K-RAS mutation in colorectal cancer

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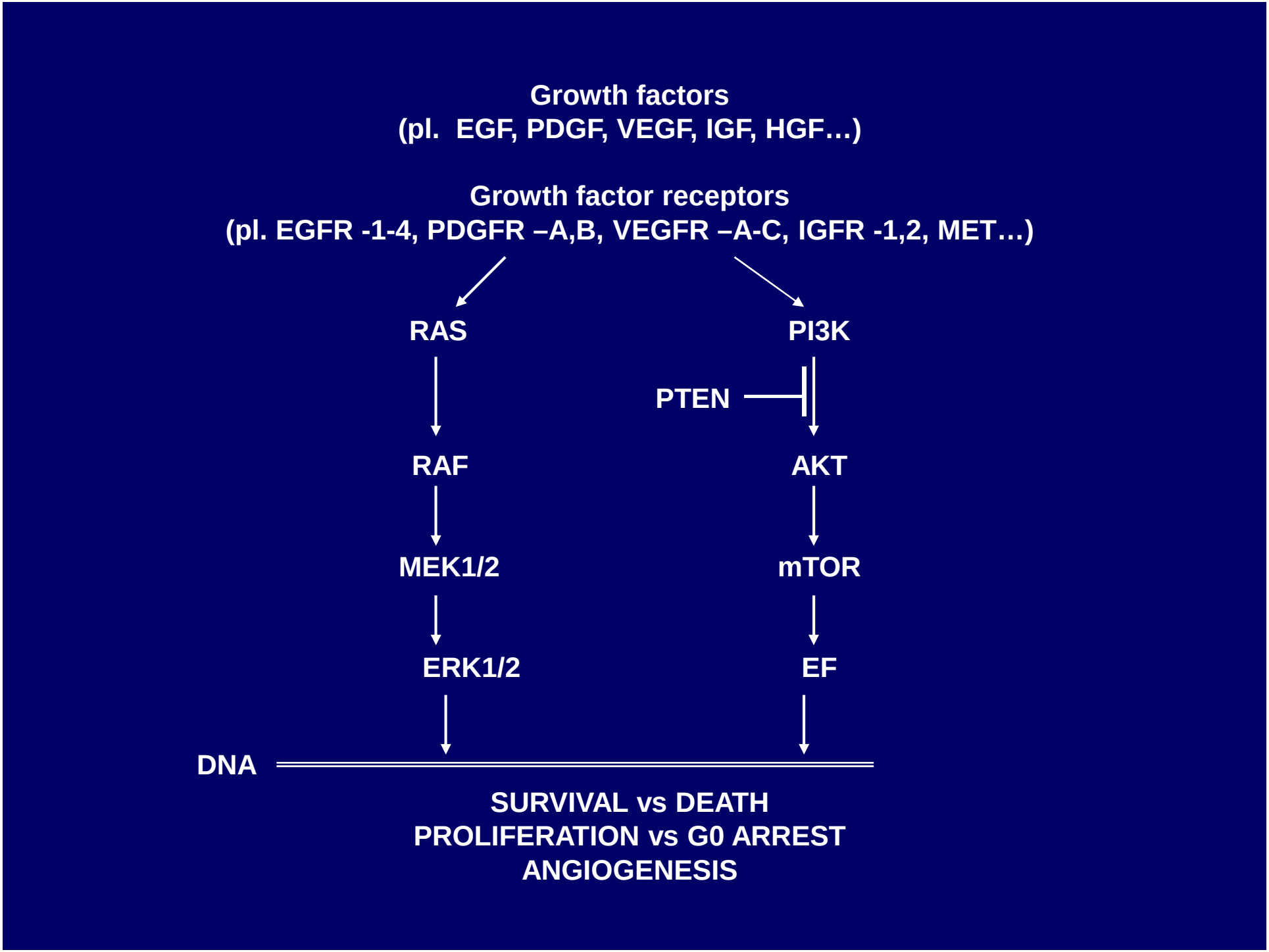
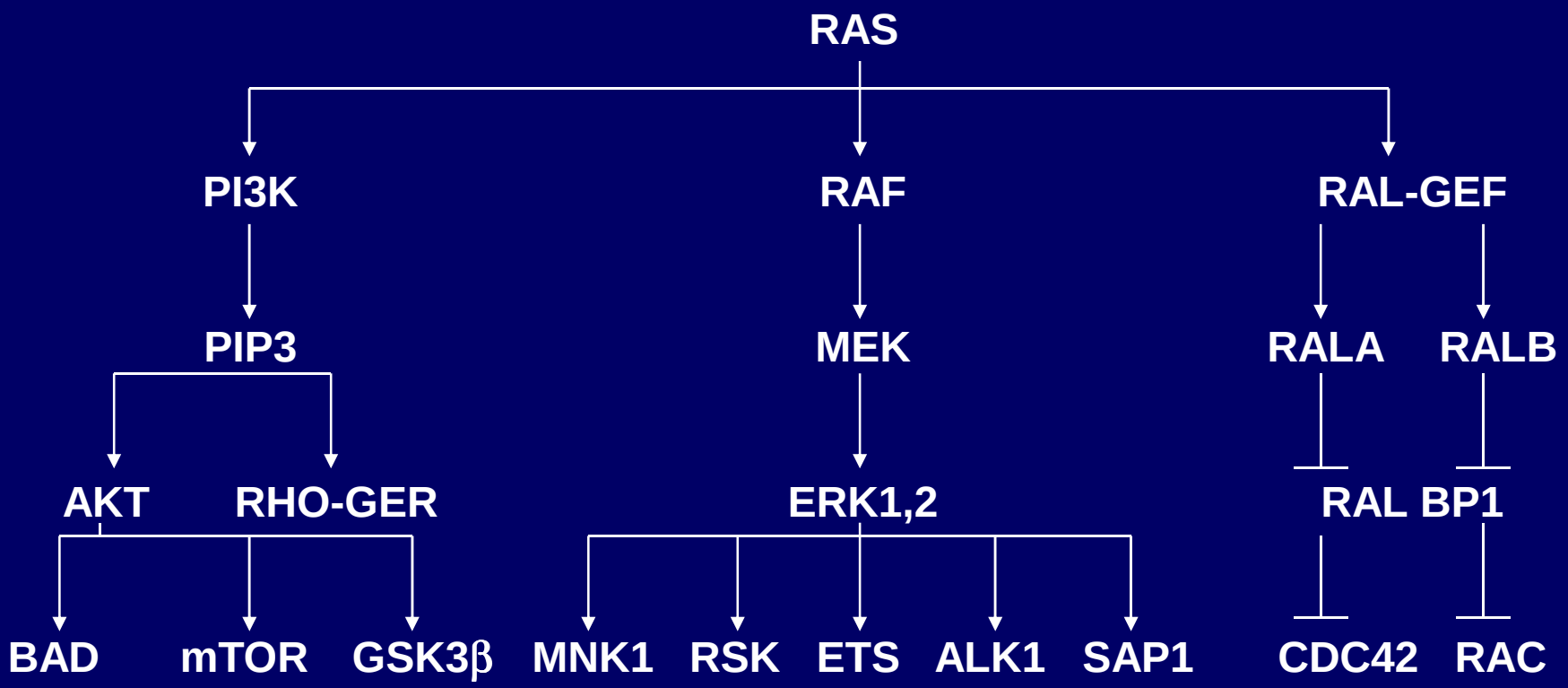
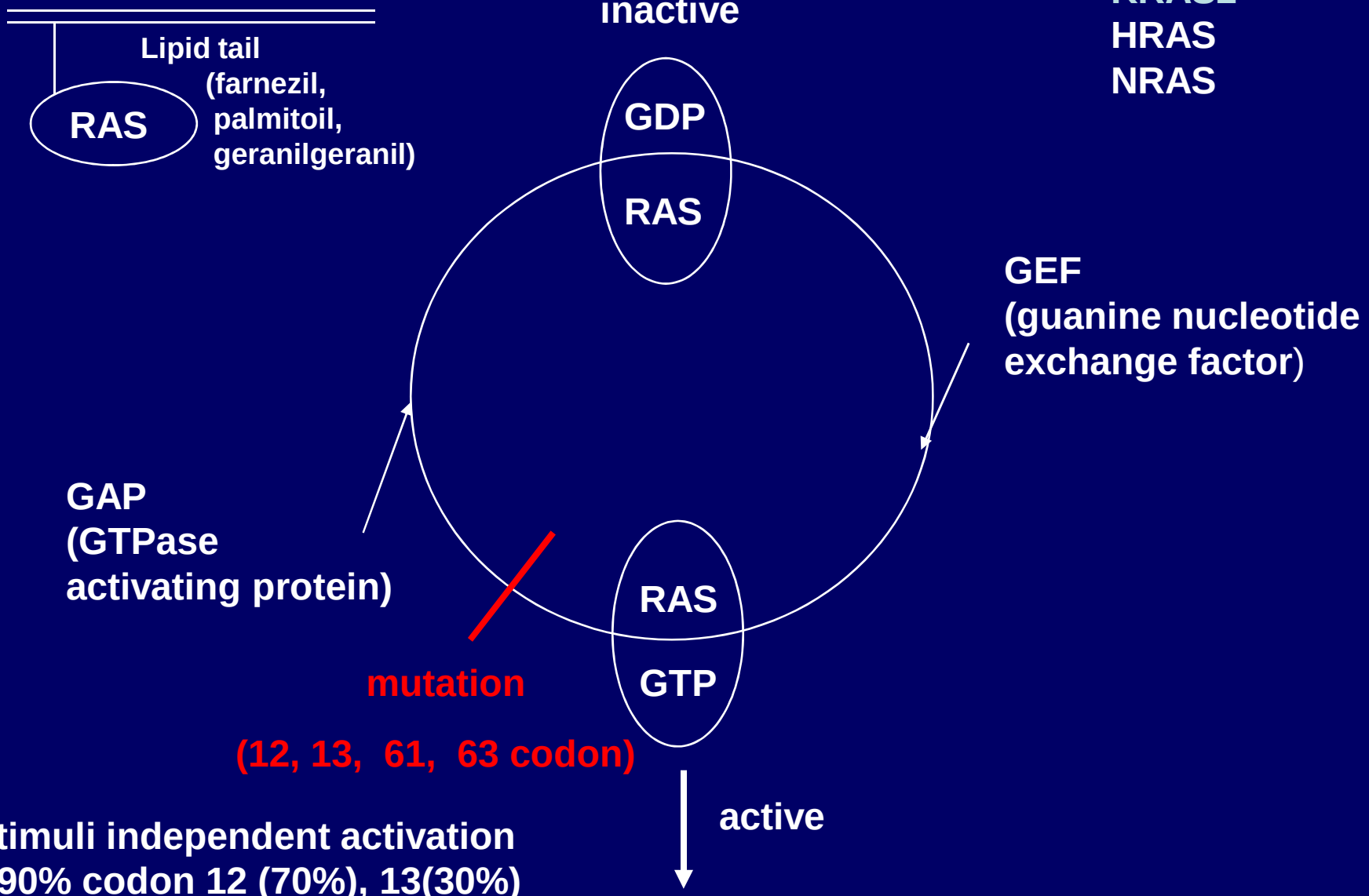

Growth factors
(pl. EGF, PDGF, VEGF, IGF, HGF…)
Growth factor receptors
(pl. EGFR -1-4, PDGFR –A,B, VEGFR –A-C, IGFR -1,2, MET…)
RAS
PI3K
PTEN
RAF
AKT
MEK1/2
mTOR
ERK1/2
EF
DNA
SURVIVAL vs DEATH
PROLIFERATION vs G0 ARREST
ANGIOGENESIS

Downstream connections of RAS



RAS function and errors

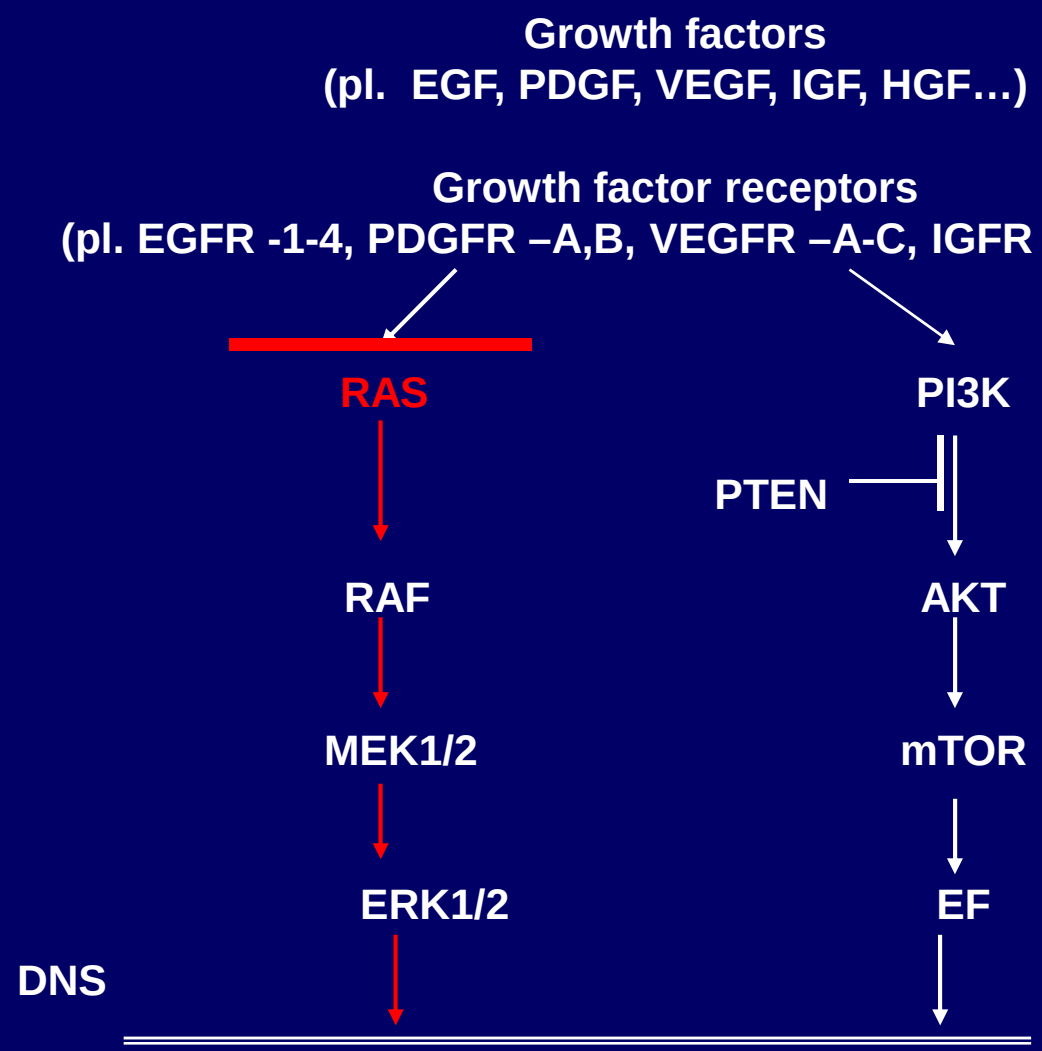


RAS family

KRAS1
KRAS2
HRAS
NRAS

stimuli independent activation
>90% codon 12 (70%), 13(30%)

When RAS is mutated the signal starts from RAS – no connection between EGFR and RAS – but the connections of EGFR with other signalis pathways are maintained



Frequency of RAS mutations

Pancreas cc	K	90%	
Thyroid gland cc	H,K,N	55-60%	
Colorectal cc	K	35-45%	P = M
Seminoma	K,N	35-45%	
NSCLC	K	15-30%	
AML, HCC	N	30%	
Melanoma	N	15%	
Urinary bladder	K	10%	
Renal cell cc	H	10%	

Anti-EGFR monoclonal antibodies

Cetuximab (Erbitux®, Merck) chimeric mouse/human antibody

- prevents ligand binding
- induces receptor internalization
- causes a direct inhibition of the receptor tyrosine kinase activity

Panitumumab (Vectibix®, Amgen) fully human antibody

Matuzumab

Zalutumumab

Nimotuzumab

Resistance to anti-EGFR1 agents

(a) Due to activation of alternative tyrosine kinase receptors that bypass the EGFR pathway (e.g. MET and IGF1R)

(b) Due to increased angiogenesis

(c) Based on the constitutive activation of downstream mediators (e.g. KRAS, PTEN and oters)

(d) Existence of specific EGFR mutations

Single arm studies of treatment of mCRC

	Treatment	No patients (w/m)	RR (w/m)	PFS (w/m)	OS (w/m)
1.	cetuximab 2nd line	65 / 24	40 / 0	31 / 10	14 / 10
2.	cetuximab alone cetux + irinotecan	57 / 46	41 / 0	12 / 12 34 / 12	27 /25 45 / 27
3.	cetuximab 2nd – 3rd line	50 /30	10 / 0		
4.	cetuximab + chemotherapy	43 / 16	28 / 0		
5.	panitumomab or cetuximab or cetuximab + chemotherapy	32 / 16	31 / 6		

1. Lievre et al, 2008 – 2. DeRoock et al, 2008 – 3. Khambata-Ford et al, 2007 – De Fiore et al, 2008 – Benvenuti et al, 2007

KRAS as a predictive factor in CRC

CRYSTAL

phase III - first line – EGFR-expressing mCRC

cetuximab + FOLFIRI

FOLFIRI

(benefit of cetuximab appeared to be restricted to patients with WT KRAS)

OPUS	**phase II**			
	WT	**cetuximab + FOLFOX**	**RR: 61%**	**PFS: 7.7 mo**
		FOLFOX	**RR: 37%**	**PFS: 7.2 mo**
	M	**cetuximab + FOLFOX**	**RR: 33%**	**PFS: 5.2 mo**
		FOLFOX	**RR: 49%**	**PFS: 8.6 mo**
			WT KRAS	
Panitumumab (3rd line)			**RR: 17%**	**PFS: 12.3**
BSC (best supportive care)			**RR: 0%**	**PFS: 7.7**

EMEA has approved panitumumab only for patients with tumors that contain WT RAS

Provisional Clinical Opinion (PCO)

Based on systematic reviews of the relevant literature, all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR antibody therapy should have their tumor tested for KRAS mutations in a CLIA-accreditd laboratory. If KRAS mutation in codon 12 or 13 detected, then patients with metastatic colorectal carcinoma should not receive anti-EGFR antibody therapy as part of their treatment

American Society of Clinical Oncology Provisional Clinical Opinion: Testing for KRAS Gene Mutations in Patients with Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy
Allegra CJ et al JCO 27 2091-2096, 2009

This PCO

Based on assays that detect mutations in codon 12 and 13 of KRAS on (although other activating mutations can occur uncommonly

Do not evaluate the difference in sensitivity and specificity among the various assays, that are available for KRAS mutation testing

Limited to the current state of knowledge about the treatment of mCRC and does not address

- **the use of anti-EGFR MoAbs for adjuvant therapy in CRC**
- **the use of small tyrosine kinase inhibitors in mCRC**
- **or assays for other alterations that have been reported to effect response to anti-MoAbs (e.g. mutations in BRAF, PI3K, PTEN)**

Summary of the College of American Pathologists report on KRAS mutation testing

Acceptable sample types

- samples should be specifically chosen by a pathologist (!tumor cells)
- freshly extracted from the patient
  provided fresh or in solution as RNAlater
  rapidly frozen and stored frozen
- neutral buffered formalin fixed and paraffun embedded, area of interest selected specifically by the pathologist

Acceptable assay types

DNA is first extracted

- RT-PCR – for the most common mutations in codon 12 and 13
- direct sequencing – sequencing of exon 1, identifies all possible mutations in the exon
- US: laboratory-developed tests (there is no FDA-approved test) – outside US (e.g. TheraScreen).

Assay reporting

KRAS normal – No mutation was identified. Report will specify assay type and controls used

KRAS abnormal – Treatment with anti-EGFR MoAbs is not recommended. Mutation was found. Report will specify what mutation was found, what assay was done and what controls were used

NCCN Practice Guidelines in Oncology – Colon Cancer
Principle of patologic review
KRAS mutation testing

- **Mutation** in codons 12 and 13 in exon 2 of the coding region of the **KRAS** gene product **lack of response** to therapy with **antibodies to the EGFR**

- **Testing for mutations** in codons 12 and 13 should be performed **only in laboratories** that are certified under the laboratory improvement amendements (CLIA-1988) as **qualified** to perform high complex laboratory (**molecular pathology**) testing. **No specific methodology** is recommended.

- The testing can be performed on **formalin fixed paraffin embedded** tissue. The testing can be performed on primary colorectal cancers and/or the metastasis as literature has shown that the **KRAS mutations are similar** in both specimen types

Recommendation for KRAS mutation testing

Sensitivity	detection limit	1% tumor cells for allel-specific PCR 25-30% for direct sequencing
Specificity	test should be detect 7 common mutation in codons 12 and 13	
Method validation	the laboratory should use a validated method for KRAS mutation testing – the objectives of the validation are to: ……	
Analysis success rate	95% of samples with successful DNA extraction 97% of samples with correct KRAS test results	
Costs	should be calculated and documented for national reimbursement schemes	

KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program
Van Krieken et al Virchows Arch DOI 10.1007/s00428-008-0665-y

Case 1

	Tumor ratio	KRAS	codon	sequence	aminoacid
A	35*	w			
B	20	w			
C	25	w			
D	40	w			
E	40	w			
F		w			
G	15-20	mut	12		G12A

Case 2

A	60*	mut	12	G35T	G12V
B	no DNA	w			
C	50	mut	12	GTT/WT	
D	70	mut	12		G12V
E	50	mut	12	GTT	G12V
F		mut		GCCAAC/GTTGGC	
G	20-25	w			

Case 3

A	45*	mut	13	G37C	G13R
B	25	mut	13	GCGCGC	
C	60	mut	13	CGC	
D	50	mut	13		G13D
E	60	mut	13	CGC	G13R
F		mut		GCGACC/GGTCGC	
G	25-30	mut	13		G13R

W	W	W	W	W	W	MUT
MUT	W	MUT	MUT	MUT	MUT	W
MUT	MUT	MUT	MUT	MUT	MUT	MUT
MUT	W	W	W	W		W
W	W	W	W	W	W	
MUT	MUT	MUT	MUT	MUT	MUT	MUT
MUT	MUT	MUT	MUT	MUT	MUT	MUT
W	W	W	W	W	?	MUT
W	W	W	W	W	W	MUT
W	W	W	W	W	W	W
MUT	MUT	MUT	MUT	MUT	MUT	MUT
MUT	MUT	MUT	MUT	MUT	MUT	W
MUT	MUT	MUT	MUT	MUT	MUT	MUT
MUT	W	MUT	MUT	MUT		
W	W	W	W	W	W	W
W	W	W	MUT	MUT	MUT	?
W	W	W	W	W	MUT	?
MUT	MUT	MUT	MUT	MUT	MUT	MUT
W	W	W	MUT	W	W	
MUT	MUT	MUT	MUT	MUT	MUT	MUT
MUT	MUT	MUT	MUT	MUT	MUT	MUT
MUT	MUT	MUT	MUT	MUT	MUT	W
W	W	W	MUT	W	W	W
W	W	W	W	W	?	W
MUT	W	MUT	MUT			W

97% correct testing **95% successful DNA extraction**

24/25 **96%**

		- 4 és - 6
agreement - total	**11 / 25**	**11 / 23nt**
with 1 difference	**8 / 25**	**8 / 23**
with 2 differences	**4 / 25**	**4 / 23**
with major differences	**2 / 25**	

	difference in 23 samples	(+4)	(+4,16w)	(+4,16mut)
A	0	0	0	1
B	2	3	3	4
C	0	1	1	2
D	2	3	4	3
E	0	1	2	1
F	3	4	5	4
G	8	9	10	10

How to increase responses rate in KRAS WT patients?

EGFR gene copy number
patients with elevated EGFR GCN obtained more benefit from cetuximab however reproducibility concerns regarding the cutoff points for GCN are still problematic
EGFR gene mutations are rare in CRC and have no clinical relevance with regard to anti-EGFR therapy

Are there novel therapeutic alternatives to KRAS mutant patients?

Inhibiting farnesyl transferase failed
Inhibition of targets downstream of RAS has failed e.g. in pancreatic cancer

??? Inhibition of protective proteins (e.g. HSP90)
??? Taking advantage of differential imunogenicity of mutated RAS

BRAF – mutations 0-12.5% (mutually exclusive with KRAS mutation)
PI3K – mutations 11.4 -17.7% (not correlated with KRAS mutation)
PTEN – loss 31-41% (loss of PTEN and KRAS mutation 36%)

KRAS mutations and sensitivity to epidermal growth factor receptor inhibitors in colorectal cancer: practical application of patient selection – Jimeno A, et al JCO 27, 1130-1136, 2009

KRAS mutation

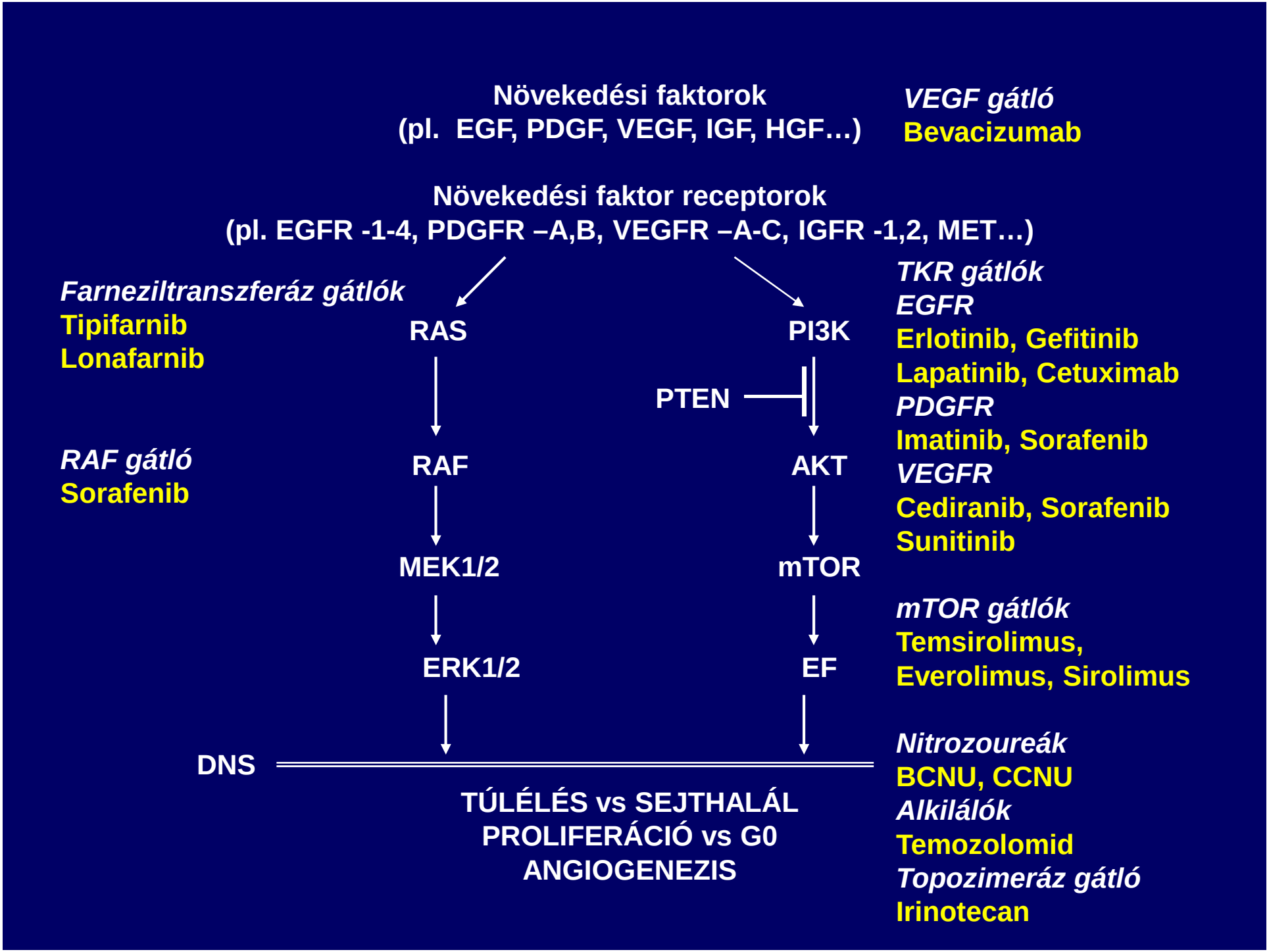
reagáló betegek aránya

RAF mutation

PI3K-path mutant

TGFb-path mutant

??? PTEN, IGF1R, MET, WNT…


Növekedési faktorok
(pl. EGF, PDGF, VEGF, IGF, HGF…)
VEGF gátló
Bevacizumab
Növekedési faktor receptorok
(pl. EGFR -1-4, PDGFR –A,B, VEGFR –A-C, IGFR -1,2, MET…)
Farneziltranszferáz gátlók
Tipifarnib
Lonafarnib
RAS
PI3K
TKR gátlók
EGFR
Erlotinib, Gefitinib
Lapatinib, Cetuximab
PDGFR
Imatinib, Sorafenib
VEGFR
Cediranib, Sorafenib
Sunitinib
PTEN
RAF gátló
Sorafenib
RAF
AKT
MEK1/2
mTOR
mTOR gátlók
Temsirolimus,
Everolimus, Sirolimus
ERK1/2
EF
Nitrozoureák
BCNU, CCNU
Alkilálók
Temozolomid
Topozimeráz gátló
Irinotecan
DNS
TÚLÉLÉS vs SEJTHALÁL
PROLIFERÁCIÓ vs G0
ANGIOGENEZIS

COLORECTAL CANCER - markers

KRAS mutáció	33%	+ EGFR (génkópiaszám > 2.83) - rossz prognózis
KRAS mutáció	42%	PFS – w/mut – FOLFOX 7.7/8.6 hó, FOLFOX + cetuximab 7.7/5.5 hó
KRAS mutáció	25%	RR – w/mut – cetuximab (250 mg/m2) + irinotecan 21%, (500 mg/m2) 46%
KRAS mutáció	38%	cetuximab, mCRC – PR + CR 27% (csak a w reagált)
KRAS mutáció	19%	nincs v. csekély a terápiás válasz EGFR ampl (kópiaszám/FISH) – terápiás válasz (ampl nélkül nincs válasz)
KRAS mutáció	45%	MEK, ERK, AKT közül MEK fokozott expresszió – rossz prognózis (KRAS mut-val még rosszabb)

EGFR polimorfia (497G/A) – EGFR aktivitás csökken – cetuximab és panitumumabban szemben rezisztencia nő (PFS: 3.7 hó, OS 7.1 hó)

PTEN, pAKT (IHC) – primer és met egyezett: 60-67% - primer tumoron PTEN aktivitás nem függött össze a PFS-sel, a met-ban igen – AKT nem függött össze

BRAF mutáció összefügg a cetuximab rezisztenciával, a MET és IGF1R génexpresszió nem

DCC allélvesztés korai rezisztenciát jelenthet cetuximabbal szemben

(K(irsten)RAS, H(arvey)RAS, NRAS

Integrators and not just tranducer - negative and positive feed-back loops

Activation: prenylation by farnesyl transferase – RAS more sticky – adherence to the inner aspect of cytoplasmic membrane – ADP (ATPase) / ATP – signaling pathways

Mutation : stimuli independent activation
>90% codon 12 (70%), 13(30%) and codon 61, 63 prognosis
m12 (pG12V), m13 worse overall

Mutation in solid tumors

CRC	40%
NSCLC	27%
esophagus	30%
stomach	15%
biliary tract	26%
cholangiocc	45%
pancreas	95%

concordance of KRAS mutation analysis was 95% in primary and related metastases

Methods to identify mutated RAS

direct sequencing
- **dideoxy method**
- **pyrosequencing**

allele specific PCR
- **ARMS-PCR or SnaPshot-PCR**
- **4 allele specific probes plus melting curve analysis**

generally there is no best method for detection of KRAS