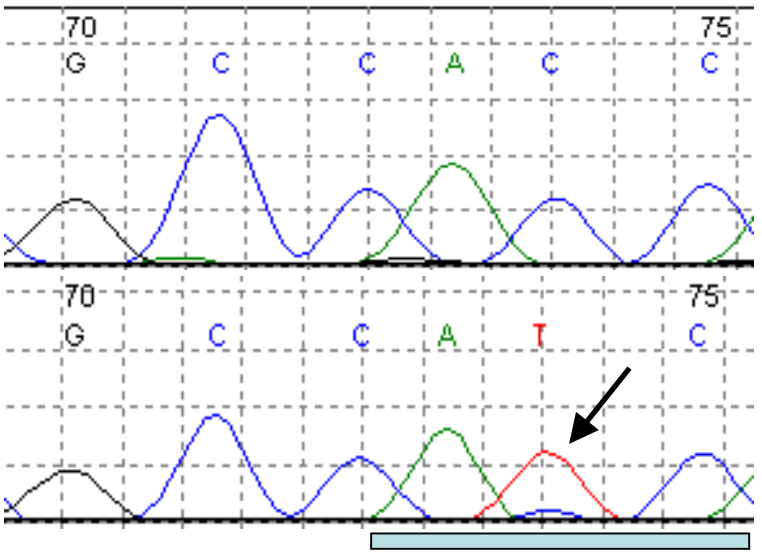
Extremely sensitive techniques are needed

Direct sequencing of the PCR products


A.
Current
apillar
Detector
–
+
Sample
Buffer
B.
–
Compute
Laser
Detector
+
C.
1040
1120
1200
1280
1360
1440
1520
1600
T T G G C G T A A T C A T G G T C A T A G C T G T T T C C T G T G T G A A A T T G T T A T C C
90
100
110
120
130

Codone 12 (GGT)

Tessuto normale

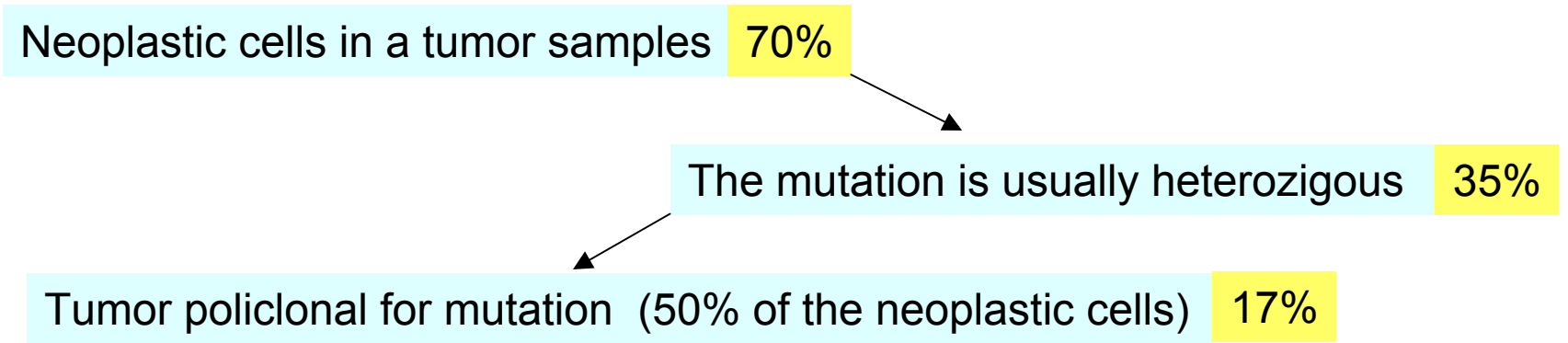


Tumore

Codone 12 (GAT)

The direct sequencing is not the the most sensitive method available

Detection limit of sequencing = 1:5 (20%)



Molecular Diagnostic Technologies Genetic Analysis Services Contact Us News

DxS Diagnostic Innovations

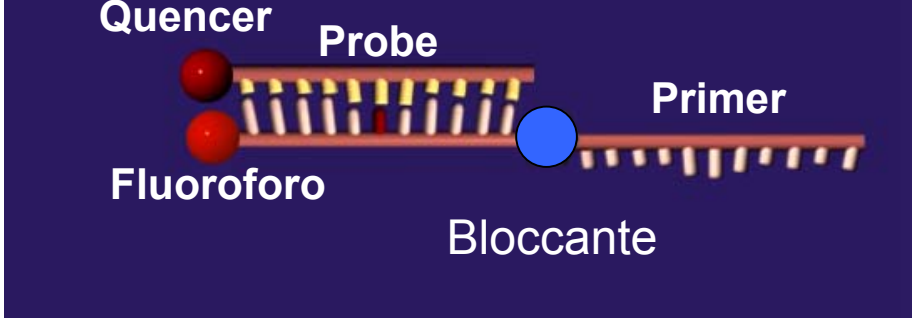
THERASCREEN: K-RAS MUTATION TEST KIT

TheraScreen: K-RAS Mutation kit is the first molecular companion diagnostic to support targeted therapies in colorectal cancer. The kit detects seven mutations in codons 12 and 13 of the K-RAS oncogene.

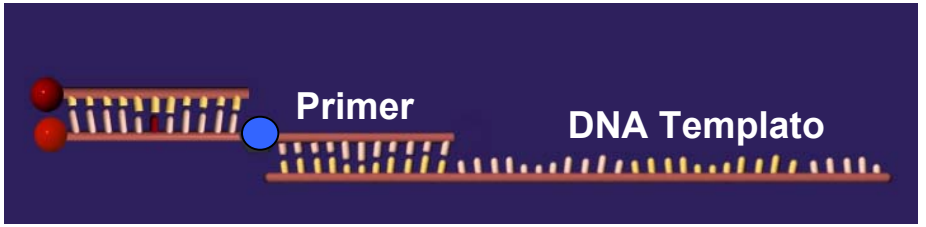
Technological principles

The Kit combines ARMS (allele specific PCR) with the scorpions real time PCR technolog

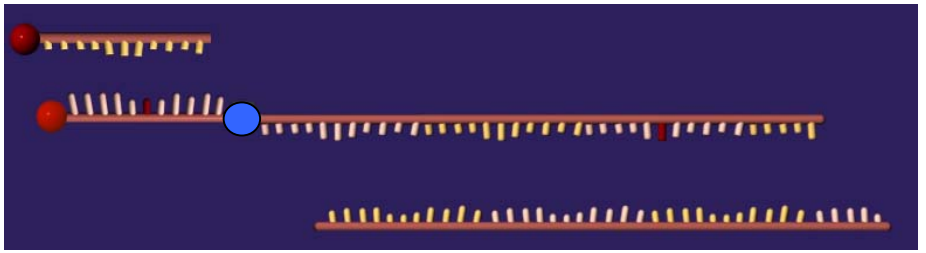
A Sonda scorpion



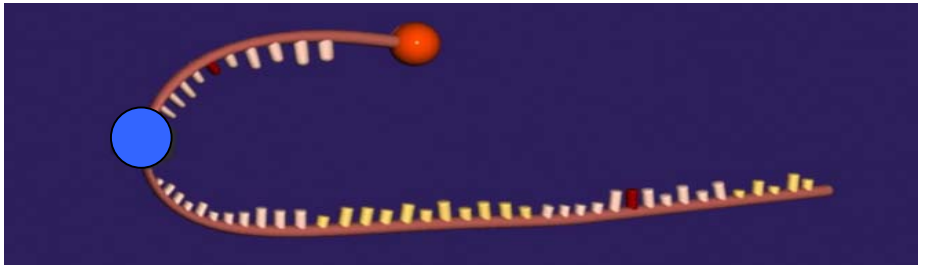
B Ibridazione al templato



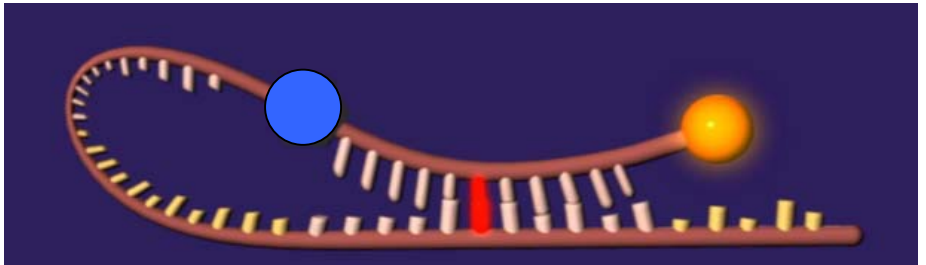
C Denaturazione



D Formazione dell'ansa



E Ibridazione ed emissione di fluorescenza



SENSITIVITY

The TERASCREEN assays can detect 1% of mutant in a background of wild type genomic DNA.

The test identifies 7 somatic mutations in codons 12 and 13

Codon 12	**Gly 12 Asp** **Gly 12 Ala** **Gly 12 Val** **Gly 12 Ser** **Gly 12 Arg** **Gly 12 Cys**
Codon 13	**Gly 13 Asp**

Amado R et al. *JCO:26 (10) April* 2008

39% of K-ras mutations

15% **in codon 13**

K- ras mutations in CRC revealed by direct sequencing

Studio	n° casi studiati	n° mutazioni	%	Codone 12 (%)	Codone 13 (%)
Urosevic et al. [1993]	37	17	46		
Breivik et al. [1994]	251	99	39		
Andreyev et al. [1993]	679	225	33		
Rajagopalan et al. [2002]	330	169	51		
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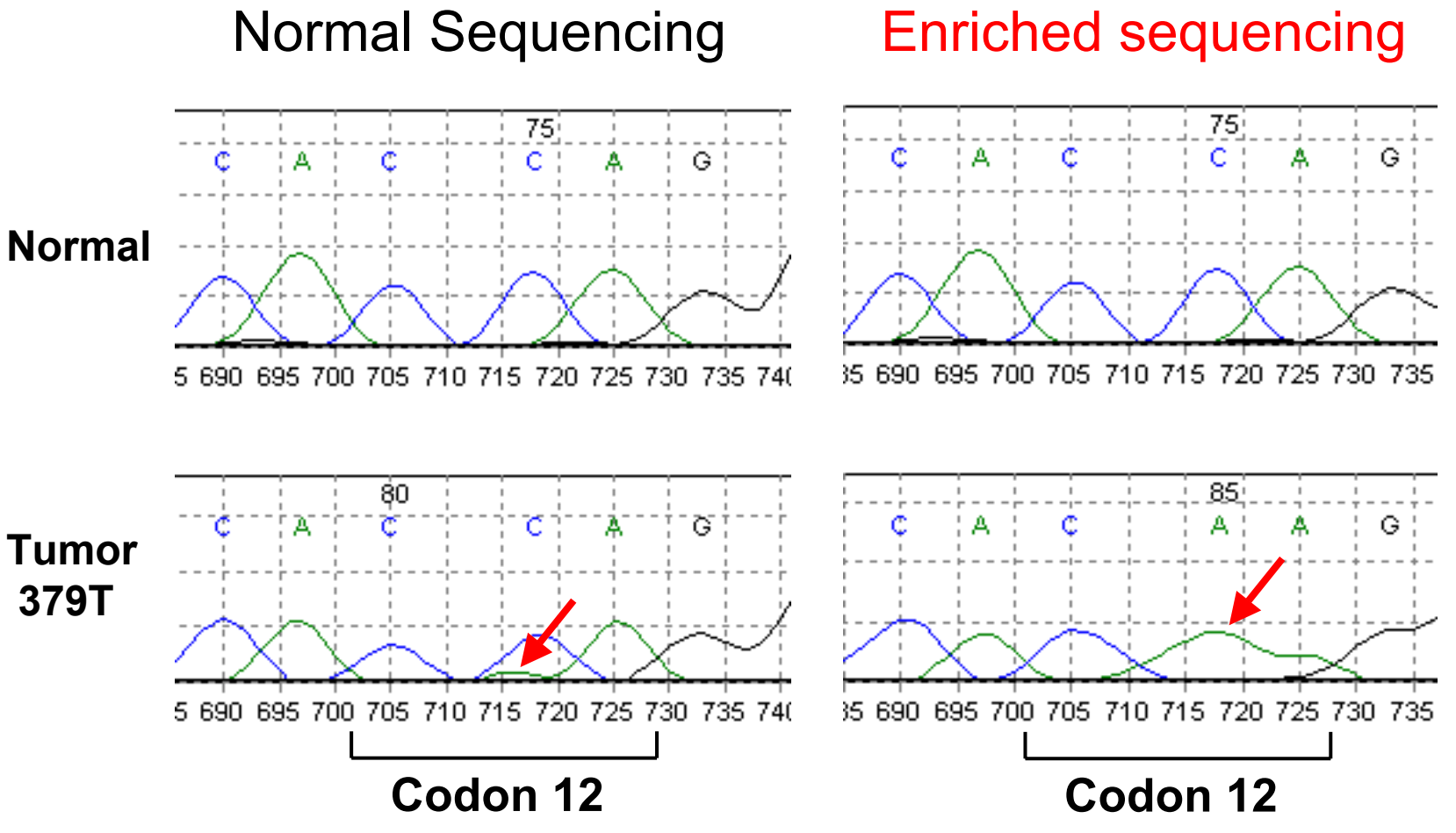
Moroni et al. [2005]	31	10	32	60	40
Ogino et al. [2005]	30	10	33	60	40
Lièvre et al. [2006]	30	13	43		
Benvenuti et al. [2007]	48	16	33	63	37
Wojcik et al. [2008]	163	57	35	66	22
Benvenuti et al. [2008]	175	70	44		
Freeman et al. [2008]	62	24	39		
Totale	**2573**	**981**	**38**	**64**	**32**

Sensitive detection of K-ras mutations

Enriched sequencing

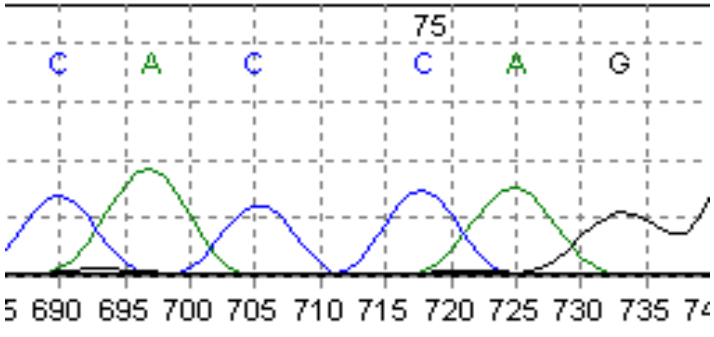


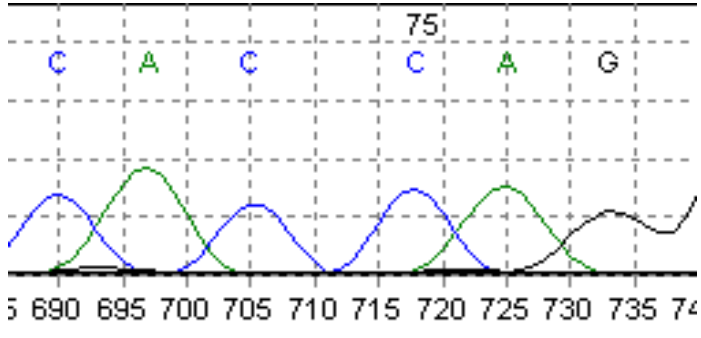
Sensitive detection of K-ras mutations

Normal Sequencing

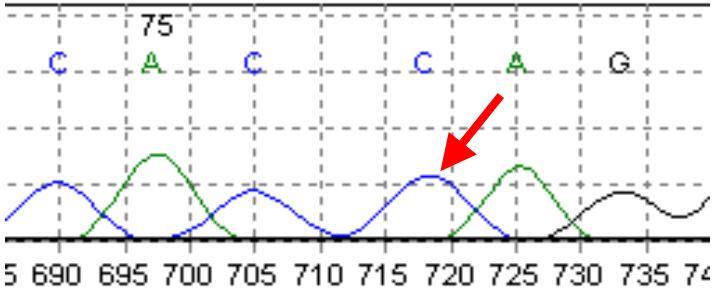
Enriched sequencing

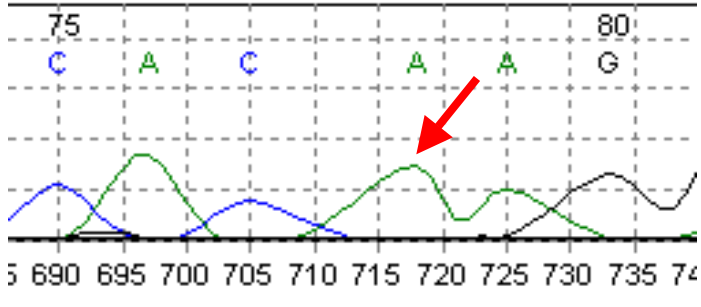
Normal





Tumor 382T





Detection limit of Enriched sequencing = 1:1000 (0,001%)

In a series of 90 colorectal carcinomas

METHOD	(%) of K-ras mutations
Direct sequencing:	39 %
Enriched sequencing	54 %

In a series of 126 NSCLC treated with Erlotinib :

METHOD	(%) of K-ras mutations
Direct sequencing:	17 %
Enriched sequencing	30 %

verall survival
(multivariate analysis)

	HR (95% CI)	*P*
KRAS mut (Direct sequencing)	2.45 (0.92-6.319)	0.044
KRAS mut (Enriched)	3.19 (1.43-7.16)	**0.005**

Ferdinand I (June 2, 1423 – January 25, 1494),

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Ferdinand I of Naples

From Wikipedia, the free encyclopedia

Ferdinand I of Naples should not be confused with Ferdinand I of the Two Sicilies, a latter king of Naples.

Ferdinand I (June 2, 1423 – January 25, 1494), also called **Don Ferrante**, was the King of Naples from 1458 to 1494. He was the natural son of Alfonso V of Aragon.

Contents [hide]

1 Biography
2 Ferdinand's reputation
3 Marriages and children
4 Notes
5 External links

Biography [edit]

In order to arrange a good future for Ferdinand, King Alfonso had him married in 1444 to a feudal heiress, Isabella of Taranto, who besides being the elder daughter of Tristan di Chiaramonte (Tristan de Clermont-Lodeve), Count of Copertino, and Catherine of Baux Orsini, was the niece and heiress presumptive of childless prince Giovanni Antonio del Balzo Orsini of Taranto. She was a granddaughter of Queen Mary of Enghien (mother of Giovanni and Catherine), who had been Queen Consort of Naples (Queen of Jerusalem and Sicily) in 1406-14.

Ferdinand I of Naples.

"He was autopsied by an Italian team. They concluded that King Ferrante I had died of a large pelvic tumor, either prostate cancer or colorectal cancer.

Two years later, in 1996, genetic research of a small part of the tumor revealed a *KRAS* mutation, which is rare in prostate cancer but is frequently encountered in colorectal tumors. Concluded is that King Ferrante I of Naples died from a colorectal tumor and is therefore the earliest documented person in history who died from this cancer."

THE LANCET

K-ras mutations in the tumour of Ferrante I of Aragon, King of Naples

A. Marchetti , S Pellegrini, G. Bevilacqua, G. Fornaciari.

Saturday 4 May 1996, vol. 347 No. 9010

K-ras mutation: G→A