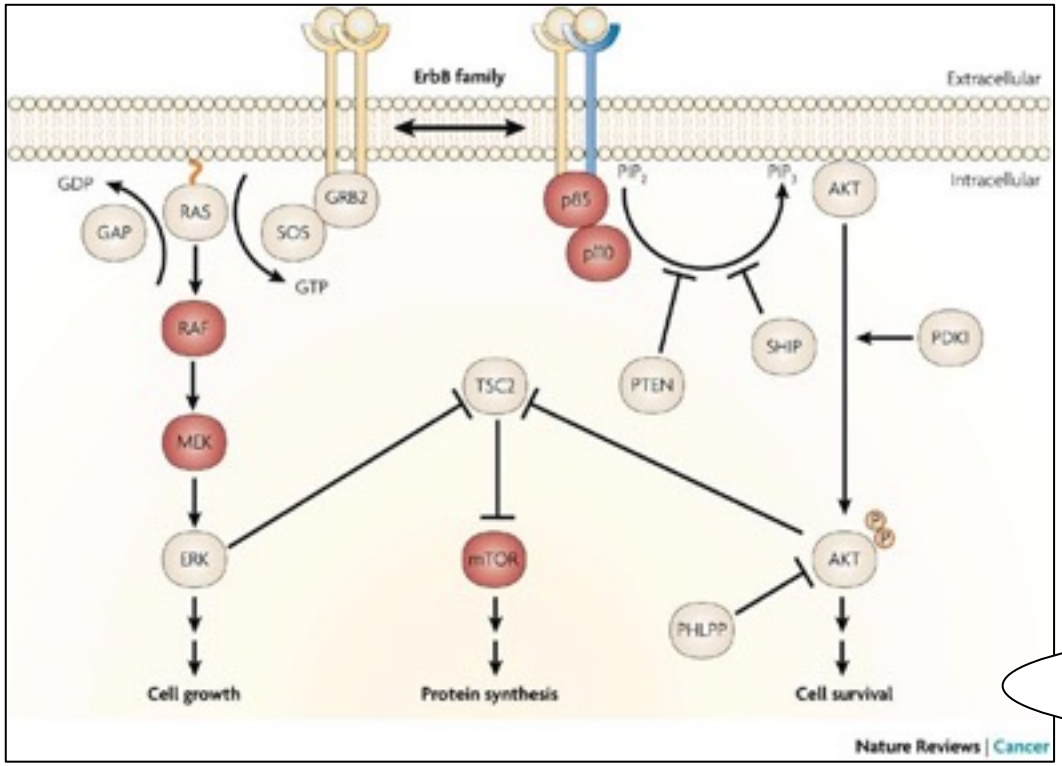
Cancer cells are dependent on the activation or the expression of one specific oncogene

Oncogene addiction

(Weinstein et al, 2000)



c-KIT/ PDGFRα in sarcomas

BCR-ABL in leukemias

HER-2/neu in breast cancer

EGFR in lung cancers

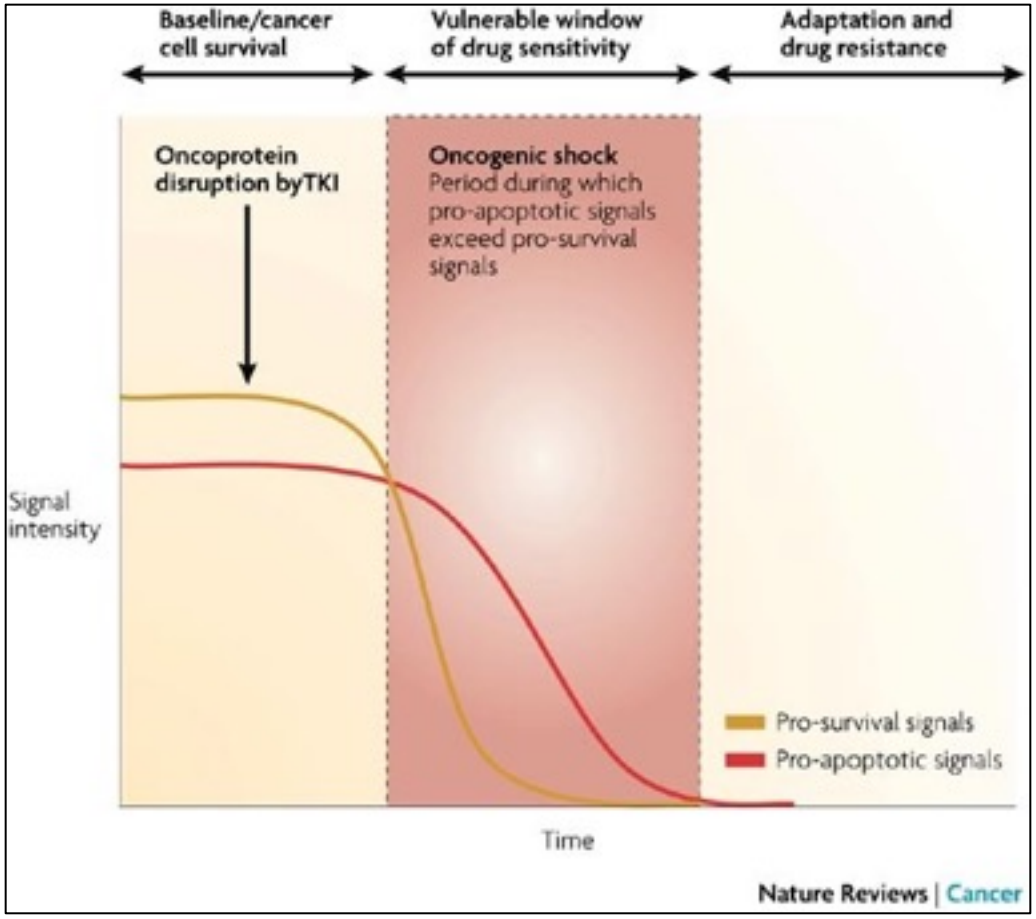
Transduction alterations inducing drugs sensibility

" Target therapy "

Sharma et al., Nature Rev, 2007

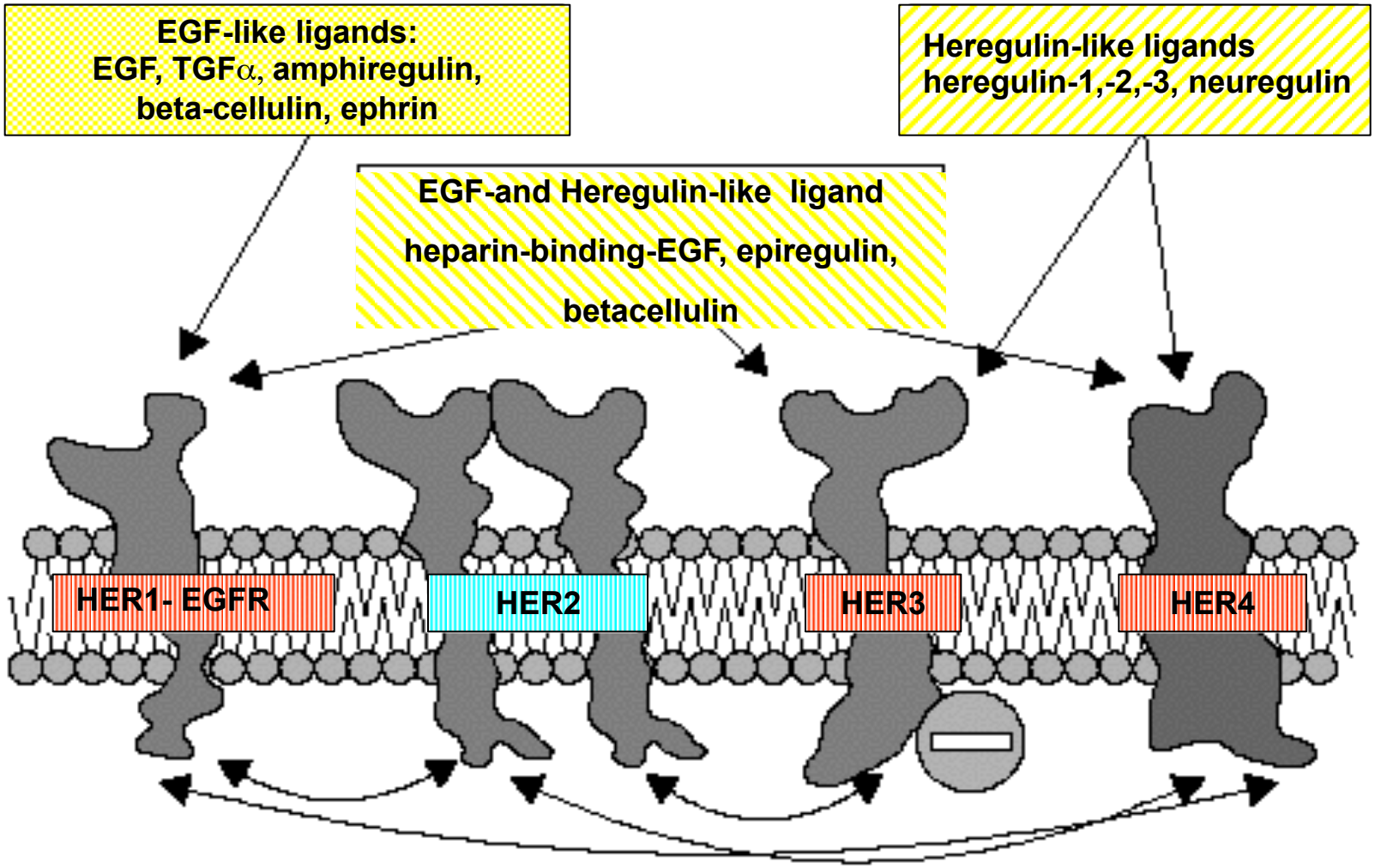
Oncogene addiction

Sharma et al., Nature Rev, 2007



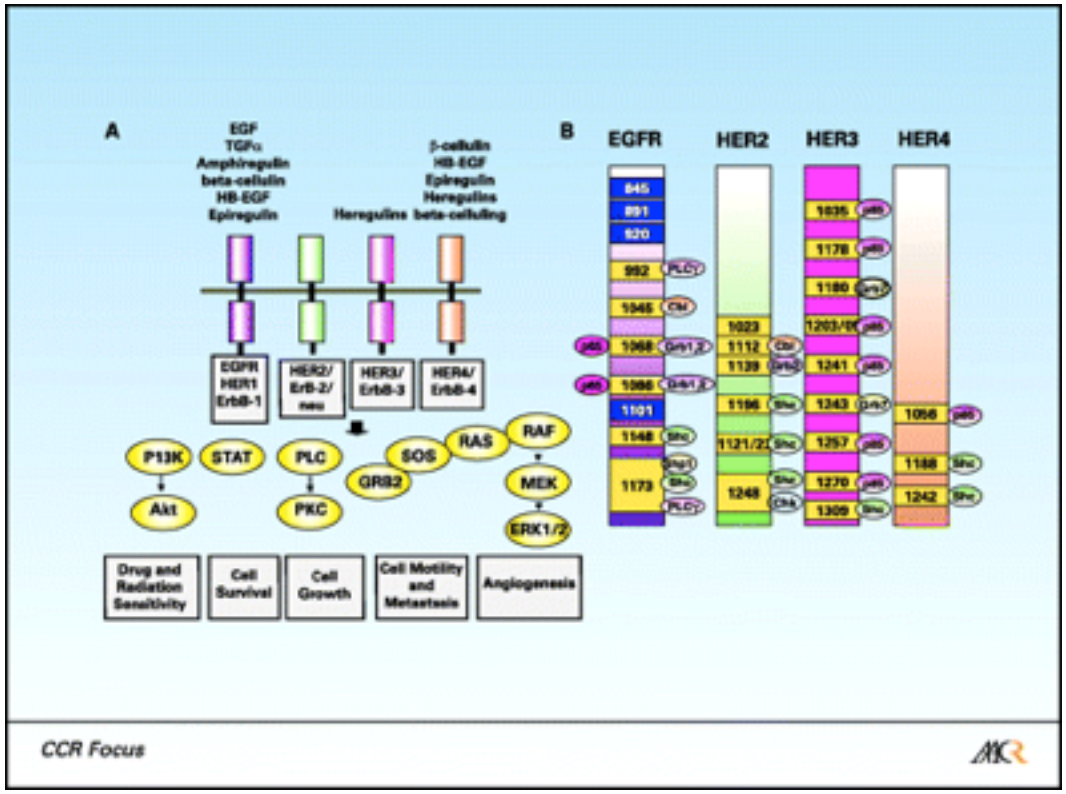
Target drugs induce an imbalance between pro-apoptotic and pro-survival signals

Epidermal Growth Factor Receptors



EGFR family pathway

Ono et al., Clin Cancer Res, 2006



Tyrosin kinase receptors

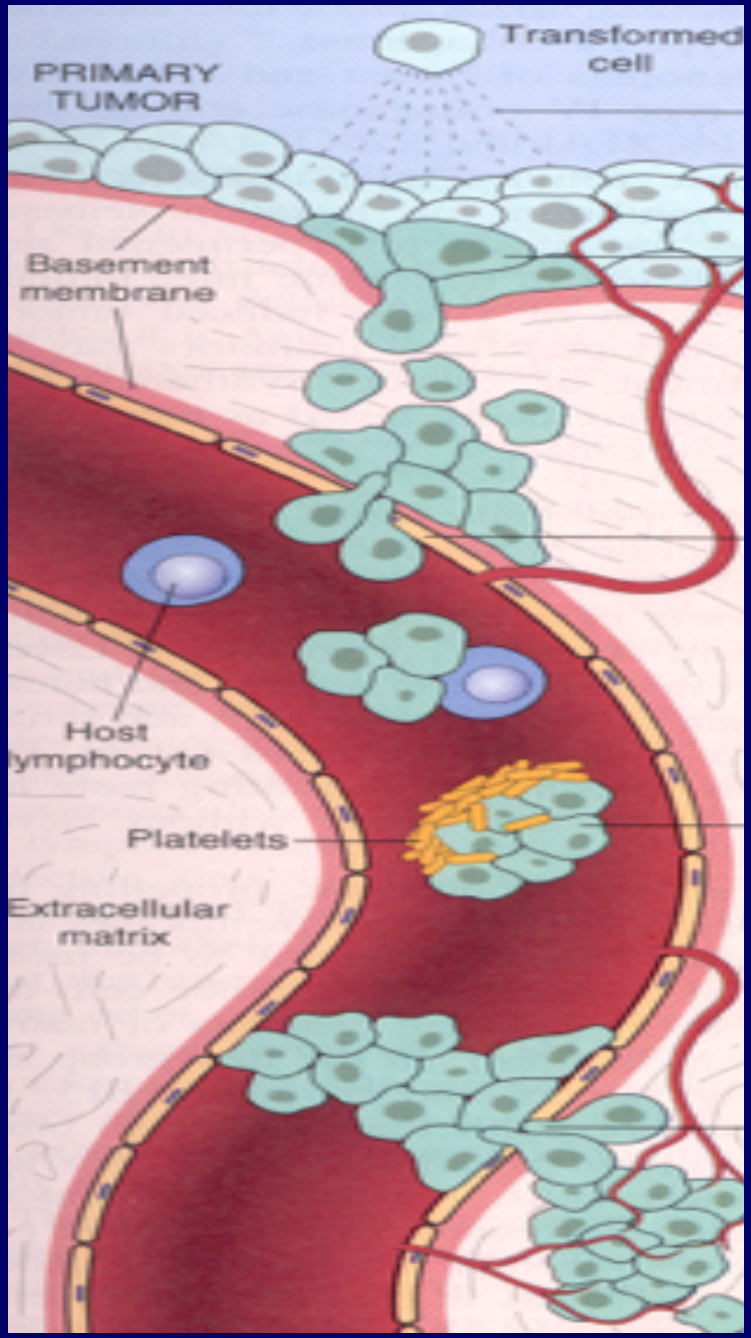
Activation

- Homo/dimerization
- Amplificaton
- Mutation

Cell transformation control

EGFR expression has been reported indifferent neoplasia

Head-neck, gastric, colon,breast,lung cancers…..



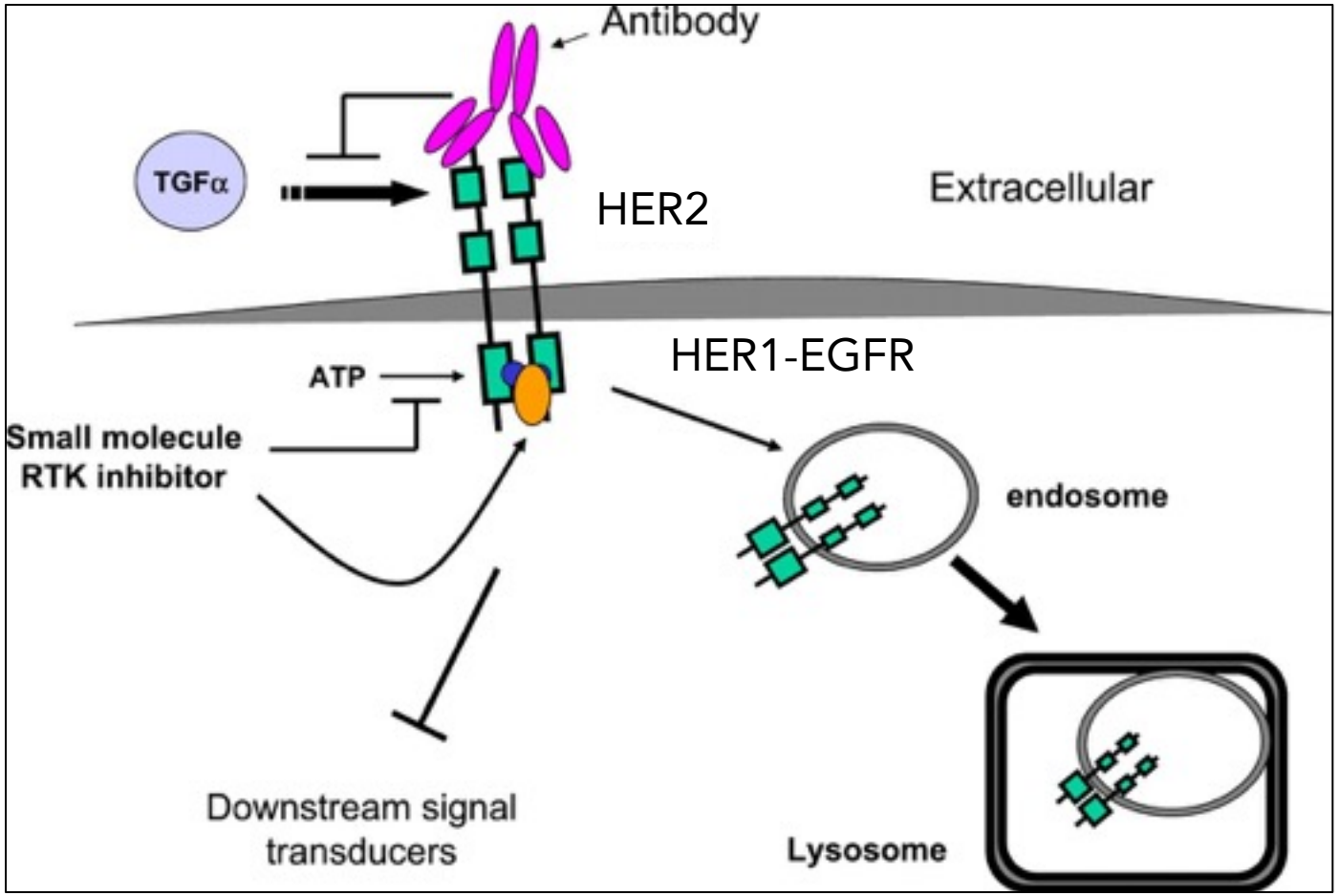
- Trasformation
- Hyperproliferation
- Apoptosis inhibition
- Invasion
- Metastatizazione
- Angiogenesis

Anti-HER2 and HER1/EGFR TKi

Monoclonal Antibodies (MAb)

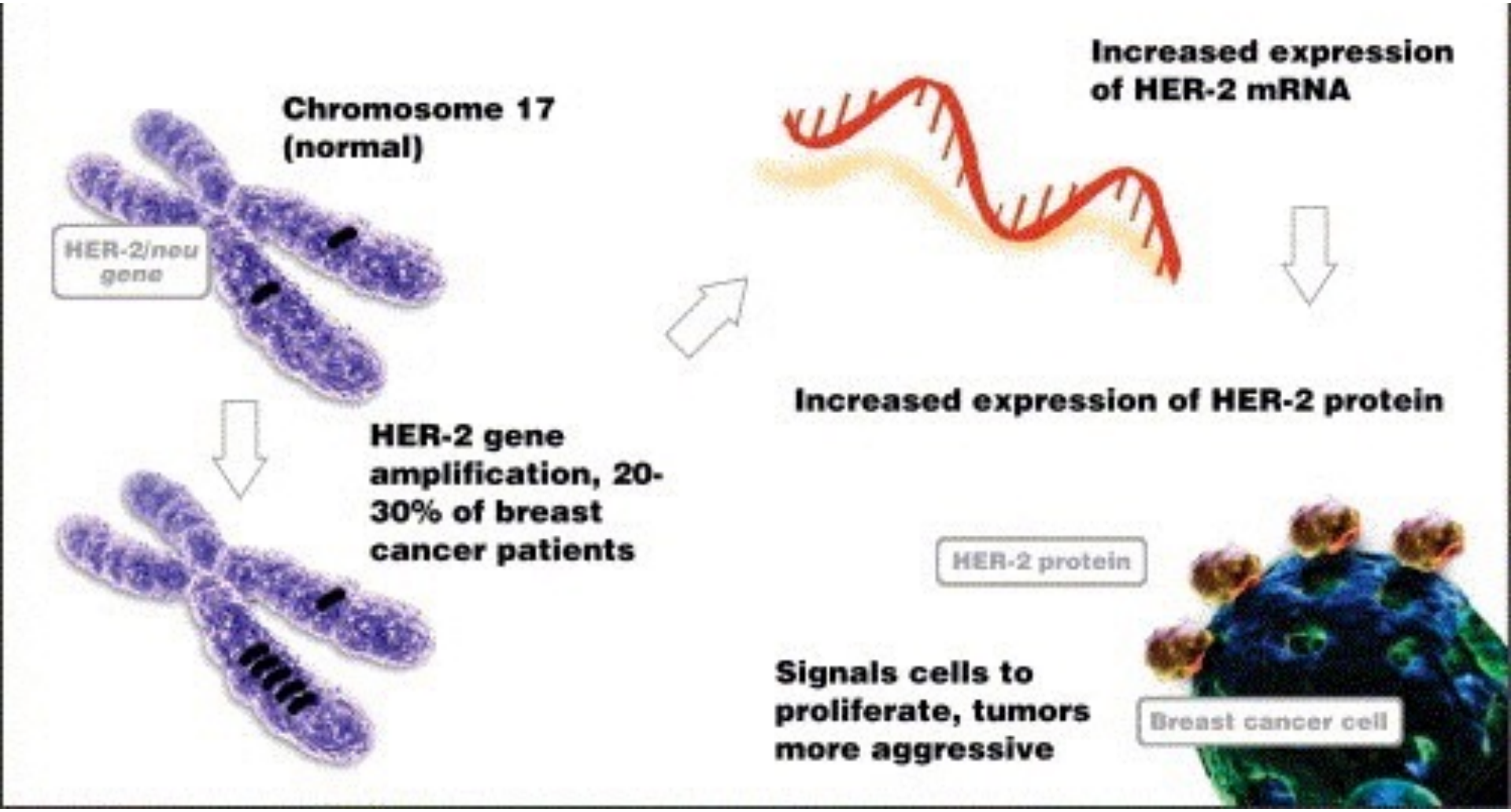
Tyrosin-Kinase Inhibitors (TKIs)

Mechanisms of action of EGFR inhibitors



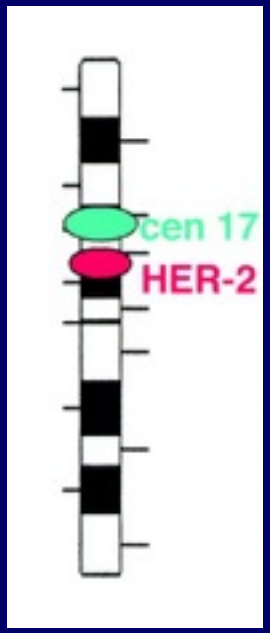
Arteaga, C. L. J Clin Oncol; 19:32s-40s 2001

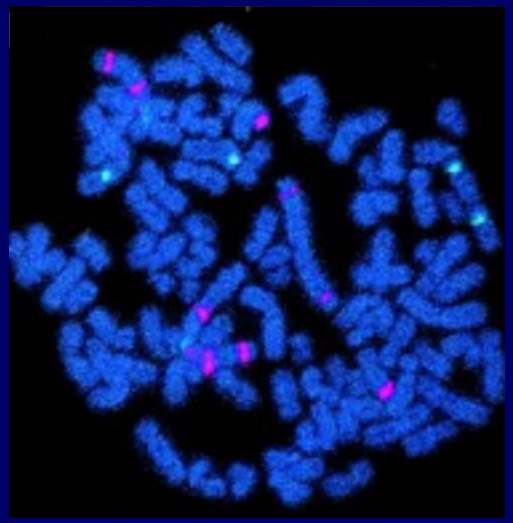
HER-2 gene in breast cancer

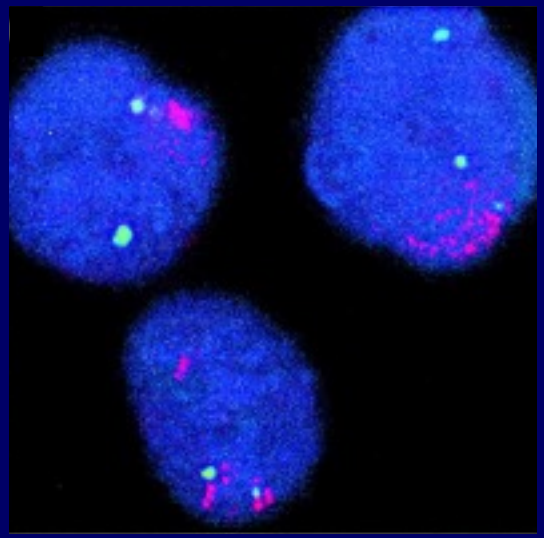


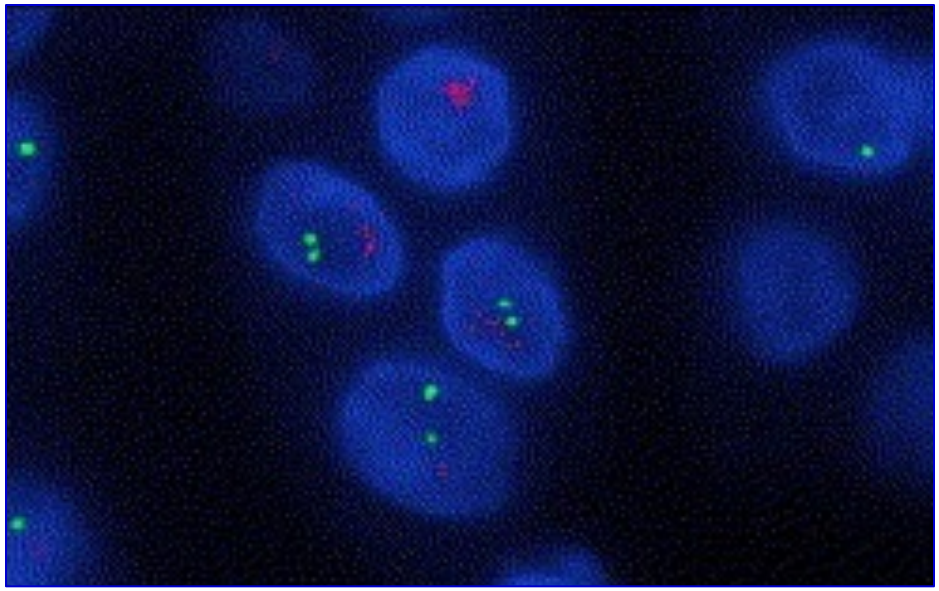
HER-2 gene in breast cancer

Dual Color FISH

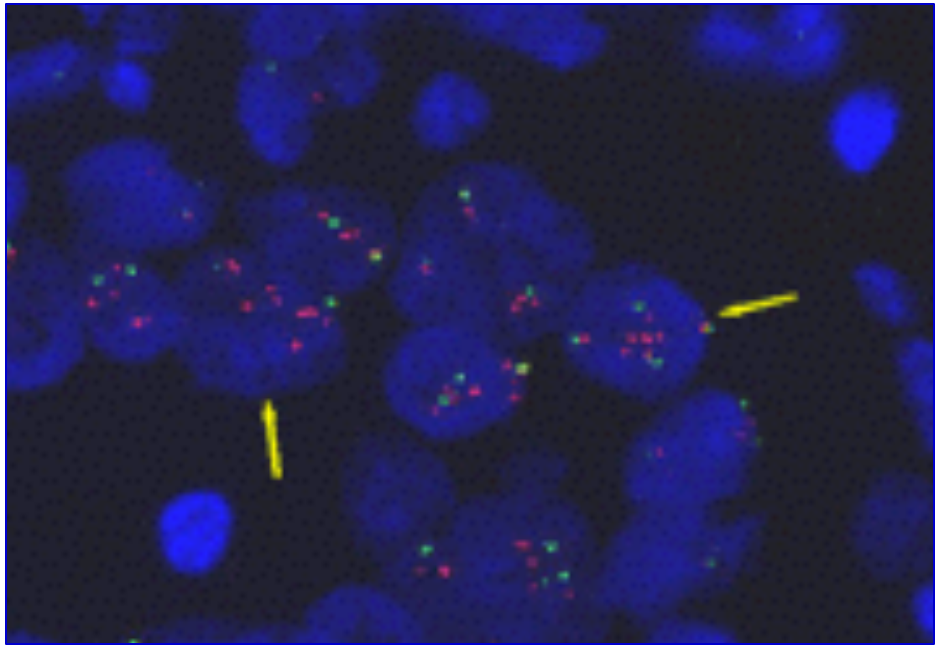


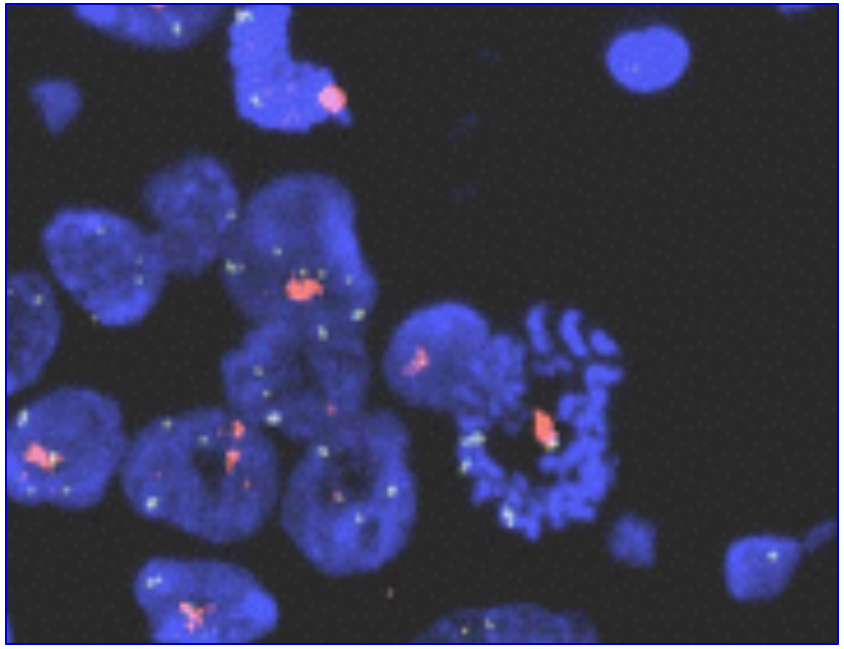




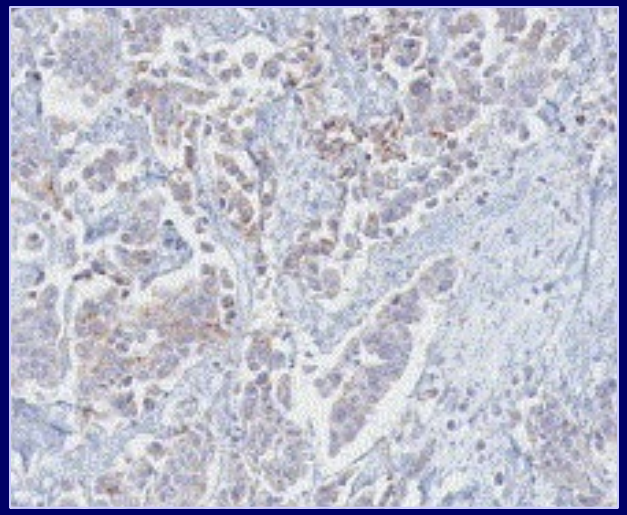


HER-2 amplification





HER-2 protein expression in breast cancer



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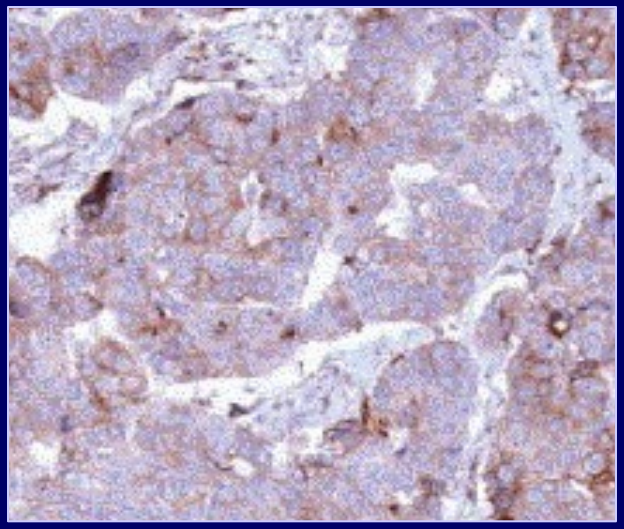
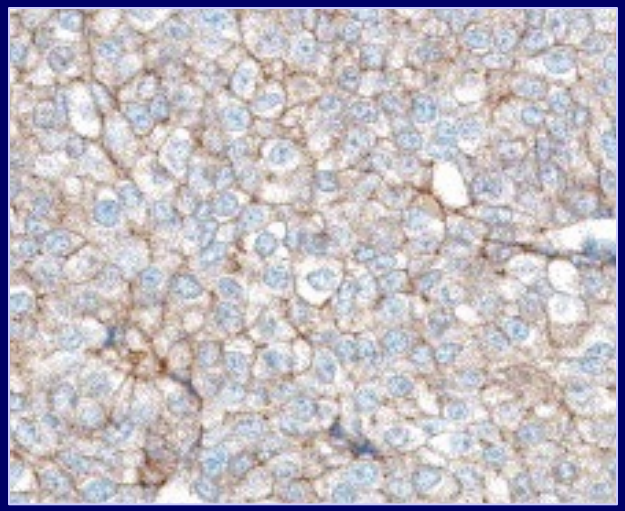
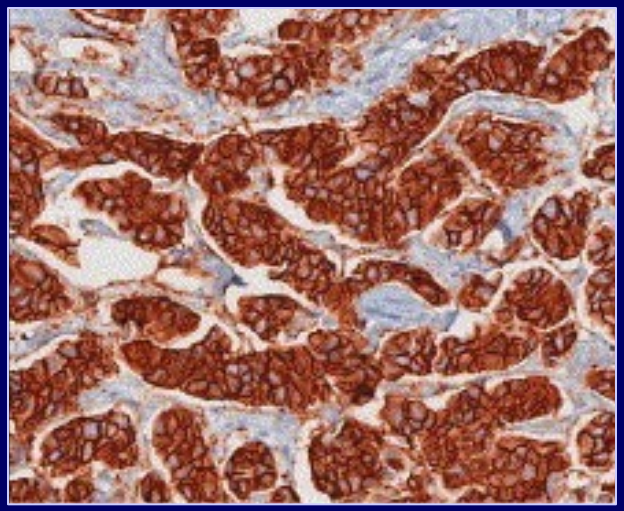


1+



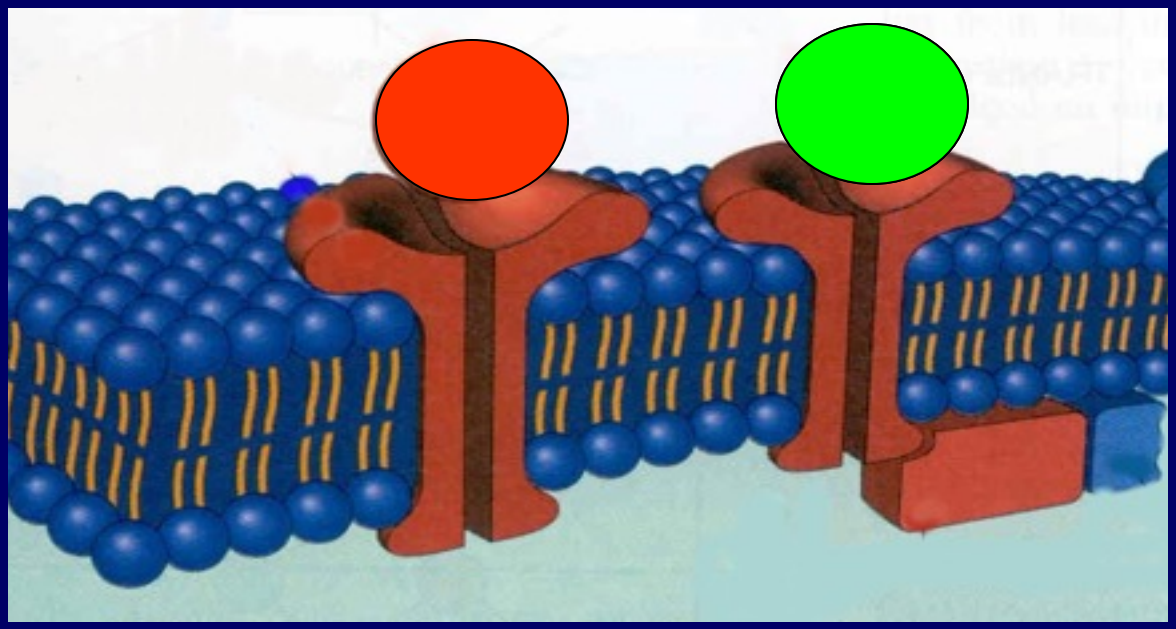
2+



3+

Inhibition by MAbs

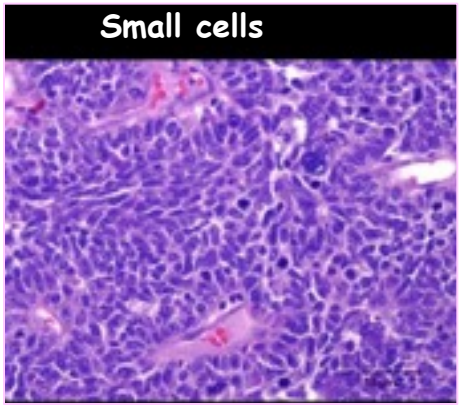
Growth factor



Monoclonal Antibody

Lung Cancer

Is the leading cause of cancer deaths worldwide



Small Cell Lung Carcinoma – SCLC

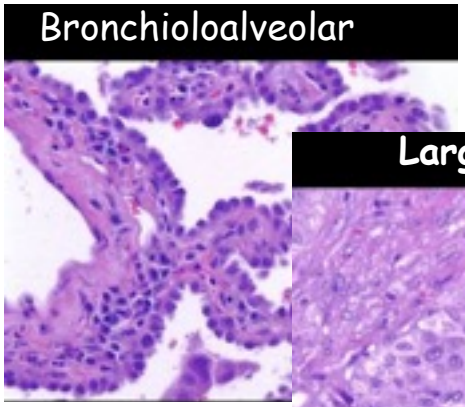
15%

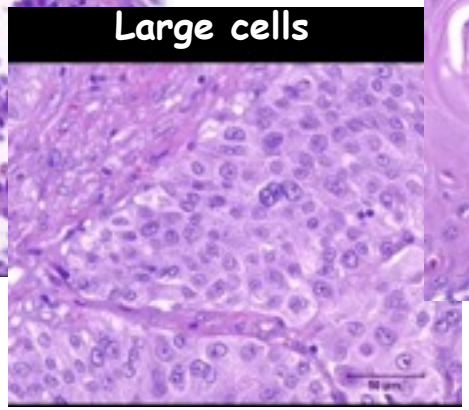
"Neuroendocrine"

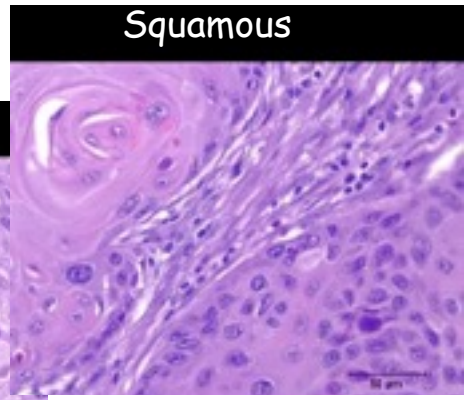
Non Small Cell Lung Carcinoma – NSCLC

85%

Lung epithelial cells





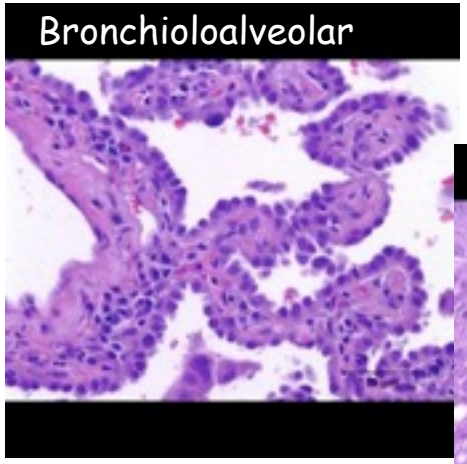


- **Adenocarcinoma**
- **Squamous cell**
- **Large cell**
- **…..**

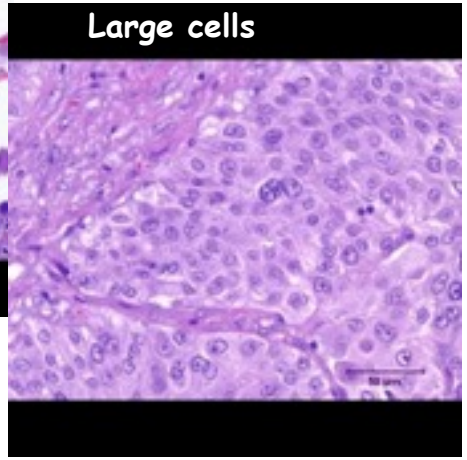
It is often diagnosed at an advanced stage…

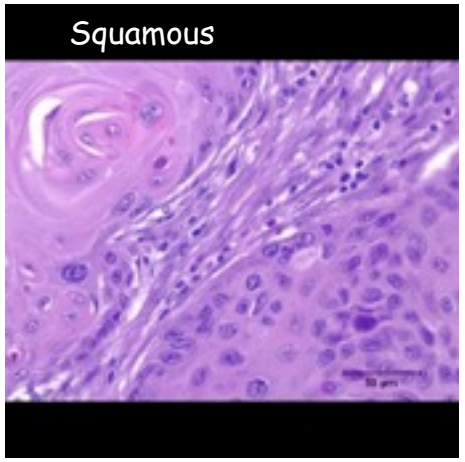
What we know about

Non Small Cell Lung Cancer



- Adenocarcinoma is the most common type of NSCLC



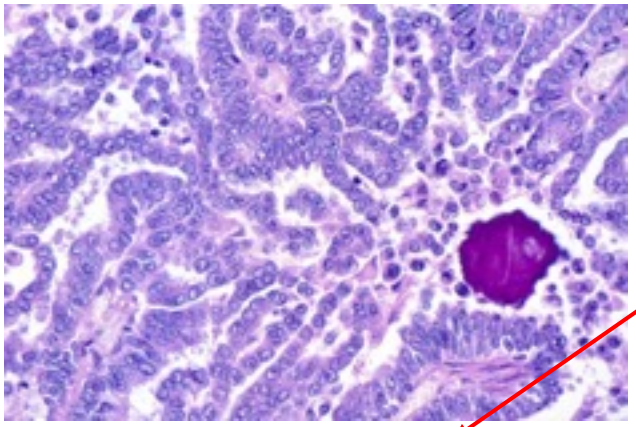


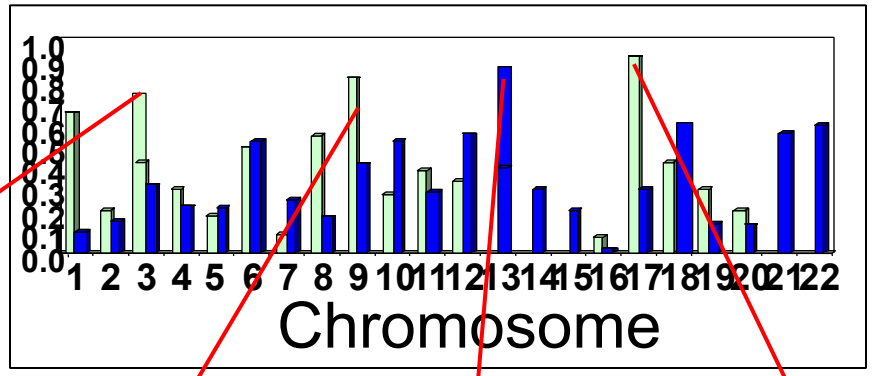
- Smoking causes all types of lung cancer but it is more related to SCLC and squamous-cell carcinoma

In NSCLC molecular origins and progression are still debated

What we know about that ?

Allelotype of NSCLC



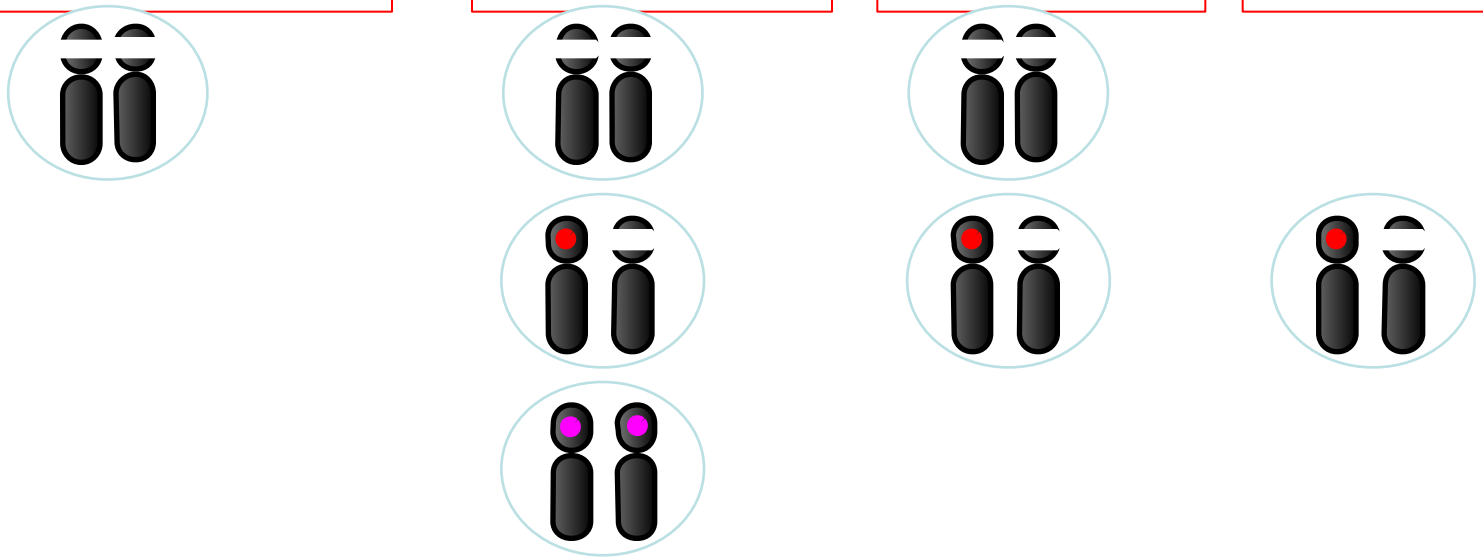


FHIT = 80%

P16 = 70%

Rb = 80%

P53 = 50%



NSCLCs can exhibit a strikingly different molecular profiles....

smoking

other causes

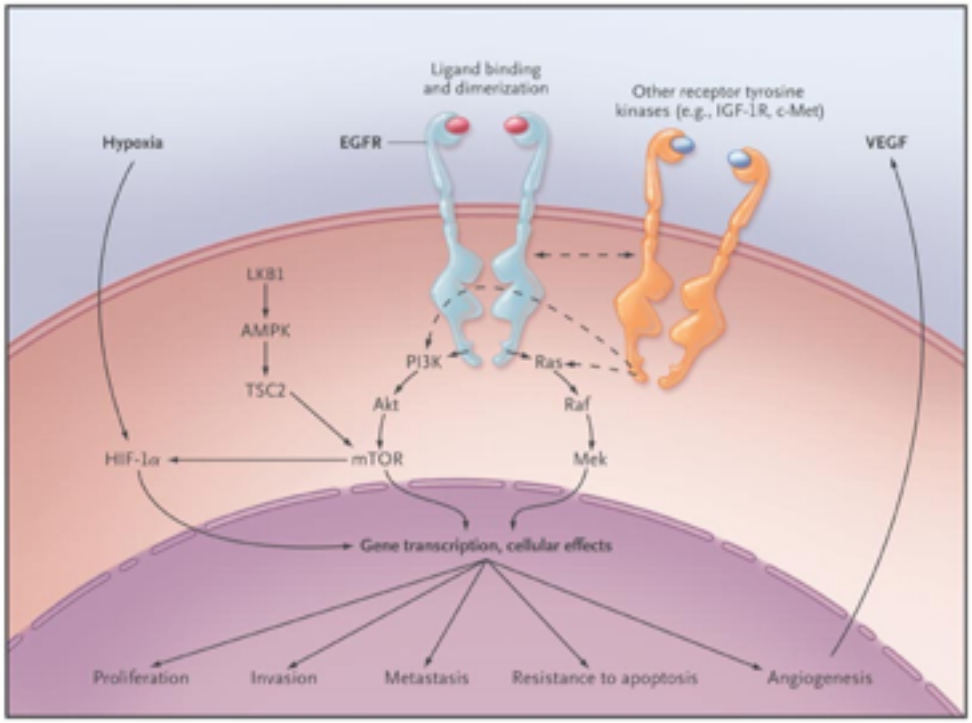
Table 1. Genetic Abnormalities Specific in the Lung to Non–Small-Cell Lung Cancer and Small-Cell Lung Cancer.*

Abnormality	Non–Small-Cell Lung Cancer		Small-Cell Lung Cancer
Precursor	Squamous-Cell Carcinoma	Adenocarcinoma	
Lesion	Known (dysplasia)	Probable (atypical adenomatous hyperplasia)	Possible (neuroendocrine field)†
Genetic change	*p53* mutation	*KRAS* mutation (atypical adenomatous hyperplasia in smokers), *EGFR* kinase domain mutation (in nonsmokers)	Overexpression of c-MET
Cancer			
KRAS mutation	Very rare	10 to 30%‡	Very rare
BRAF mutation	3%	2%	Very rare
EGFR			
Kinase domain mutation	Very rare	10 to 40%‡	Very rare
Amplification§	30%	15%	Very rare
Variant III mutation	5%¶	Very rare	Very rare
HER2			
Kinase domain mutation	Very rare	4%	Very rare
Amplification	2%	6%	Not known
ALK fusion‖	Very rare	7%	Not known
MET			
Mutation	12%	14%	13%
Amplification	21%	20%	Not known
TITF-1 amplification	15%	15%	Very rare
p53 mutation	60 to 70%	50 to 70%‡	75%
LKB1 mutation	19%	34%	Very rare
PIK3CA			
Mutation	2%	2%	Very rare
Amplification	33%	6%	4%

* Non–small-cell lung cancer includes squamous-cell carcinoma and adenocarcinoma.
† Neuroendocrine fields have been detected only in tissue surrounding tumors and have been characterized by extremely high rates of allelic loss and by c-MET overexpression (Salgia R: personal communication).
‡ Variations are based in part on smoking profiles.
§ The percentages include increased gene copy numbers from amplification or polysomy and represent percentages from resected cancers. The percentages are higher in primary tumors from patients with metastatic disease. Increased copy numbers have been reported in squamous dysplastic lesions but not in adenocarcinoma precursors.
¶ Genomic *EGFR* variant III mutations have been detected only in lung squamous-cell carcinoma, and these tumors are sensitive preclinically to irreversible EGFR tyrosine kinase inhibitors. The incidence of 5% is substantially lower than that of 30 to 40% for the detection in squamous-cell carcinoma or adenocarcinoma by immunohistochemical analysis or other techniques.
‖ The anaplastic lymphoma kinase (*ALK*) fusion gene (involving chromosome 2p), consisting of parts of *EML4* and *ALK*, is transforming in fibroblasts and occurs in adenocarcinoma but not in other types of non–small-cell lung cancer or other nonlung cancers.

Herbst et al., N Engl J Med, 2008

EGFR pathway and "target therapy" in lung cancer



EGFR was identified as a target of drug inhibitors in '90

Reversible inhibitors

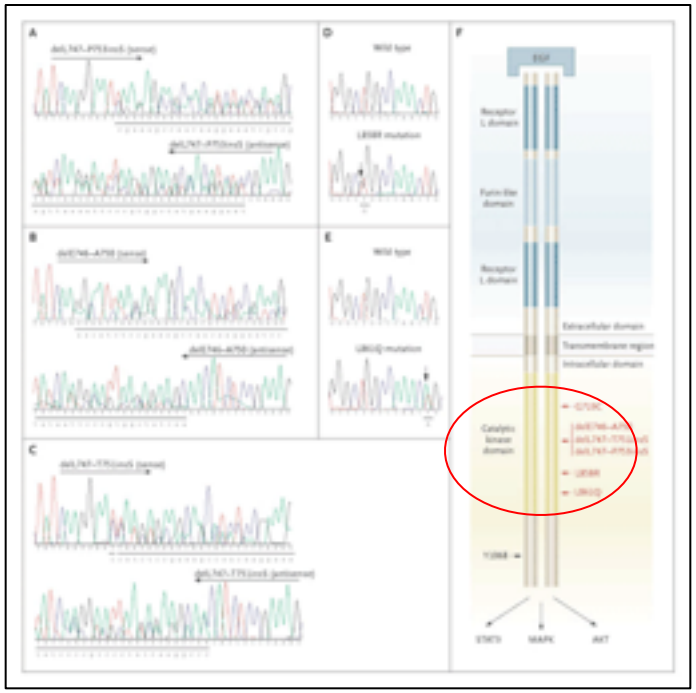
Gefitinib (Iressa)

Erlotinib (Tarceva)

were approved by FDA in 2003 and 2004

Poor results

Lynch et al., NEJM, 2004



Responsiveness to TKIs was associated with the presence of mutations in tyrosine kinase domain of EGFR

EGFR pathway must be activated to be useful as TKIs target

Responsive patients were only a subset of NSCLC (15%):

adenocarcinomas, women, non-smoking

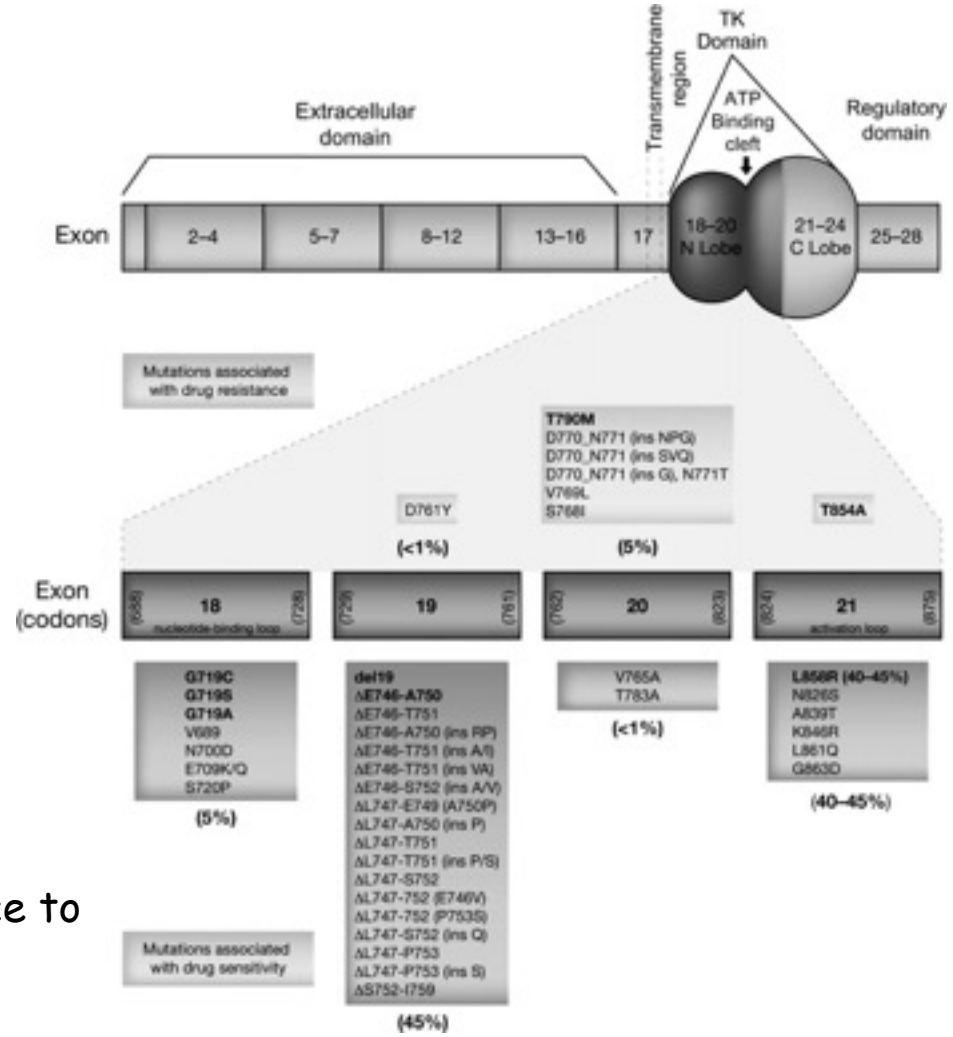
The history of EGFR mutations in NSCLC 2004-2014

Most responsive mutations are (90%):

- in-frame deletions in exon 19
- missense mutations L858R in exon 21

Rare mutations in exons 18-21 are also associated with TKI responsiveness

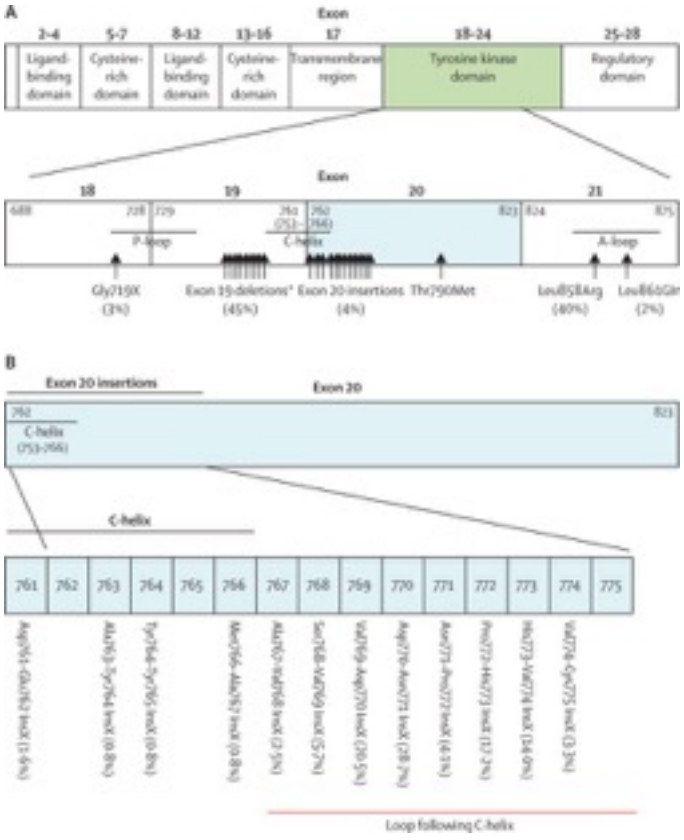
Mutations in exon 20 confer resistance to TKIs treatment



Gately K et al., J Clin Pathol 2012;65:1-7

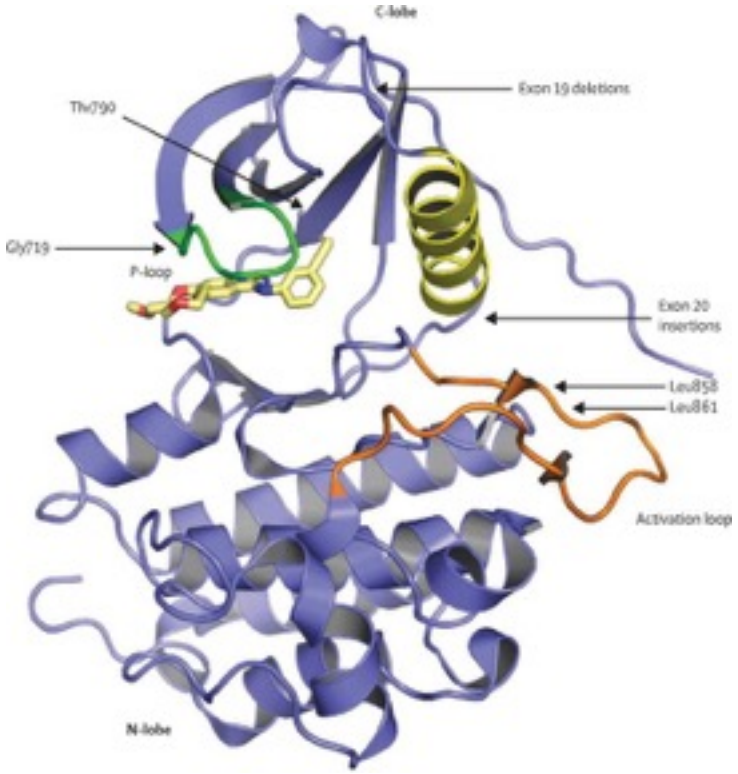
The history of EGFR mutations in NSCLC (2004-2014)

New mutations…



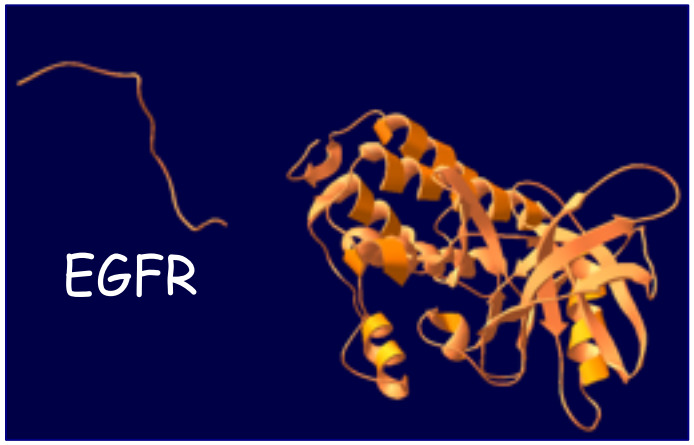
Yasuda et al., Lancet Oncol.,2012

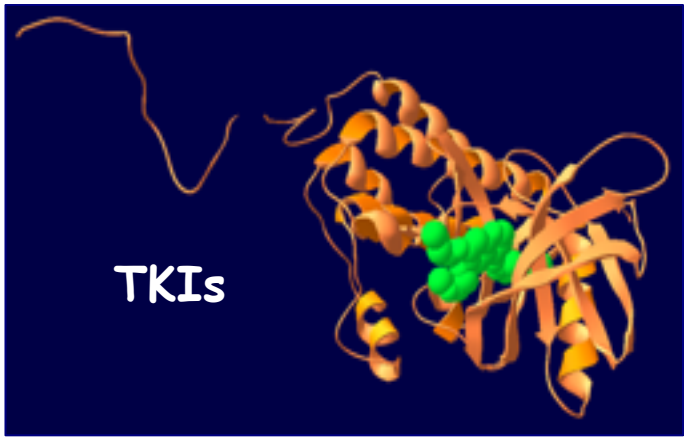
Insertions in exon 20 account for 4%

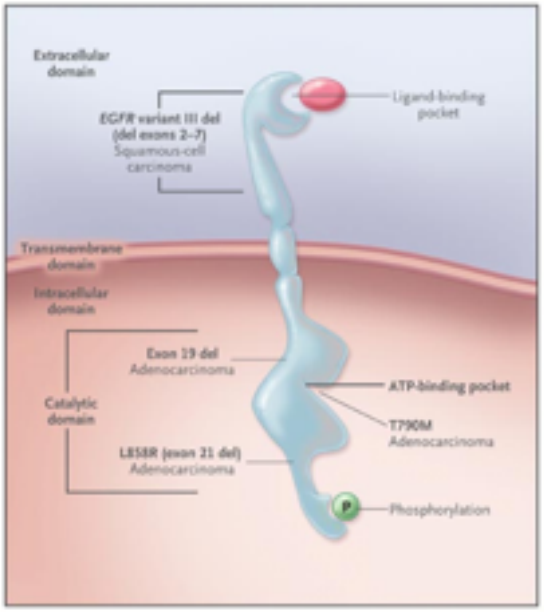


No responsiveness to TKi treatment ?

Why only some mutations confer TKI sensitivity ?



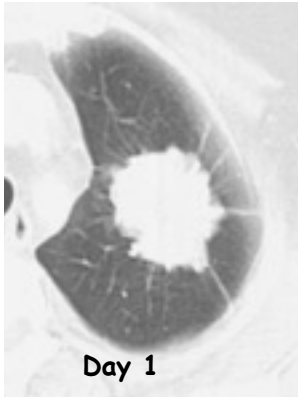


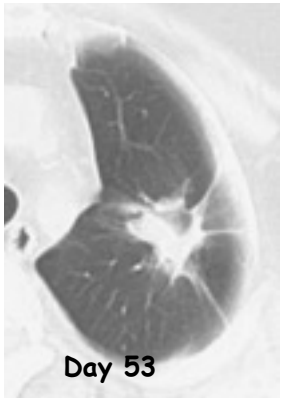


Gefitinib/erlotinib compete with ATP for the same site in the ATP-binding pocket.

Exon 19 del and L858R are located near this site

TKIs treatment causes a shrinkage of the primary lesions and metastasis

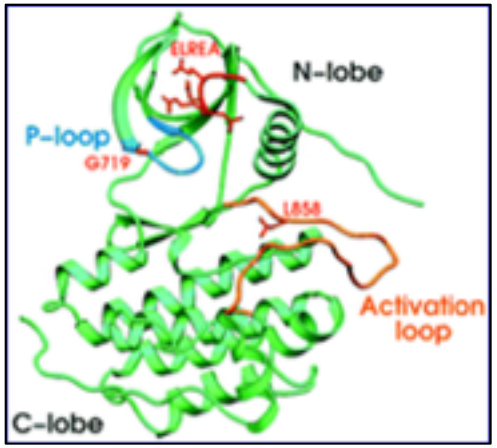




adenocarcinoma with exon 19 Δ in 77 years old woman

Responsiveness to TKIs depends on the type of the EGFR mutation

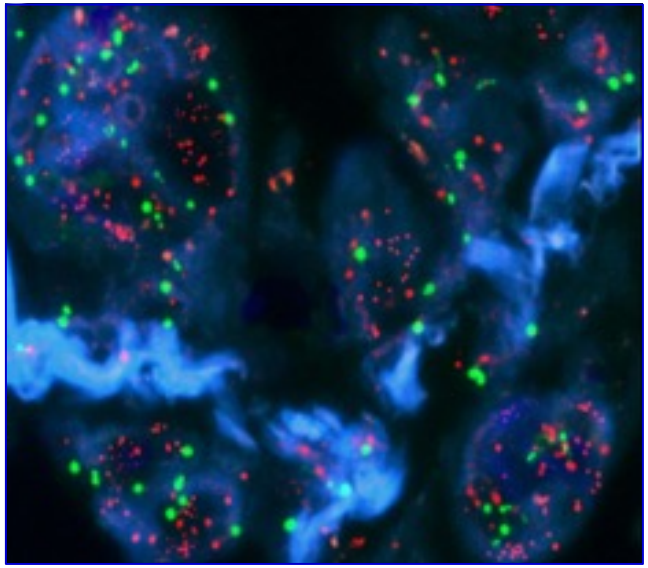
Disease-free survival (DFS)		Overall-survival (OS):	
exon 19 Δ	12 months	Exon 19 Δ	36 months
L858R	5 months	L858R	8 months



This difference depends on the ability of the drugs to inhibit the downstream phosphorylation of mutated EGFR

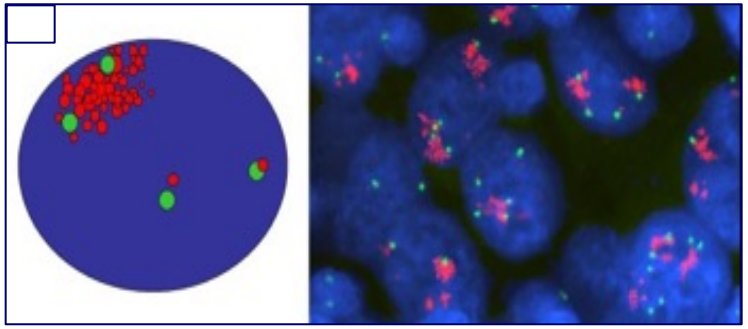
EGFR and TKi treatment

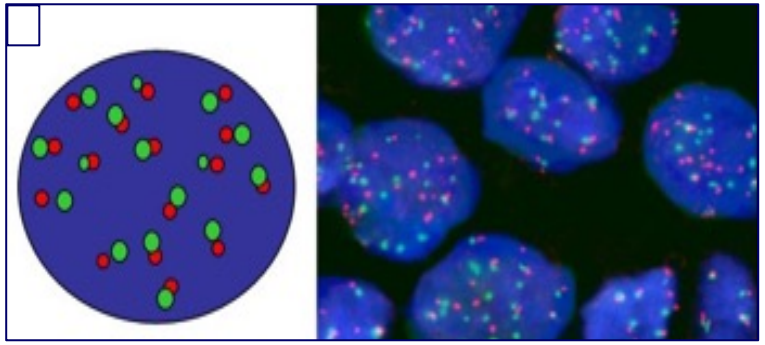
EGFR increased copy number (ICN) in addition to EGFR mutations



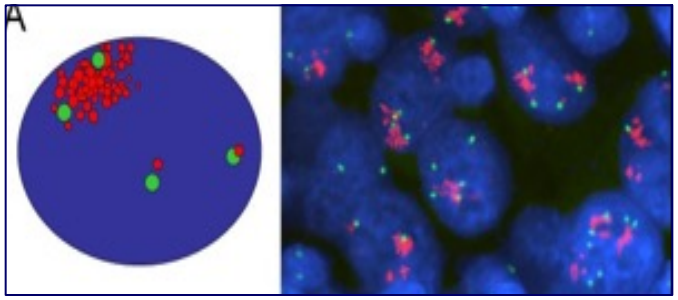
Cappuzzo et al., J Natl Cancer Inst 2005

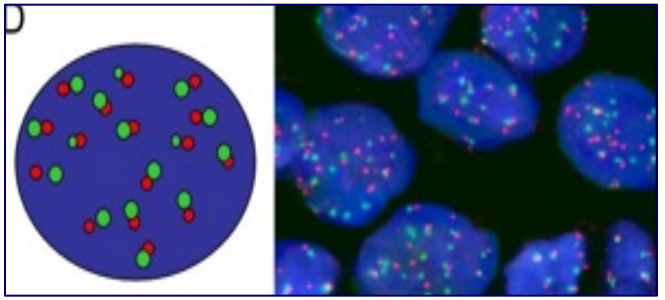
EGFR amplification and chromosome 7p polysomy may be associated with TKIs responsiveness





Amplification and polysomy are different molecular mechanisms !

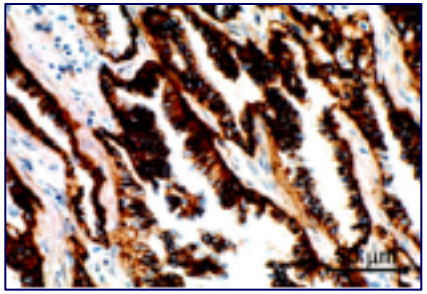


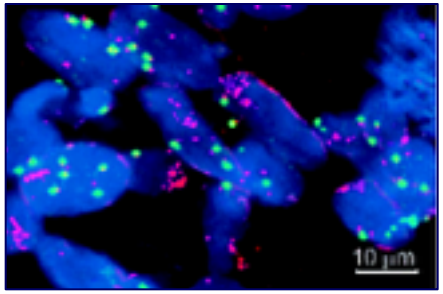


University of Colorado Cancer Center (UCCC) criteria

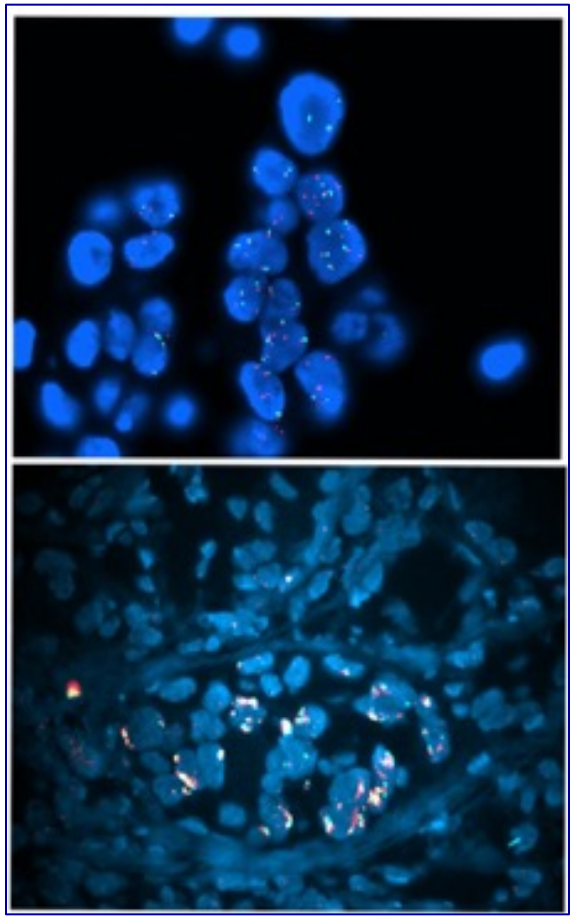
- **Polysomy** > 4 EGFR copies in > 40% of the cells,
- **Gene amplification** a) EGFR/centromero 7 > 2, b) presence of gene clusters (4-10 copies) in >10% cells, c) brighter EGFR signals in >10% of the cells , d) >15 copies of EGFR signals in >10% of the cells

It has not been found a good correlation between IIC expression and FISH





Contrasting data between EGFR ICN and TKI sensitivity



Different studies have been affected by some limitations since only few of these compared EGFR mutations and FISH

A good correlation between mutations and amplifications on the same allele has been shown

Takano et al., JCO, 2005

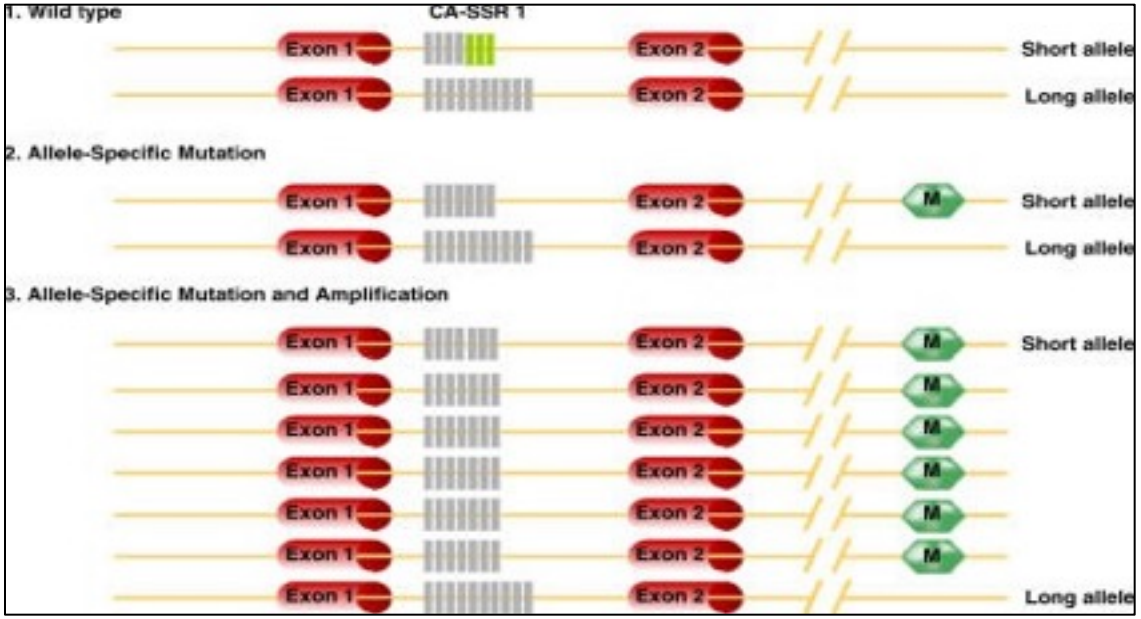
Okabe et al., Cancer Res., 2007

Sholl et al.,Cancer Res.,2009

What mechanisms underlying the association of mutation and amplification in the same EGFR allele ?

Three polymorphisms have been found associated with increased EGFR protein production - shorter CA-SSR1 length, in intron 1, and the variant forms of SNPs - 216 and – 191 in the promoter-

EGFR mutations were found to favor the shorter allele of CA-SSR1, and selective amplification of the shorter allele of CA-SSR1 occurred frequently in tumors harboring a mutation.

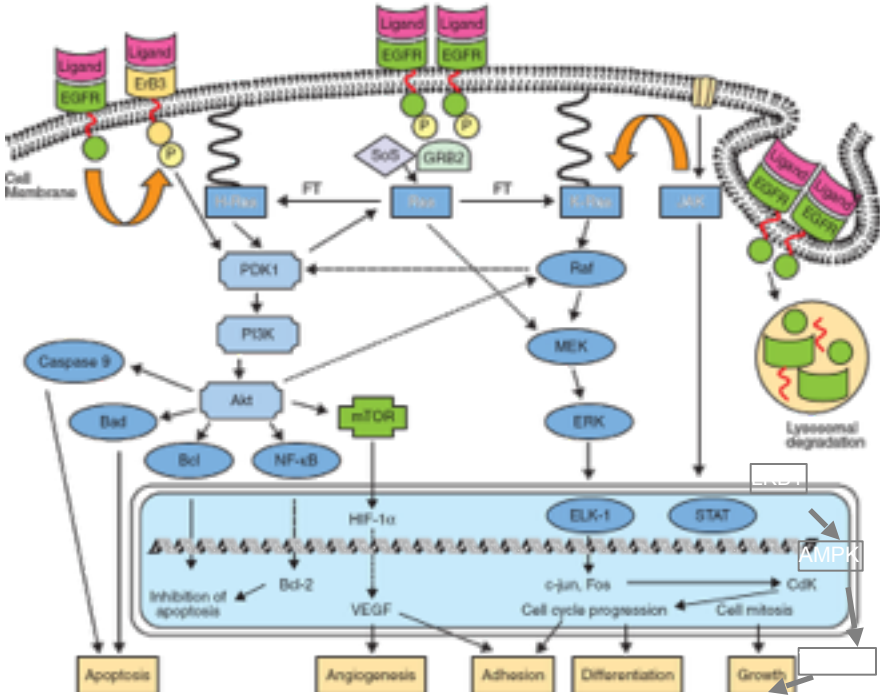


Nomura et al., Plos Med, 2007

Similar associations have been reported in other oncogenes - cKIT or KRAS

Primary resistance to TKIs treatment

TKi responsiveness is abrogated by the acquisition of genetic alterations affecting genes in other pathway or in the same pathway



MAPK pathway gene mutations

MET gene amplifications

HER2 mutations

Alternative drugs targeting

- pathway P13K-mTOR-AKT
- pathway RAS-RAF-MEK-ERK-ERK

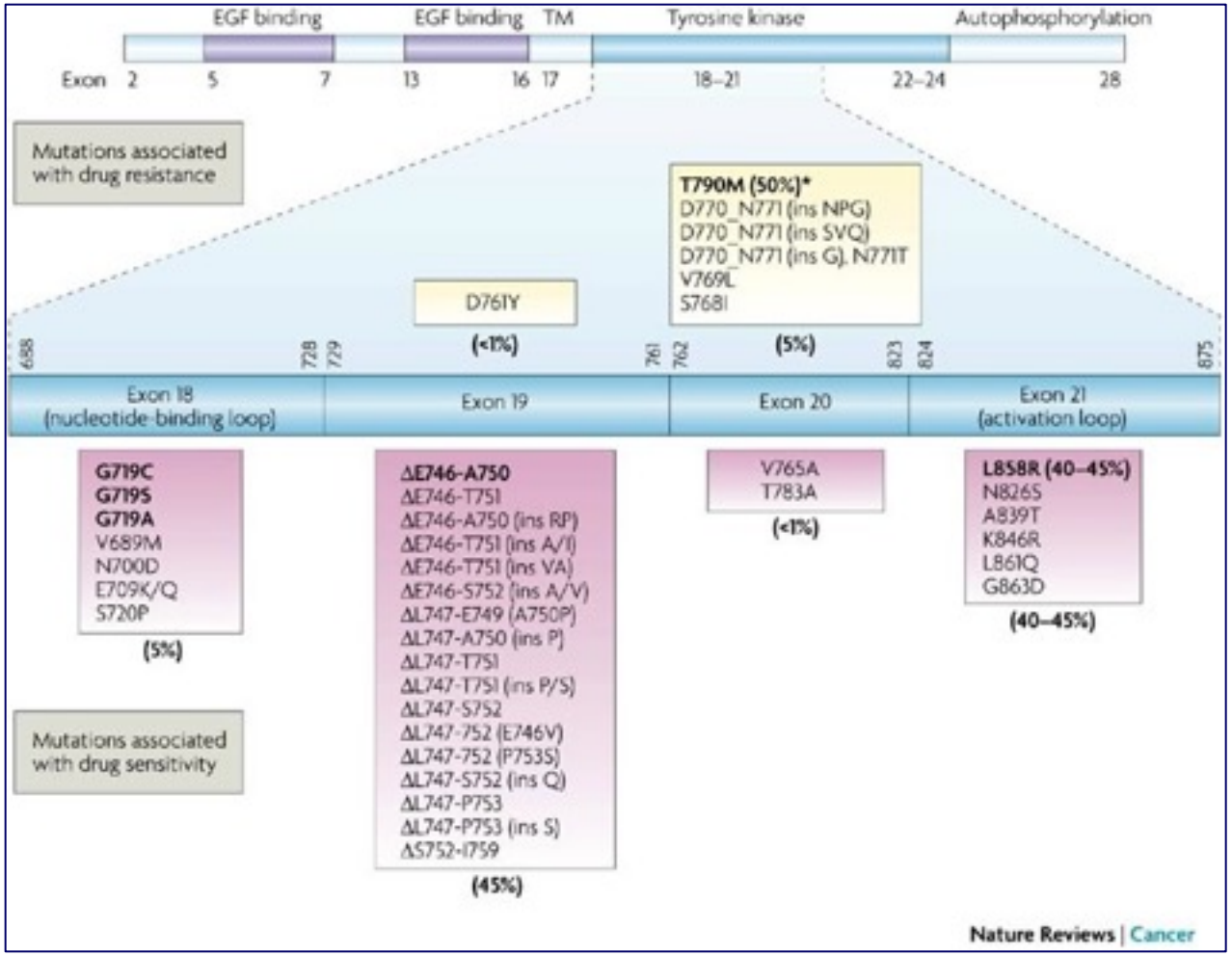
Secondary resistance to TKIs treatment

Second EGFR mutations on exon 20, particularly T790M

Responsiveness to TKIs can be abrogated by secondary mutations in EGFR kinase domain

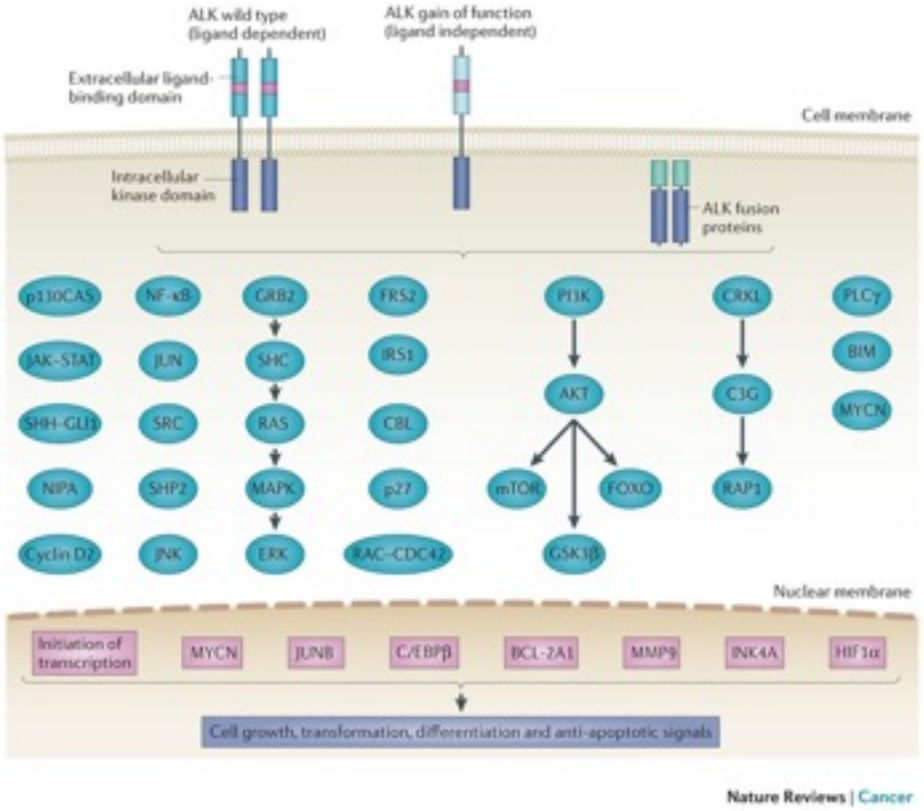
the story of T790M

T790M is selected by TKI treatment



Other genetic abonormalities with «driver» characteristics in NSCLC : anaplastic lymphoma kinase (ALK) gene rearrangements

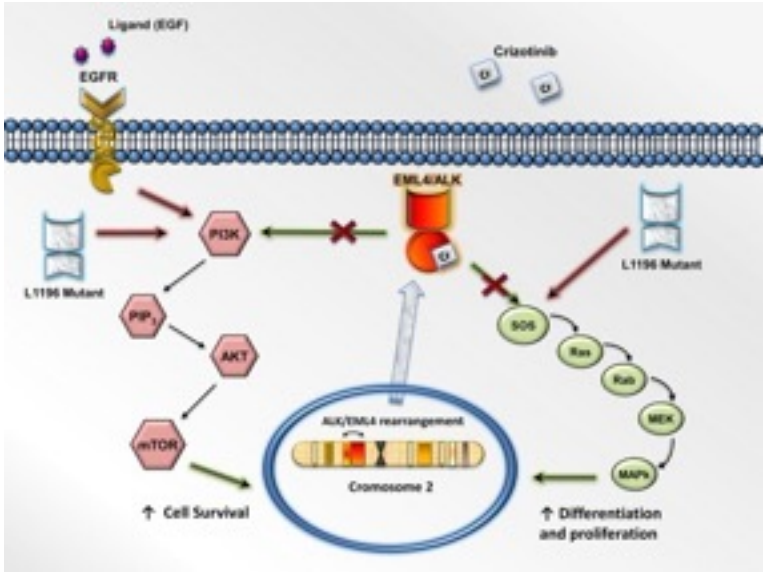
chromosome 2p inversion leading to a fusion with echinoderm microtubule–associated protein like 4 (EML4) gene

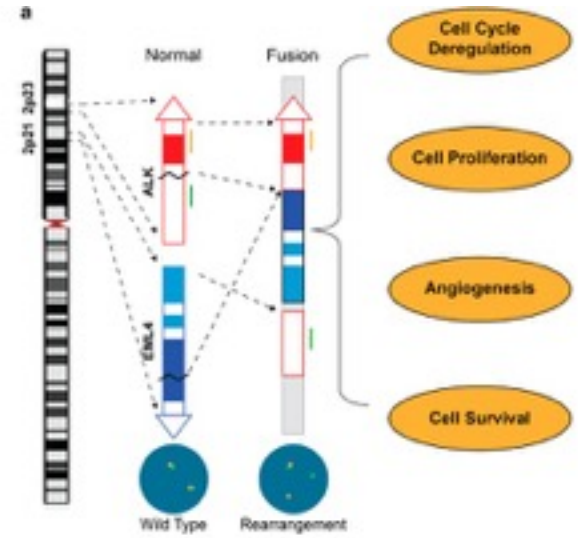


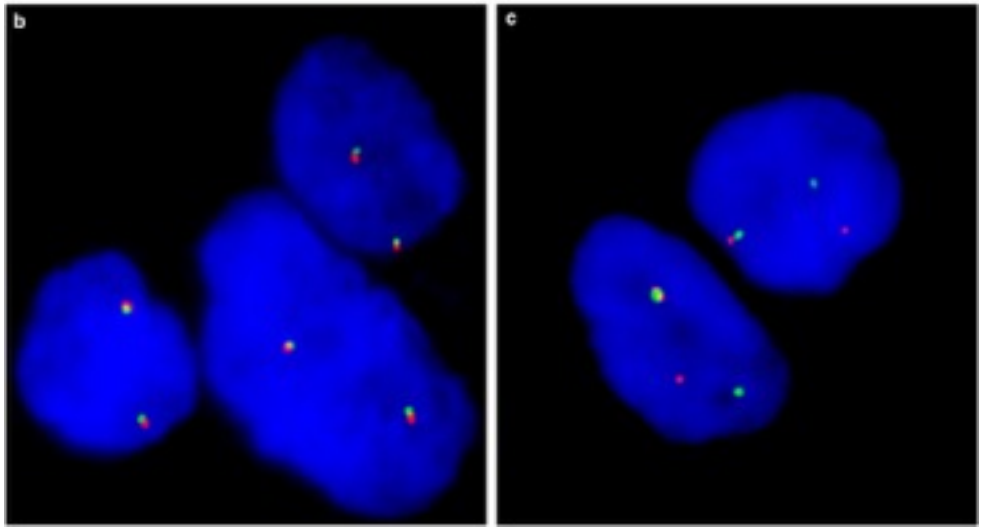
Hallberg and Palmer, Nat Rev Cancer, 2013

This rearrangement occurs in **2-5%** NSCLC, 50 years or younger, never or former smokers with adenocarcinoma, independently of EGFR or KRAS gene mutations

A fluorescent in situ hybridization assay was approved by the US Food and Drug Administration (FDA) as the standard method for the detection of ALK gene rearrangement







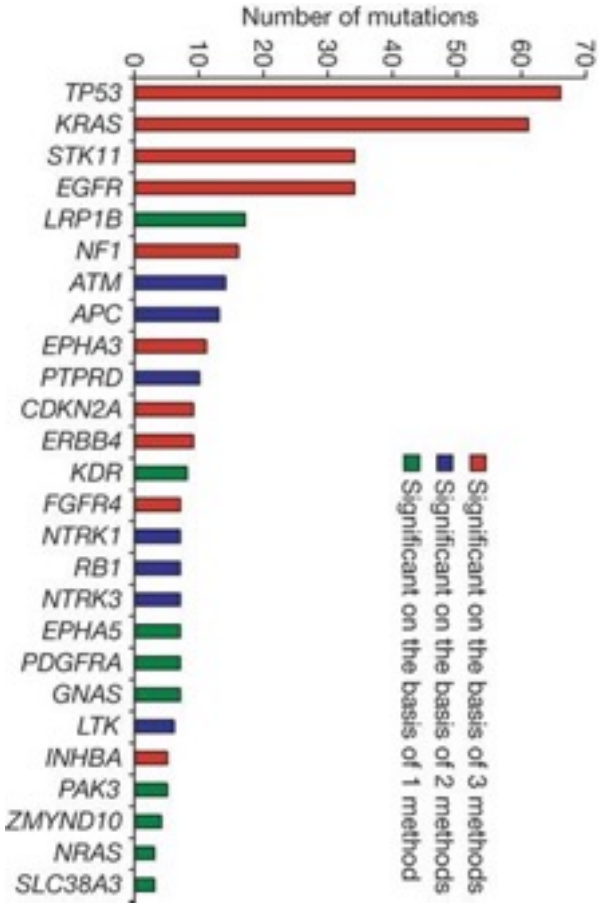
Crizotinib, a first-in-class dual ALK and c-MET inhibitor, has been shown to be particularly effective against ALK positive NSCLC

The genomic approach…

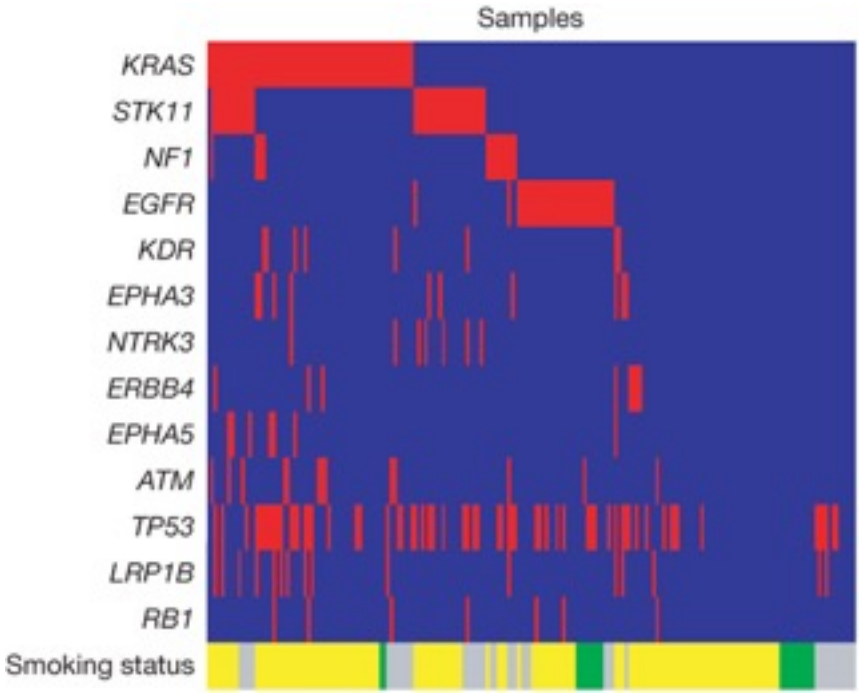
L Ding *et al. Nature* **455**, 1069-1075 (2008)

188 primaries screened for 623 candidate genes

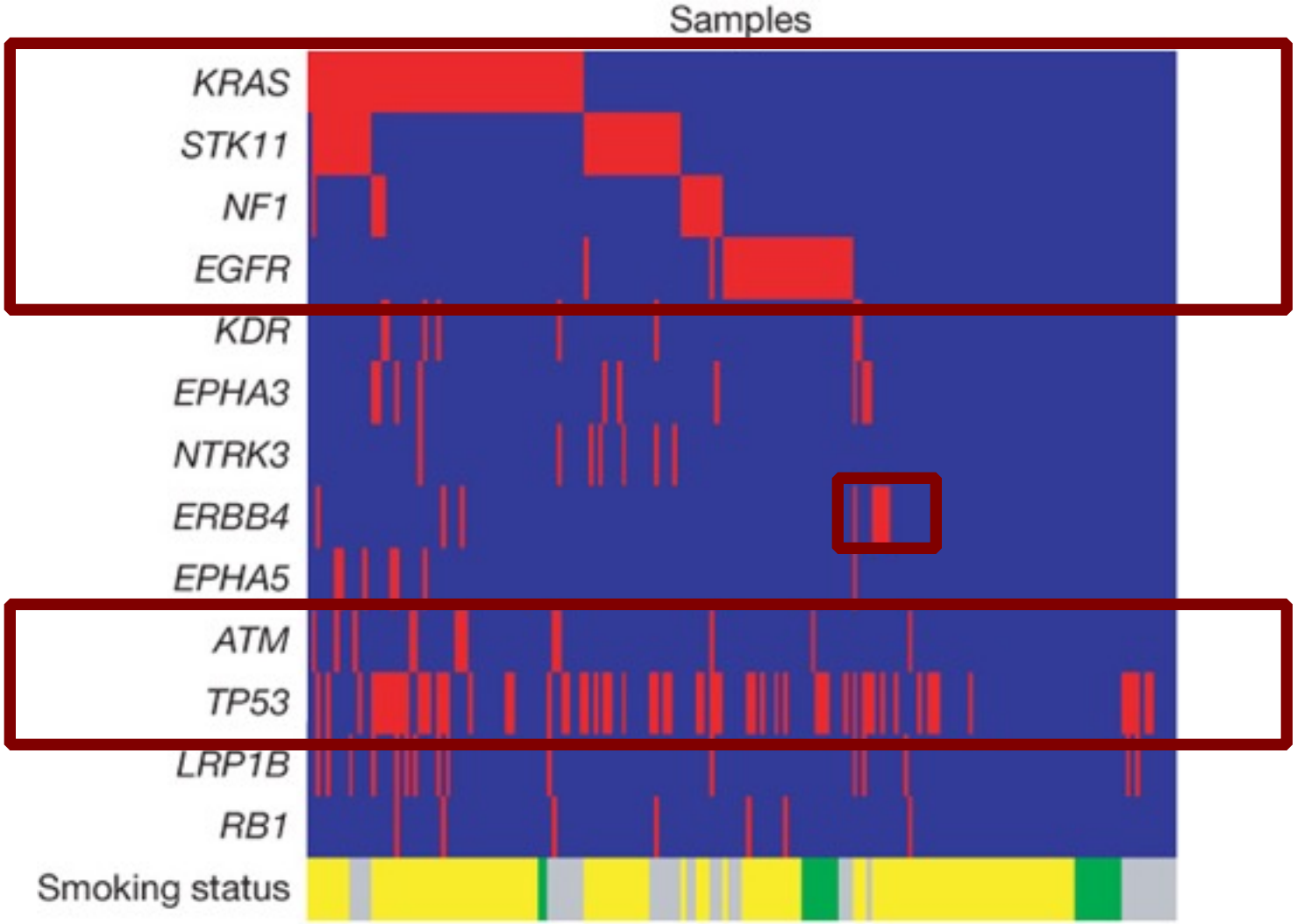
26 significantly mutated genes in lung adenocarcinomas



Concurrent and mutual exclusion of mutations observed across genes in lung adenocarcinomas.



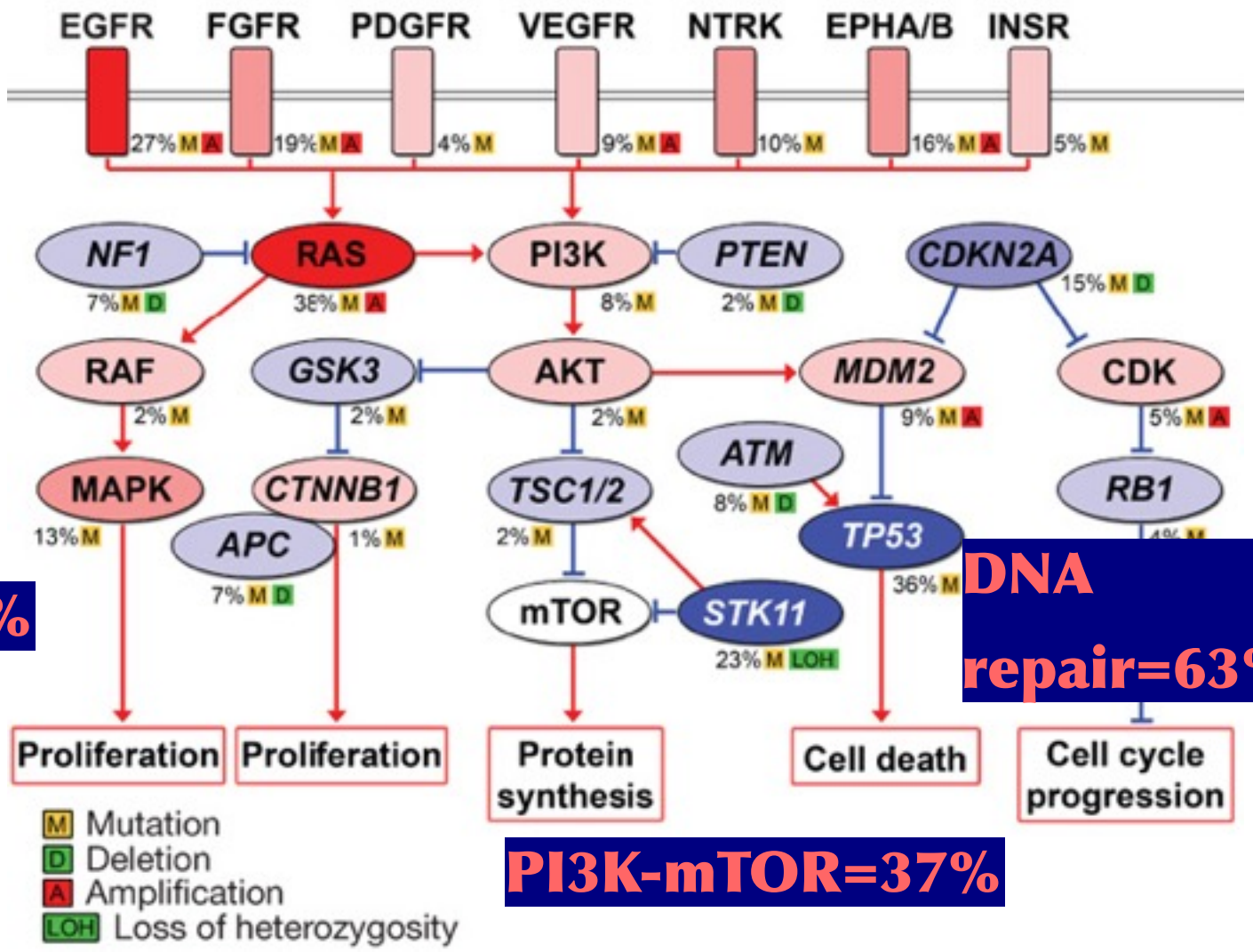
Concurrent and mutual exclusion of mutations observed across genes in lung adenocarcinomas



Ding et al., Nature, 2008

Significantly mutated pathways in lung adenocarcinomas

RTK=90%



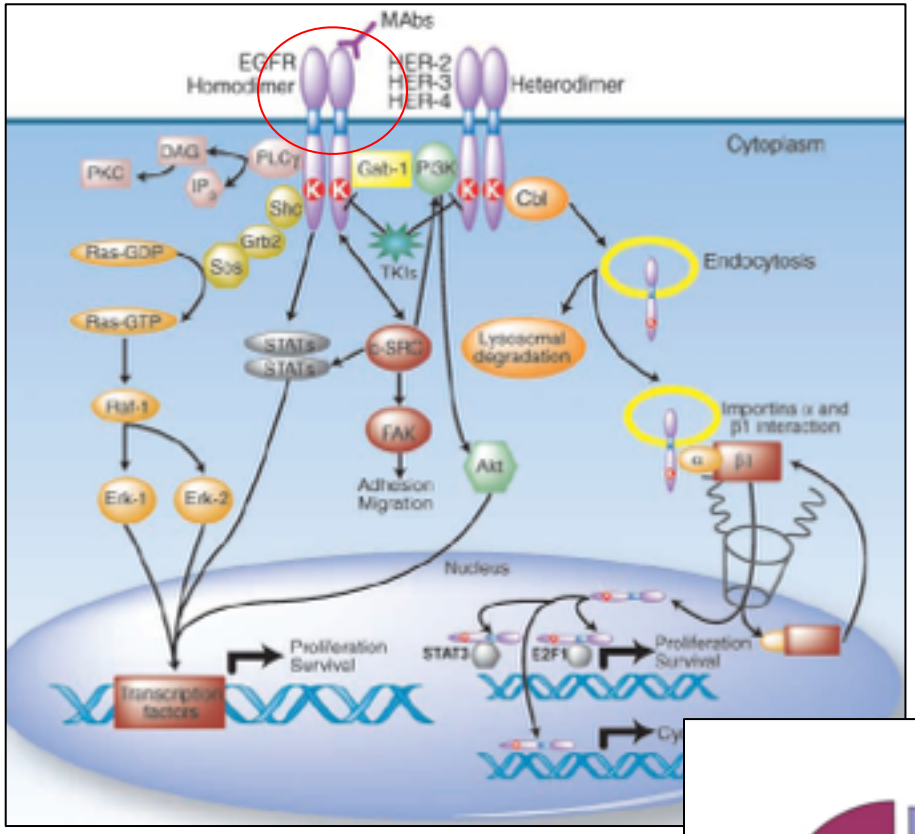
MAPK=60%

DNA repair=63%

PI3K-mTOR=37%

Target therapy and colorectal cancer

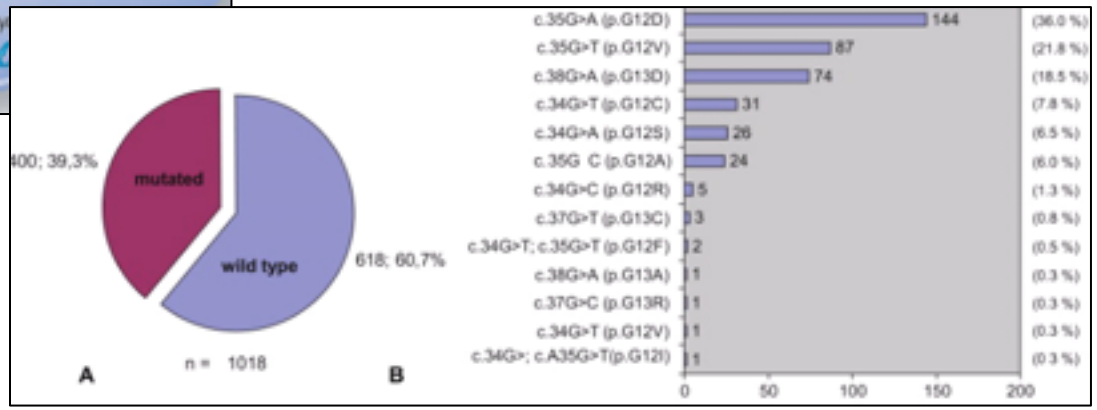
MoAbs targeting EGFR amplifications in CRC



KRAS mutations activate the EGFR pathways independently of EGFR status….

In this case the EGFR MoAB treatment is useless

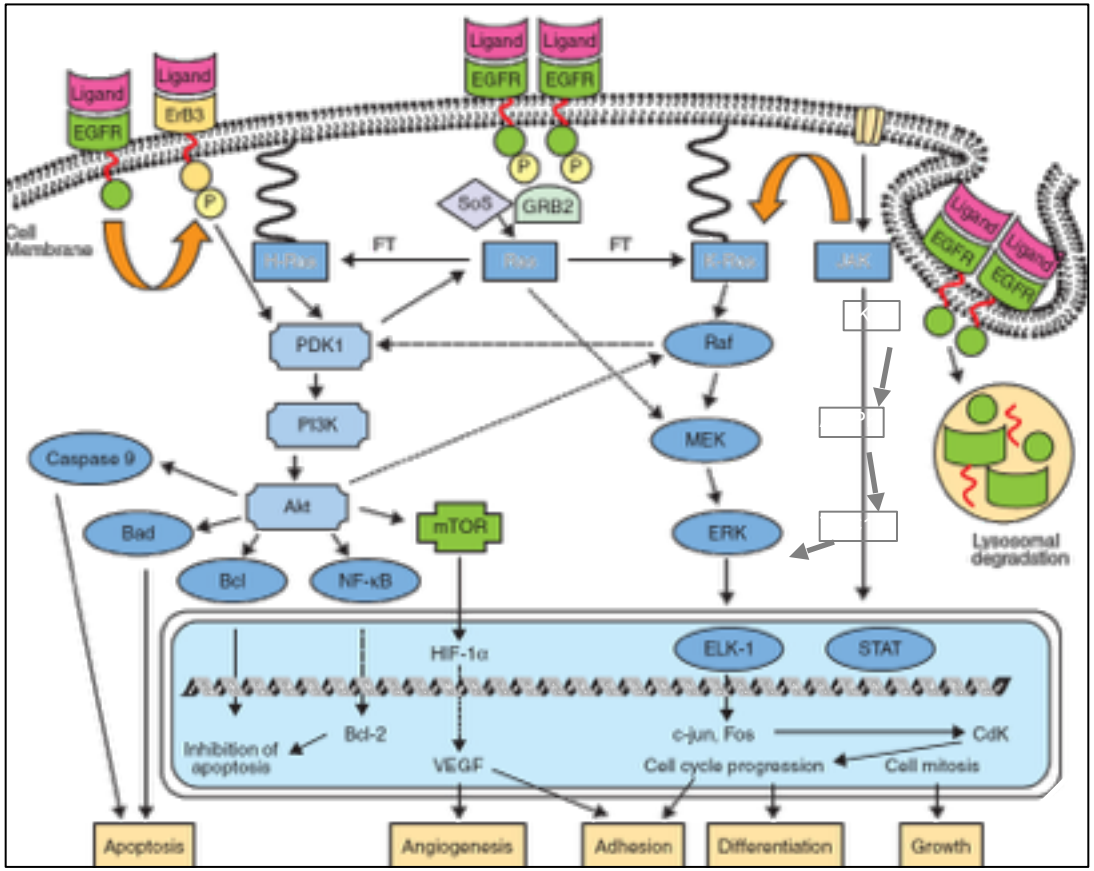
CRCs show KRAS mutations in 50% of cases are



Neumann et al., Pathol. Res. Practise, 2009

NRAS, BRAF and PI3KCA mutations can activate the MAPK pathway

(Rocha-Lima et al., 2007).

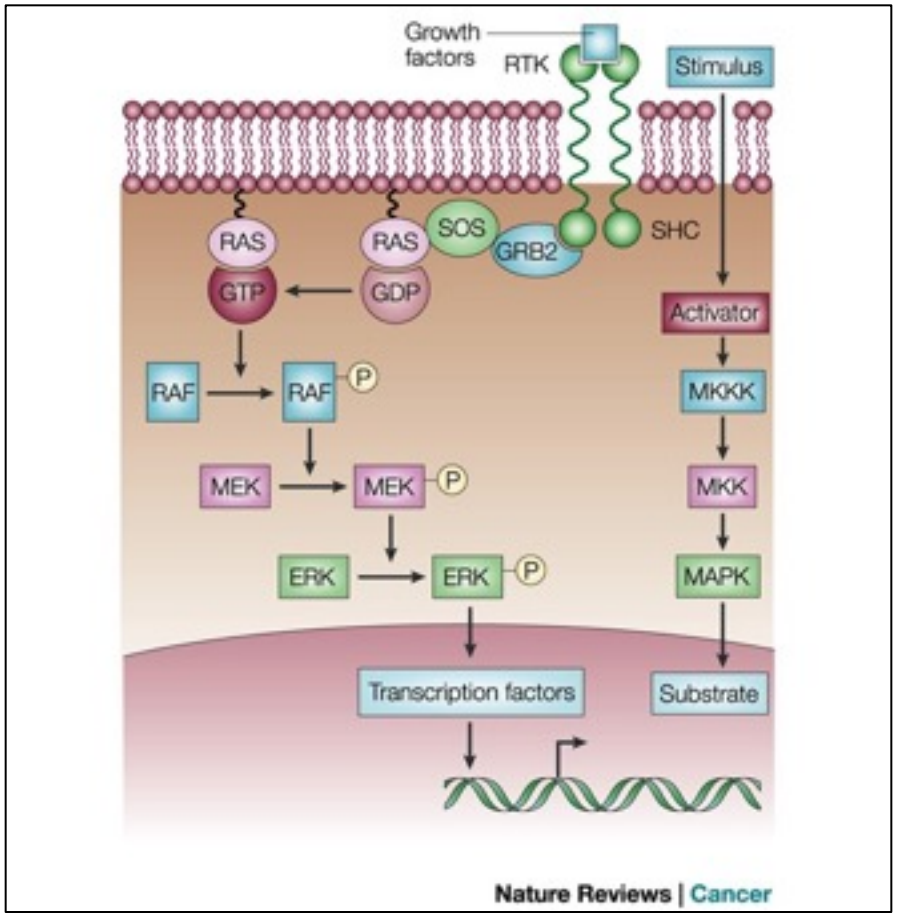


Also in this case the EGFR MoAB treatment can be useless

Before EGFR MoAB treatment is **compelling** to analyze the metastatic colorectal cancer for the presence of KRAS and NRAS mutations on selected codons (12,13,59,61, 117,146)

Target therapy e melanoma

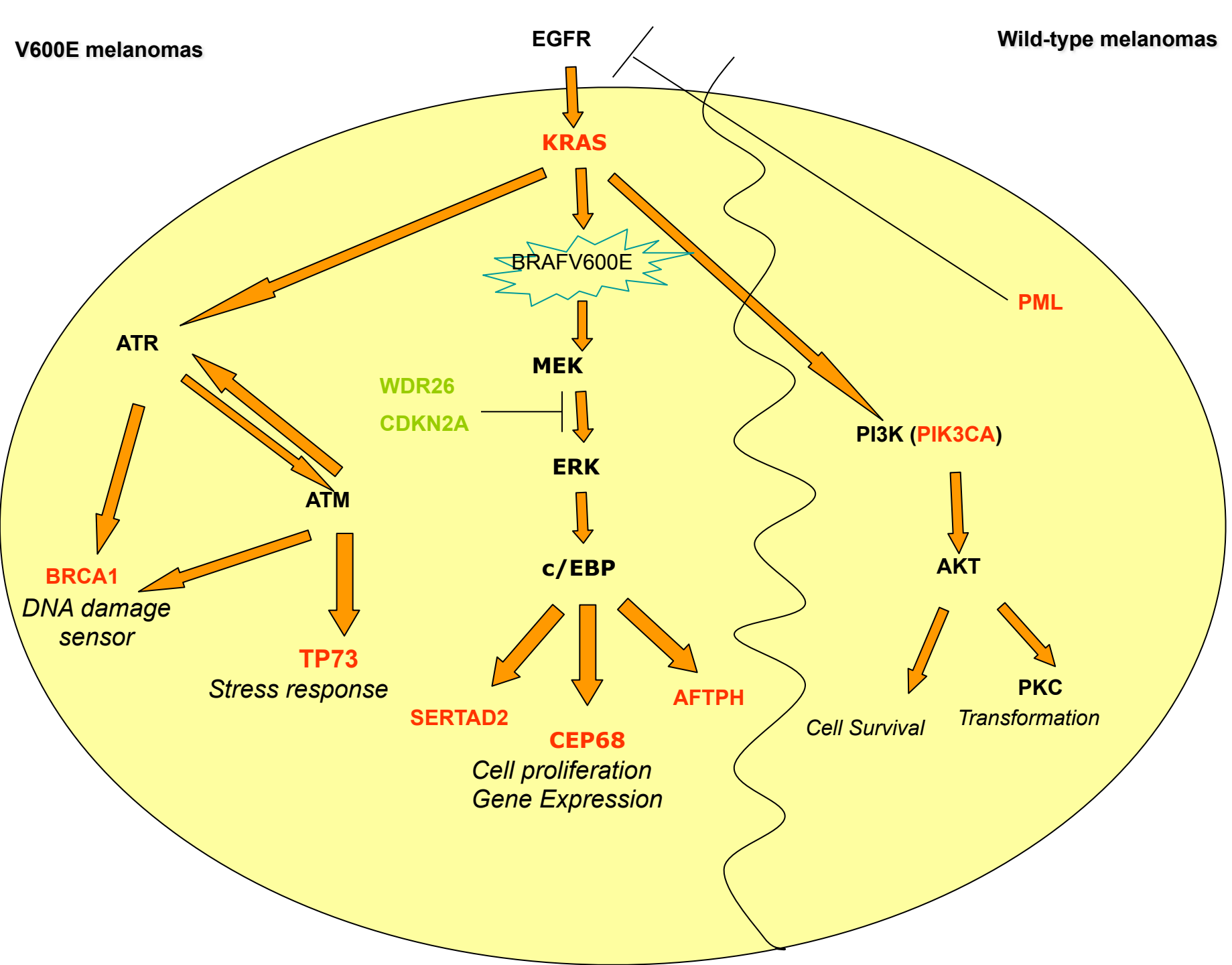
Somatic genetic alterations of the MAPK pathway play a role for the onset of most melanomas



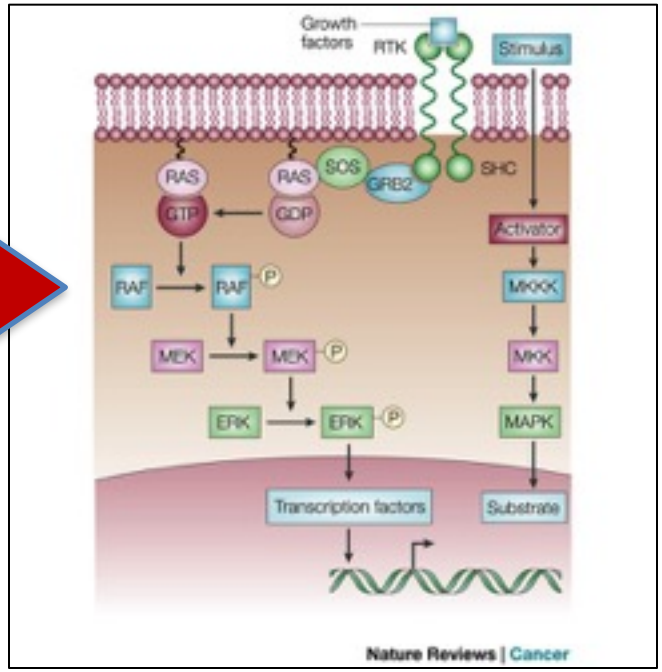
BRAF mutations are the most frequent alterations, prevalently due to T/A substitution on codon 600 (>90%)

Alterations involving NRAS, mostly on codon 61, have been also associated with melanomagenesis, but to a lesser extent

Mutated BRAF TKis have been developed and successfully used in the treatment of several melanomas

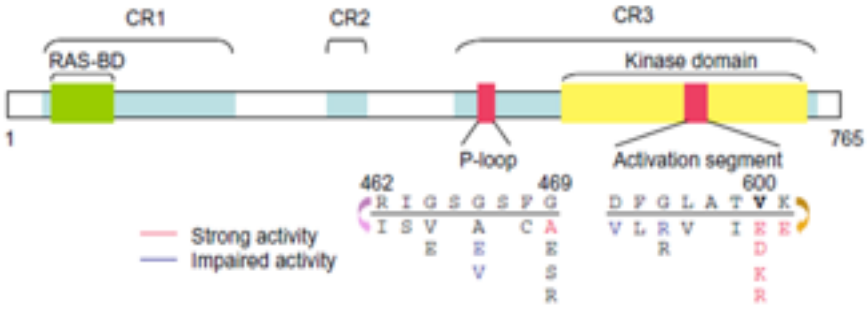

V600E melanomas
EGFR
Wild-type melanomas
KRAS
BRAFV600E
PML
ATR
MEK
WDR26
CDKN2A
PI3K (PIK3CA)
ATM
ERK
BRCA1
DNA damage sensor
c/EBP
AKT
TP73
Stress response
SERTAD2
CEP68
AFTPH
Cell proliferation
Gene Expression
Cell Survival
PKC
Transformation

BRAF mutations have been reported in 45% of cutaneous melanomas from intermittently sun exposed sites



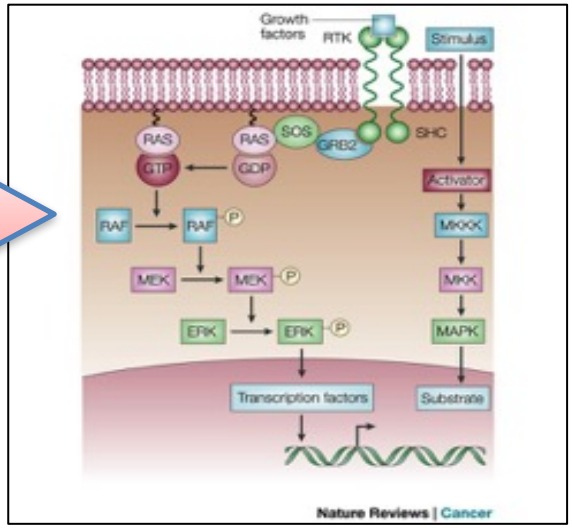
Mutations are mostly on exon 15

The most frequent mutations are :
V600E (75%) and V600K (20%)
Rarer mutations in codons 599 and 601

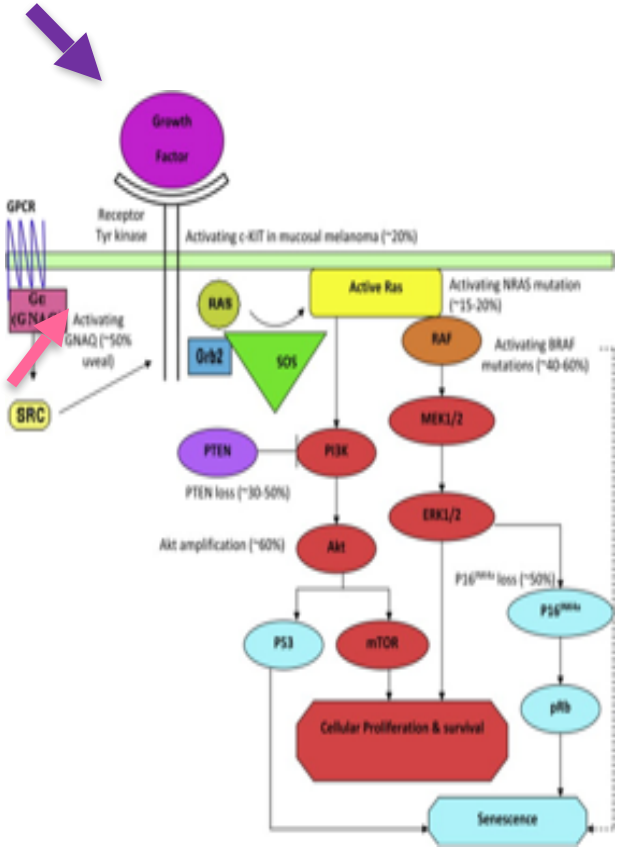


NRAS Mutations have been reported in 10-15% of cutaneous melanomas from chronically sun exposed sites

The most frequent mutations are:
Q60 and Q61 (80%) in exon 2
G12 and G13 (20 %) in exon 1.



Other mutations associated with different histology and different sites ?

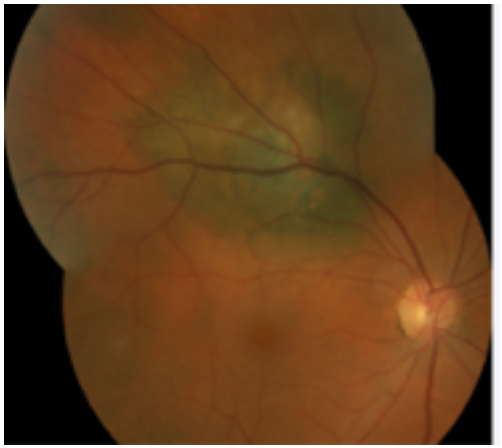


cKIT mutations have been found in 10-20% of **mucosal**, **acral** and cutaneous melanomas from **chronically** sun exposed sites

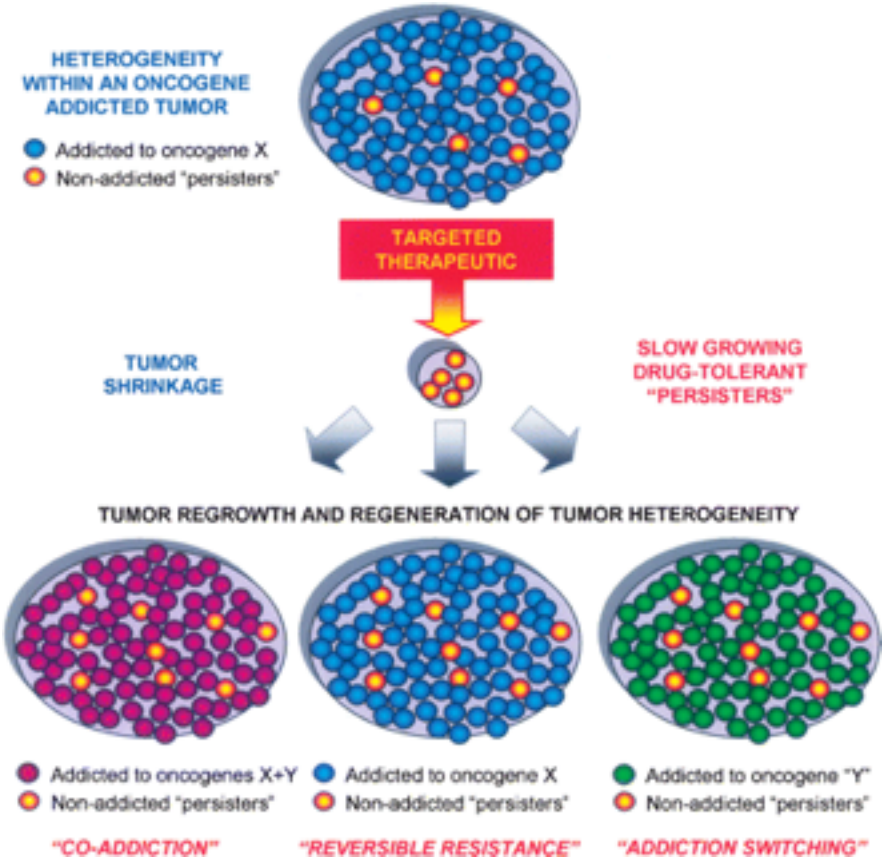
Mainly missense mutations
in exons 13, 17 and 18

This type of mutations can be used for specific treatment with cKIT TKi

Activating mutations in **GNAQ** and **GNAQ11** in 35% and 45% of uveal melanoma



Resistance to therapeutics targeting oncogene addiction: heterogeneity of tumour cells

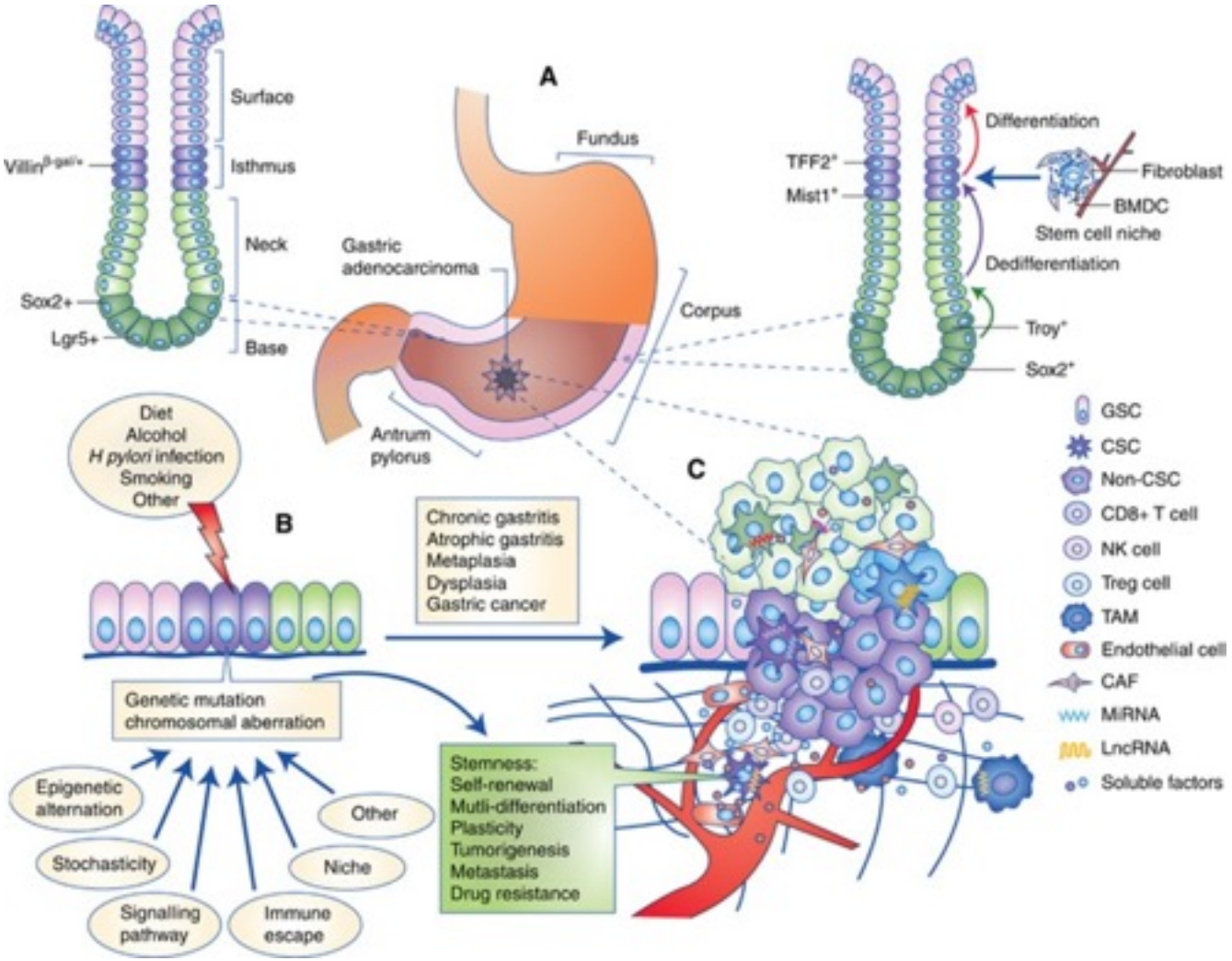


- A tumor is composed of a cell population which is addicted to a specific oncogene, but with a subpopulation of non addicted cancer cells
- Upon treatment with a TKIs, tumor shrinkage results from ablation of the addicted cells, but non addicted cells are mantained

These non-addicted cells have the capacity to regenerate

Tumour heterogeneity

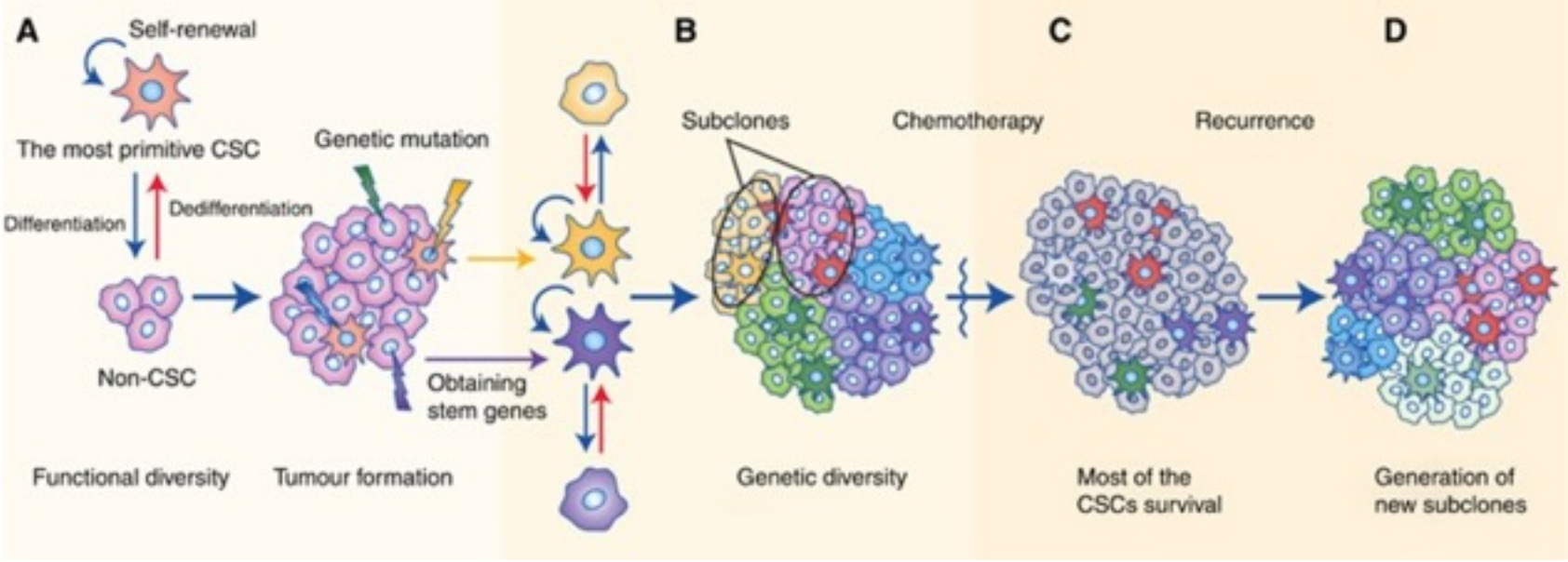
Emerging evidence has shown that some tumours display a hierarchical organisational structure, with cancer stem cells (CSCs) at the apex.



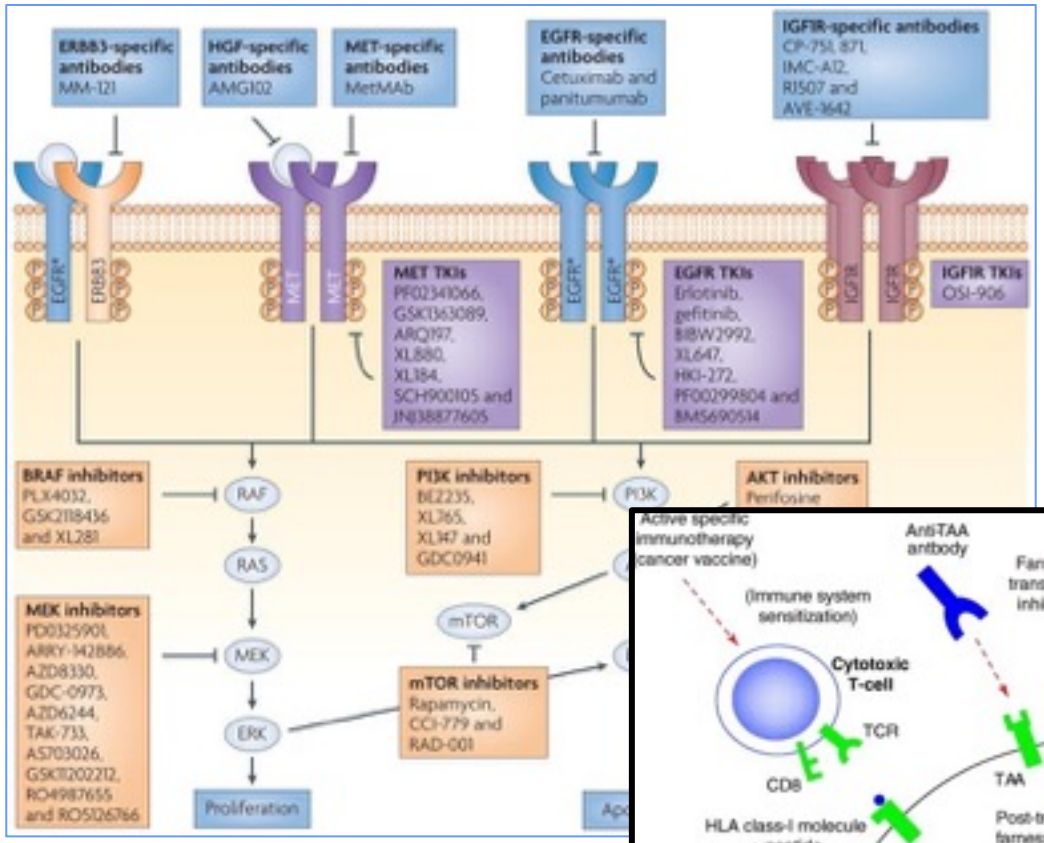
Tumour heterogeneity

Stochastic and hierarchical models are reasonable systems that have been hypothesised to describe tumour heterogeneity.

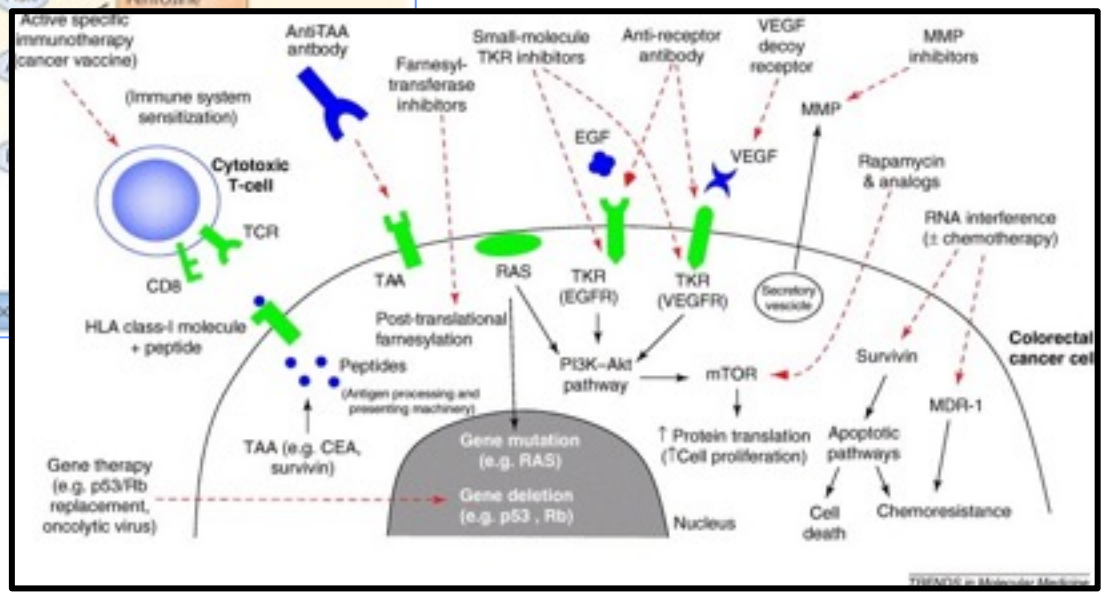
Each model alone inadequately explains tumour diversity.
The two models can be integrated to provide a more comprehensive explanation.



Potential multi-drugs approach….



Important weaknesses of the target therapy



A new role of active specific immunotherapy in different tumors

Tumoral mutational burden

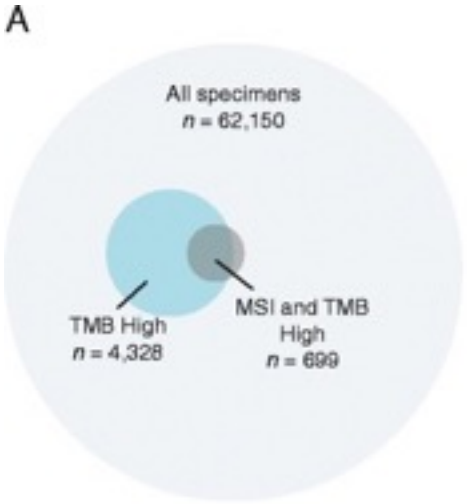
Chalmers et al. Genome Medicine (2017) 9:34
DOI 10.1186/s13073-017-0424-2

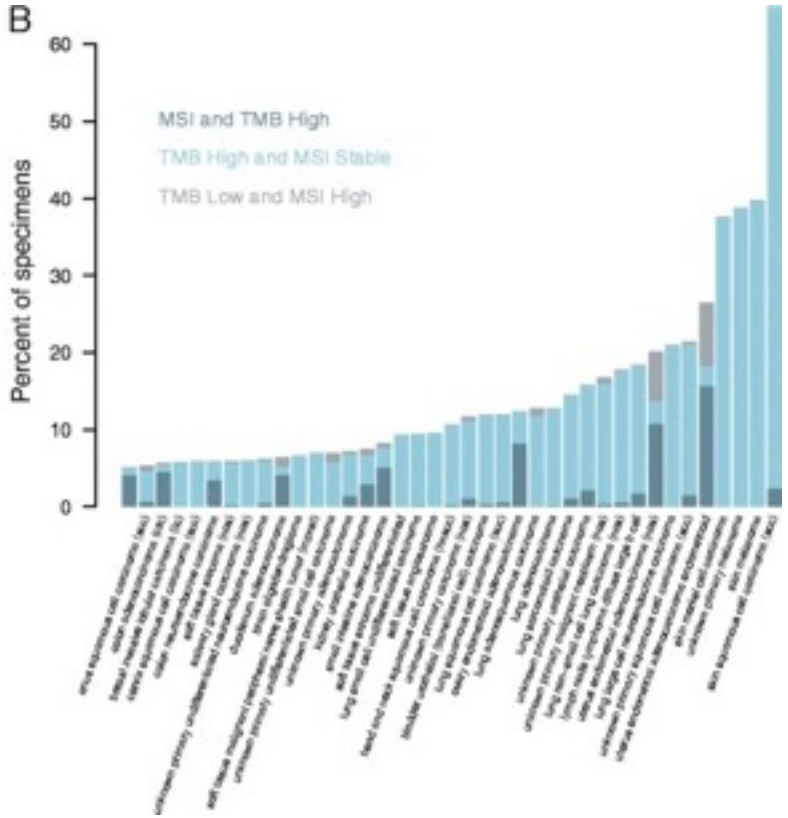
Genome Medicine

RESEARCH Open Access

Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden

Zachary R. Chalmers[1†], Caitlin F. Connelly[1†], David Fabrizio[1], Laurie Gay[1], Siraj M. Ali[1], Riley Ennis[1], Alexa Schrock[1], Brittany Campbell[1], Adam Shlien[1], Juliann Chmielecki[1], Franklin Huang[2], Yuting He[1], James Sun[1], Uri Tabori[1], Mark Kennedy[1], Daniel S. Lieber[1], Steven Roels[1], Jared White[1], Geoffrey A. Otto[1], Jeffrey S. Ross[1], Levi Garraway[2,3], Vincent A. Miller[1], Phillip J. Stephens[1] and Garrett M. Frampton[1*]

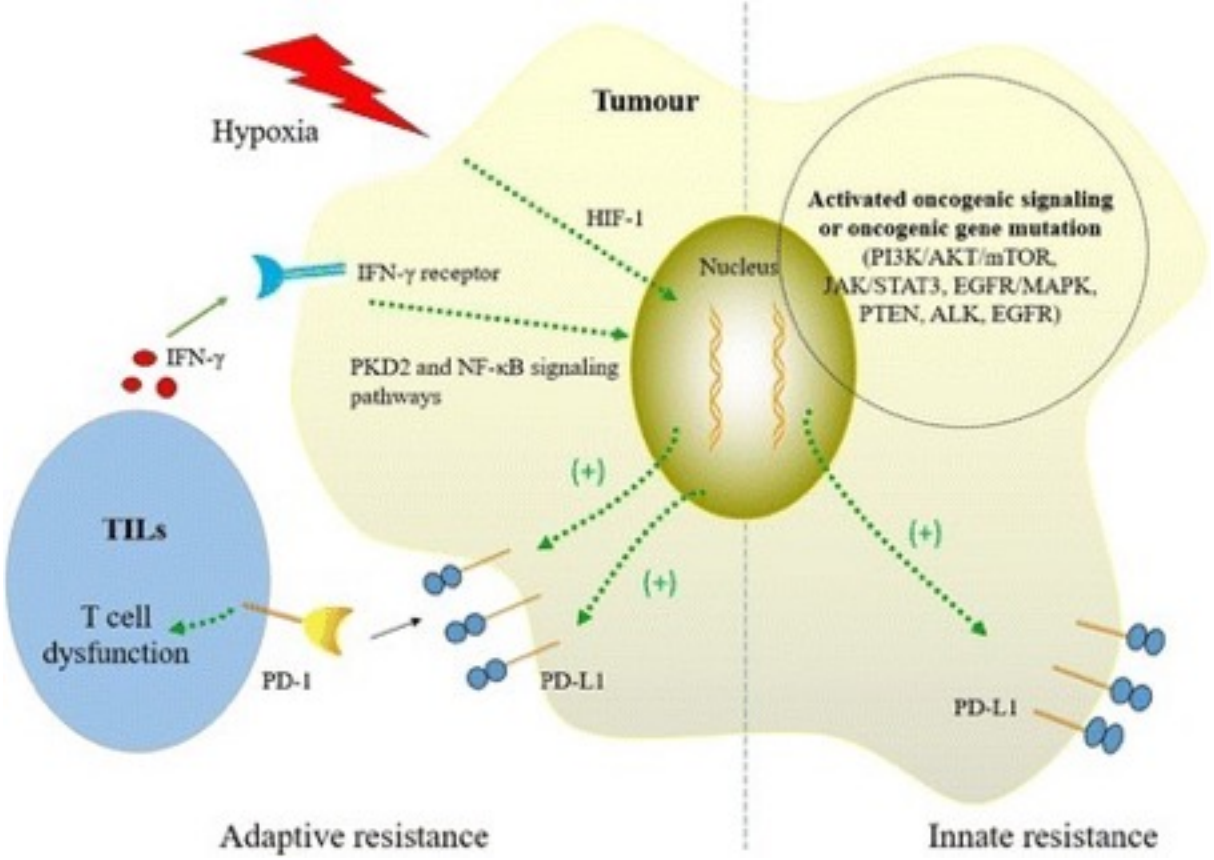




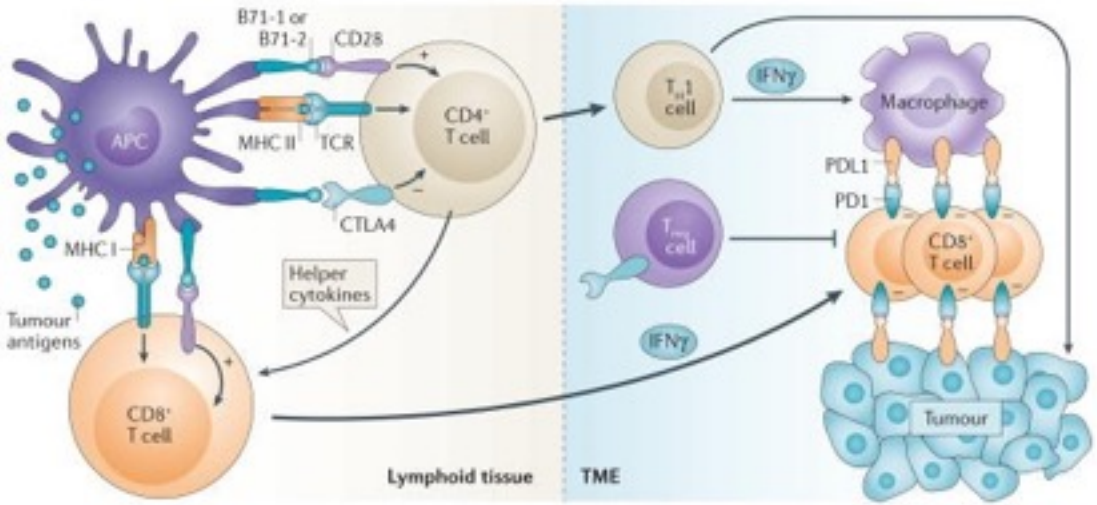
High TMB means high number of neoantigens

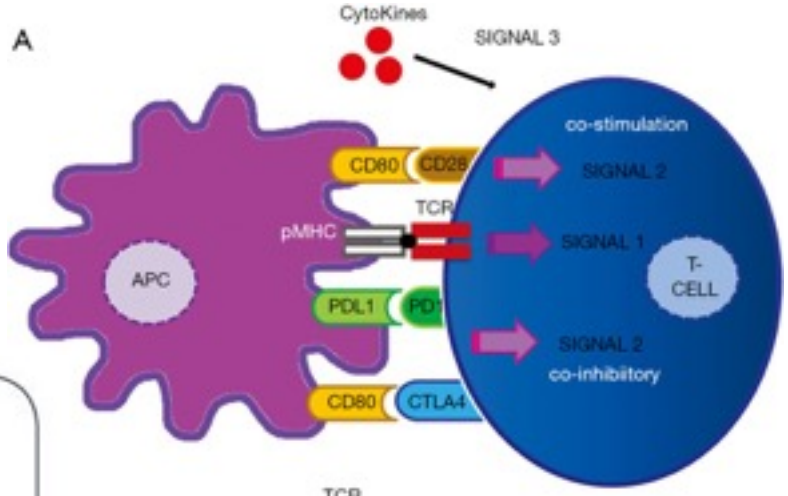
Induction of immunoresponse by the host

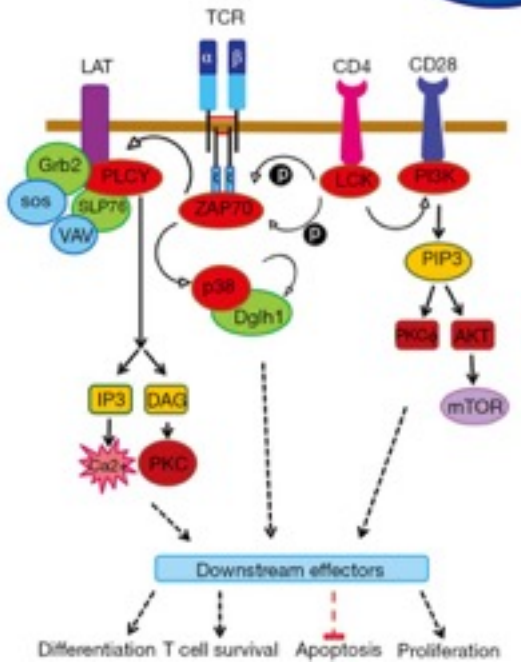
Immunotherapy : a new frontier for tumors with a high mutational burden ?

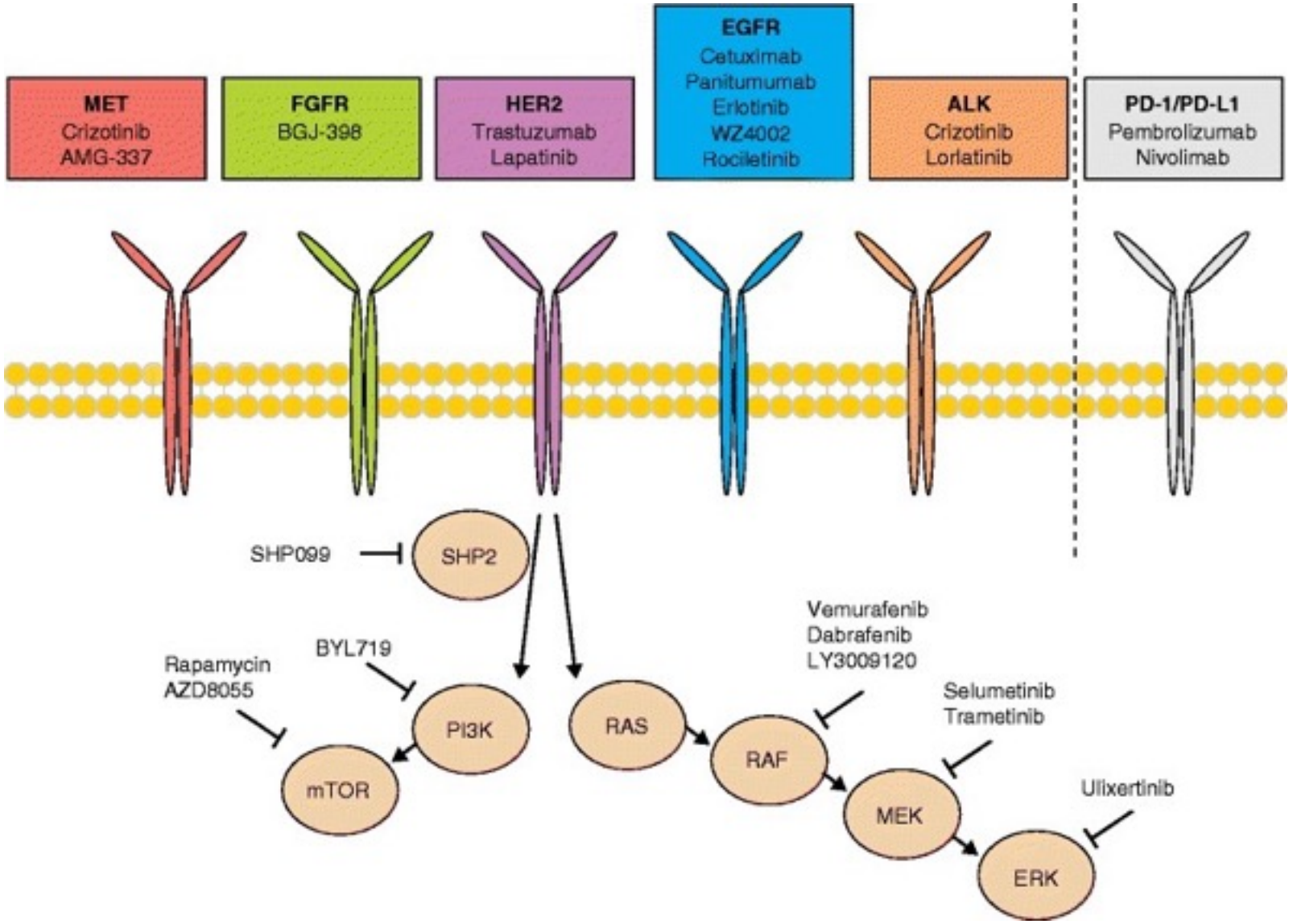


Widespread use of MoAb vs PD-1/PDL1








MET
Crizotinib
AMG-337
FGFR
BGJ-398
HER2
Trastuzumab
Lapatinib
EGFR
Cetuximab
Panitumumab
Erlotinib
WZ4002
Rociletinib
ALK
Crizotinib
Lorlatinib
PD-1/PD-L1
Pembrolizumab
Nivolimab
SHP099
SHP2
Vemurafenib
Dabrafenib
LY3009120
BYL719
Rapamycin
AZD8055
PI3K
RAS
RAF
Selumetinib
Trametinib
mTOR
MEK
Ulixertinib
ERK

What next ??