ΜΑΘΗΜΑ :ΚΛΙΝΙΚΗ ΧΗΜΕΙΑ

Molecular Oncology B

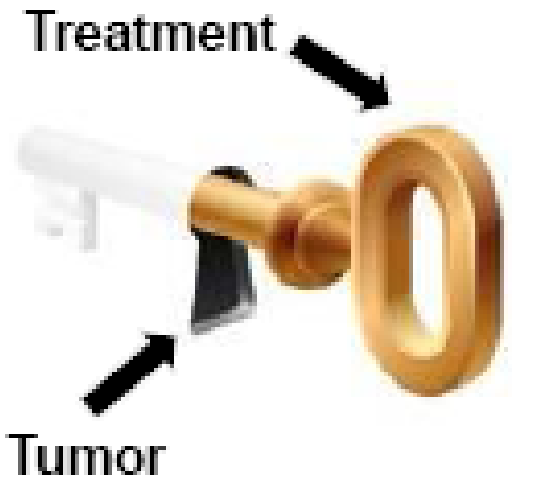
Ε. Λιανίδου, Καθηγήτρια,

Εργαστήριο Αναλυτικής Χημείας, Τμήμα Χημείας, Πανεπιστήμιο Αθηνών

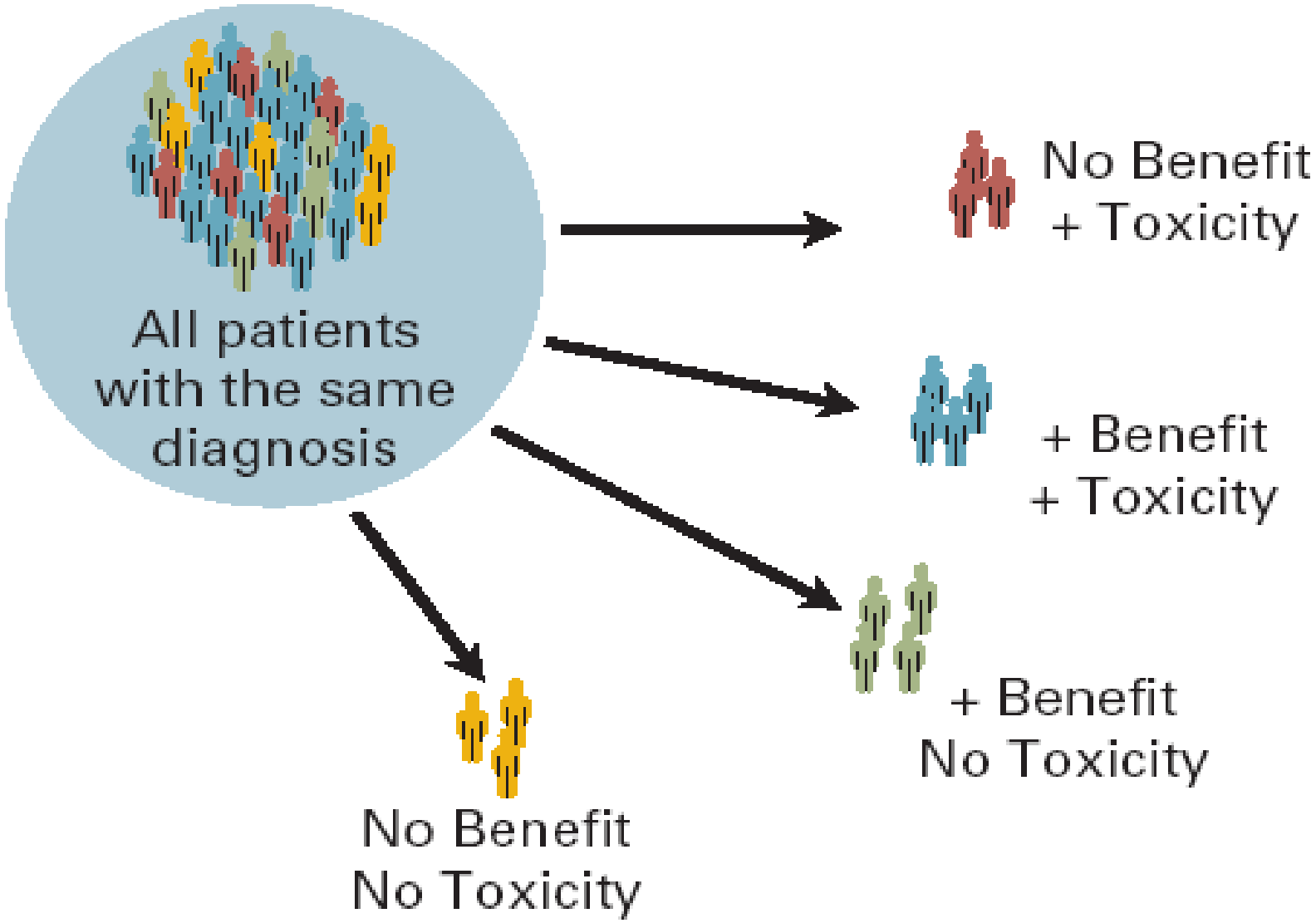
The goals of biomarkers and their role in “personalized medicine”

- **Identification of low risk patients (prognostic biomarkers)**

- **Identification of disease free and overall survival benefit from a specific treatment (predictive)**



BENEFIT – TOXICITIES / RATIO



Walgren et al, JCO 2005

What is targeted therapy?

MOLECULAR TARGETED THERAPY

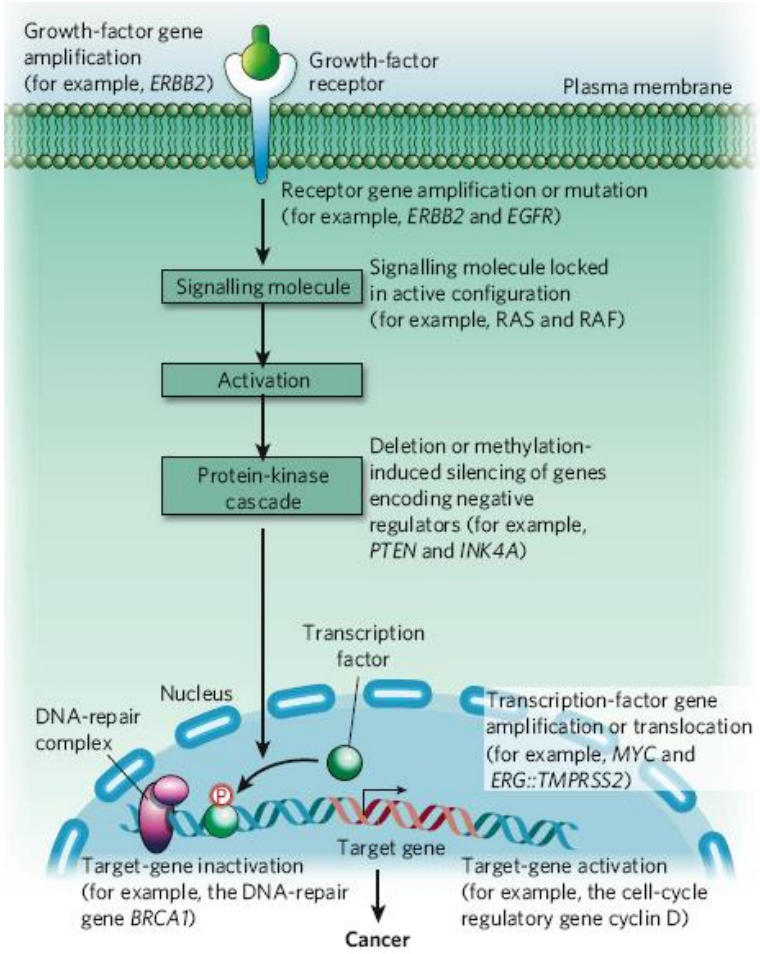
- **None generally accepted definition**

"In cancer treatment, substances that kill cancer cells by targeting key molecules involved in *cancer cell growth*"

National Cancer Institute, Dictionary of Cancer Term (www.cancer.gov)

- **But substances targeted:**
  - **Tumor micro-environment**
  - **Metastatic process**
  - **Host immune response to cancer cells**

are not targeted therapies?



Translating insights from the cancer genome into clinical practice

Lynda Chin[1] & Joe W. Gray[2]

Figure 3 | Disruption of intracellular signalling by alterations in the cancer genome. A simplified signalling pathway is depicted to highlight known examples of *bona fide* oncogenes that are subjected to dysregulation by various mechanisms. It is clear that a signalling pathway can be disrupted at multiple points, and a variety of genomic and epigenomic alterations can contribute to this, ultimately leading to cancer.

MODEL OF TARGETED THERAPY

- **Target discovery-validation**
  - **Target identification**
  - **Target importance to initiate and/or sustain malignant phenotype**
  - **Inhibition of the target – tumor regression**
  - **Primary tumors analysis**

- **Substance/molecule preparation and validation**
  - **Confirmation of target inhibition *in vitro* and *in vivo***
  - **Early clinical/translational trials (phase 0/I studies)**
    - **Surrogate markers**
    - **Treatment interactions**
    - **Patients selection**

- **Registration trials**

Examples of Personalized Medicine in Oncology

1. Breast Cancer

HER-2 : trastuzumab

HER2 and Transtuzumab story

The triumphal narrative of translational research

- ***HER2/neu*** **was discovered in a rat model of chemically induced carcinogenesis**
- **Sequence resembled that of the normal cellular human gene**
- ***HER2/neu*** **when overexpressed transforms normal cells into cancer cells**
- **overexpression of the gene was found in a subset (20-25%) of human breast cancer**
- **overexpression was correlated with poor prognosis**
- **a novel antibody therapy that targets the overabundant HER2 protein was developed**
- **this antibody has redefined the natural history of the disease**

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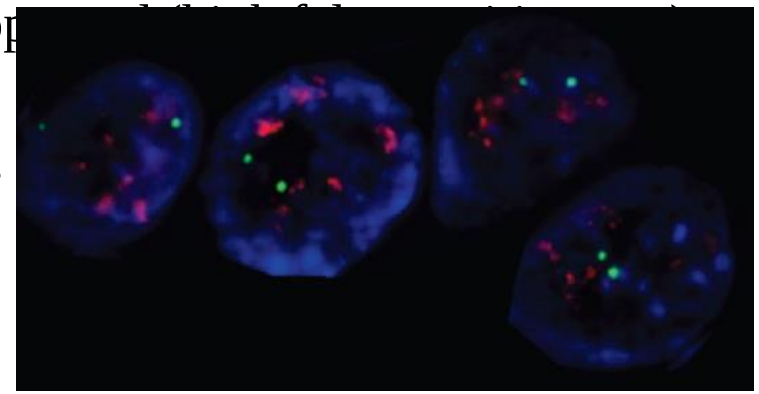
Her-2/Neu Testing

- **Her-2/Neu [homology to epidermal growth factor receptor; neu → neuroblastoma]**
  - **Herceptin (Trastuzumab, Genentech, Roche) is an antibody against the extracellular domain of Her-2/Neu protein**
  - **Patients with amplified Her-2/Neu gene respond better to Herceptin alone or Herceptin plus chemotherapy**
  - **FDA approved the test for predicting therapy with Herceptin**

Slamon et al., N Engl J Med 2001;344:783-

Methods for Assessing Her-2/Neu Status

- Immunohistochemistry for protein (Dako Hercep Test). FDA app
- FISH analysis



Resistance to Trastuzumab in Breast cancer,
Pohlmann et al,
Clin Cancer Research Focus, Dec 2009

CCR FOCUS

Resistance to Trastuzumab in Breast Cancer

Paula R. Pohlmann,[1] Ingrid A. Mayer,[1,2] and Ray Mernaugh[3]

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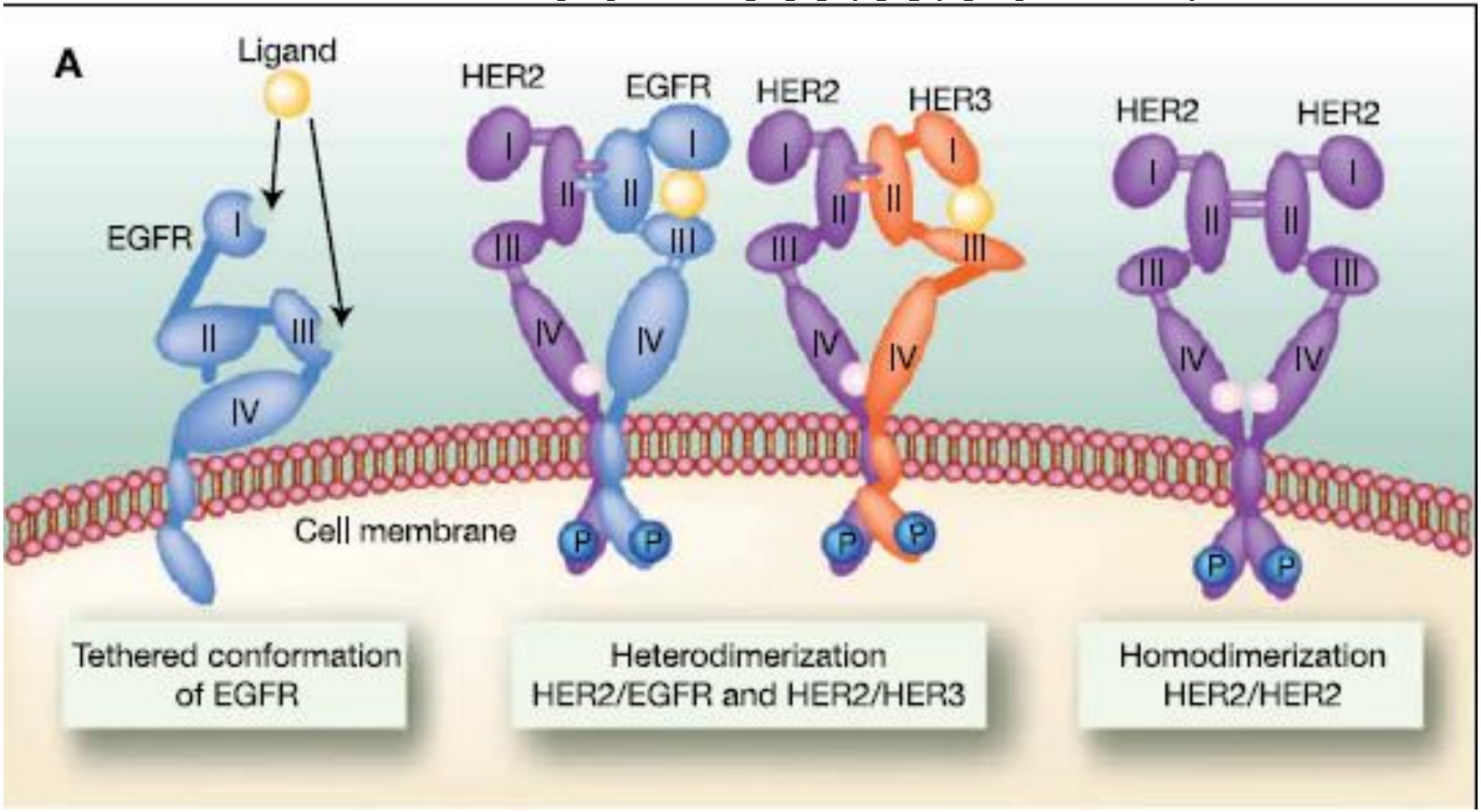
A, Receptor dimerization



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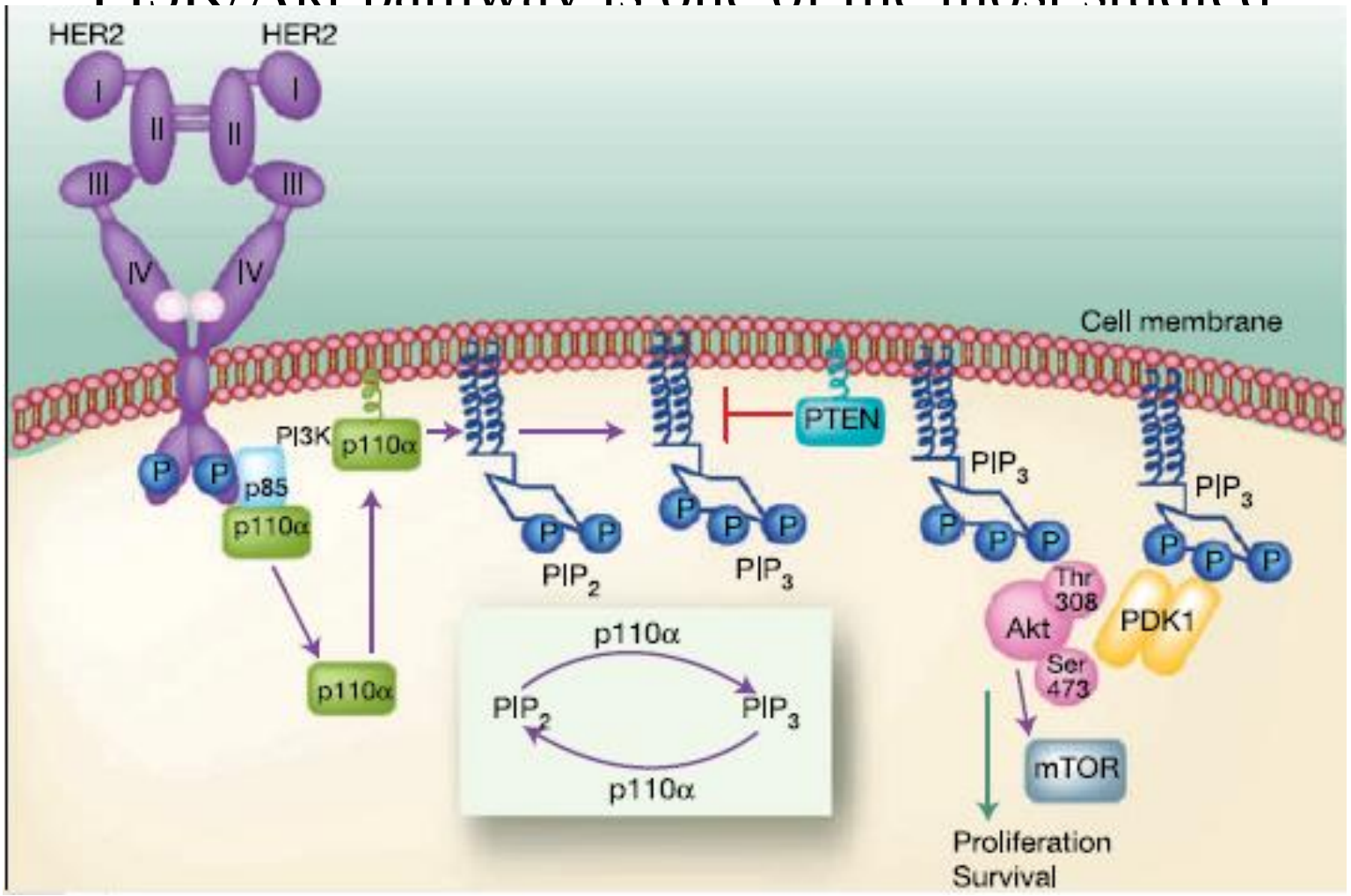
B, Activation of
PI3K/Akt pathway is one of the most studied



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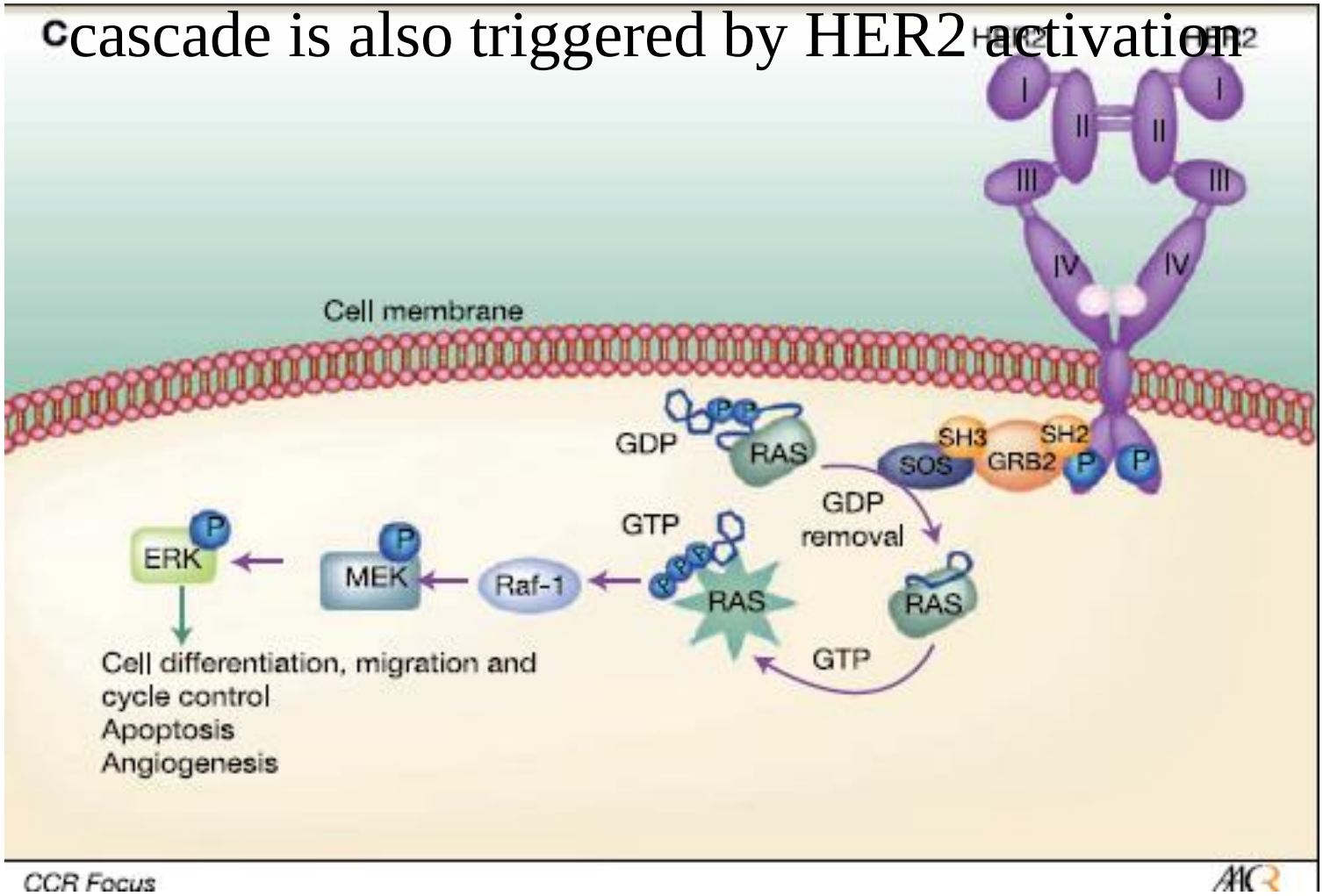
C. The RAS/Raf/MAPK signaling cascade is also triggered by HER2 activation



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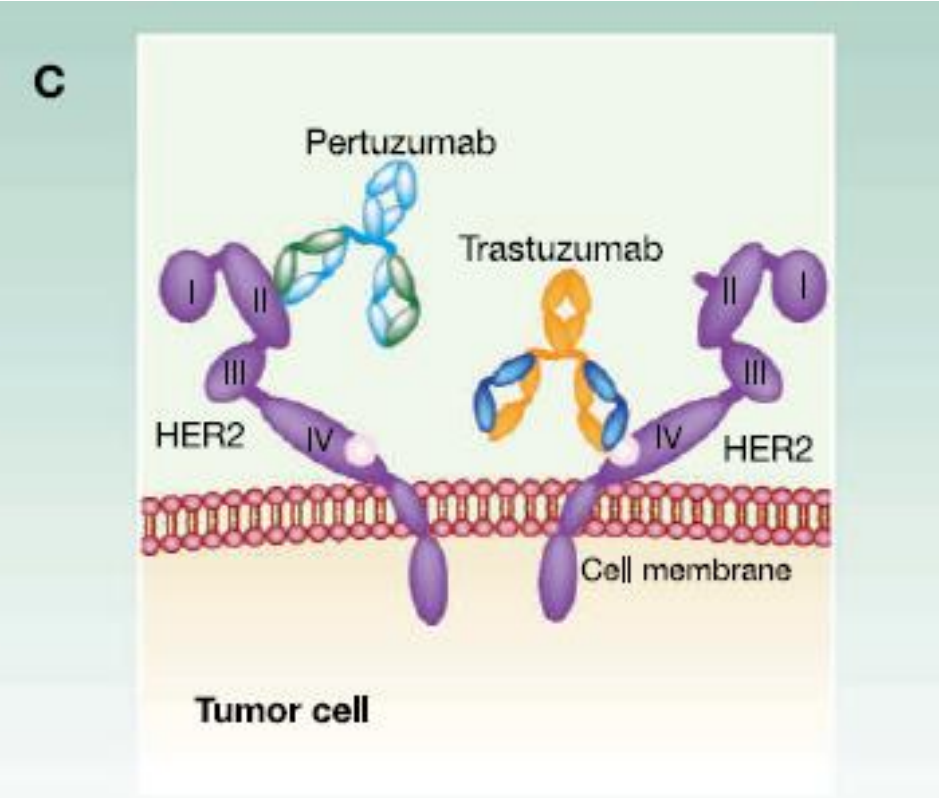
•C, trastuzumab Fab-related function results from its binding to domain IV of HER2.

HER2 indicates the human EGFR 2 (in purple).

Pertuzumab, another anti-HER2 humanized mAb, binds to an epitope present on domain II of HER2



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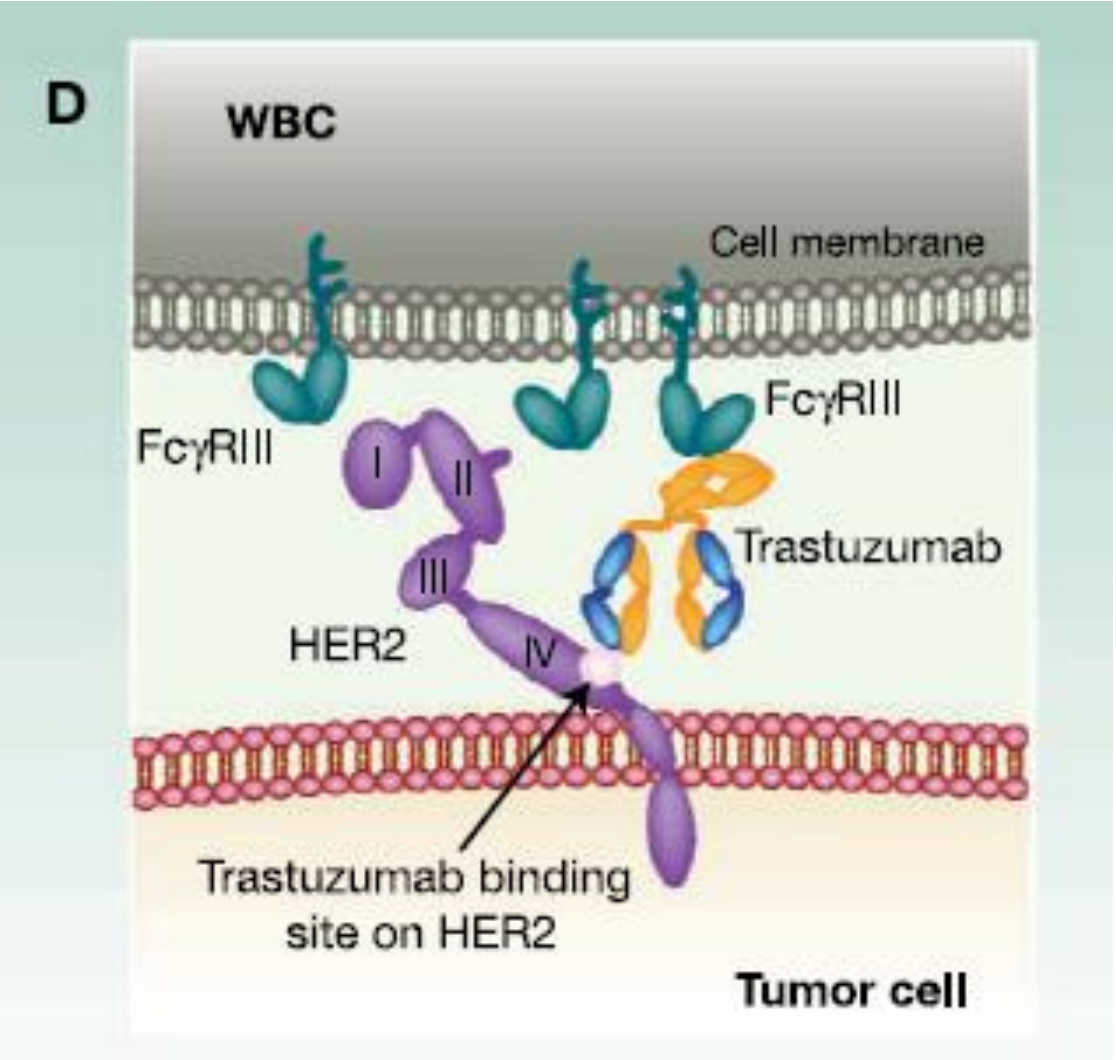
D, trastuzumab Fc-related functions result from the binding of its Fc portion to other cells that express Fc receptors, such as immune cells, hepatocytes, and endothelial cells.

The Fc region of trastuzumab can bind to Fcγ receptor III (RIII) present on the surface of effector cells from the immune system and trigger
tumor cell death via ADCC



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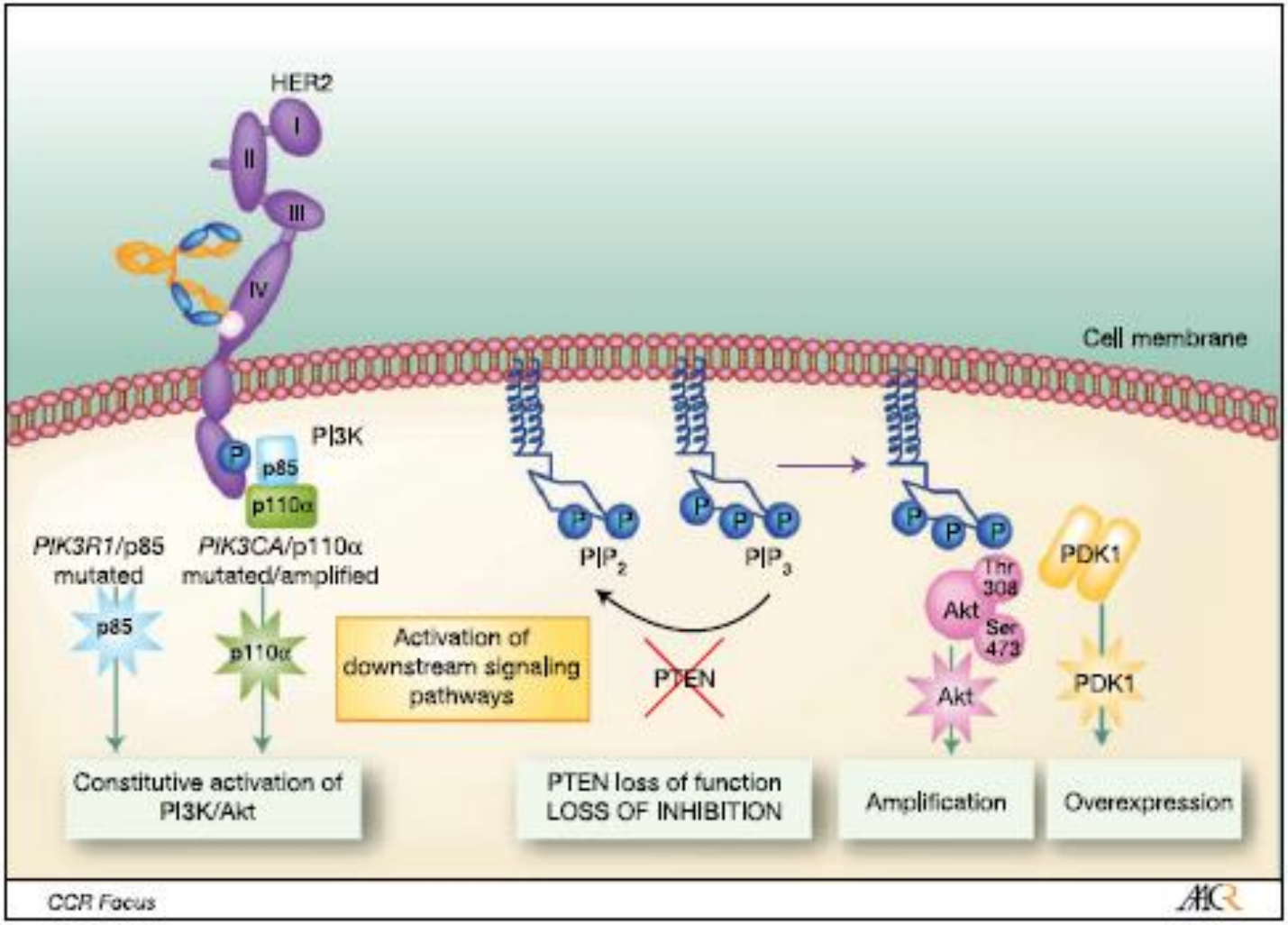
PIK3CA mutations and



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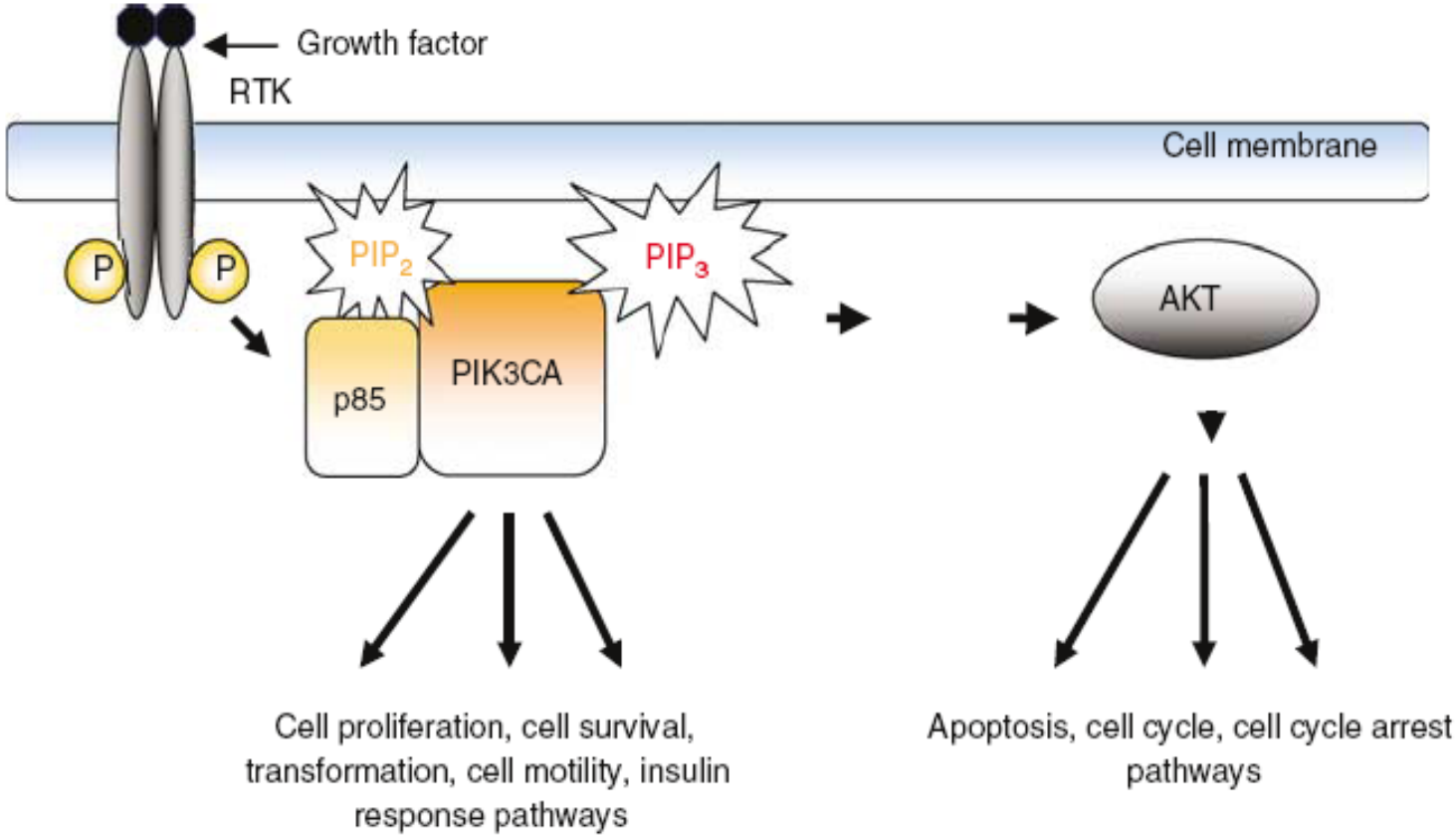
PIK3CA



(Karakas B, et al. British Journal of Cancer, 94, 455-459, 2006)

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PIK3CA

Cancer Cell
Report

Cell PRESS

A Functional Genetic Approach Identifies the PI3K Pathway as a Major Determinant of Trastuzumab Resistance in Breast Cancer

Katrien Berns,[1,6] Hugo M. Horlings,[2,6] Bryan T. Hennessy,[5] Mandy Madiredjo,[1] E. Marielle Hijmans,[1] Karin Beelen,[3] Sabine C. Linn,[3] Ana Maria Gonzalez-Angulo,[5] Katherine Stemke-Hale,[5] Michael Hauptmann,[4] Roderick L. Beijersbergen,[1] Gordon B. Mills,[5] Marc J. van de Vijver,[2] and René Bernards[1,*]

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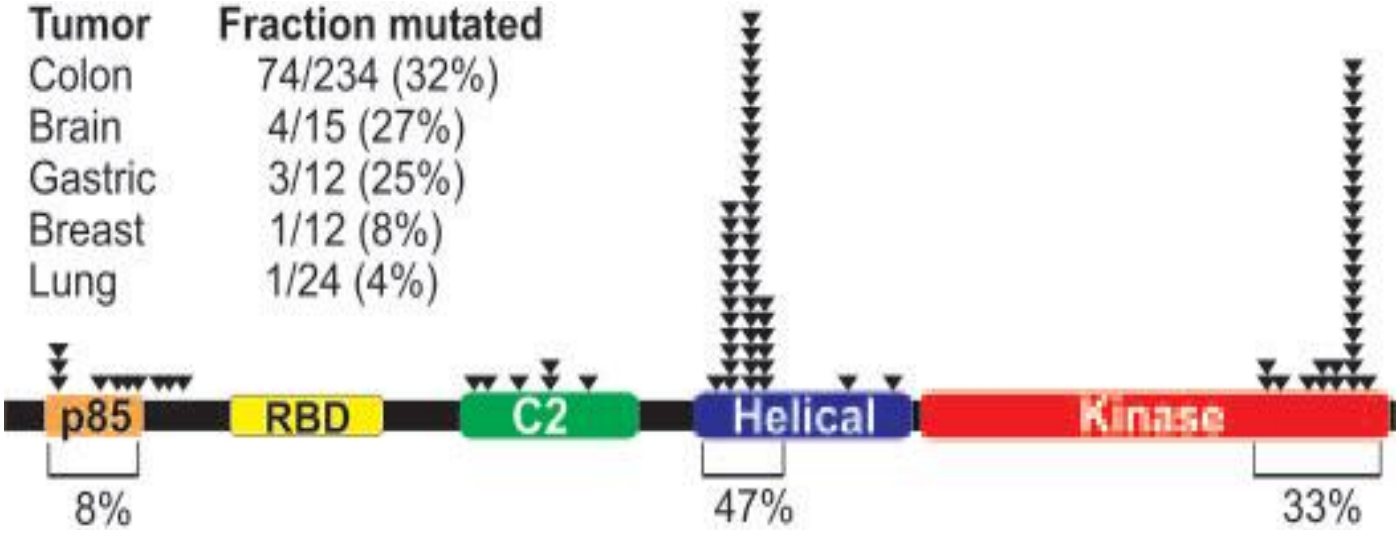
PIK3CA hot spot mutations



(Samuels Y et al. Science, Vol 304, 554, 2004)

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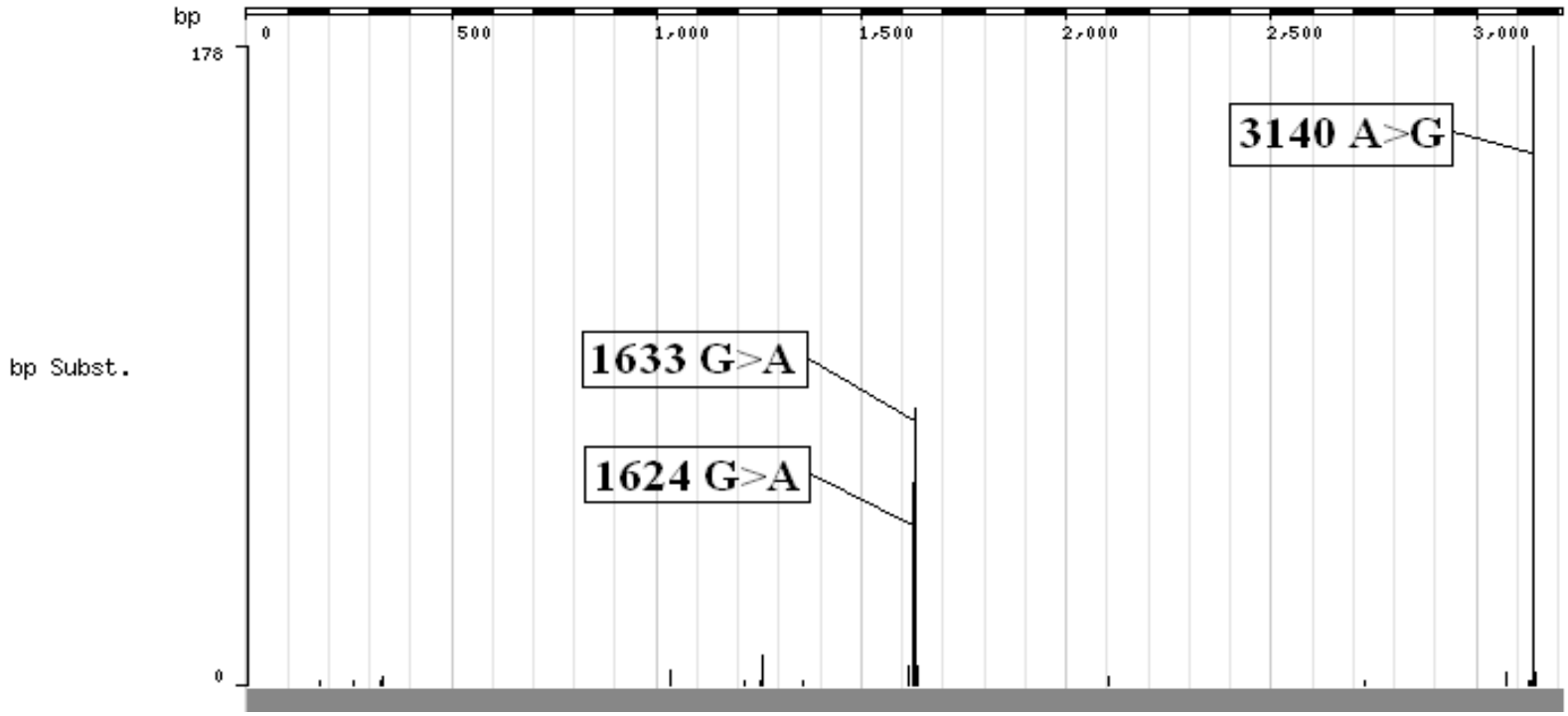
PIK3CA hot spot mutations

1624G>A (E542K)

1. **1633G>A (E545K)**
2. **3140A>G (A3140G)**



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Examples of Personalized Medicine in Oncology

2. NSCLC: EGFR mutations

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EGFR pathway

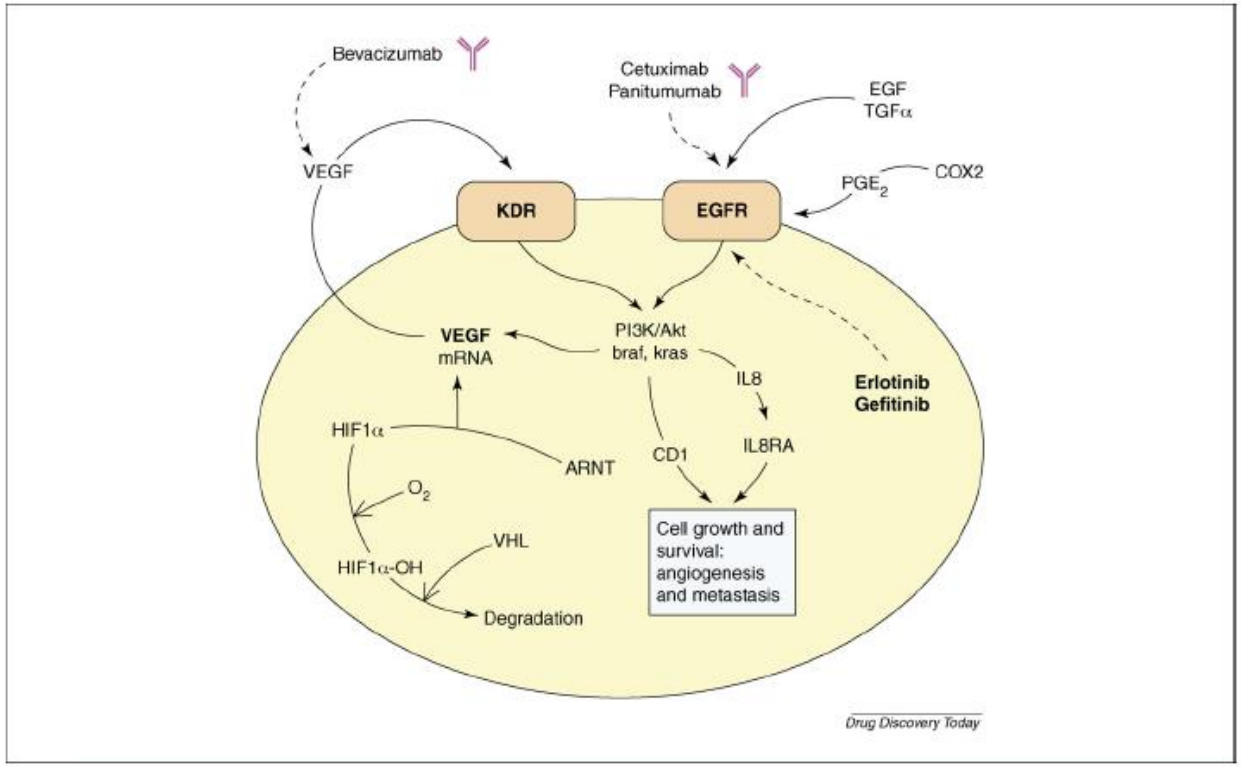

FIGURE 1

Simplified overview of the EGF and VEGF pathways. *Abbreviations* – ARNT: aryl hydrocarbon receptor nuclear translocator; braf: protein kinase b-raf; CD1: cyclin D1; COX2: cyclooxygenase 2; EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; HIF1α: hypoxia inducible factor 1α; HIF1α-OH: hydroxylated hypoxia inducible factor 1α; IL8: interleukin 8; IL8RA: interleukin 8 receptor A; KDR: kinase domain receptor; kras: small G protein k-ras; PGE_2: prostaglandin E_2; PI3K/Akt: phosphoinositide 3-kinase/akt protein kinase; TGFα: transforming growth factor α; VEGF: vascular endothelial growth factor; VHL: von Hippel-Landau tumour suppressor.

Pander et al, Drug discovery today, 2007

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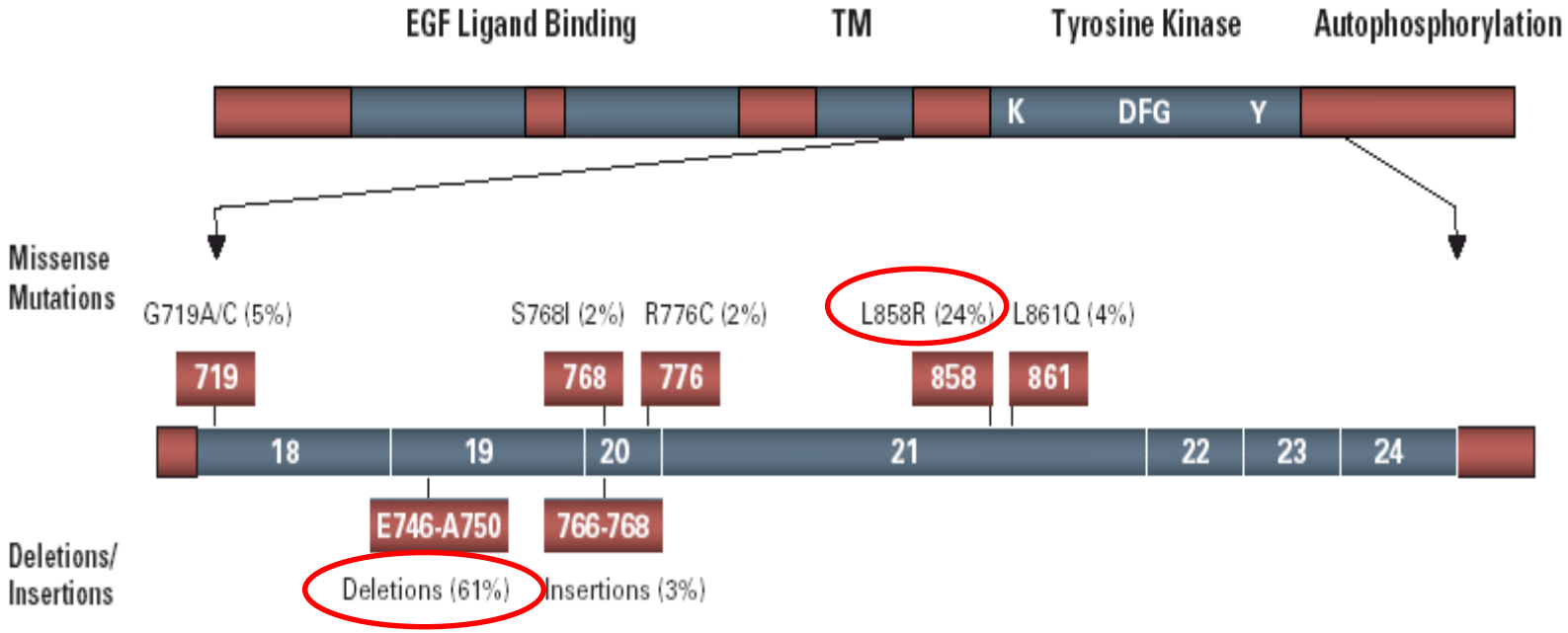
EGFR mutations in NSCLC



Most common mutations (almost 90%)

- **Exon 19 deletions (E746_A750)**
- **Missense mutations, exons 18 and 21 (L858R)**

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EGFR Mutations

- 29 somatic mutations in EGFR
- DNA samples isolated from fresh tissues, or FFPEs
- Patients with Non Small cell Lung cancer (NSCLC)
- EGFR mutations are related to therapy response in Tyrosine Kinase Inhibitors, like gefinib (Iressa, Astra Zeneca) and erlotinib (Tarceva, Roche),
- T790M mutation is correlated with resistance in Tyrosine Kinase inhibitors

IPASS Trial , Mok et al, SATURN Trial Capuzzo et al

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

◄ Previous Volume 361:947-957 September 3, 2009 Number 10 Next ►

Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

Tony S. Mok, M.D., Yi-Long Wu, M.D., F.A.C.S., Sumitra Thongprasert, M.D., Chih-Hsin Yang, M.D., Ph.D., Da-Tong Chu, M.D., Nagahiro Saijo, M.D., Ph.D., Patrapim Sunpaweravong, M.D., Baohui Han, M.D., Benjamin Margono, M.D., Ph.D., F.C.C.P., Yukito Ichinose, M.D., Yutaka Nishiwaki, M.D., Ph.D., Yuichiro Ohe, M.D., Ph.D., Jin-Ji Yang, M.D., Busyamas Chewaskulyong, M.D., Haiyi Jiang, M.D., Emma L. Duffield, M.Sc., Claire L. Watkins, M.Sc., Alison A. Armour, F.R.C.R., and Masahiro Fukuoka, M.D., Ph.D.

Conclusions Gefitinib is superior to carboplatin–paclitaxel as an initial treatment for pulmonary adenocarcinoma among nonsmokers or former light smokers in East Asia. The presence in the tumor of a mutation of the *EGFR* gene is a strong predictor of a better outcome with gefitinib. (ClinicalTrials.gov number, NCT00322452 [ClinicalTrials.gov].)

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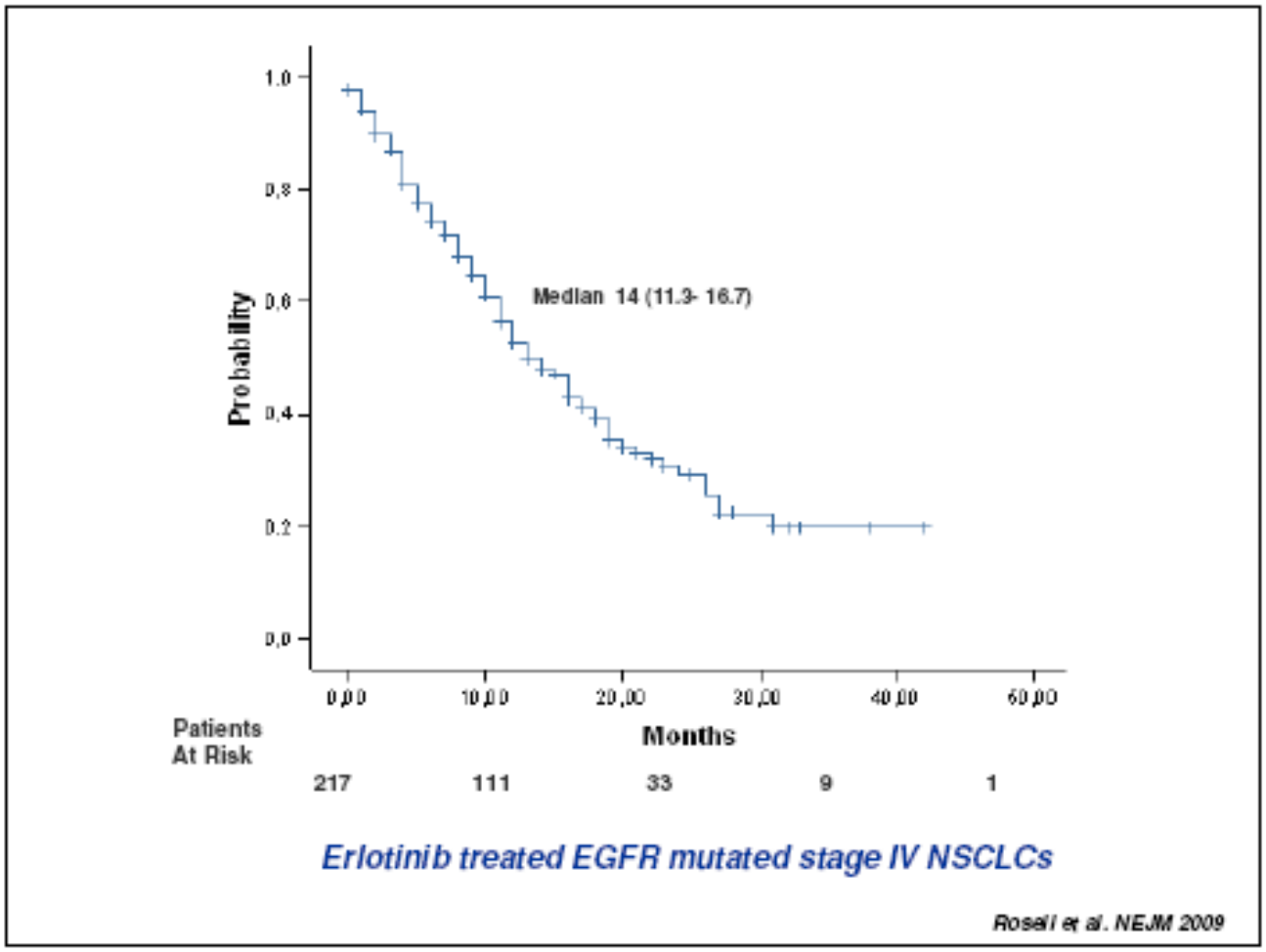

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Examples of Personalized Medicine in Oncology

3. NSCLC: EML4-ALK fusion gene

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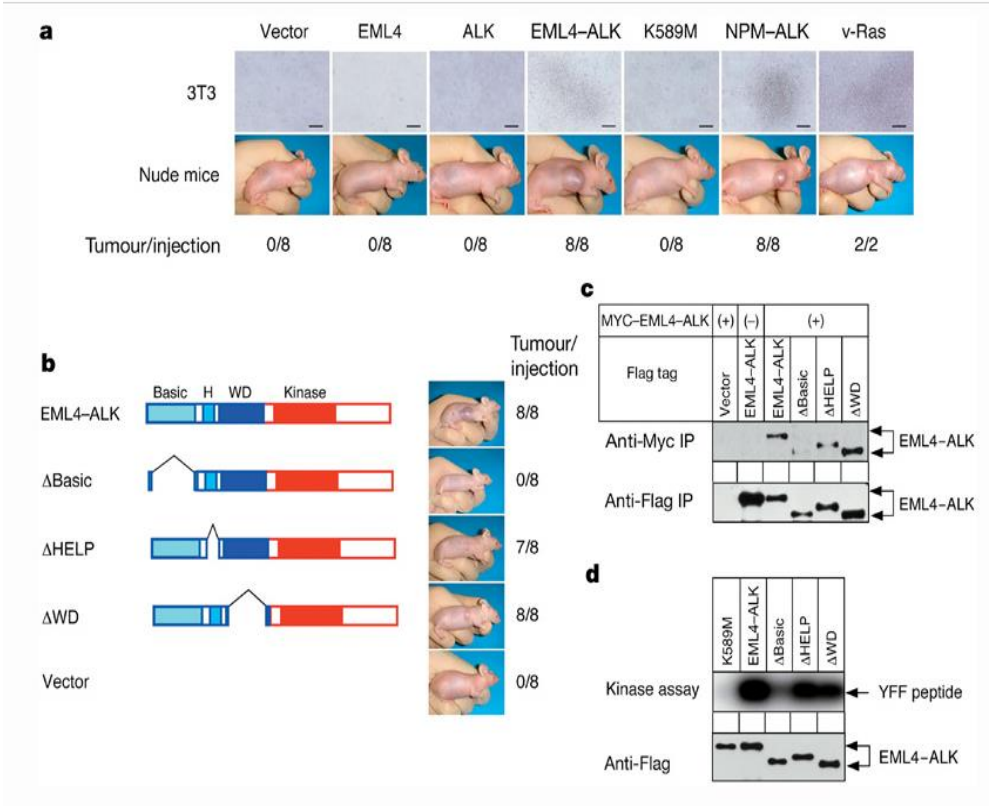
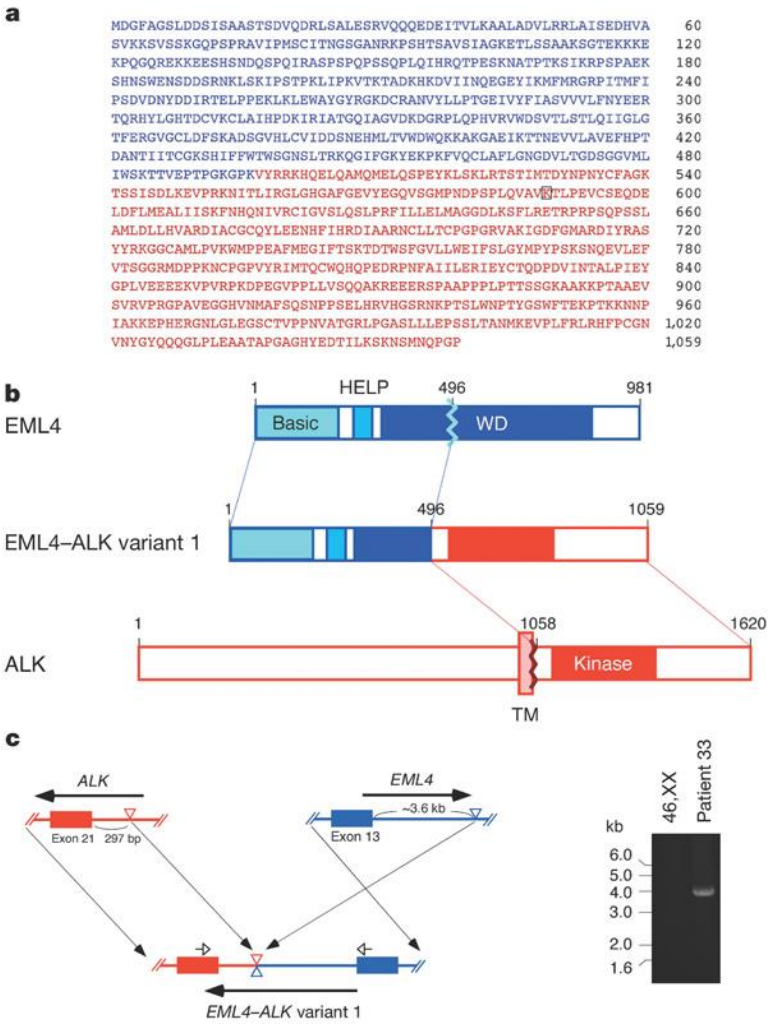
2007 Discovery

EML4–ALK fusion gene in NSCLC





Soda M et al Nature 448, 561-566 (2 August 2007)

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2010 inhibitor- clinical trial

EML4-ALK rearrangement in Non Small Cell Lung Cancer

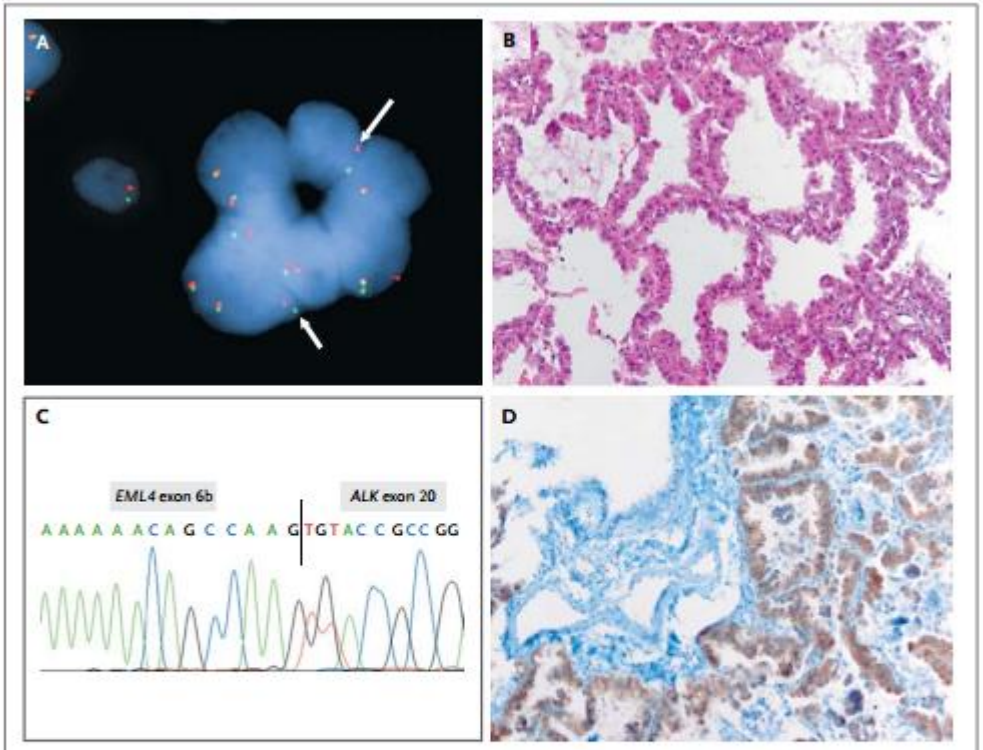


Figure 1. Diagnosis of an *EML4-ALK*–Positive Non–Small-Cell Lung Cancer in a Single Representative Patient.

Panel A shows the results of a break-apart fluorescence in situ hybridization assay of tumor cells from a patient with rearrangement of the gene encoding anaplastic lymphoma kinase (*ALK*). The green probe hybridizes to the region immediately 5′ to *ALK*, and the red probe to the 3′ region. The separation of red and green probe signals (arrows) indicates a chromosomal rearrangement involving *ALK*. Close apposition of red and green probe signals indicates an intact wild-type copy of *ALK*. The probe that was used was the Vysis LSI ALK Dual Color, Break Apart Rearrangement Probe (Abbott Molecular). Panel B shows a light micrograph of the same tumor, revealing adenocarcinoma (hematoxylin and eosin). Panel C shows a representative sequence electropherogram of a reverse-transcriptase–polymerase-chain-reaction assay of *EML4-ALK*. The sequence of a junction between *EML4* exon 6b and *ALK* exon 20 is shown. Panel D shows immunohistochemical analysis of ALK protein expression in tumor cells (brown) but not in adjacent normal bronchial epithelium (diaminobenzidine).

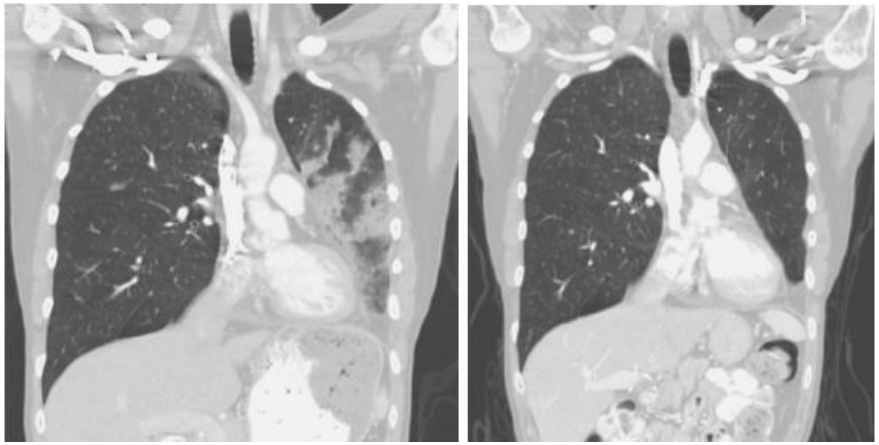
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 28, 2010 VOL. 363 NO. 18

Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

Crizotinib: inhibitor

Before after



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2011 FDA approval of the drug!!!

EML4-ALK gene fusion detection (Companion Diagnostic test)
FDA approval at the same time!!!

- Crizotinib (Pfizer) is a new medicine that can have a dramatic impact in a small minority of lung cancer patients.
- 60% of patients on this drug were alive after two years, compared to 9% in historical controls
- EML4- ALK fusion: very rare
- Only 5% of patients with non-small cell lung cancer have this rearrangement
- However, lung cancer is so common that this still represents some 9,000 people each year.
- In one study of 136 patients, half of patients responded to the drug, based on scans of their tumors.
- In a second study, 61% of patients responded. Three patients saw their tumors disappear from the scans.
- Read more:
- ***http://www.nejm.org/doi/full/10.1056/NEJMoa1006448***
- ***http://www.forbes.com/sites/robertlangreth/2010/10/28/pfizer-drug-powers-cancer-treatment-into-the-dna-age/***

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Clinical Activity of the Oral ALK Inhibitor, Crizotinib (PF-02341066), in Patients with *ALK*-positive Non-small Cell Lung Cancer

Y Bang et al.

- **Study aim**
- This study set out to investigate the efficacy and safety of the oral anaplastic lymphoma kinase (ALK) inhibitor, crizotinib, in patients with ALK-positive NSCLC.

- *Phase I*

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Examples of Personalized Medicine in Oncology

4. Metastatic colorectal cancer: K-RAS mutations

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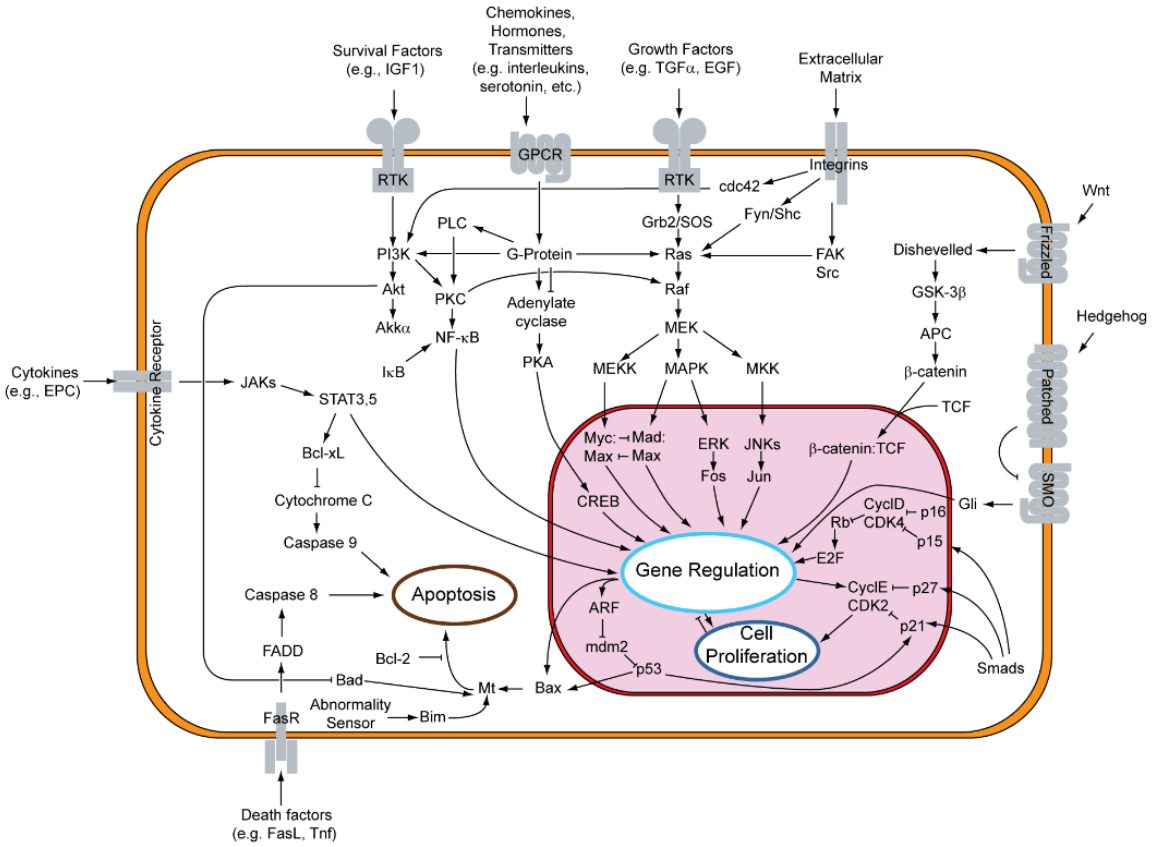
KRAS
(V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog)

- **KRAS acts as a molecular on/off switch**
- **KRAS binds to *GTP* in the active state and possesses an intrinsic enzymatic activity which cleaves the terminal phosphate of the nucleotide converting it to *GDP***
- **The rate of conversion is usually slow but can be sped up dramatically by an accessory protein of the GTPase activating protein (GAP) class, for example *RasGAP***
- **Other members of the Ras family include: *HRAS NRAS* and *RRAS***



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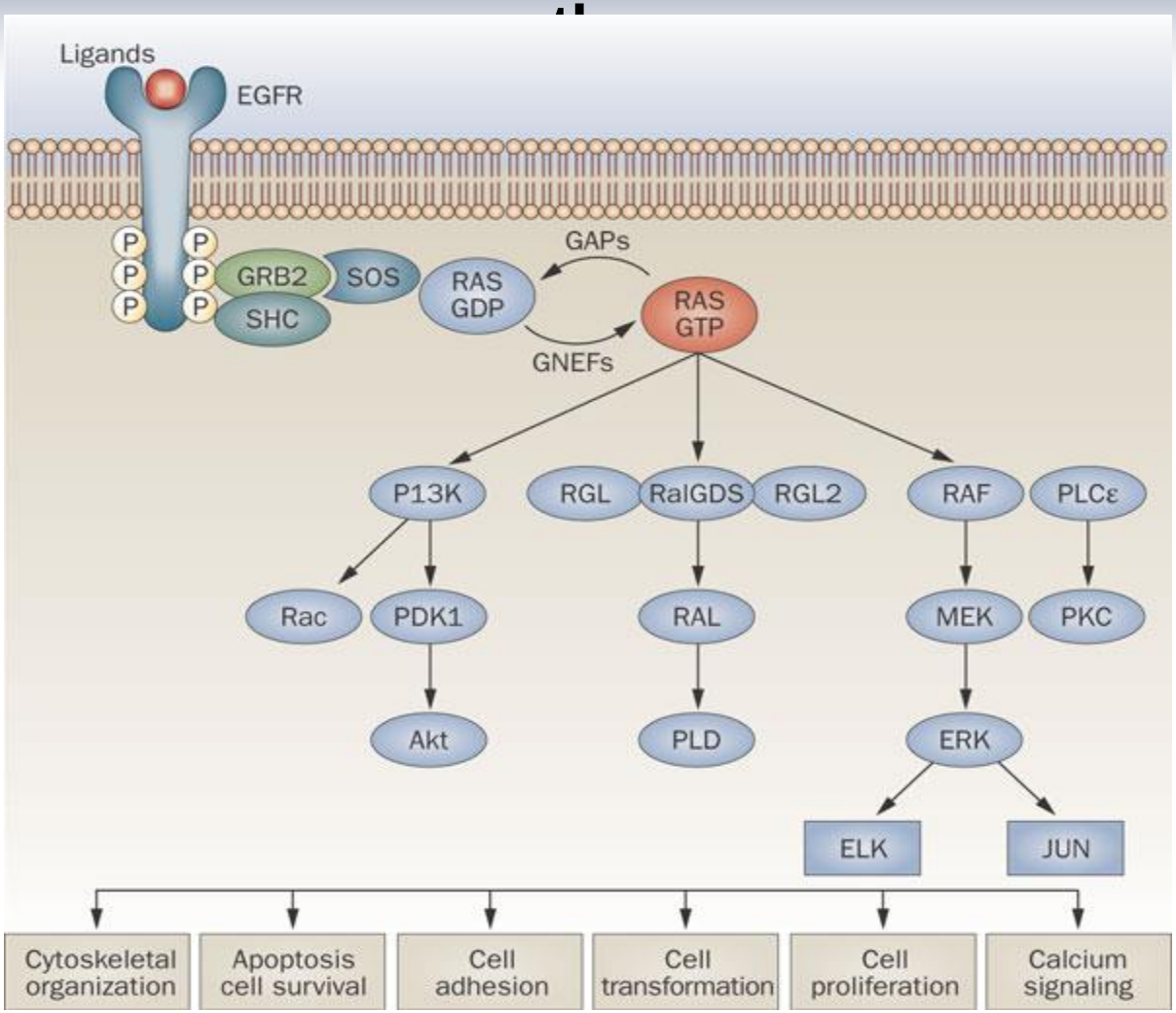
RAS mediated intracellular signal transduction



Normanno, N. *et al.* (2009) Implications for *KRAS* status and EGFR-targeted therapies in metastatic CRC

Nat. Rev. Clin. Oncol. **doi:10.1038/nrclinonc.2009.111**

nature REVIEWS CLINICAL ONCOLOGY

British Journal of Cancer (2010), 1–11

www.bjcancer.com

Full Paper

Activating *K-Ras* mutations outwith 'hotspot' codons in sporadic colorectal tumours – implications for personalised cancer medicine

G Smith[1,5], R Bounds[1,5], H Wolf[1], RJC Steele[2], FA Carey[3] and CR Wolf[*,1,4]

[1]Biomedical Research Institute, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK; [2]Department of Surgery and Molecular Oncology, University of Dundee, Dundee DD1 9SY, UK; [3]Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK; [4]CRUK Molecular Pharmacology Unit, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK

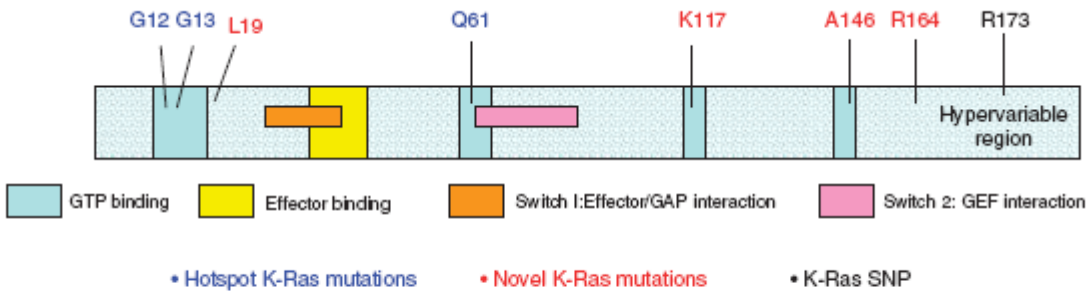


Figure 2 Location of novel and hotspot Kirsten-Ras (*K-Ras*) mutations. The location of new (red) and hotspot (blue) *K-Ras* mutations are illustrated on a representation of the *K-Ras* protein sequence, together with the position of the novel *K-Ras* single-nucleotide polymorphism (SNP) (black). Putative GTP and effector binding sites and GAP and GEF interaction domains are highlighted.

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Frequencies of hot spot and novel *K-ras* mutations in colorectal tumors

Table 2 Frequencies of hotspot and novel mutations in Kirsten-Ras (*K-Ras*) in colorectal tumours

Mutation	Nucleotide change	Amino acid change	Frequency (%)
K-Ras			
(A) Hotspot codon mutations			
Codon			
12			23/106 (21.7%)
	G->A	$Gly_{12}Ser$	4/106 (3.8%)
	G->C	$Gly_{12}Ala$	3/106 (2.8%)
	G->T	$Gly_{12}Cys$	2/106 (1.9%)
	G->T	$Gly_{12}Val$	6/106 (5.7%)
	G->C	$Gly_{12}Arg$	1/106 (<1%)
	G->A	$Gly_{12}Asp$	7/106 (6.6%)
13	G->A	$Gly_{13}Asp$	6/106 (5.7%)
61	None detected	None detected	0/106
Mutation total			29/106 (27.4%)
(B) Novel codon mutations			
Codon			
19	G->T	$Leu_{19}Phe$	1/106 (<1%)
117	A->C	$Lys_{117}Asn$	1/106 (<1%)
146	G->A	$Ala_{146}Thr$	7/106 (6.6%)
164	G->A	$Arg_{164}Gln$	1/106 (<1%)
173[a]	T->C	No change	39/106 (36.8%)
New mutation total			39/106 (36.8%)
B-Raf			
Codon			
600	T->A	Val600Glu	1/106 (<1%)
Final mutation total			40/106 (37.8%)

[a]The sequence change at codon 173 is a single-nucleotide polymorphism, not a tumour-specific mutation.

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K-RAS Mutation Testing

- **Detection of 7 *somatic* mutations in codons 12 and 13 of k-ras oncogene in DNA samples from patients with metastatic colorectal cancer**

- **Mutation detection sensitivity should be at the level of 1% in the presence of wt DNA**

- **K-RAS mutation detection is important to identify patients with metastatic colorectal cancer that do not respond to anti EGFR therapies like cetuximab (*Erbitux, Merck) &* panitumumab (*Vectibix, Amgen).***

- **K-RAS mutations should be tested before the administration of *Erbitux* και *Vectibix*, according to most recent guidelines of FDA and European Society of Pathology**

[1] Amado et al, JCO, Apr 2008, Van Cutsem et al, JCO, May 2008

K-RAS Mutations

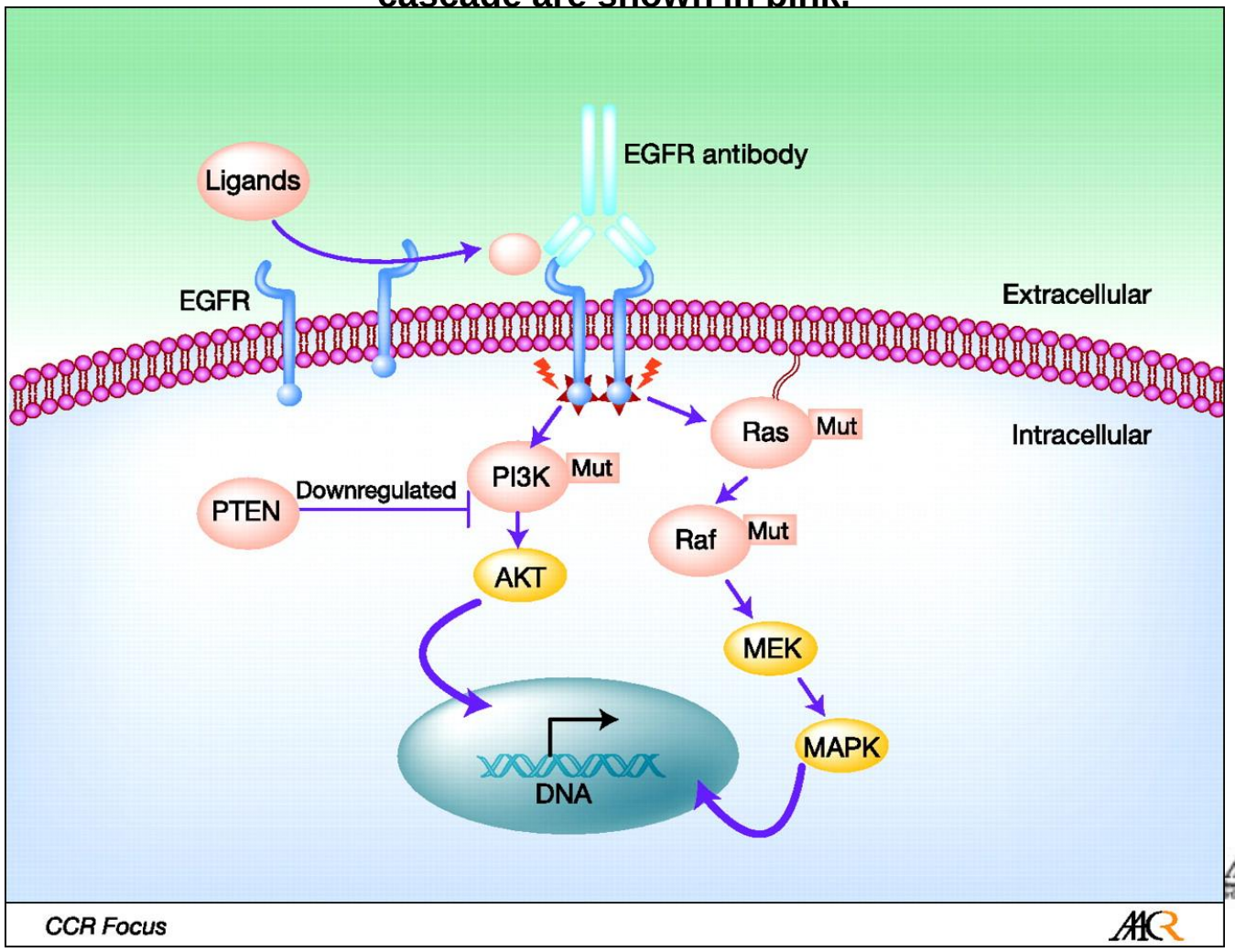
Mutation	Base Change	Cosmic ID
12 Asp	Gly12Asp (GGT>GAT)	521
12 Ala	Gly12Ala (GGT>GCT)	522
12 Arg	Gly12Arg (GGT>CGT)	518
12 Cys	Gly12Cys (GGT>TGT)	516
12 Ser	Gly12Ser (GGT>AGT)	517
12 Val	Gly12Val (GGT>GTT)	520
13 Asp	Gly13Asp (GGC>GAC)	532

> 97.0%of K-RAS mutations are found in codons 12 and 13

Examples of Personalized Medicine in Oncology

5. Colorectal cancer (CRC): beyond kRAS mutations

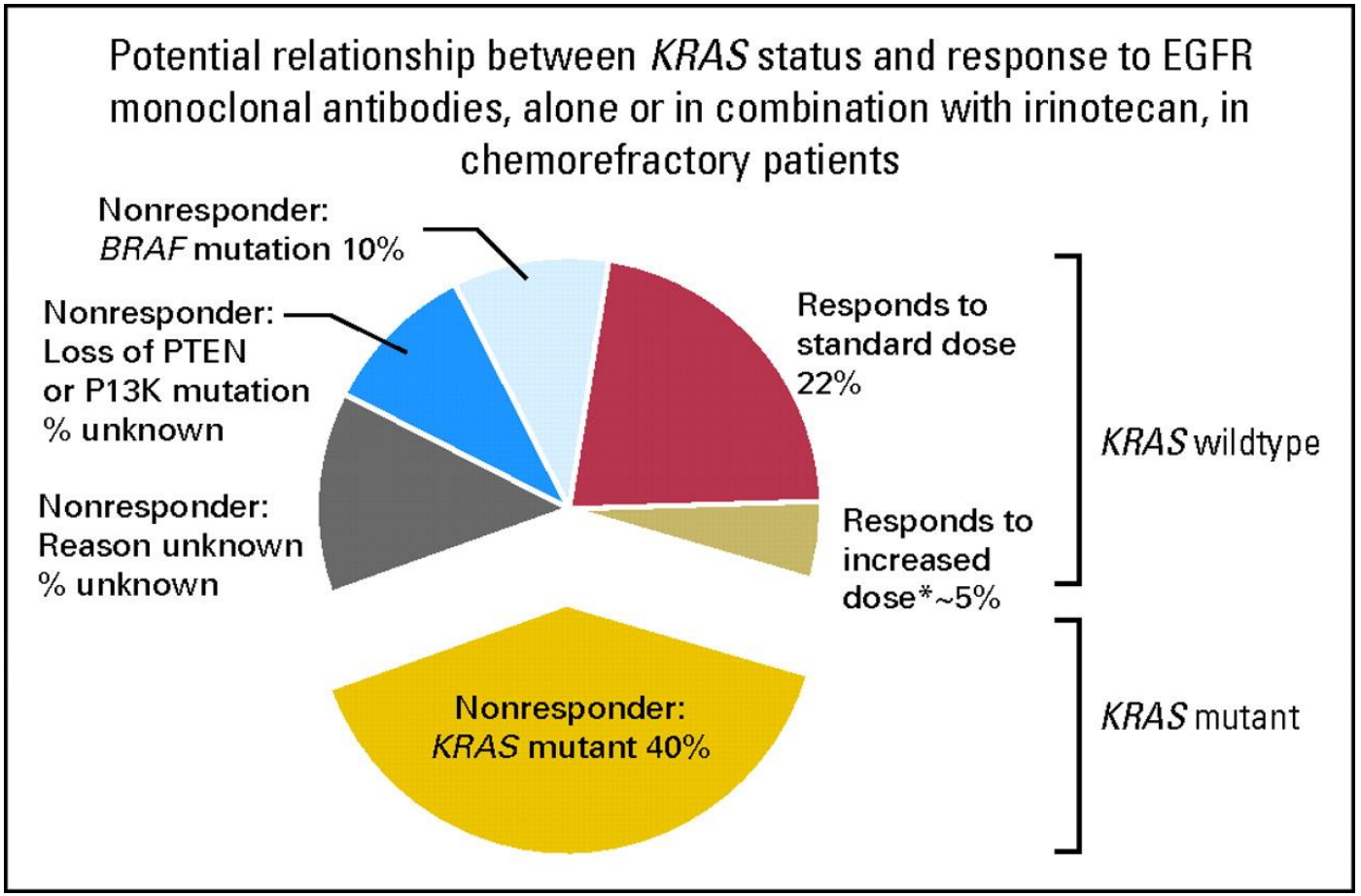
EGFR signaling pathway and candidate predictive molecular markers for the activity of EGFR antibodies in CRC. Candidate predictive biomarkers that are also part of the EGFR signaling cascade are shown in pink.



Clinical Cancer Research

Banck M S , Grothey A Clin Cancer Res 2009;15:7492-7501

BEYOND *KRAS* MUTATIONS



Wong, R. et al. J Clin Oncol; 26:5668-5670 2008

VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF)

- **Key regulator of angiogenesis.**
- **Potent and specific mitogen for endothelial cells.**
- **Also known as Vascular Permeability Factor (VPF) based on its ability to induce vascular leakage.**
- **Potently inhibits the differentiation of dendritic cells.**
- **The human VEGF gene is located in chromosome 6p21.3 and is organized in 8 exons.**
- **Alternative exon splicing of a single VEGF gene results in the formation of various isoforms.**

Examples of Personalized Medicine in Oncology

melanoma

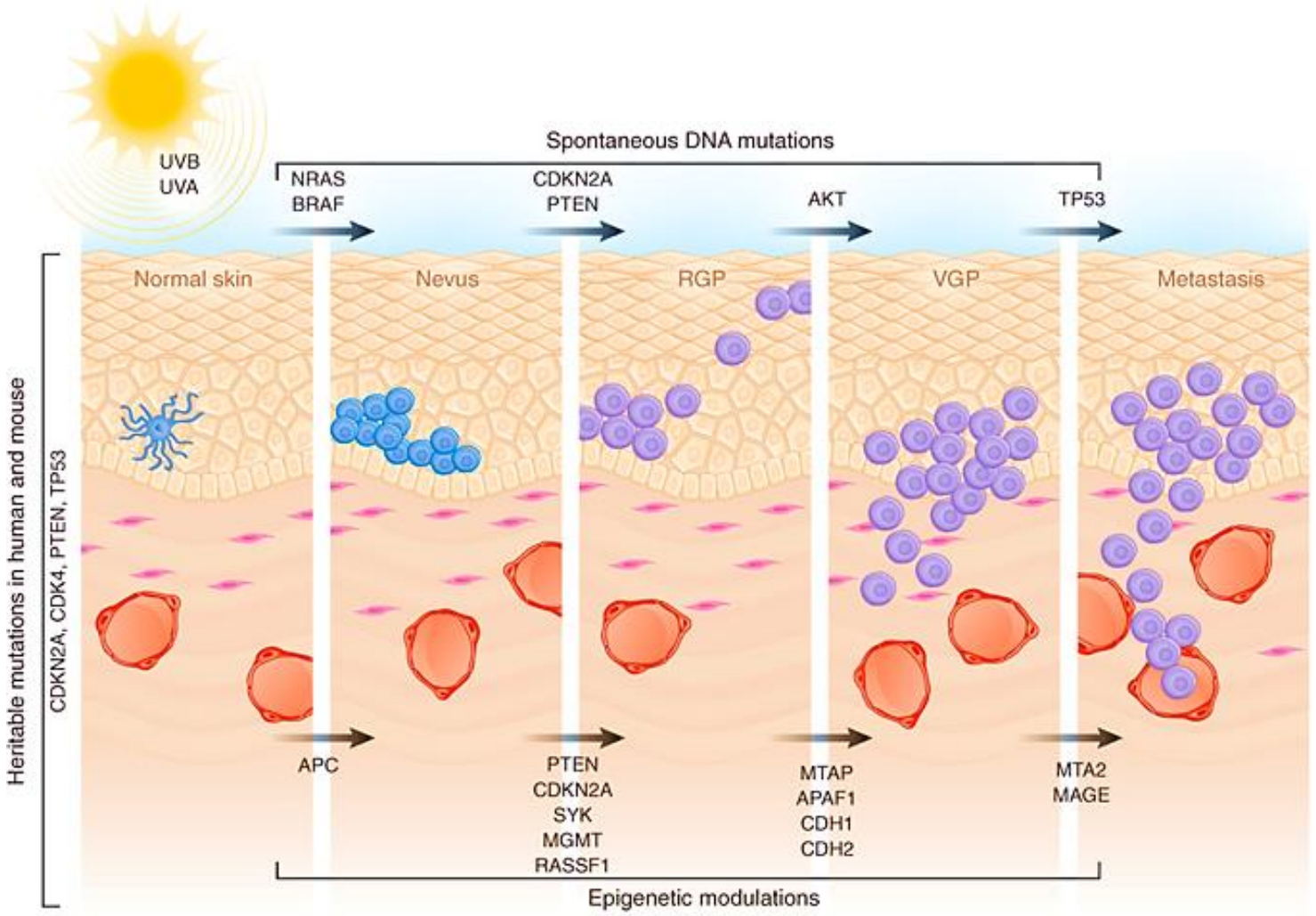
6. BRAF mutations

Evi Lianidou

Melanoma

- is the fifth most common solid malignancy in the United States, affecting 76,000 individuals each year
- the survival rate between the stages of disease is decreasing significantly
- most melanoma patients are cured after surgical excision of the primary tumor, but 10-20% of patients progress to develop metastatic disease
- new treatments have been recently developed, including targeted therapies and immune modulators in patients with advanced disease
- risk factors: ultraviolet exposure, genetic predisposition, immunosuppression

Melanoma metastatic process



Zaidi MR et al. *Journal of Investigative Dermatology* 2008 128, 2381–2391

Melanoma Molecular Biomarkers (1)

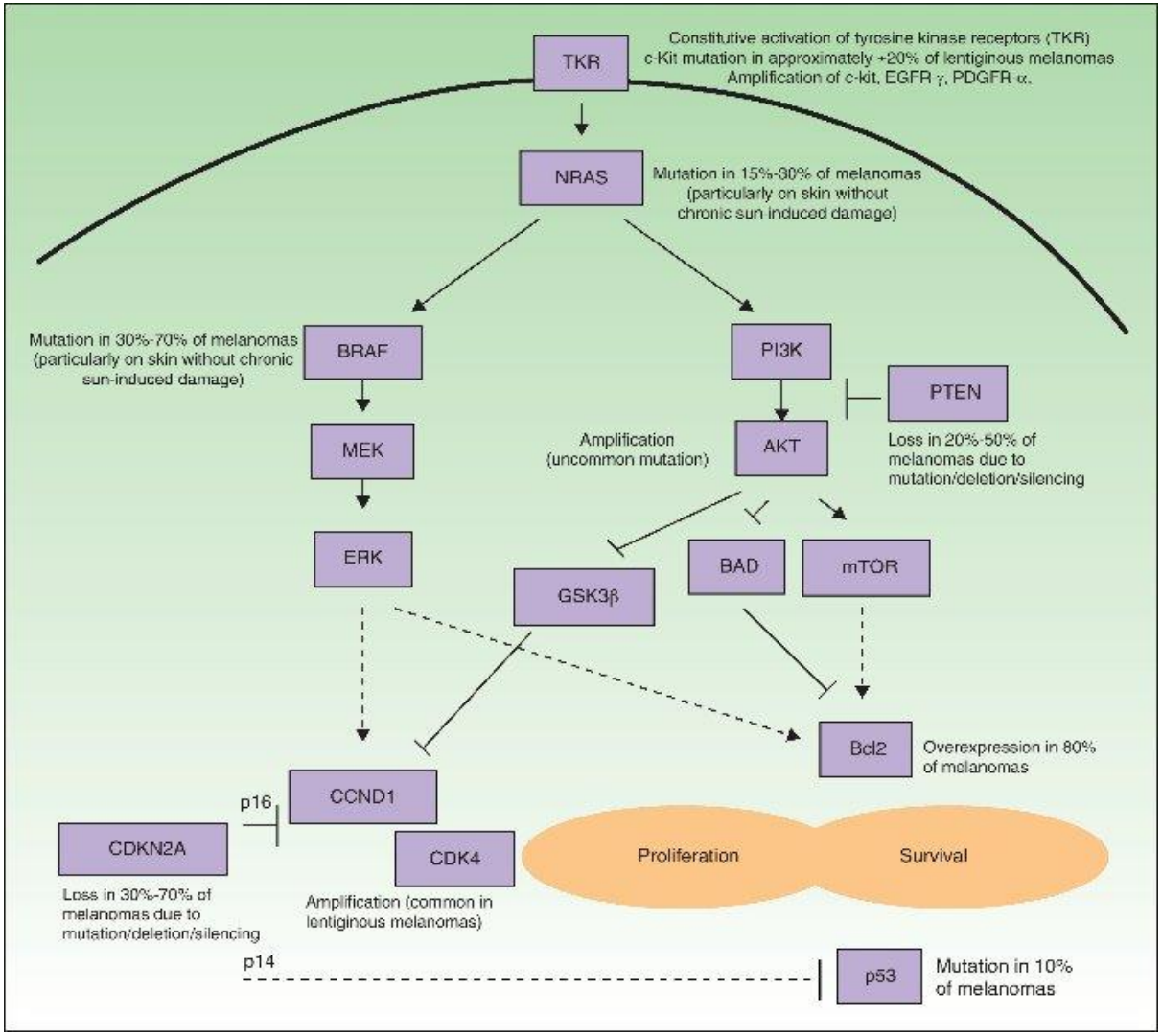
MARKER	CHARACTERISTICS	CLINICAL RELEVANCE
mRNA BM		
MART-1	a frequent melanoma associated antigen specific for melanoma	Diagnostic/Prognostic: Combination of these biomarkers can be used in the diagnosis of SLN to upstage melanoma patients and for detection of CTC during treatment or follow-up.
MAGE-A3	cancer-testis antigen not found in normal tissues except testis and placenta	
GalNac-T	key enzyme involved in gangliosides GM2 and GD2 synthesis	
PAX3	involved in the regulation of melanin synthesis, migration and anti-apoptosis well-expressed in melanomas	
MITF	essential for the development and postnatal survival of melanocytes	
HMW-MAA	melanoma chondroitin sulfate proteoglycan	Diagnostic: Improve desmoplastic melanoma diagnosis
FABP-7	involved in lipid-metabolism	Prognostic: independent poor-prognostic factor for DFS and OS if found in tumor
Survivin	inhibitor of apoptosis protein family	Prognostic: expression in tumor is correlated to good prognosis among stage IV patients who received postoperative immunotherapy
CXCR4	chemokine receptor	Tumor characterization: the most common chemokine receptor expressed in PE liver melanoma metastases
CCR-9	chemokine receptor	Tumor characterization: Expression in tumor may facilitate metastasis to the small intestine

Melanoma Molecular Biomarkers (2)

MARKER	CHARACTERISTICS	CLINICAL RELEVANCE
Genomic BM		
BRAF	a component of the Ras-MAPK-ERK pathway	Diagnostic/Prognostic: V600E mutation detected in patient serum can predict disease outcome and therapeutic response
RET	receptor tyrosine kinase	Diagnostic: G691S polymorphism improves desmoplastic melanoma diagnosis
Apaf-1	tumor-suppressor gene mediating p53-induced apoptosis	Prognostic: LOH detection in tumor and serum associated with poor prognosis in patient
FABP7	Lipid-metabolizing capacity associated with fatty acids	Prognostic: LOH detection in tumor is associated with poor prognosis in patient
RASSF1A/RARb(beta)	tumor suppressor gene	Prognostic: Detection of hypermethylated RASSF1A in patient serum is associated with worse survival in patients receiving biochemotherapy
RARβ	tumor suppressor gene	
estrogen recepter-α	sex hormone receptor	Prognostic: Hypermethylation of ER-αfound in serum is associated with poor prognosis
MINT31	multiple noncoding, methylated-in-tumor loci	Prognostic: Hypermethylation of MINT31 found in tumor is linked to good prognosis in stage III melanoma
DNMT3	DNA methyltransferase	Prognostic: high level of DNMT3 in LN metastatic tumor is associated with poor prognosis in patients
miR-29c	microRNA	Prognostic: high level of miR-29c in LN metastatic tumor is associated with better prognosis in patients
miR-532-5p	microRNA	Tumor characterization: may contribute to melanoma progression by downregulation of RUNX3 expression

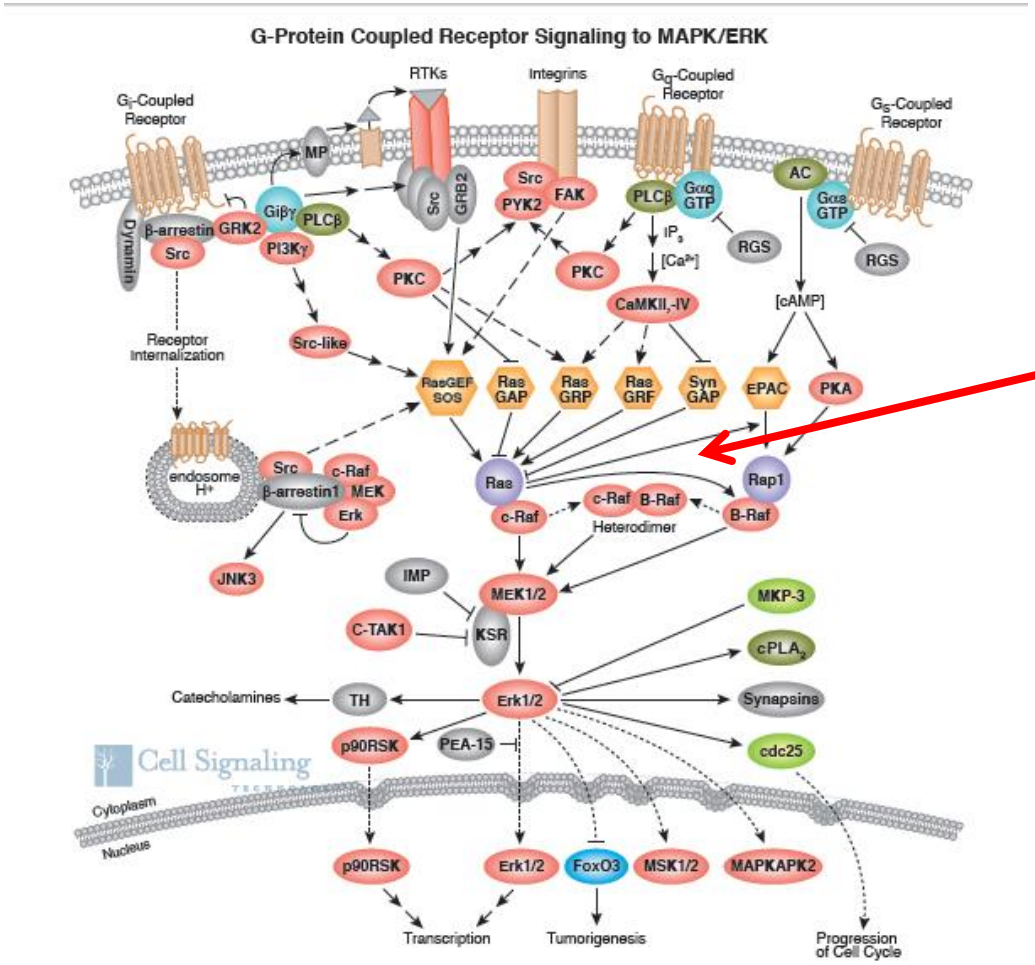
Tanaka R et al. J Surg Oncol. 2011 Sep;104(4):438-46

Molecular Pathways of melanoma



Marti RM et al. Actas Dermosifiliogr.2012;103(7) :579---590

RAS-RAF pathway



BRAF gene

- 7q24
- 18 exons (190280 bp)
- mRNA : 2,478 nt

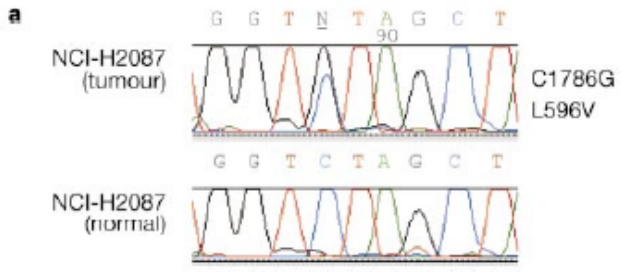
BRAF mutations

Mutations of the *BRAF* gene in human cancer

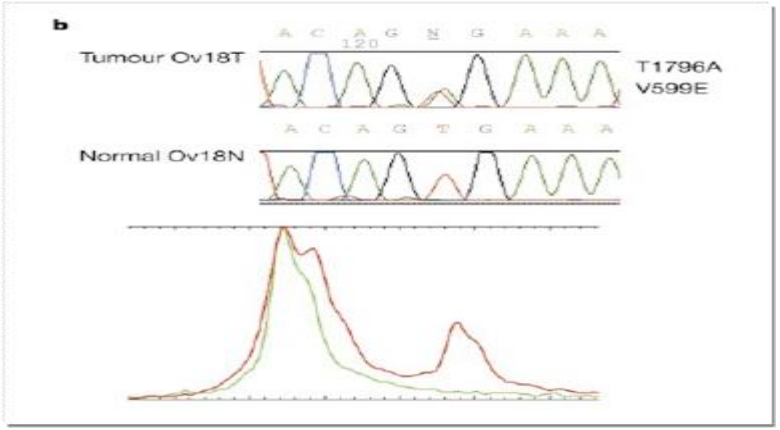
Helen Davies[1,2], Graham R. Bignell[1,2], Charles Cox[1,2], Philip Stephens[1,2], Sarah Edkins[1], Sheila Clegg[1], Jon Teague[1], Hayley Woffendin[1], Mathew J. Garnett[3], William Bottomley[1], Neil Davis[1], Ed Dicks[1], Rebecca Ewing[1], Yvonne Floyd[1], Kristian Gray[1], Sarah Hall[1], Rachel Hawes[1], Jaime Hughes[1], Vivian Kosmidou[1], Andrew Menzies[1], Catherine Mould[1], Adrian Parker[1], Claire Stevens[1], Stephen Watt[1], Steven Hooper[3], Rebecca Wilson[3], Hiran Jayatilake[4], Barry A. Gusterson[5], Colin Cooper[6], Janet Shipley[6], Darren Hargrave[7], Katherine Pritchard-Jones[7], Norman Maitland[8], Georgia Chenevix-Trench[9], Gregory J. Riggins[10], Darell D. Bigner[10], Giuseppe Palmieri[11], Antonio Cossu[12], Adrienne Flanagan[13], Andrew Nicholson[14] Judy W. C. Ho[15], Suet Y. Leung[16], Siu T. Yuen[16], Barbara L. Weber[17], Hilliard F. Seigler[18], Timothy L. Darrow[18], Hugh Paterson[3], Richard Marais[3], Christopher J. Marshall[3], Richard Wooster[1,6], Michael R. Stratton[1,4] & P. Andrew Futreal[1]

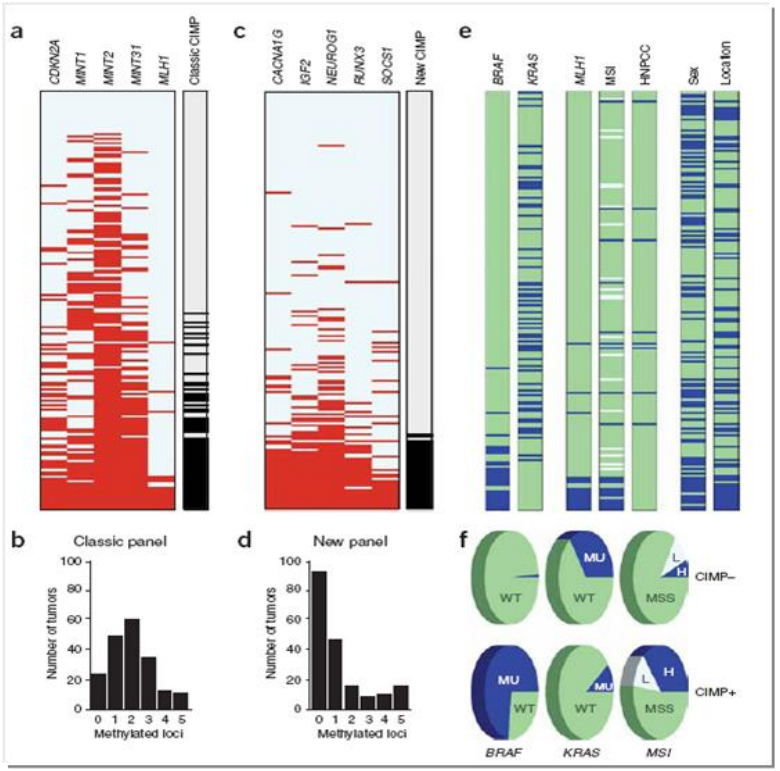
[1]*Cancer Genome Project, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, CB10 1SA, UK*
[3]*Cancer Research UK Centre for Cell and Molecular Biology, Chester Beatty Labs, Institute of Cancer Research, London SW3 6JB, UK*
[4]*Section of Cancer Genetics;* [6]*Section of Molecular Carcinogenesis; and* [7]*Section of Paediatrics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, UK*
[5]*Department of Pathology, Western Infirmary, University of Glasgow, S11 6NT, UK*

phoblastoid cell lines from the same individuals were screened for sequence variants through the coding exons and intron–exon junctions of the *BRAF* gene using a capillary-based modified heteroduplex method followed by direct sequencing of polymerase chain reaction products. (Exon 1, containing 135 base pairs (bp) of coding sequence, failed to amplify despite the use of five different primer sets.) Three single-base substitutions were detected. Two were in *BRAF* exon 15: T1796A leading to a substitution of valine by glutamic acid at position 599 (V599E) in the melanoma cell line Colo-829, and C1786G leading to L596V in the NSCLC cell line NCI-H2087 (Fig. 1). A further mutation was found in exon 11: G1403C leading to G468A in the NSCLC cell line NCI-H1395. None of the three changes were present in the lymphoblastoid cell lines from the same individuals, indicating that the variants were somatically acquired mutations.



BRAF Mutations





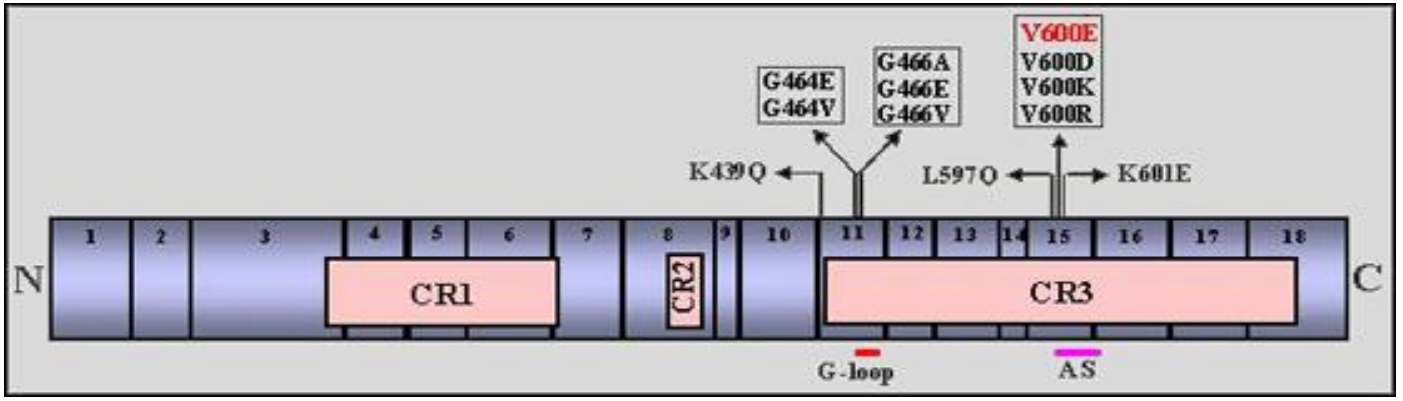
- ***BRAF* Mutations Frequency**
  - Melanoma 70%
  - Papillary Thyroid Ca 50%
  - CRC 10%
    - 50% in MSI-H
    - <5% in MSS
    - 96% of the cases V600E

Davies H et al Nature 2002

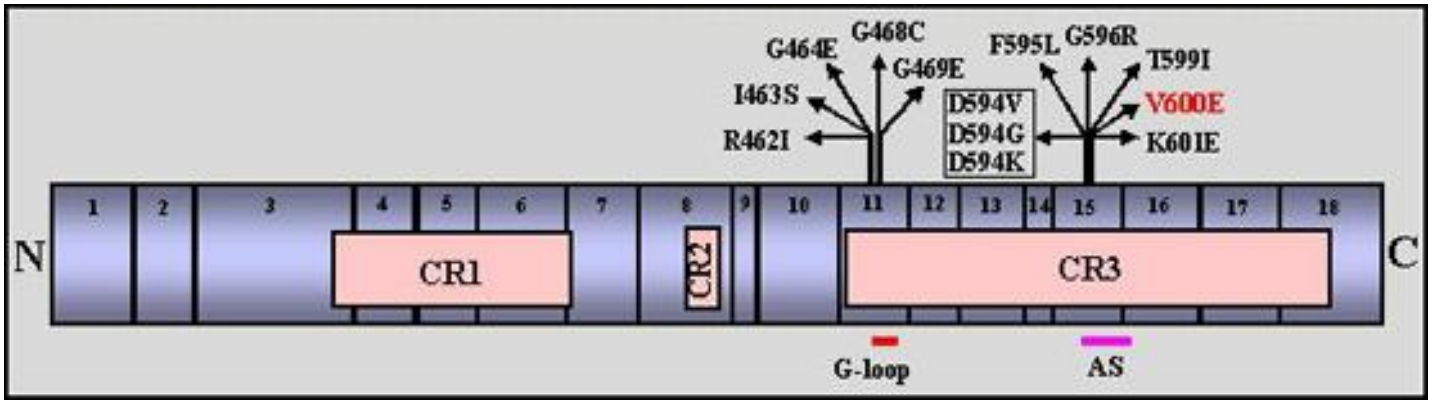
Weisenberger DJ et al Nature Genomics 2006

Main BRAF mutations
(V600E →1796 T>A)

www.ncbi.nlm.nih.gov



Μεταλλάξεις στο μελάνωμα.



BRAF mutations and personalized medicine

- ***BRAF* is the most frequently mutated oncogene in melanoma**
- **Selective BRAF inhibitors have produced tumor regression in the vast majority of patients with metastatic melanoma whose tumors harbor activating *BRAF* mutations**
- **Selective BRAF inhibitors are associated with the appearance of keratinocyte proliferations in patients; upregulation of the MAPK pathway in normal cells observed *in vitro* may explain this observation**
- **Additional oncogenic events are associated with *BRAF* mutations and may provide rational additional targets for combination therapy**
- **Preliminary evidence suggests that selective BRAF inhibitors may complement immunotherapy by upregulating antigen expression but without inhibiting T-cell function**

Targeted Therapy based on BRAF mutations

Melanoma :

- ***PLX4032*** (difluoropenylsulfonamine)
- BS590885(triarymidazole) (BRAF inhibitor)

Colorectal cancer:

- *BAY43-9006* (BRAF and CRAF inhibitor)

- MEK inhibitors

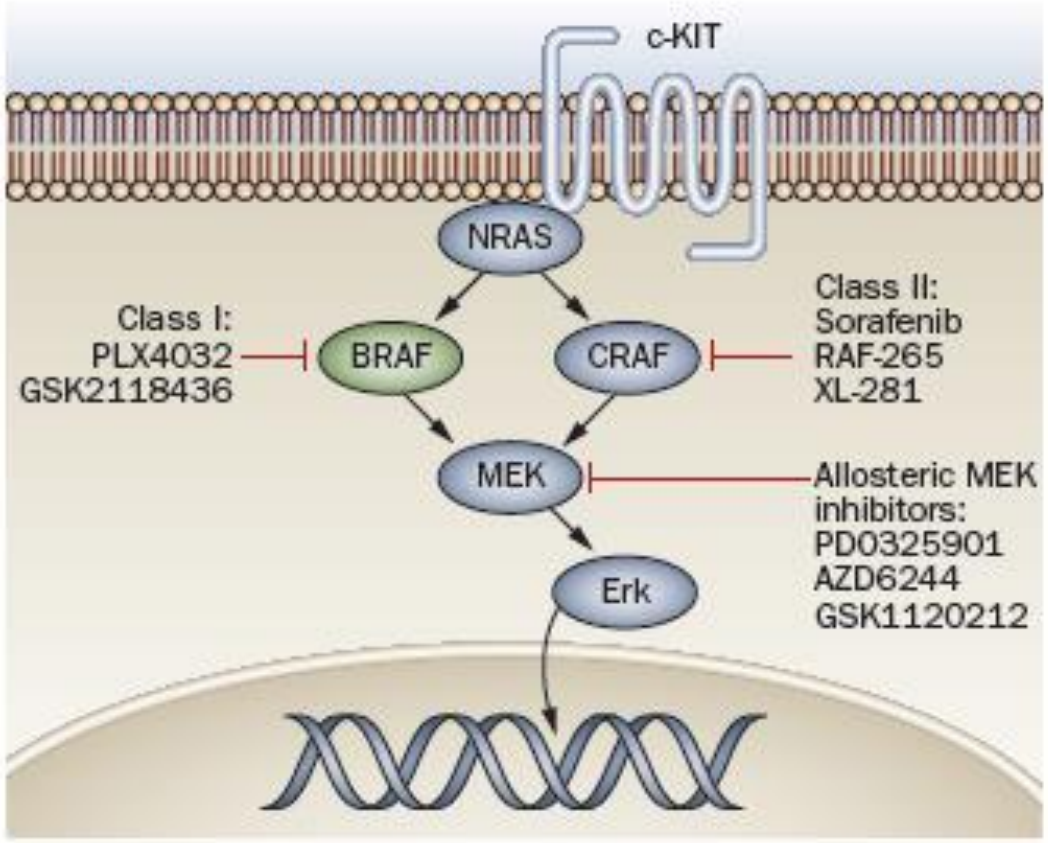
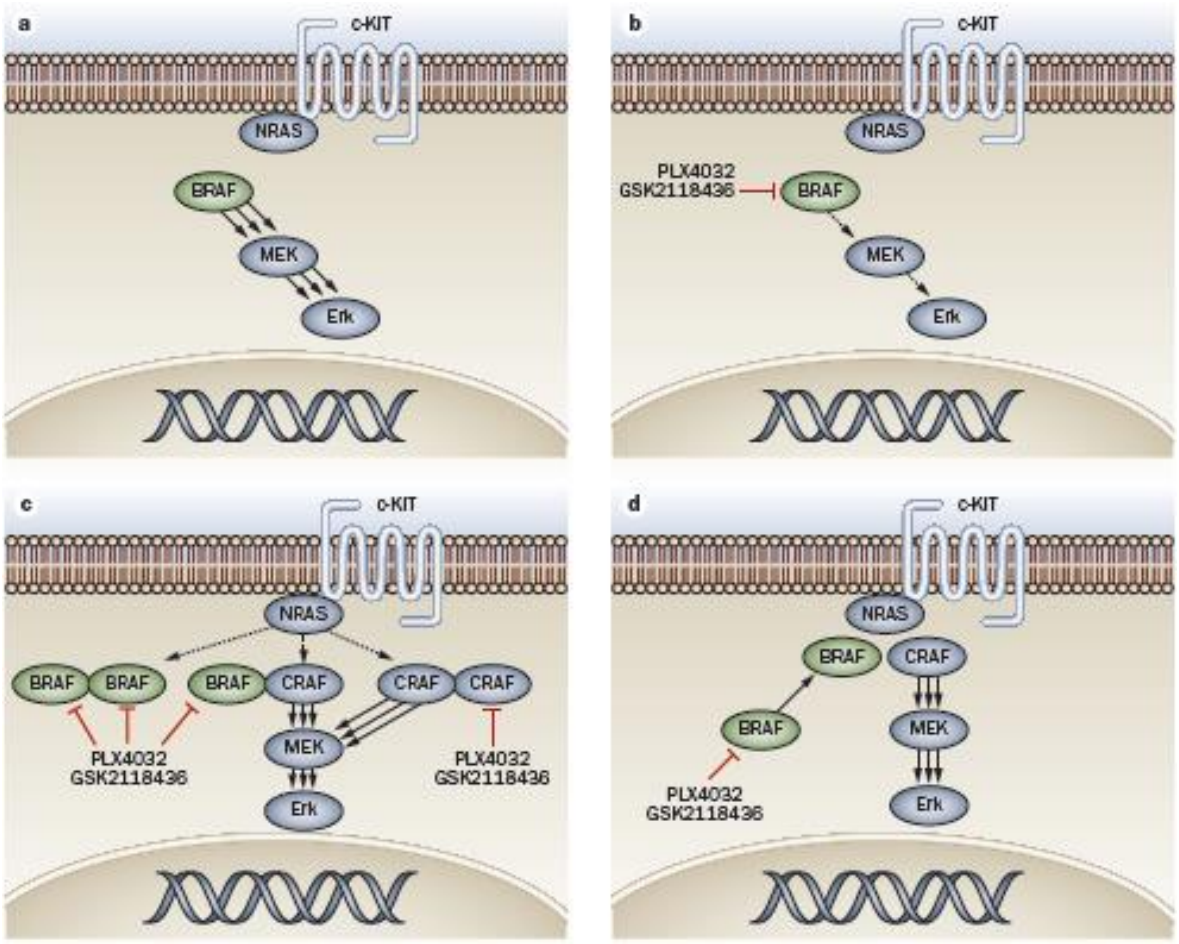


Figure 1 | The ERK/MAPK pathway and the pharmacologic agents in clinical development targeting RAF kinases or MEK.

Signal transduction through the MAP kinase pathway and the effect of BRAF inhibitors



Antoni Ribas & Keith T. Flaherty
Nature Reviews Clinical Oncology 8, 426-433 (July 2011)

BRAF mutations and melanoma molecular treatments

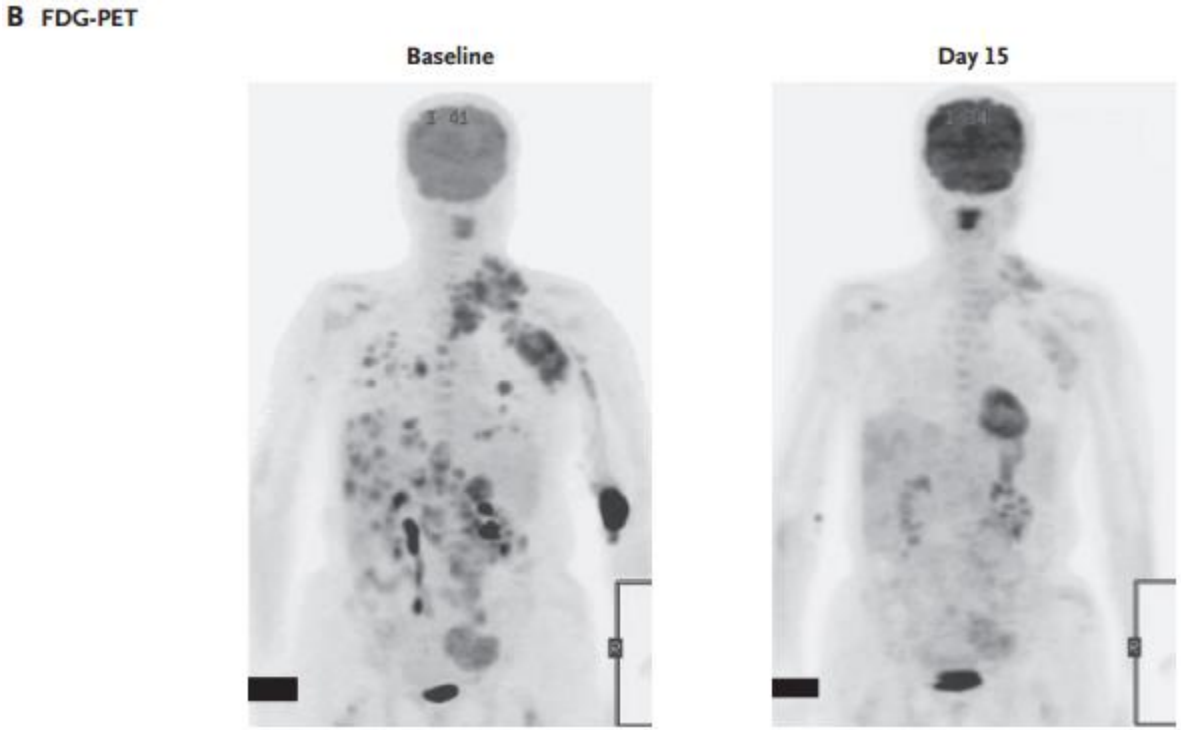
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 26, 2010 VOL. 363 NO. 9

Inhibition of Mutated, Activated BRAF in Metastatic Melanoma

Keith T. Flaherty, M.D., Igor Puzanov, M.D., Kevin B. Kim, M.D., Antoni Ribas, M.D., Grant A. McArthur, M.B., B.S., Ph.D., Jeffrey A. Sosman, M.D., Peter J. O'Dwyer, M.D., Richard J. Lee, M.D., Ph.D., Joseph F. Grippo, Ph.D., Keith Nolop, M.D., and Paul B. Chapman, M.D.

Metastatic Melanoma patient before and after 2 weeks of [illegible] administration



Flaherty et al, NEJM 2010

Inhibition of mutated, activated BRAF in metastatic melanoma

- **The identification of somatic mutations in the gene encoding the serine-threonine protein kinase B-RAF (BRAF) in the majority of melanomas offers an opportunity to test oncogene-targeted therapy for this disease.**

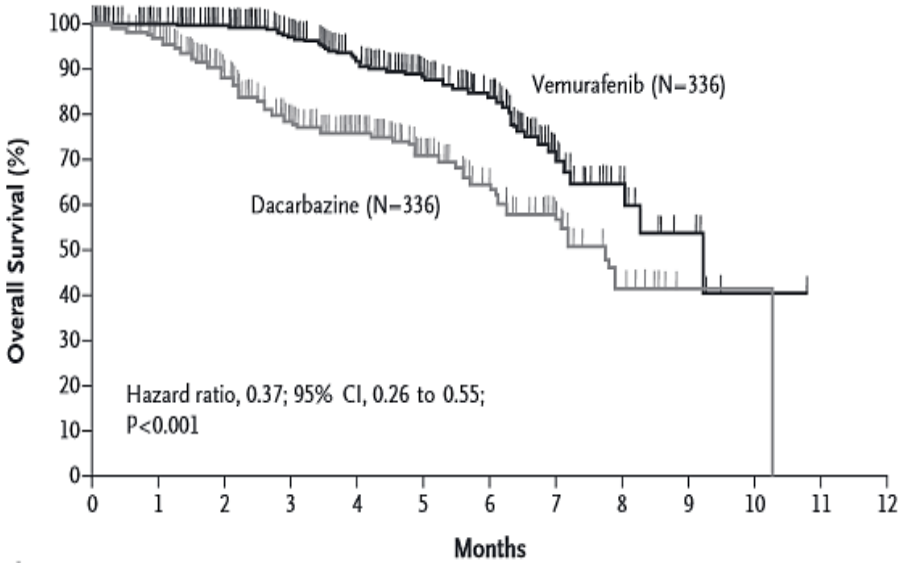
- **Multicenter, phase 1, dose-escalation trial of PLX4032 (also known as RG7204)**

- **orally available inhibitor of mutated BRAF**

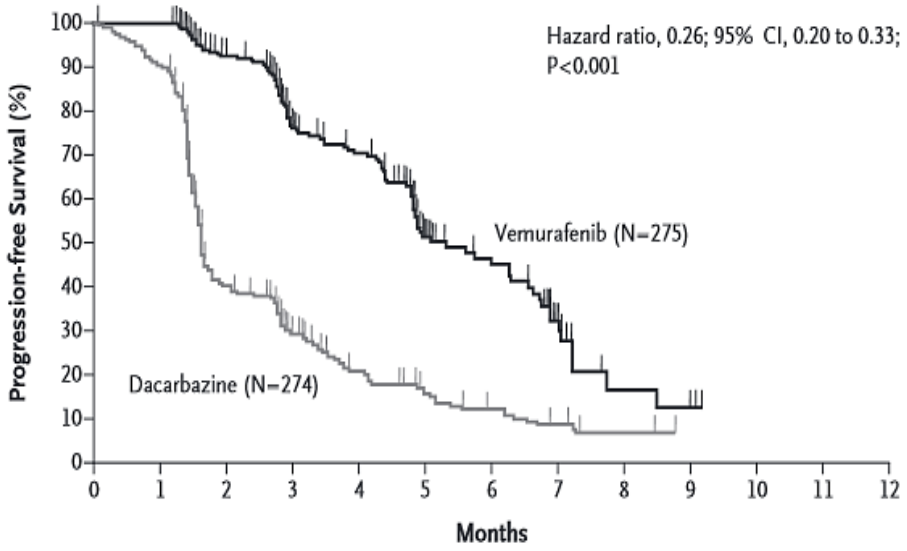
- **Treatment of metastatic melanoma with PLX4032 in patients with tumors that carry the V600E BRAF mutation resulted in complete or partial tumor regression in the majority of patients**
- ***Flaherty KT et al, N Engl J Med. 2010 Aug 26;363(9):809-19***

Vemurafenib: Phase III

Overall Survival



Progression-free Survival



Chapman PB et al. N Engl J Med 2011;364:2507-16

Targeted Therapy based on BRAF mutations
Vemurafenib, (ZELBORAF):
FDA (17/8/2011) approval for patients with unresectable or metastatic melanoma

National Cancer Institute
at the National Institutes of Health

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Cancer Drug Information

Posted: 08/17/2011

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- NCI Drug Dictionary
- Search for Clinical Trials

FDA Approval for Vemurafenib

Brand name: Zelboraf

- Approved to treat late-stage or unresectable melanoma.

Full prescribing information is available, including clinical trial information, safety, dosing, drug-drug interactions and contraindications.

On August 17, 2011, the Food and Drug Administration (FDA) approved vemurafenib tablets (ZELBORAF, made by Hoffmann-La Roche Inc.) for the treatment of patients with unresectable or metastatic melanoma with the $BRAF^{V600E}$ mutation as detected by an FDA-approved test.

Related Pages

Melanoma Home Page
NCI's gateway for information about melanoma.

Cancer Drug Information
List of cancer drug information summaries from NCI. These summaries have information about cancer drugs and cancer drug combinations.

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Companion Diagnostics
BRAF inhibitor and BRAF V600E mutation detection
FDA approval at the same time!!!

- **These days, it is often not enough for pharmaceutical companies simply to bring a drug to market.**

- **Regulators and insurers are also prodding the companies to develop tests to pinpoint which patients are most likely to benefit from a drug, thereby sparing other patients from needless side effects and expense.**

- **July 2011: The F.D.A. issued guidance to the industry on companion diagnostics, including its preference for having the test ready for approval at the same time as the drug.**

- **Zelboraf, (Roche and Plexxikon), can produce remarkable improvements, but only for the roughly half of melanoma patients whose tumors have BRAFV600E mutation.**
- **The F.D.A. approved a test from Roche's diagnostics division to detect that mutation.**

Evi Lianidou

Targeted Therapy based on BRAF mutations
Vemurafenib, (ZELBORAF):
FDA (17/8/2011) approval for patients with unresectable or metastatic melanoma

- http://www.cancer.gov/cancertopics/druginfo/fda-vemurafenib

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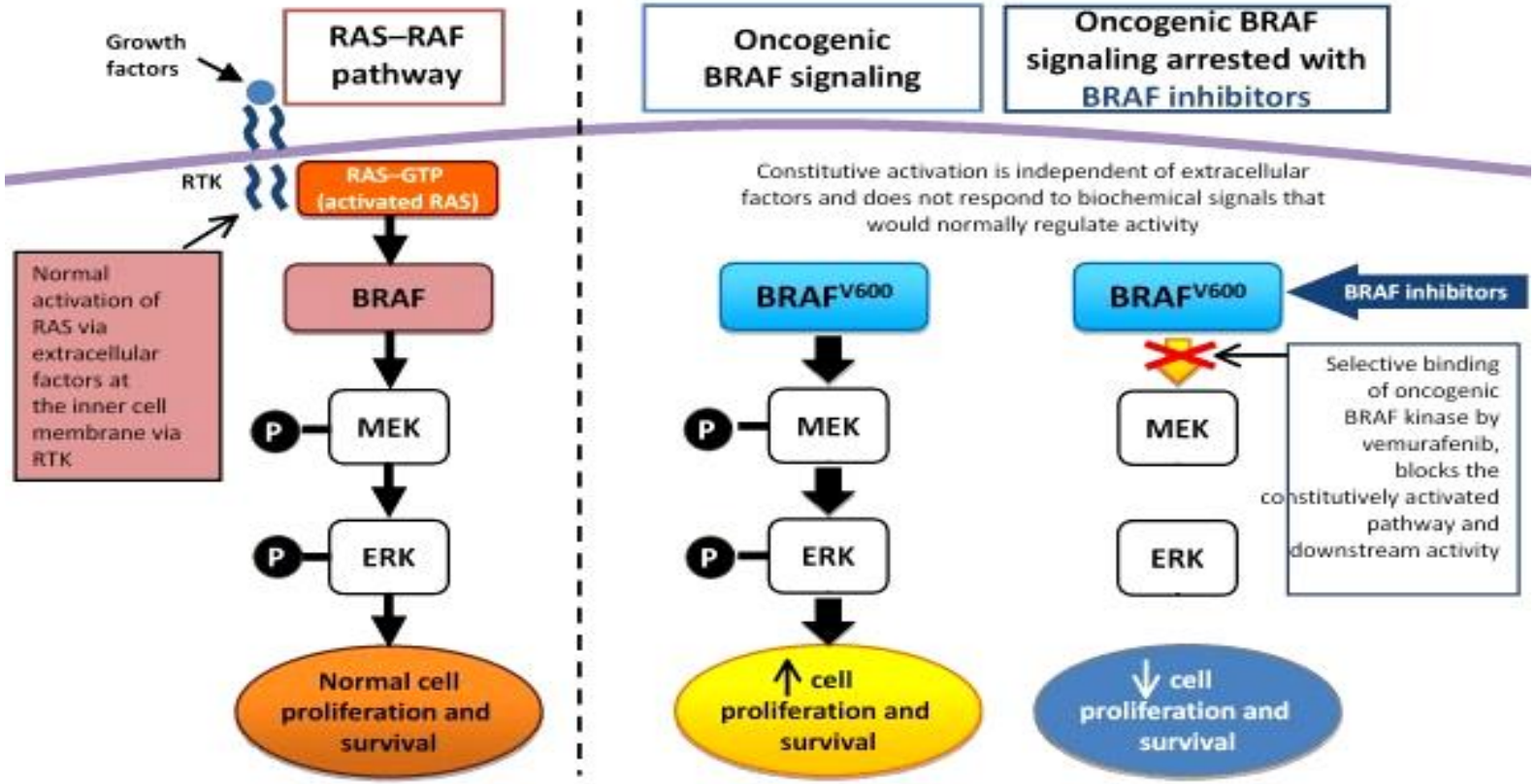
Predictive biomarker: BRAF V600E



Ascierto PA et al. J Transl Med. 2012 Jul 9;10:85

Examples of Personalized Medicine in Oncology

7. melanoma

other molecular targets

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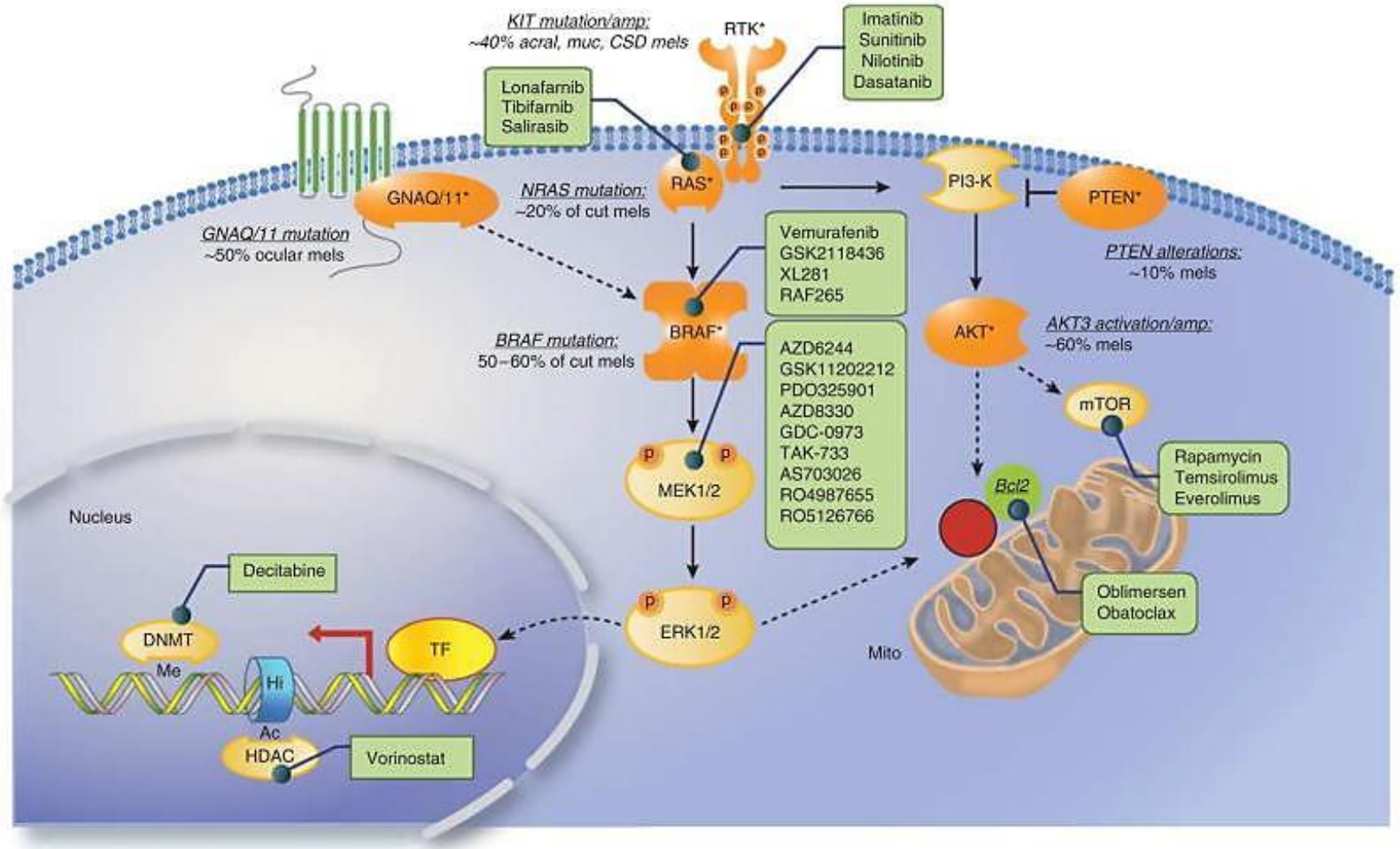
Therapeutic targets in melanoma



Nikolaou VA et al. J Invest Dermatol. 2012;132(3 Pt 2):854-63

Evi Lianidou

And then there were five: Immunotherapy!!!

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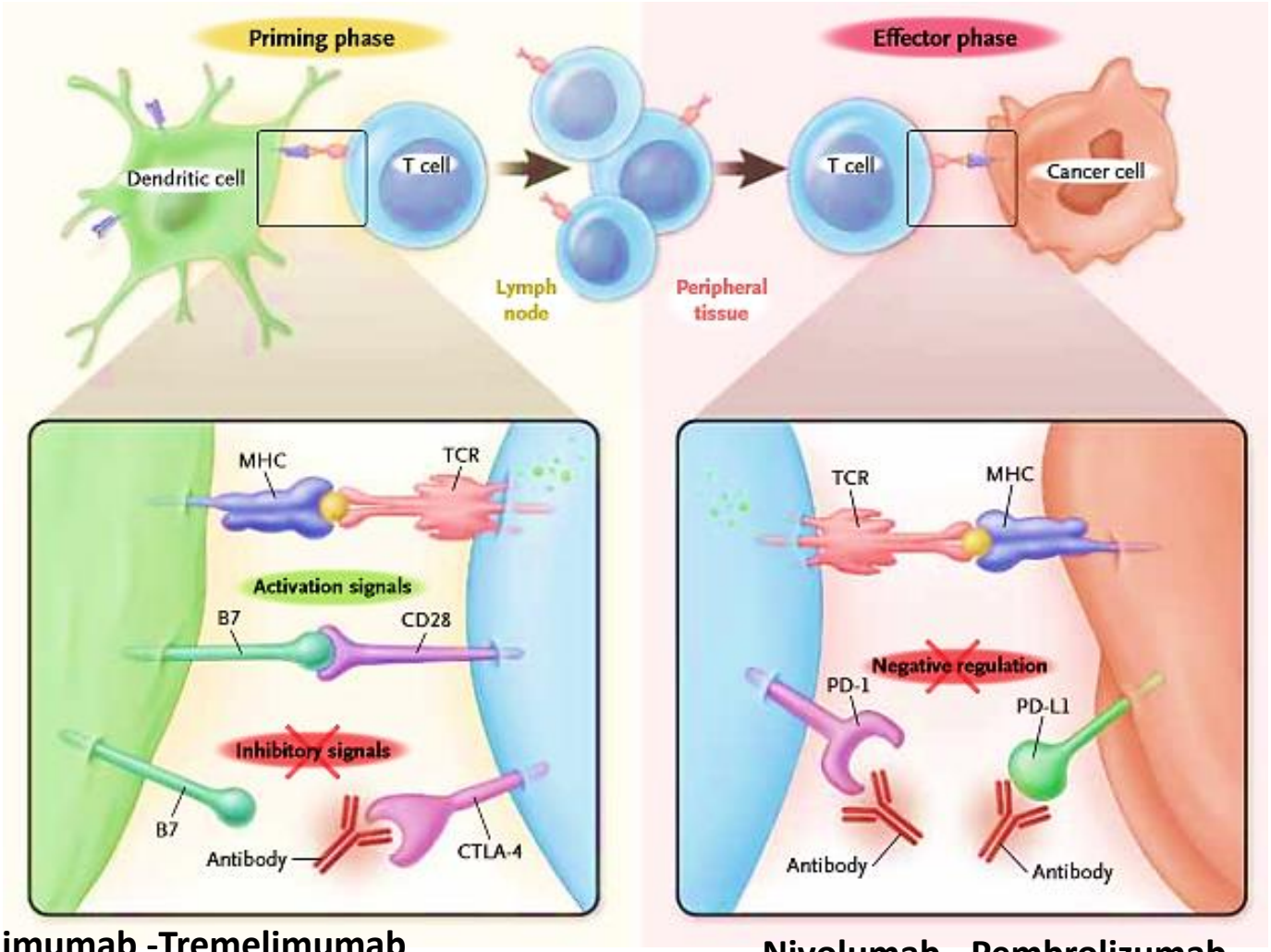
Immunotherapy in melanoma



Ipilimumab -Tremelimumab

Nivolumab - Pembrolizumab

Ribas A et al. N Engl J Med. 2012;366(26):2517-9

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Nivolumab and Ipilimumab in Untreated Melanoma

Larkin J et al. N Engl J Med. 2015 May 31

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma

J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.J. Grob, C.L. Cowey, C.D. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P.F. Ferrucci, A. Hill, J. Wagstaff, M.S. Carlino, J.B. Haanen, M. Maio, I. Marquez-Rodas, G.A. McArthur, P.A. Ascierto, G.V. Long, M.K. Callahan, M.A. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L.M. Rollin, C. Horak, F.S. Hodi, and J.D. Wolchok

METHODS

We assigned, in a 1:1:1 ratio, 945 previously untreated patients with unresectable stage III or IV melanoma to nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. Progression-free survival and overall survival were coprimary end points. Results regarding progression-free survival are presented here.

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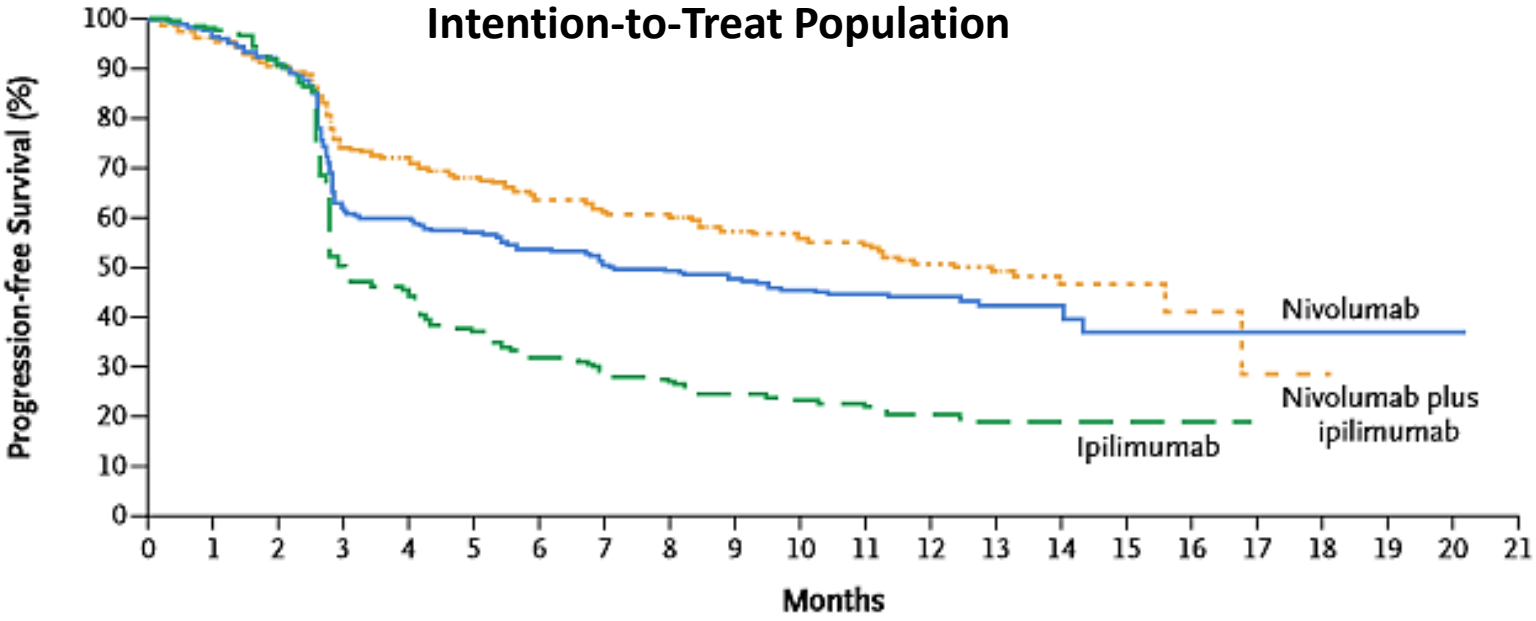
Nivolumab and Ipilimumab in Untreated Melanoma

Larkin J et al. N Engl J Med. 2015 May 31



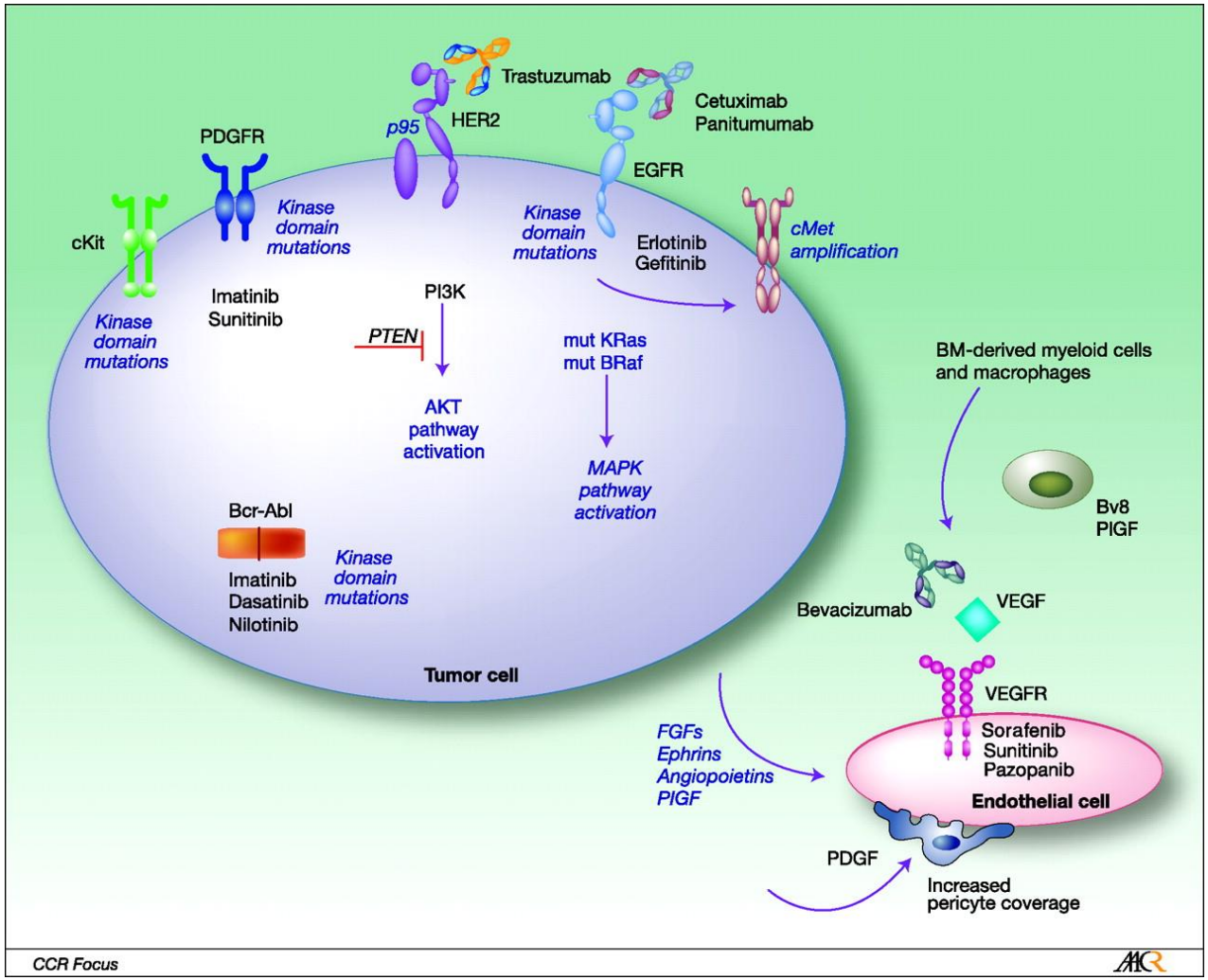
- **PD-L1 (+):** the median progression-free survival was 14.0 months in the nivolumab-plus-ipilimumab group and in the nivolumab group and 3.9 months in the ipilimumab group
- **PD-L1 (-):** progression-free survival was longer with the combination therapy than with nivolumab alone (11.2 months vs. 5.3 months)

Evi Lianidou

Conclusions

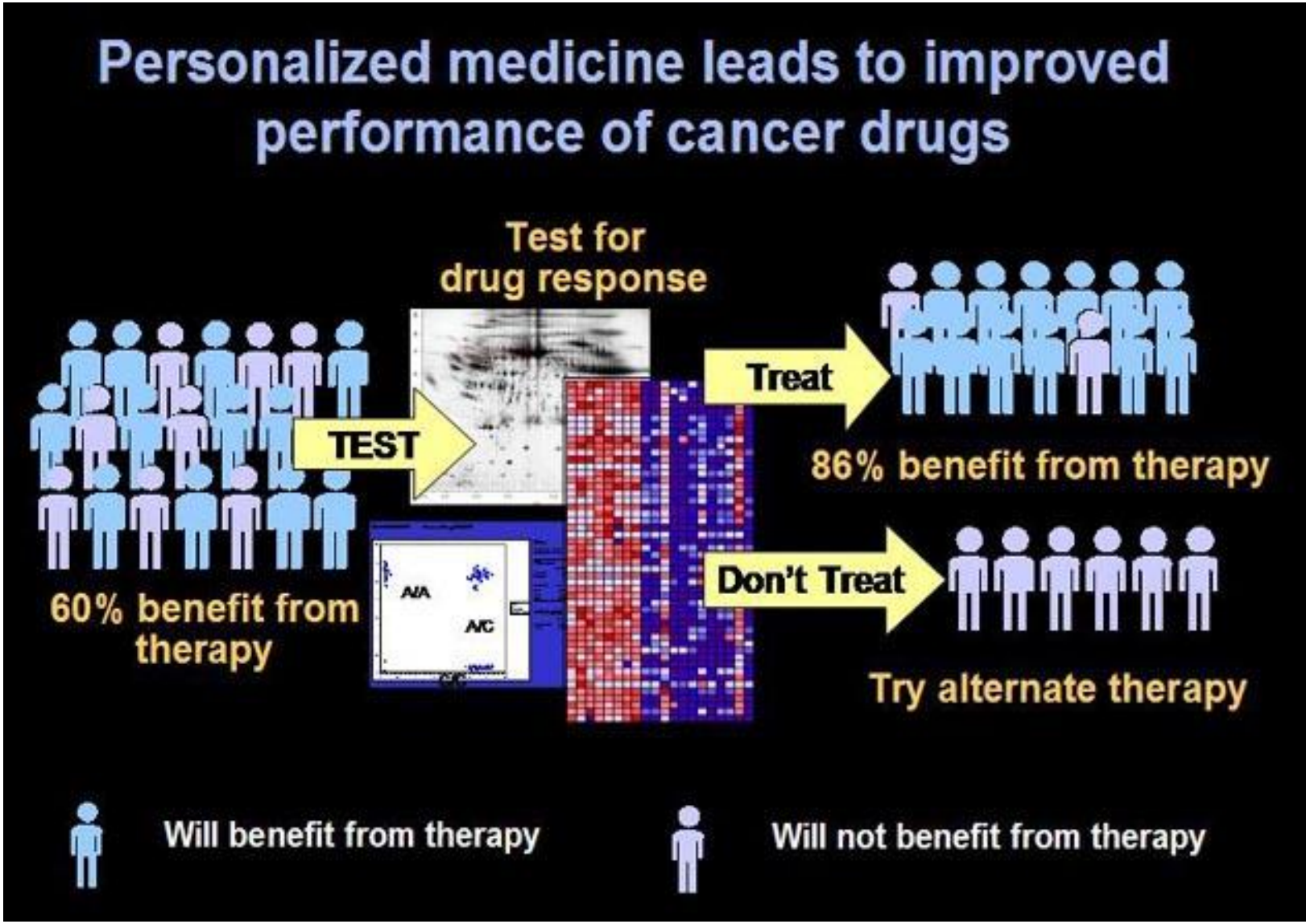
- Understanding of the molecular mechanisms of cancer is important for cancer therapy
- Personalized medicine leads to improved performance of cancer drugs
- Collaboration of basic research and clinicians

Overview of resistance pathways to targeted therapies.



Ellis L M , Hicklin D J Clin Cancer Res 2009;15:7471-7478

Evi Lianidou



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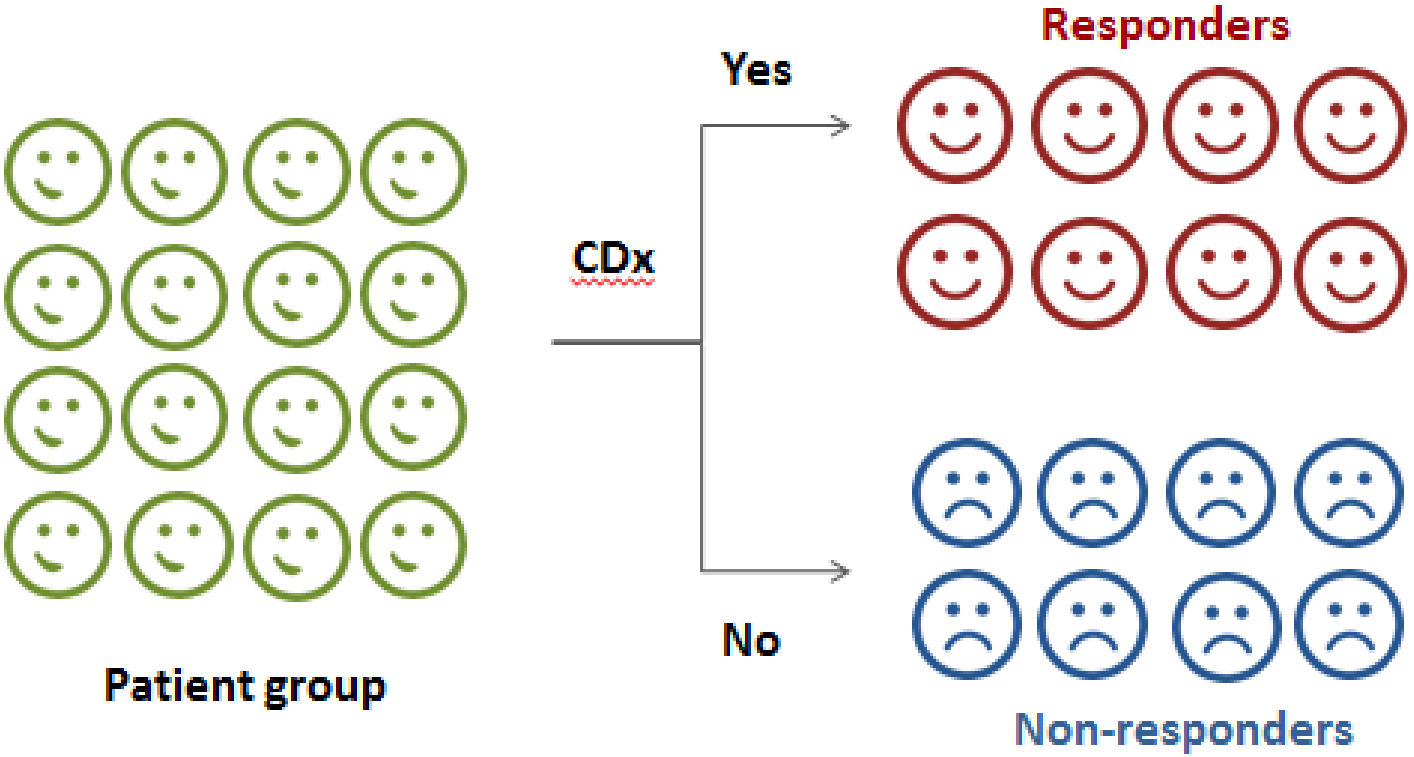
Which is the most appropriate diagnostic test that will predict the response to treatment of patients?



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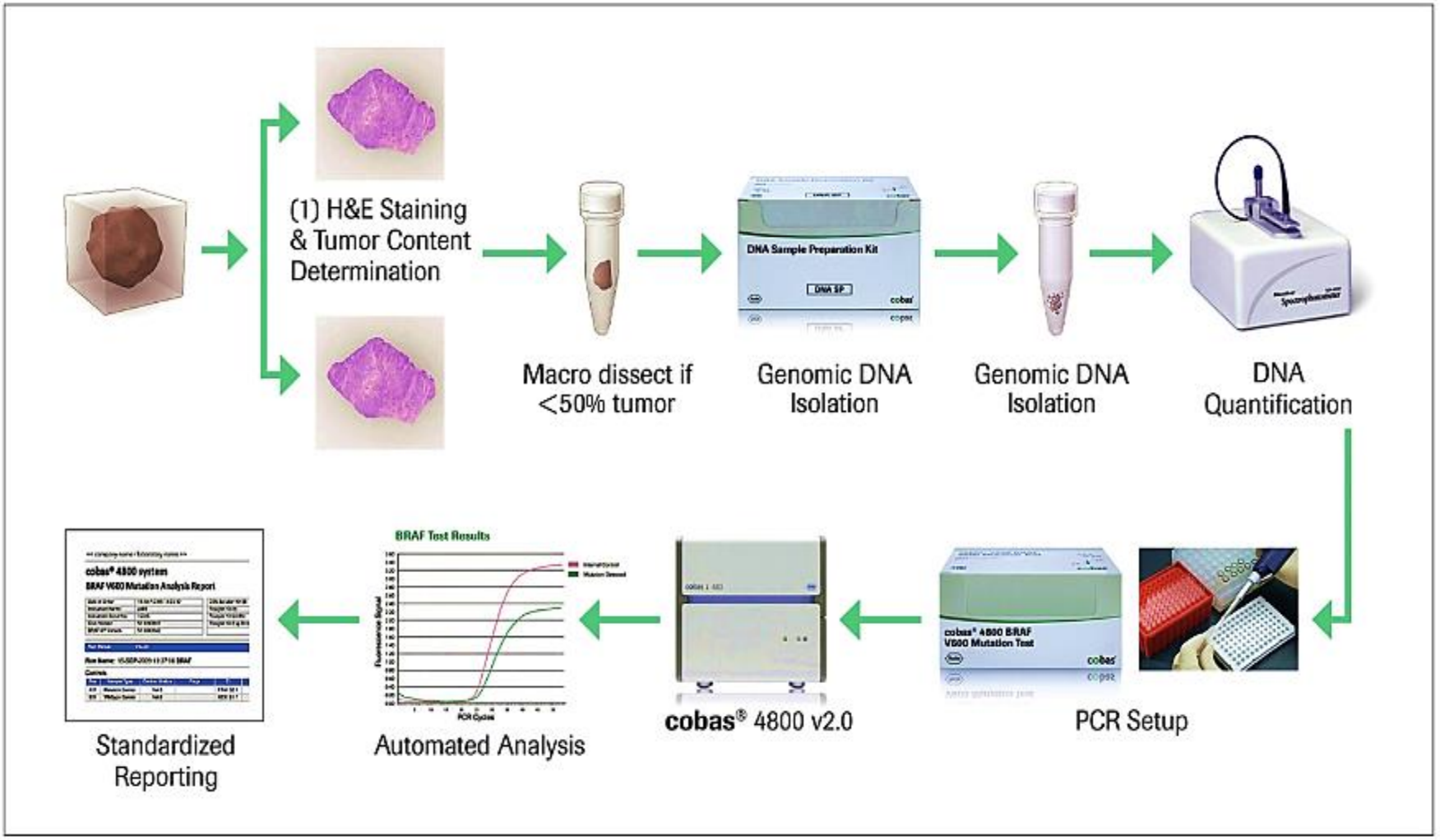
Companion Diagnostic test (CDx)



Cheng S et al. N Biotechnol 2012, 29(6):682-8

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paradigms of precision medicine in cancer

FDA-approved anti-cancer drugs that target mutant genes					
Drug (brand name, manufacturer)	**Mechanism of action**	**Known target-protein**	**Diagnostic test**	**Disease**	**Year of approval**
Trastuzumab (Herceptin, Genentech)	Antibody	HER2	Immunohistochemistry, FISH	Breast cancer	1998
Gefitinib (Iressa, Astra-Zeneca)	Tyrosine kinase inhibitor	EGFR	Sequencing	NSCLC	2003
Erlotinib (Tarceva, Genentech)	Tyrosine kinase inhibitor	EGFR	Sequencing	NSCLC	2003
Imatinib mesylate (Gleevec, Novartis)	Tyrosine kinase inhibitor	BCR-ABL	Cytogenetic, FISH, PCR	CML	2005
Cetuximab (Erbitux, ImClone Systems)	Antibody	EGFR	Immunohistochemistry, FISH	Colorectal cancer	2006
Vemurafenib (Zelboraf, Hoffmann-La Roche Limited)	Protein kinase inhibitor	BRAF V600E	Real-Time PCR, FISH	Melanoma	2011
Crizotinib (Xalkori, Pfizer)	EML-ALK Tyrosine kinase inhibitor	ALK	Immunohistochemistry, FISH	NSCLC	2011

REDIFINE THE TARGET OF TARGETED THERAPIES

“Selection”

- **Selection of the target**
- **Selection of the patients population**
- **Selectio**

Precision Medicine

Evi Lianidou