Biomarkers:

a myriad; generally poorly sensitive and specific

Cancer biomarkers are not sufficient for the diagnosis of cancer;

-**Most markers can be expressed by normal cells as well as cancer cells;**

-**A number of non-malignant diseases can induce high levels of so-called cancer biomarkers;**

-**Not every cancer patient has a high expression level of cancer biomarkers**

As of today, the biopsy remains the reference tool for the diagnosis of cancer

Mutations:

-are the basis for targeted therapies, a quantum leap in the treatment of cancer

- Leading to « personalised medicine », towards an « à la carte » treatment of cancers

Targeted therapies: how it all started

Induced reversibility of the leukemia phenotype

1966 Charlotte Friend observation princept.
The maturation block in erythroleukemia cells can be overcome by treating the cells with dimethyl sulfoxide. First observation that the leukemia phenotype can be reversed.

1978 Collins et al.
HL-60 leukemia cells can be induced to differenciate with dimethyl sulfoxide, vitamin D3, and retinoic acid.

Differentiation therapy in leukemia

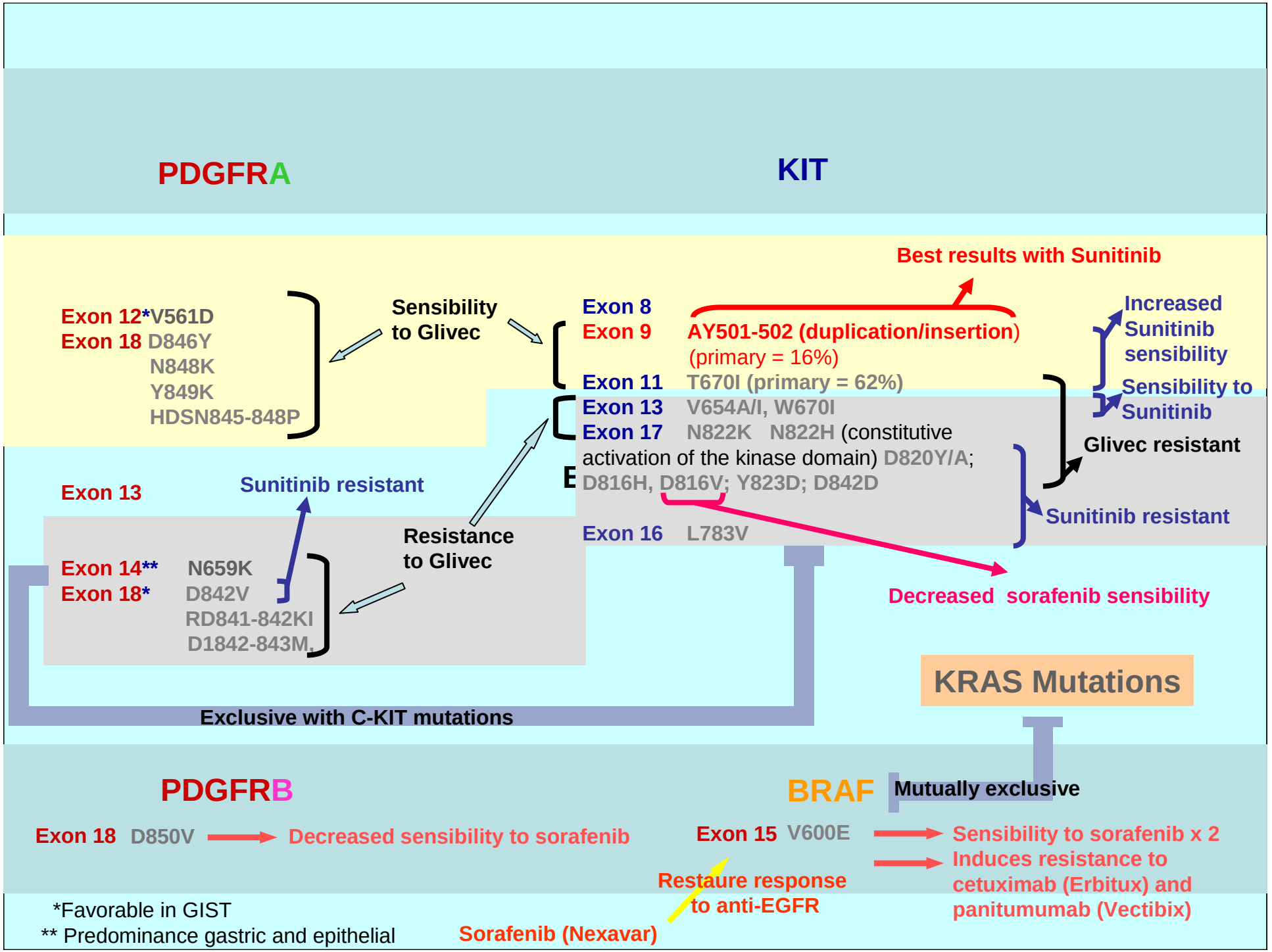
1984 Koeffler et al.
1,25(OH)2D3 induces differentiation of leukemia cells *in vivo.*

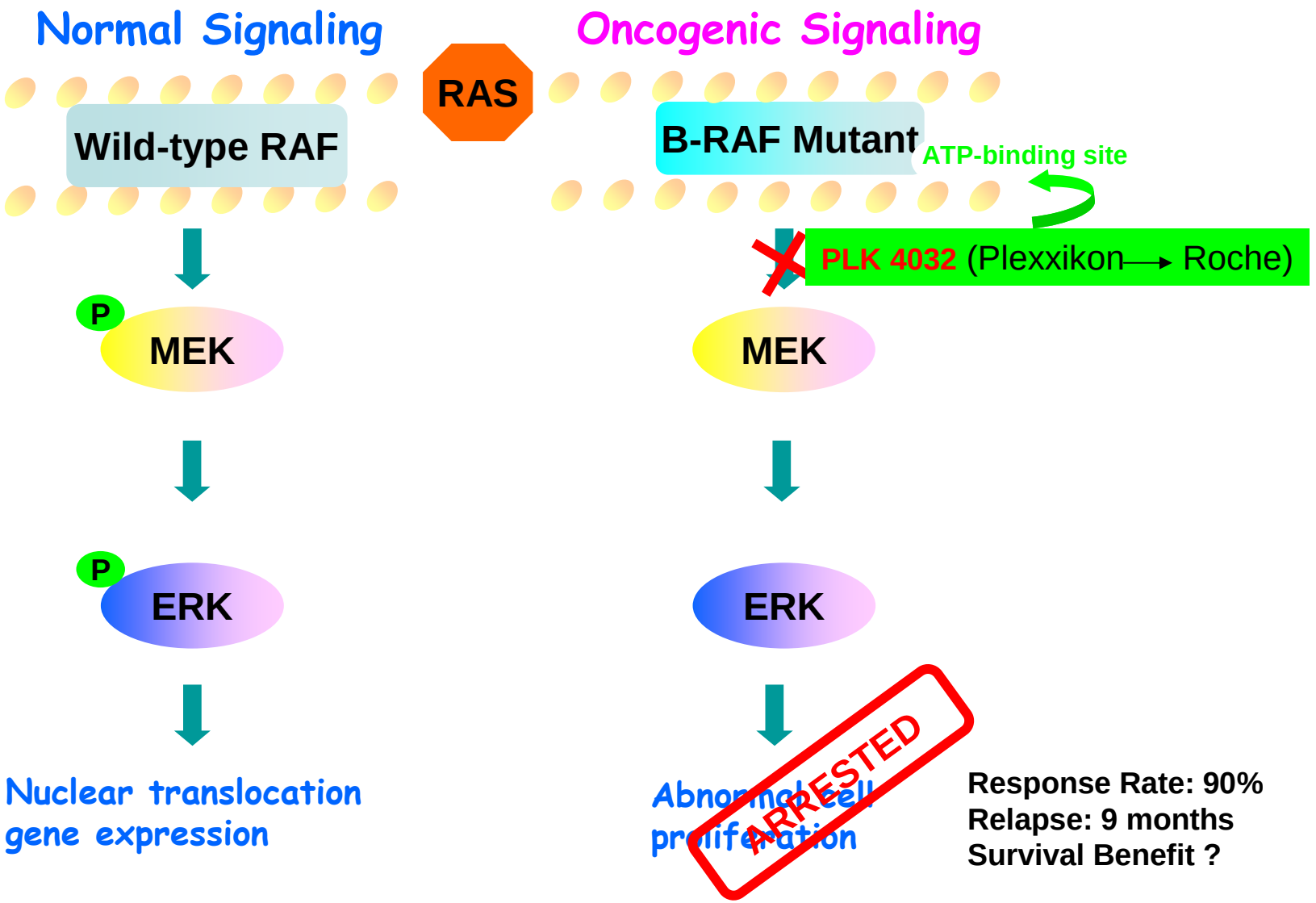
1988 Huang Meng-er et al.
First use of retinoic acide to treat acute promyelocytic leukemia.

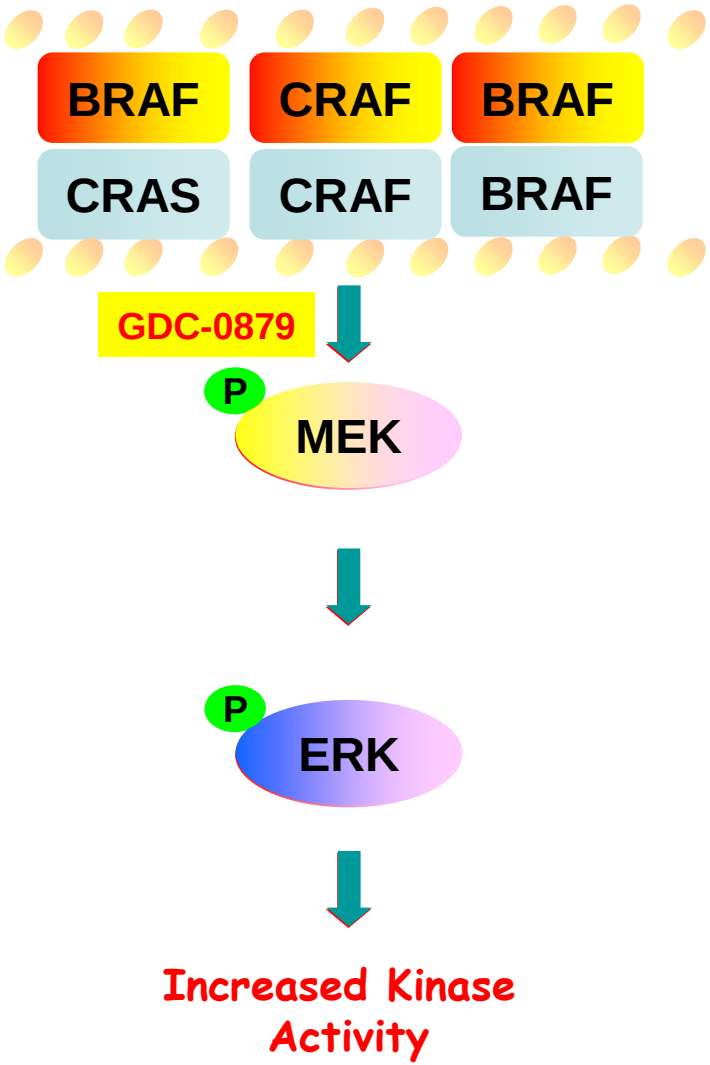
Molecular targeting in leukemia cells

1988 Novartis
Synthesis of new derivatives of 2-phénylamino-pyrimidine interacting with ATP receptors of tyrosine kinases; among them was STI-571.

1999 – 2001 Druker et al.
STI-571 become the Gleevec, first targeted anti-leukemia treatment; fastest FDA approval.


PDGFRA
KIT
Best results with Sunitinib
Exon 12*V561D
Exon 18 D846Y
N848K
Y849K
HDSN845-848P
Sensibility to Glivec
Exon 8
Exon 9
AY501-502 (duplication/insertion)
(primary = 16%)
Exon 11 T670I (primary = 62%)
Exon 13 V654A/I, W670I
Exon 17 N822K N822H (constitutive activation of the kinase domain) D820Y/A; D816H, D816V; Y823D; D842D
Exon 16 L783V
Increased Sunitinib sensibility
Sensibility to Sunitinib
Glivec resistant
Sunitinib resistant
Exon 13
Sunitinib resistant
Resistance to Glivec
Exon 14** N659K
Exon 18* D842V
RD841-842KI
D1842-843M
Decreased sorafenib sensibility
KRAS Mutations
Exclusive with C-KIT mutations
PDGFRB
BRAF
Mutually exclusive
Exon 18 D850V
Decreased sensibility to sorafenib
Exon 15 V600E
Sensibility to sorafenib x 2
Induces resistance to cetuximab (Erbitux) and panitumumab (Vectibix)
Restaure response to anti-EGFR
Sorafenib (Nexavar)
*Favorable in GIST
** Predominance gastric and epithelial


Normal Signaling
Oncogenic Signaling
RAS
Wild-type RAF
B-RAF Mutant
ATP-binding site
PLK 4032 (Plexxikon⟶ Roche)
P
MEK
MEK
P
ERK
ERK
Nuclear translocation
gene expression
ARRESTED
Response Rate: 90%
Relapse: 9 months
Survival Benefit ?


BRAF
CRAF
BRAF
CRAS
CRAF
BRAF
GDC-0879
P
MEK
P
ERK
Increased Kinase Activity

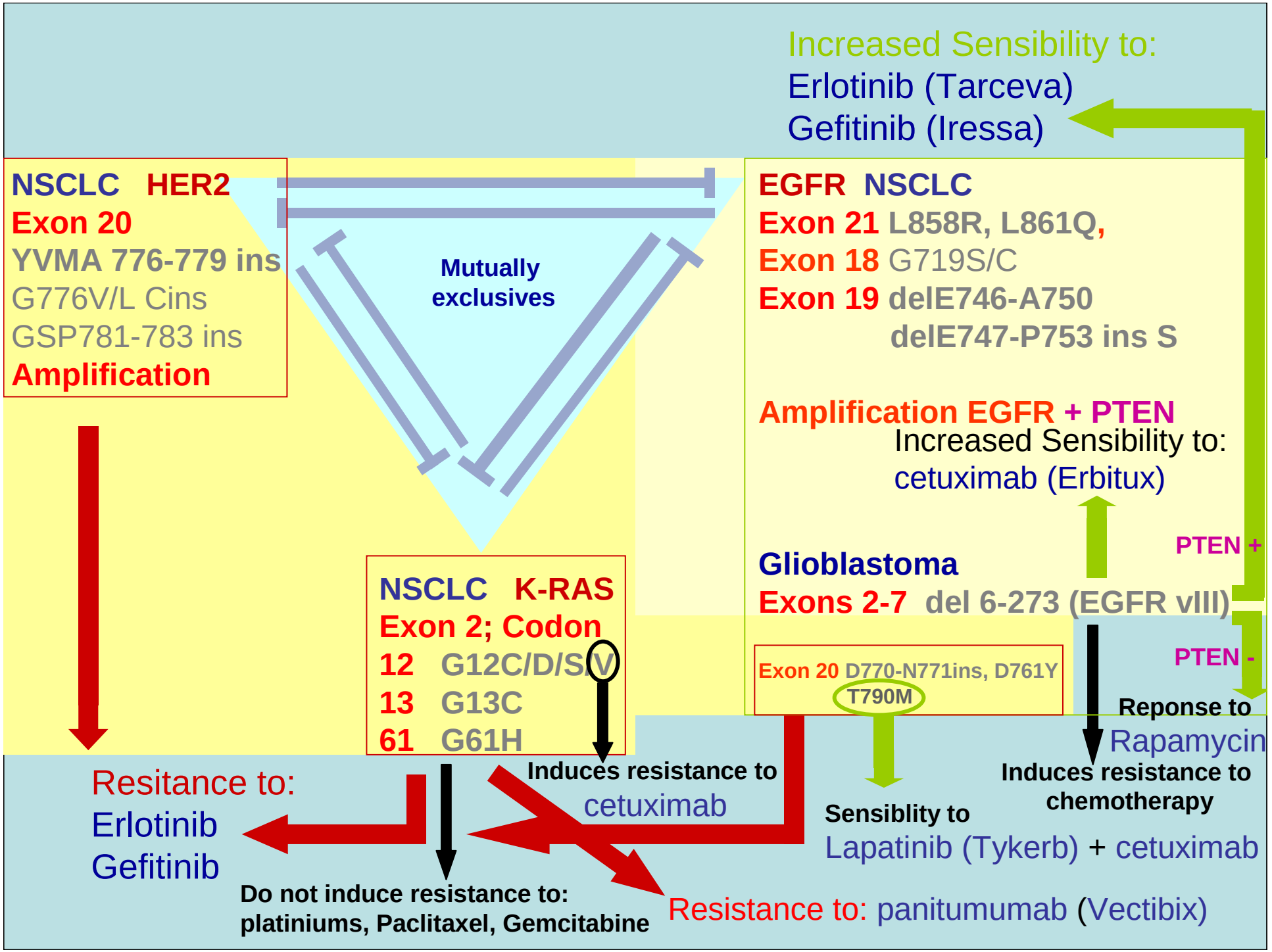
Working Hypothesis

RAF inhibitors suppress ERK signaling in BRAF(V600E) tumours because the level of RAS activation in these cells is insufficient to support transactivation of wild-type RAF

▼

Inhibition of BRAF(V600E) activity become the dominant effect of the drug.

Coexpression of mutant RAS with BRAF (V600E) to PLX4032/PLX4720 → Resistance


Increased Sensibility to:
Erlotinib (Tarceva)
Gefitinib (Iressa)
NSCLC HER2
Exon 20
YVMA 776-779 ins
G776V/L Cins
GSP781-783 ins
Amplification
Mutually exclusives
EGFR NSCLC
Exon 21 L858R, L861Q,
Exon 18 G719S/C
Exon 19 delE746-A750
delE747-P753 ins S
Amplification EGFR + PTEN
Increased Sensibility to:
cetuximab (Erbitux)
PTEN +
Glioblastoma
Exons 2-7 del 6-273 (EGFR vIII)
PTEN -
NSCLC K-RAS
Exon 2; Codon
12 G12C/D/S/V
13 G13C
61 G61H
Exon 20 D770-N771ins, D761Y
T790M
Reponse to
Rapamycin
Induces resistance to chemotherapy
Induces resistance to
cetuximab
Resitance to:
Erlotinib
Gefitinib
Sensiblity to
Lapatinib (Tykerb) + cetuximab
Do not induce resistance to:
platiniums, Paclitaxel, Gemcitabine
Resistance to: panitumumab (Vectibix)

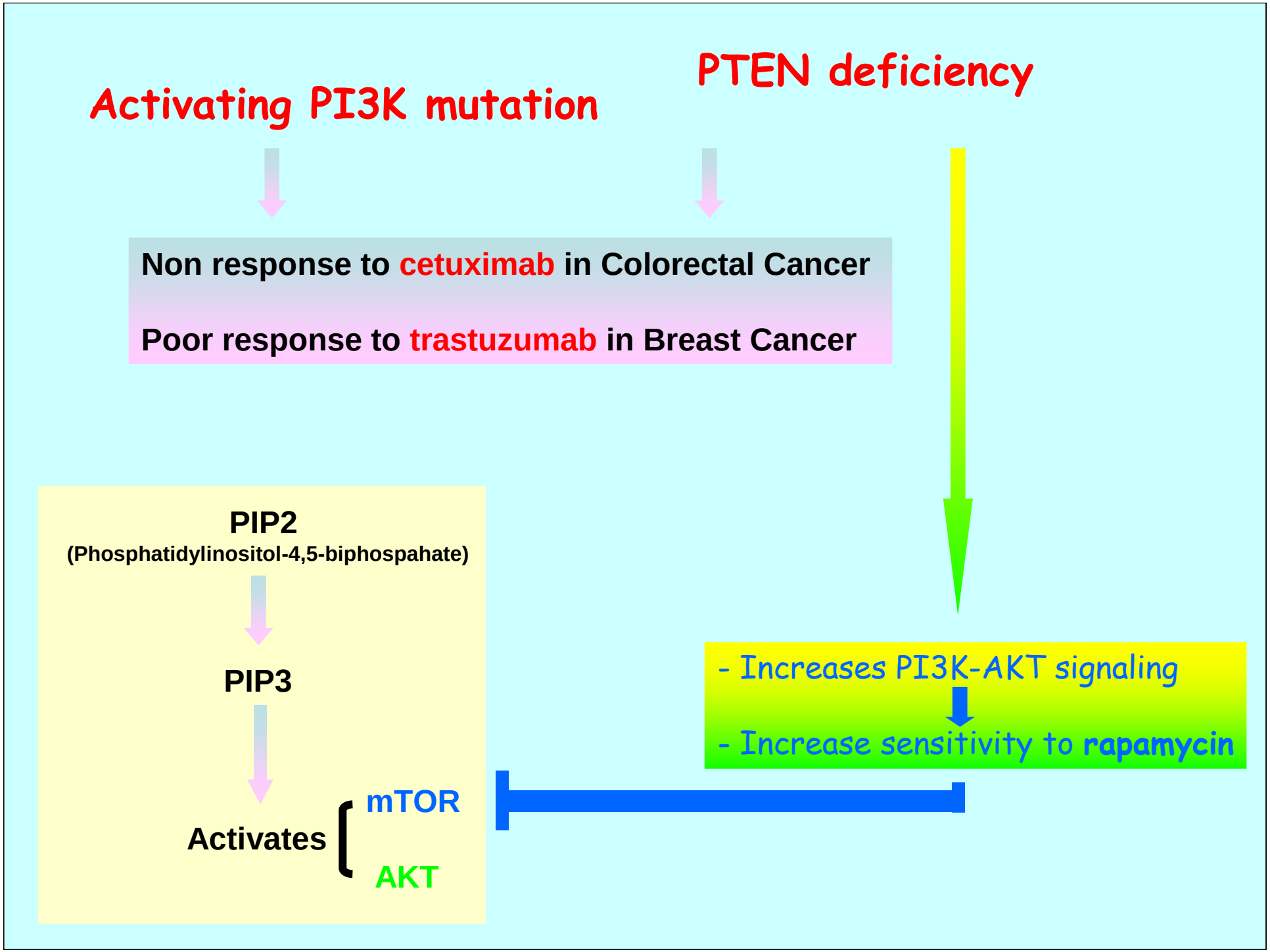
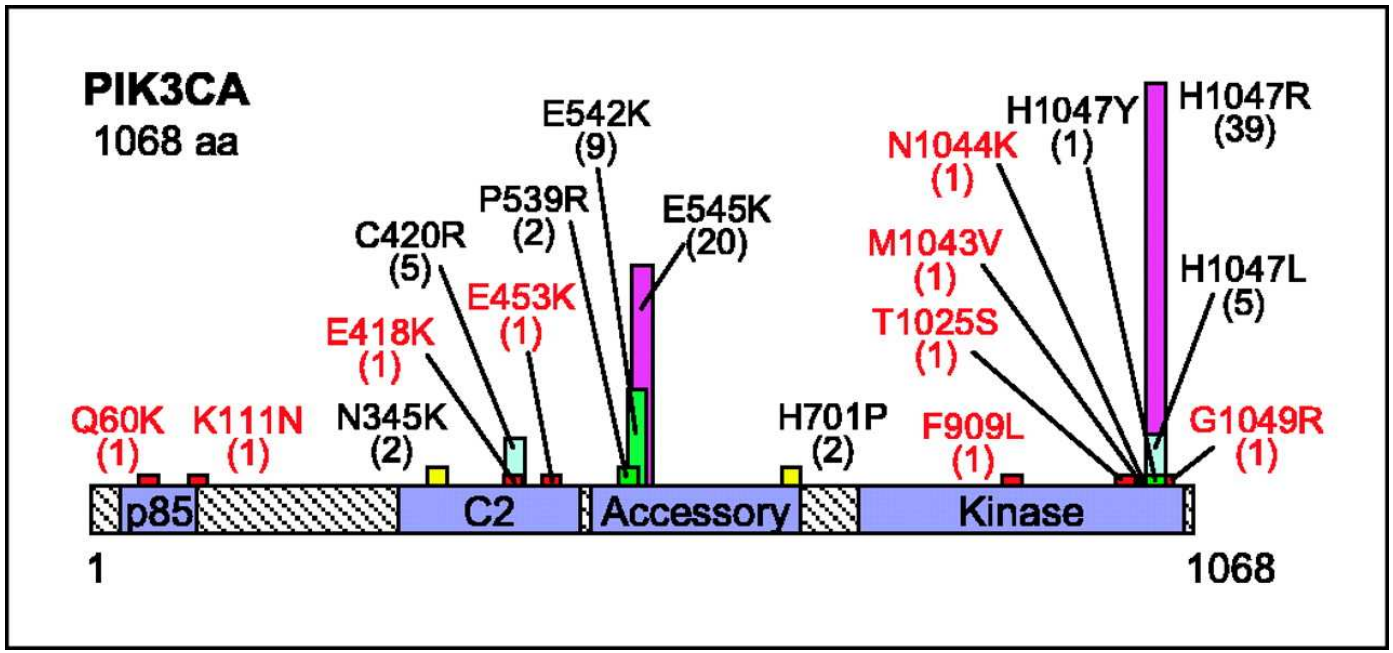

Activating PI3K mutation
PTEN deficiency
Non response to cetuximab in Colorectal Cancer
Poor response to trastuzumab in Breast Cancer
PIP2
(Phosphatidylinositol-4,5-biphospahate)
PIP3
Activates
mTOR
AKT
- Increases PI3K-AKT signaling
- Increase sensitivity to rapamycin

Figure 1. PIK3CA protein and functional domains

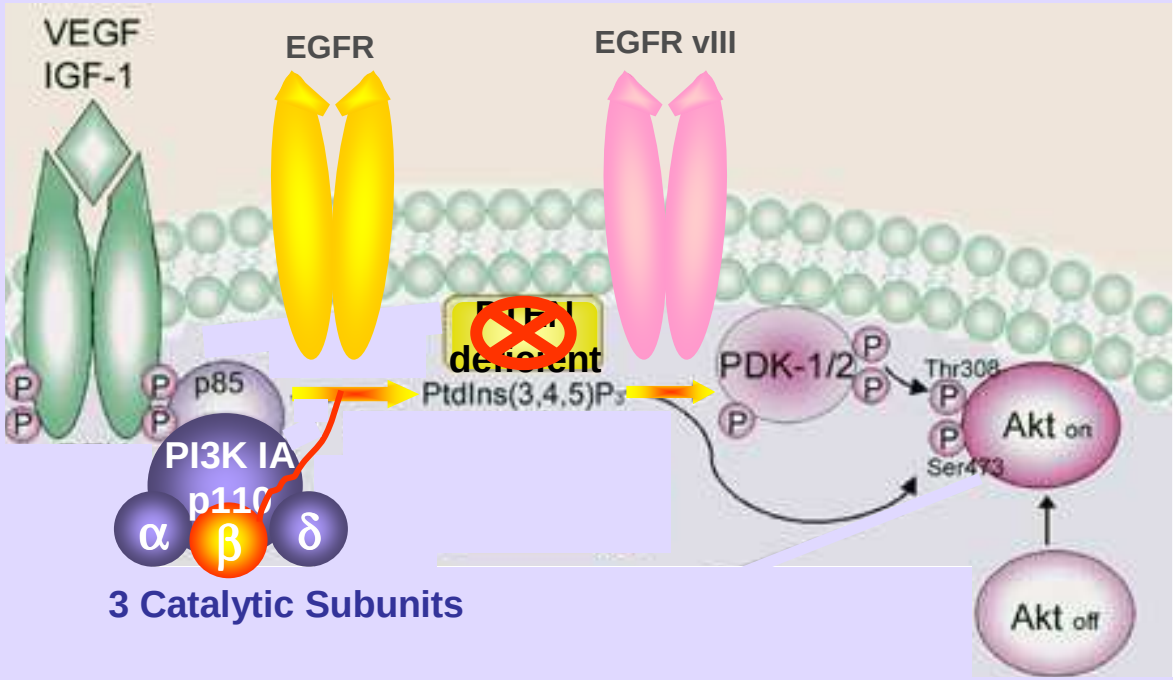


Saal, L. H. et al. Cancer Res 2005;65:2554-2559

Cancer Research

Proteasome → **Degradation**

PTEN Deficient cells



Poor response to trastuzumab
Breast cancer

Non-response to cetuximab
Colorectal cancer

High-response to rapamycin
Glioblastoma

Mutations and genetic alterations may allow:

-« real time » survey of targeted treatment in cancer patients;

- setting up alternative treatment in the case of resistance to the initial treatment;

- testing evolution of specific targets under therapeutic pressure; in the near future may apply to new targeted therapies.

but

Need a non-invasive approach to detection

Rare circulating cells can be the support of all biological tests applying to the cells they represent.

Invasive → **Non-invasive assay**

We have created and used new filter systems allowing:

A. direct extraction of genetic material from isolated rare cells (CTCs and CFCs) and subsequent use of qRT-PCR and allelic discrimination assays;

B. culture of isolated rare cells (CTCs and CFCs).