Εξατομικευμένη ογκολογική προσέγγιση ακριβείας στον Ορθοκολικό καρκίνο.

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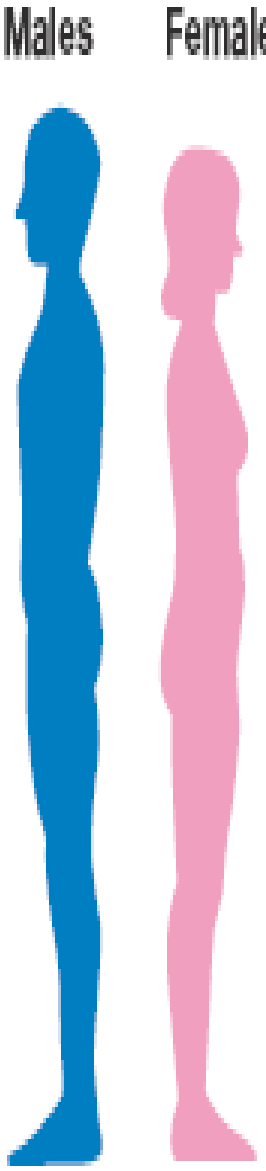
Παθολόγος Ογκολόγος

Disclosures (Conflicts of Interest)

- BMS, AMGEN, LEO

Estimated New Cases*



Males			Females		
Prostate	218,890	29%	Breast	178,480	26%
Lung & bronchus	114,760	15%	Lung & bronchus	98,620	15%
Colon & rectum	79,130	10%	Colon & rectum	74,630	11%
Urinary bladder	50,040	7%	Uterine corpus	39,080	6%
Non-Hodgkin lymphoma	34,200	4%	Non-Hodgkin lymphoma	28,990	4%
Melanoma of the skin	33,910	4%	Melanoma of the skin	26,030	4%
Kidney & renal pelvis	31,590	4%	Thyroid	25,480	4%
Leukemia	24,800	3%	Ovary	22,430	3%
Oral cavity & pharynx	24,180	3%	Kidney & renal pelvis	19,600	3%
Pancreas	18,830	2%	Leukemia	19,440	3%
All Sites	**766,860**	**100%**	**All Sites**	**678,060**	**100%**

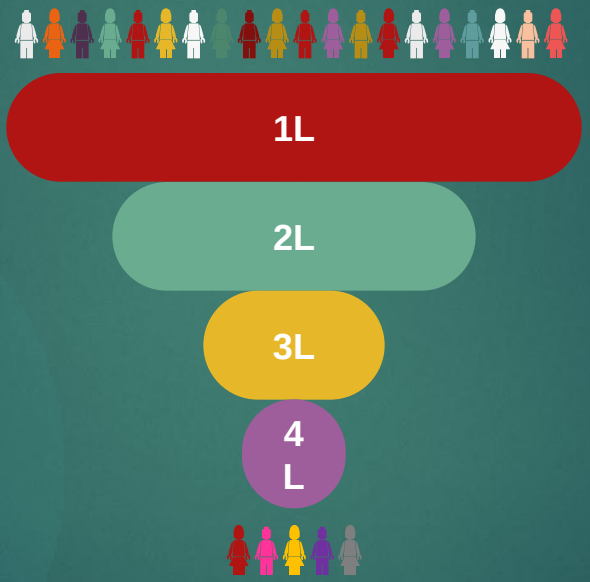
What Influences Treatment Choices in mCRC?

Patient characteristics

- Comorbidities
- Prior adjuvant treatment
- Age
- Performance status

Tumor characteristics

- Tumor burden
- Resectability
- Tumor location



Molecular characteristics

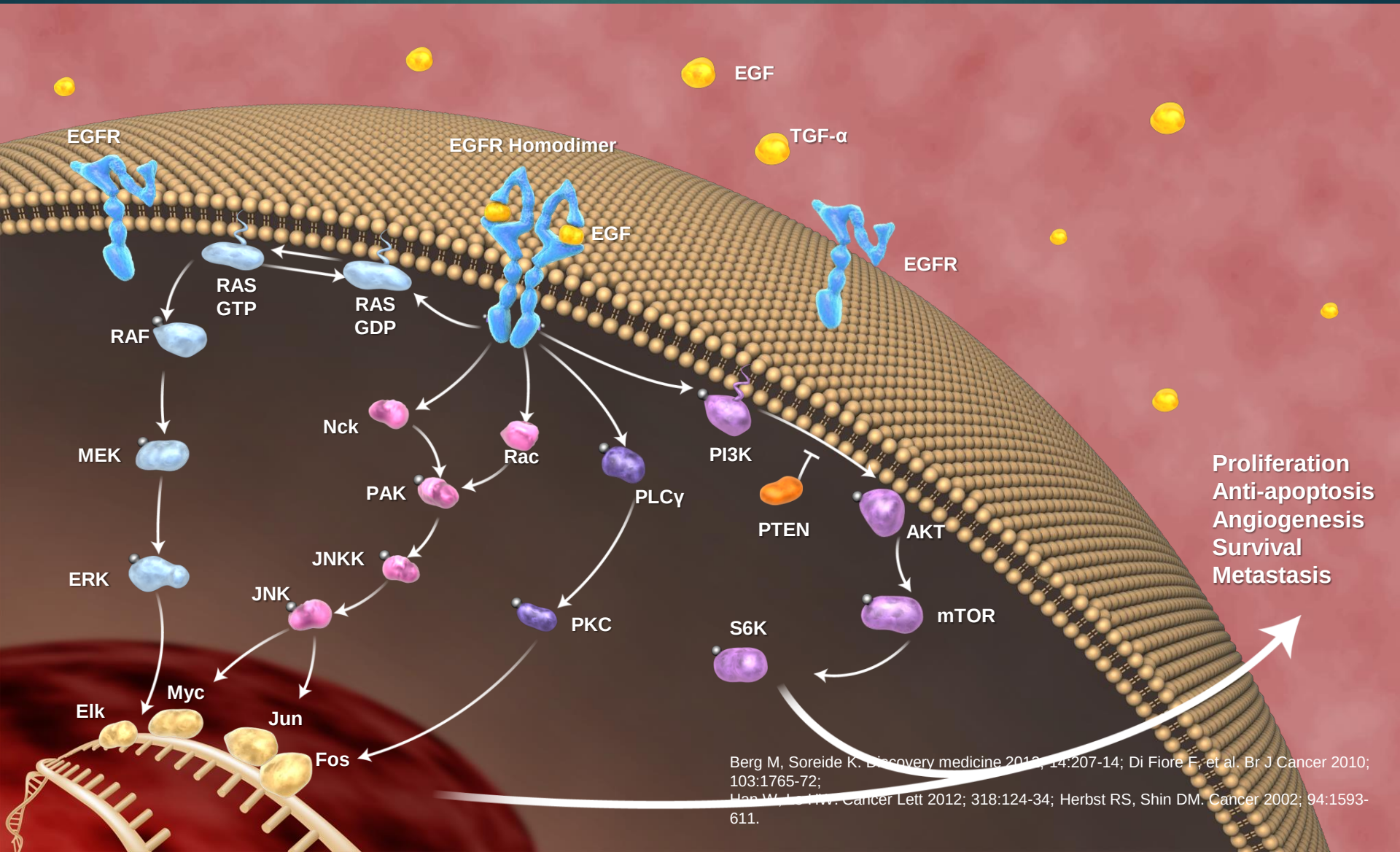
- RAS
- BRAF
- MSI-high
- HER2

Patient preference

- Quality of life
- Toxicity profile

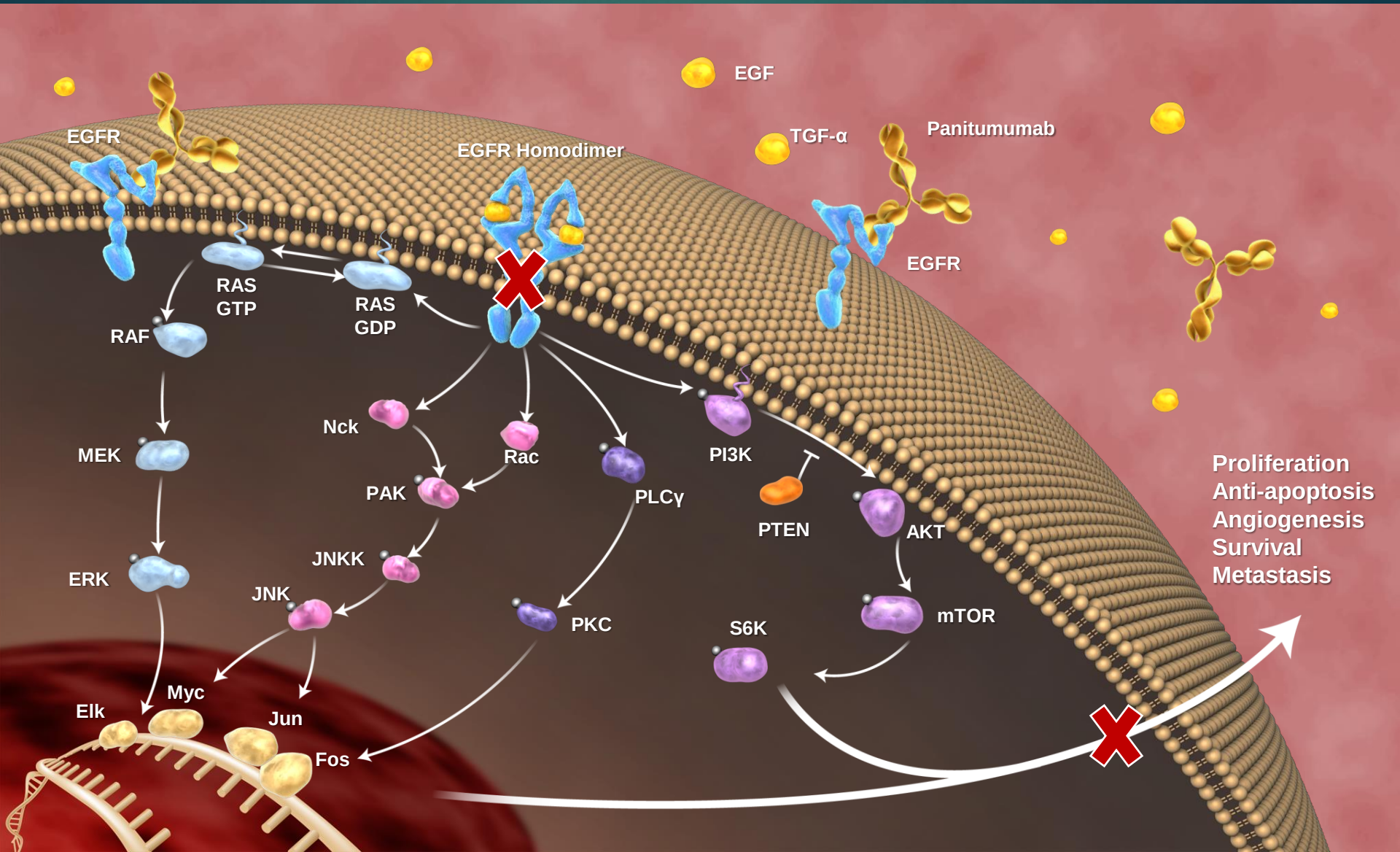
Therapy tailored according to individual patient needs

EGFR activation may involve downstream signalling pathways that include RAS proteins

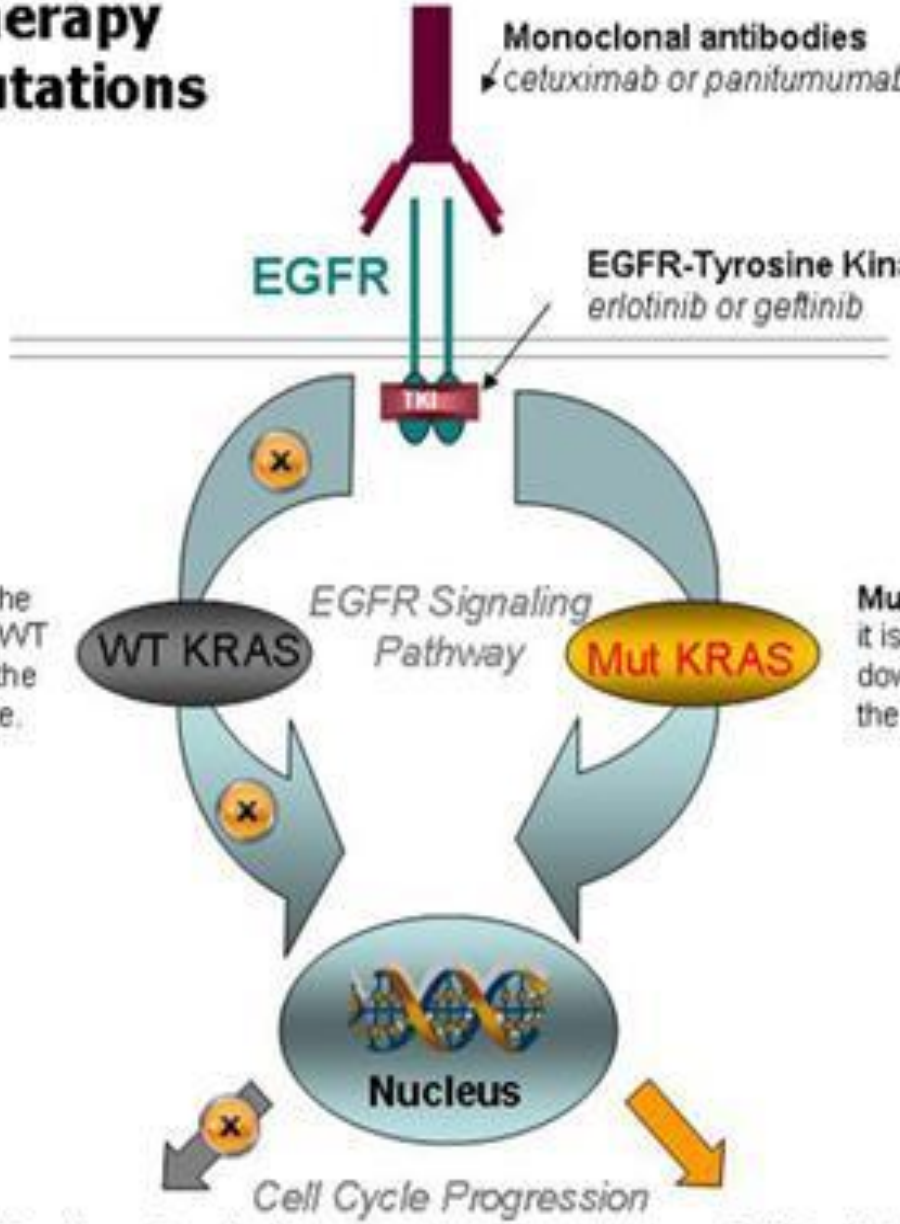


Berg M, Soreide K. Discovery medicine 2013; 14:207-14; Di Fiore F, et al. Br J Cancer 2010; 103:1765-72;
Han W, Lo HW. Cancer Lett 2012; 318:124-34; Herbst RS, Shin DM. Cancer 2002; 94:1593-611.

EGFR inhibitory Mabs inhibits EGFR dimerisation and subsequent downstream signalling



Anti-EGFR Therapy and KRAS mutations



Anti- EGFR drugs block receptor signals thus preventing downstream events such as KRAS mediated effects

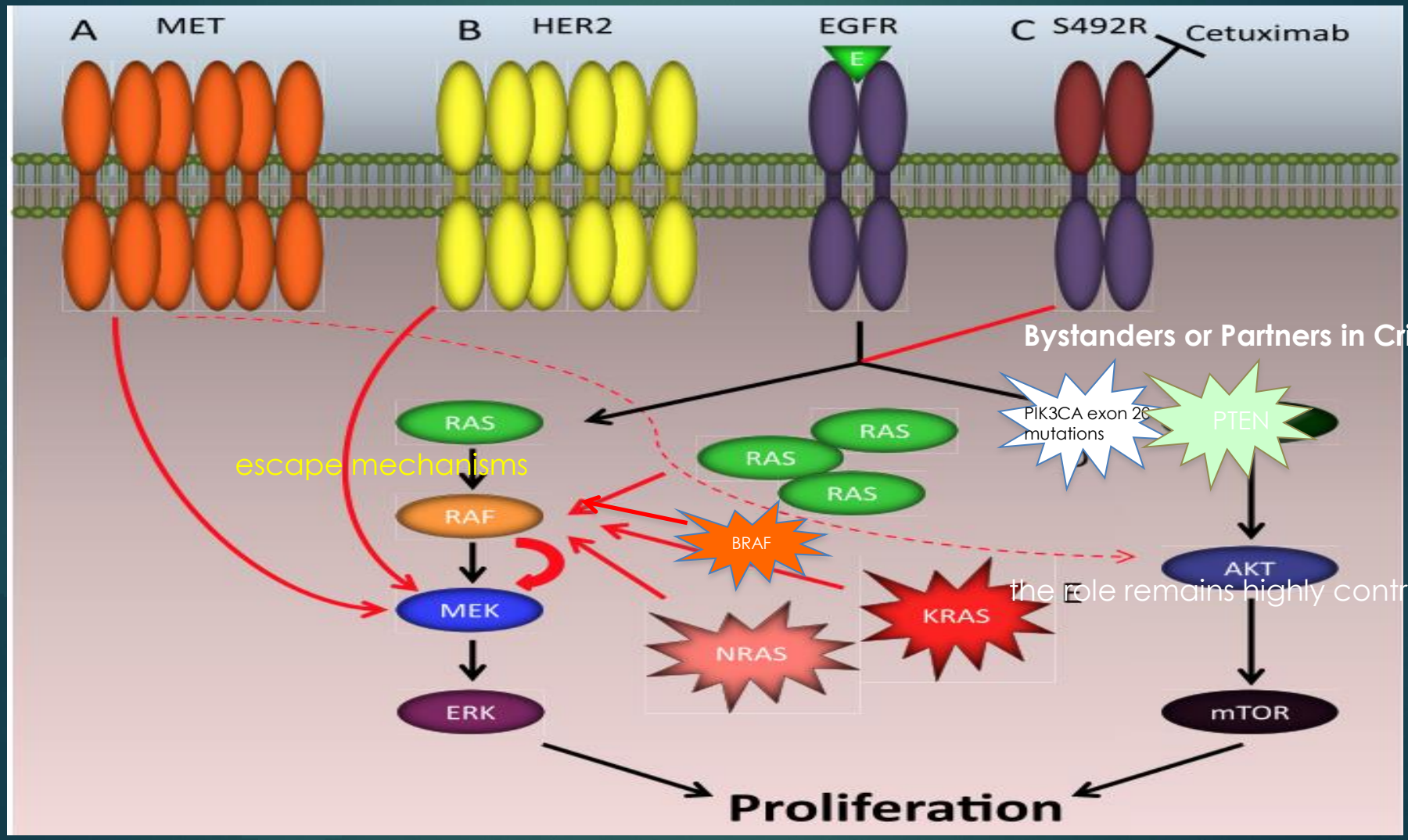
Wild-type KRAS -- When the EGFR receptor is blocked, WT KRAS does not signal and the tumor cells do not proliferate.

Mutated KRAS --When KRAS is mutated, it is permanently "turned on" and downstream events can still occur allowing the tumor to continue to proliferate.

Cell Proliferation, Survival, Invasion and Metastasis signals are blocked

Cell Cycle Progression Continues:
--Cell Proliferation
--Cell Survival
--Invasion and Metastasis

Mechanisms of resistance are genetically heterogeneous but they biochemically converge on key signaling pathways



the role remains highly controver

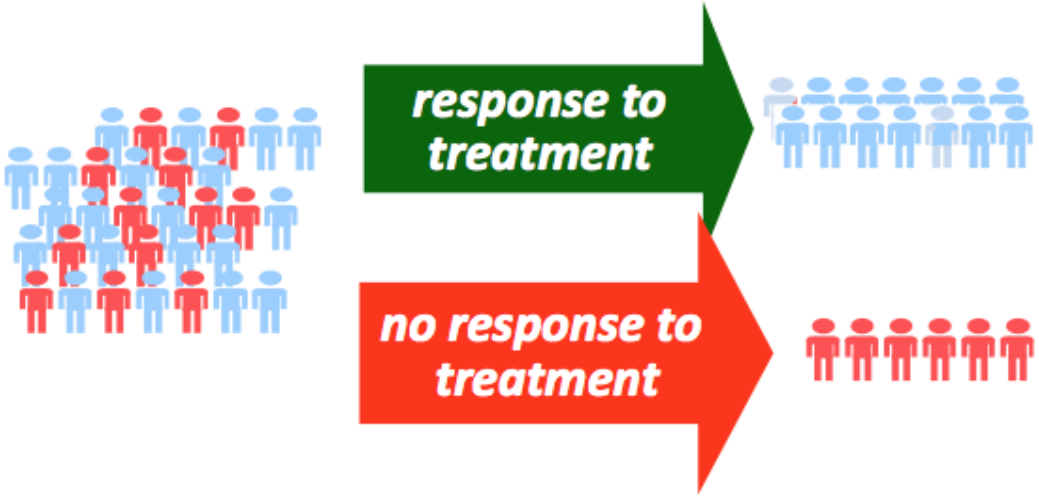
MOLECULAR ONCOLOGY 8 (2014) 1084e1094

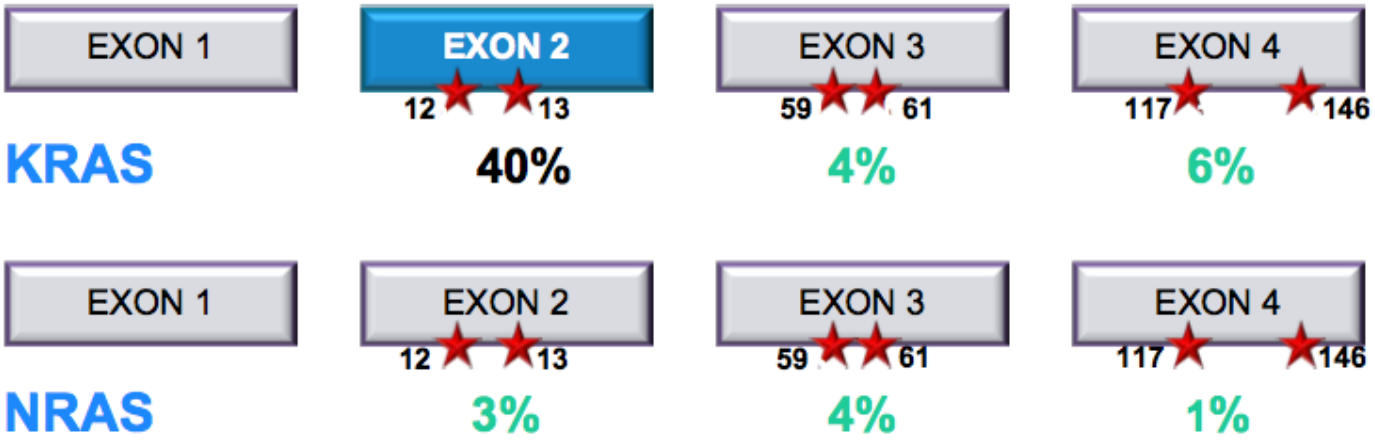
The RAS story
Major survival benefit in selected patients

Study	Comparison of targeted agents	OS HR (RAS wt)	Difference in median OS between treatment arms (months)
PRIME[1]	Panitumumab vs no	0.77	5.6 ↑
CRYSTAL[2]	Cetuximab vs no	0.69	8.2 ↑
FIRE-3[3]	Cetuximab vs bev	0.70	8.1 ↑
PEAK[4]	Panitumumab vs bev	0.63	12.4 ↑)

1. Douillard J-Y, et al. N Engl J Med 2013;369:1023–1034
2. Ciardiello F, et al. J Clin Oncol 2014;32:5s (suppl) (Abstract No. 3506)
3. Stintzing S, et al. Ann Oncol 2014;25(suppl 4):v1–v41 (Abstract No. LBA11)
4. Karthaus M, et al. EJC 2013;49 (suppl 3) (Abstract No. 2262)

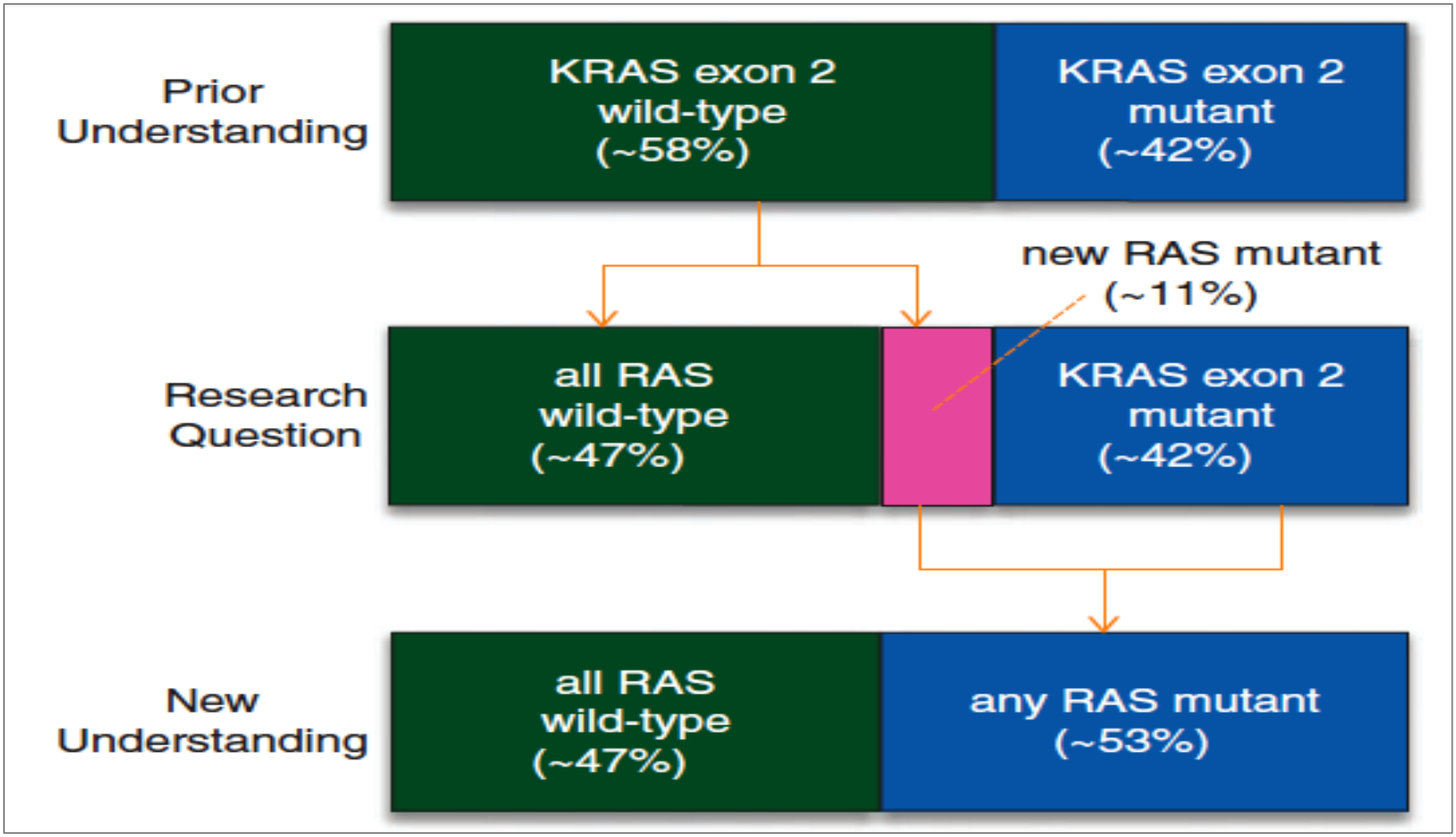
Primary resistance to anti-EGFR therapy
Mutations in KRAS and NRAS used in daily clinical practice





A meta-analysis of RCTs / 9 studies – 5948 pts

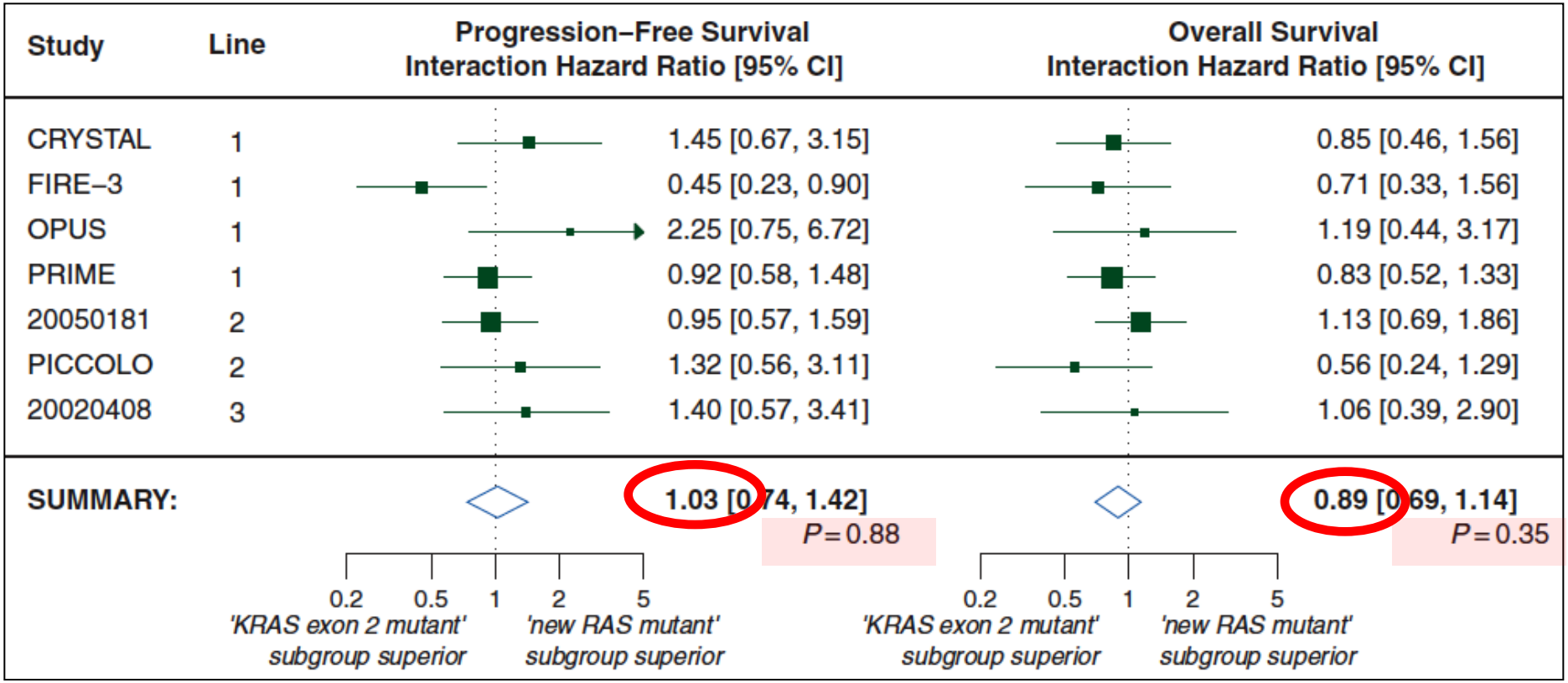
Extended RAS mutations



Annals of Oncology 26: 13–21, 2015

Extended RAS mutations: a meta-analysis

No difference in PFS or OS benefit between tumors with KRAS exon 2 mutations and tumors with the new RAS mutations

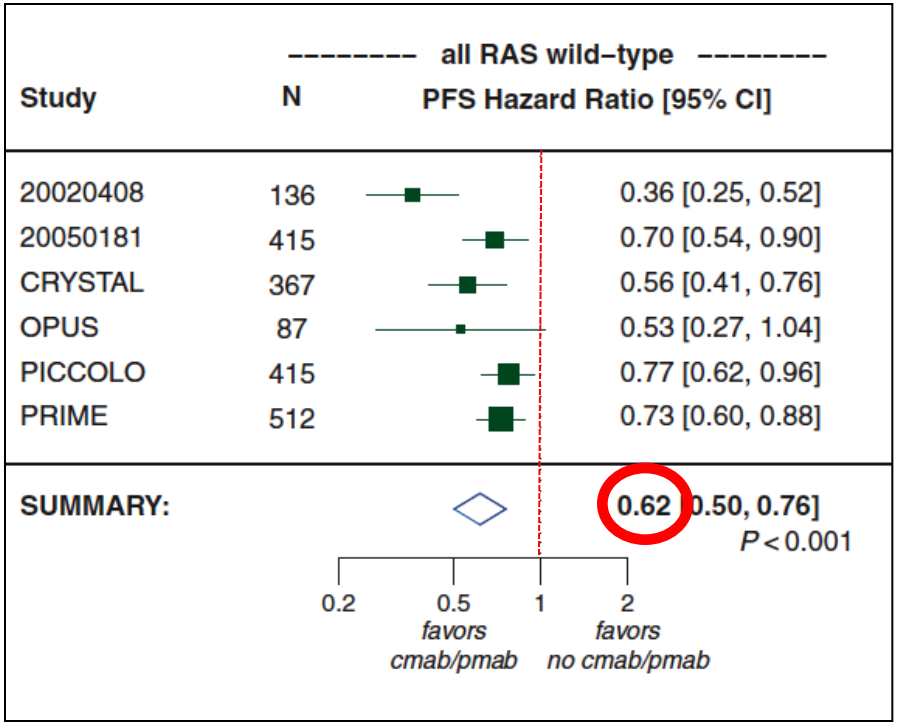


Annals of Oncology 26: 13–21, 2015

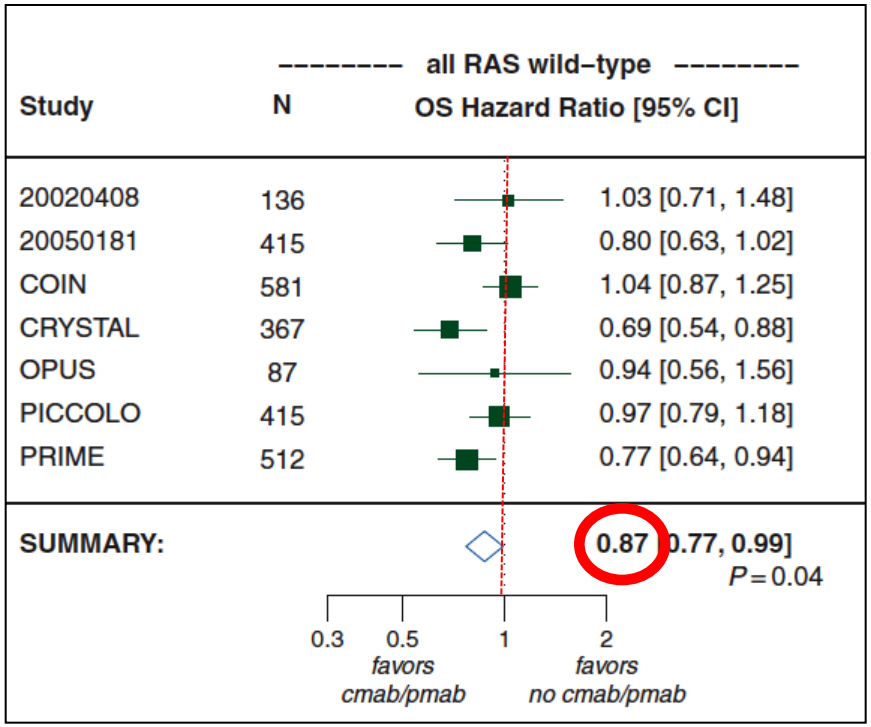
Extended RAS mutations: a meta-analysis

9 studies – 5948 pts

PFS



OS



Annals of Oncology 26: 13–21, 2015

MOLECULAR PROFILE MEANS…

ESMO consensus guidelines for the management of patients with metastatic colorectal cancer

recommendation 4: RAS testing.

- *RAS* mutational status is a negative predictive biomarker for therapeutic choices involving EGFR antibody therapies in the metastatic disease setting [I, A].
  - *RAS* testing should be carried out on all patients at the time of diagnosis of mCRC [I, A].
- *RAS* testing is mandatory before treatment with the EGFR-targeted monoclonal antibodies cetuximab and panitumumab [I, A].

recommendation 5: BRAF testing.

- Tumour *BRAF* mutation status should be assessed alongside the assessment of tumour *RAS* mutational status for prognostic assessment (and/or potential selection for clinical trials) [I, B].

recommendation 3: tissue selection.

- Tissue from either the primary tumour or a liver metastasis may be used for *RAS* mutation testing [III, A].
- Other metastatic sites such as lymph node or lung metastases may be used only if primary tumour or liver metastases samples are not available [II, B].

recommendation 9: emerging technologies.

- Although CTC number correlates with prognosis in patients with mCRC, the clinical utility of CTC assessments is not yet clear and therefore cannot be recommended [IV, D].
- The utility of liquid ctDNA biopsies to guide treatment decisions is currently under investigation in clinical trials, but cannot yet be recommended in routine practice [V, D].

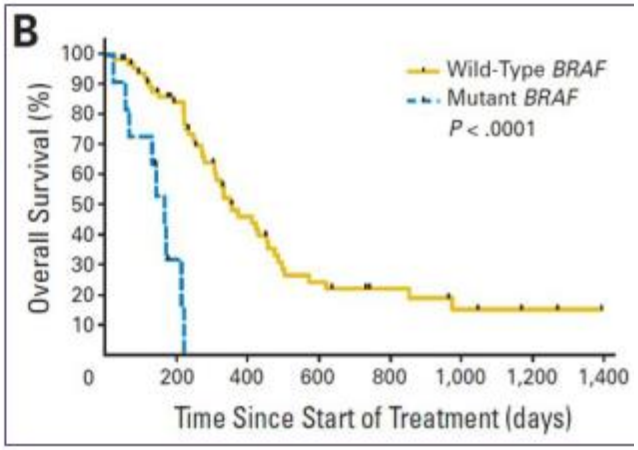
BRAF-Mutant Colorectal Cancer

BRAF V600 mutations occur in 5%-15% of CRC tumors

- Mutually exclusive of K- and N-*Ras* mutations
- Substantially worse prognosis than wild-type or *KRAS* mutant disease (OS for WT 34.7 months vs. BRAFm 10.4 months)
- *BRAF* testing is currently optional and not necessary for decision making around EGFR inhibitors, poor prognosis marker

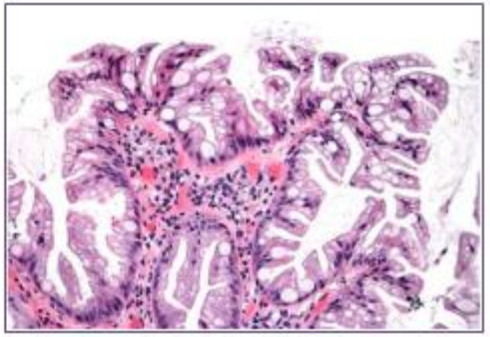
Distinct underlying biology of BRAFm metastatic CRC

- Female predominance, proximal colon lesions, poorly differentiated with increase peritoneal disease
- Unique pre-cancerous lesions (serrated adenoma)
- Hyper-mutated, MSI, and hyper-methylation in about half of the cases



Di Nicolantonio et al., JCO 2008

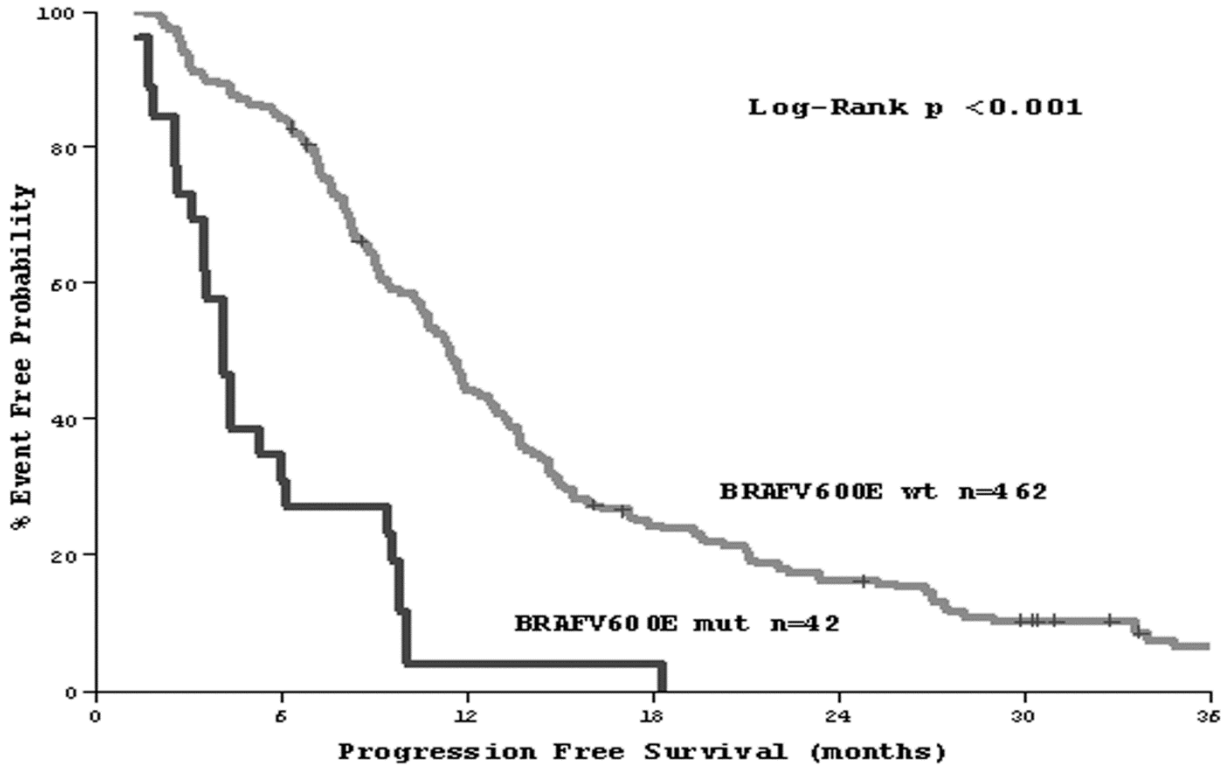
click to zoom



Serrated adenoma

Progression Free Survival according to the *BRAF*^{V600E} mutation (n=504)

Figure 1A

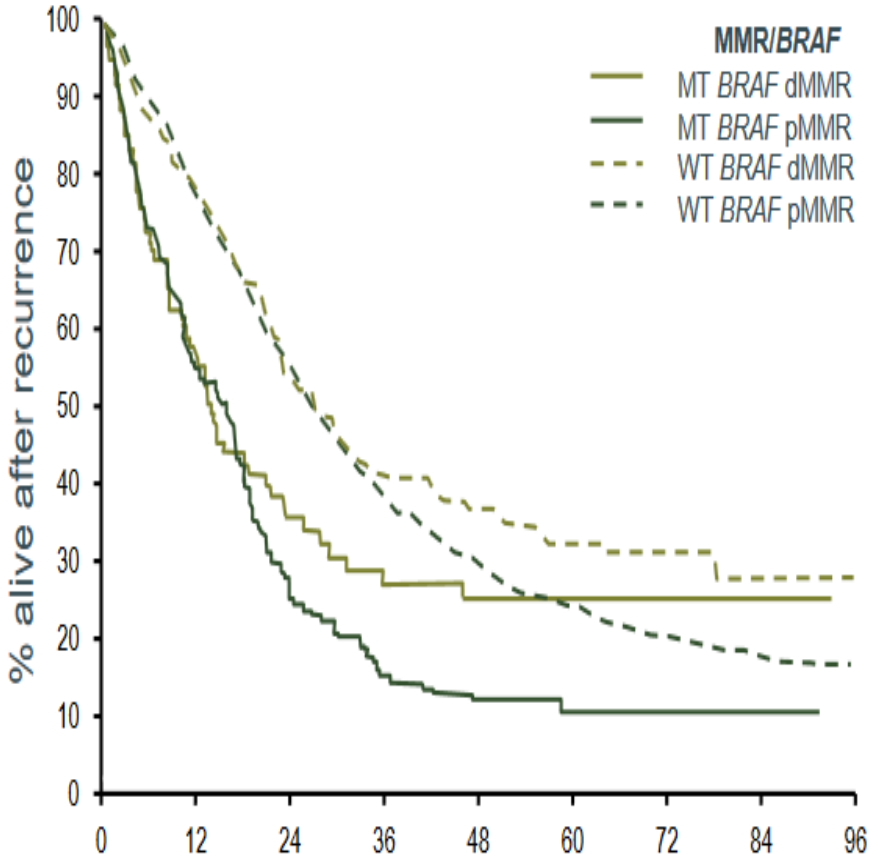


	Median (m)	95%ci
BRAF^{V600E} mut (n=42)	4.1	2.66-6.20
BRAF^{V600E} wt	11.6	

PROGNOSTIC ROLE OF *BRAFV600E* GUIDE DECISIONS

- Small population: 8 to 12% CRC[1]
- Crossover of BRAF and sporadic MSI (promoter methylation) occurs in 30% of cases, with prognostic consequences[2]
- S III (ACCENT database): 2,600 patients with metastatic recurrence (7 adjuvant trials, 271 dMMR, 303 with BRAF mutation

BRAF mutation is a poor prognostic factor in patients with MSS or MSI cancers



Patients at risk

MT *BRAF* dMMR	77	33	17	11	10	6	4	2	0
MT *BRAF* pMMR	167	77	28	11	7	3	3	3	0
WT *BRAF* dMMR	143	102	60	40	30	16	12	5	1
WT *BRAF* pMMR	1600	1202	818	524	322	174	89	38	10

BRAF mutation

Anti-EGFR therapy / chemotherapy refractory disease

No objective responses have been reported for panitumumab or cetuximab monotherapy (NCI-CO17, ASPECCT)

J Clin Oncol 2008;26:5705–12

Anti-EGFR therapy / second line

PICCOLO trial (Subgroup of 131 pts)
A trend toward worse OS with the addition of panitumumab to irinotecan (HR, 1.4; 95% CI, 0.82 to 2.39)

Lancet Oncol 14:749-759, 2013

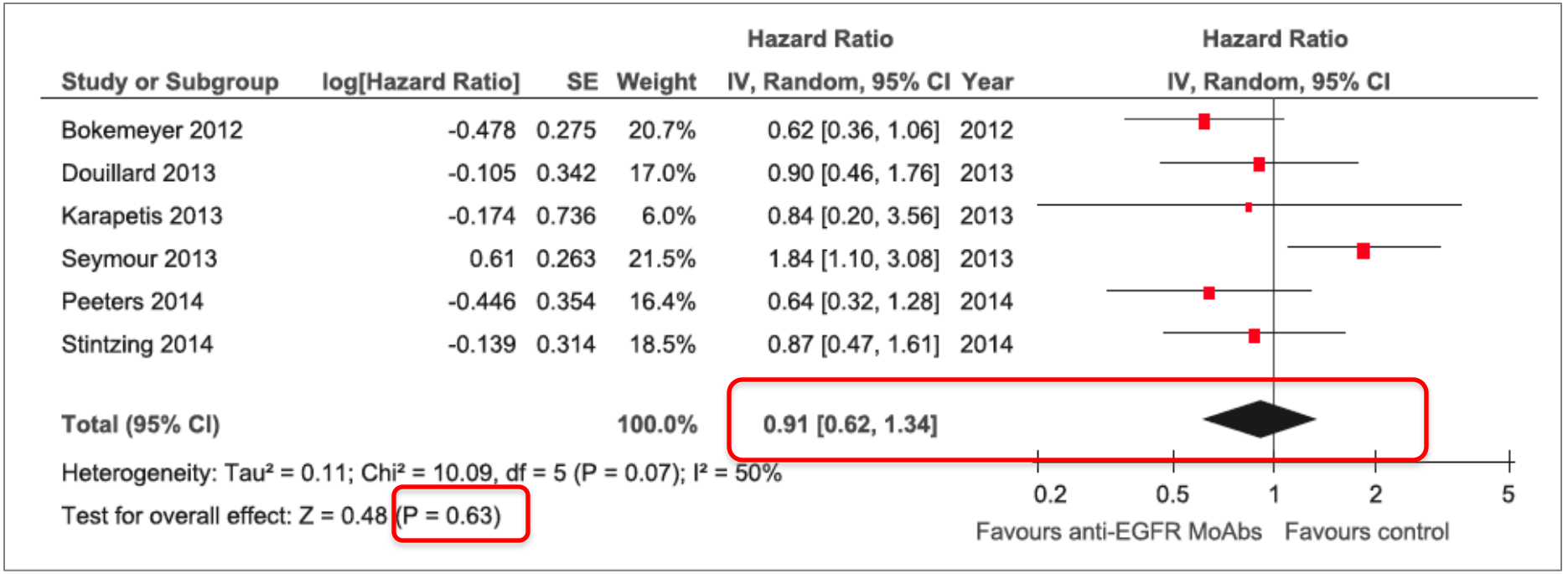
Update of the 20050181
Dismal outcomes in the BRAF-mut mCRC
FOLFIRI-panitumumab vs FOLFIRI (median OS of 4.7 vs 5.7 months)

J Clin Oncol 32, 2014 (suppl 5s; abstr 3568)

Predictive role of BRAF mutations in patients with advanced CRC receiving cetuximab and panitumumab: A meta-analysis

- 9 phase III trials / 1 phase II trial / **463 RAS-wt/BRAF-mut**
- 6 trials 1st-line and 2 second-line, 2 trials chemo-refractory patients

Forest plots showing HR for **overall survival** for anti-EGFR treatment in BRAF-mt colorectal cancer



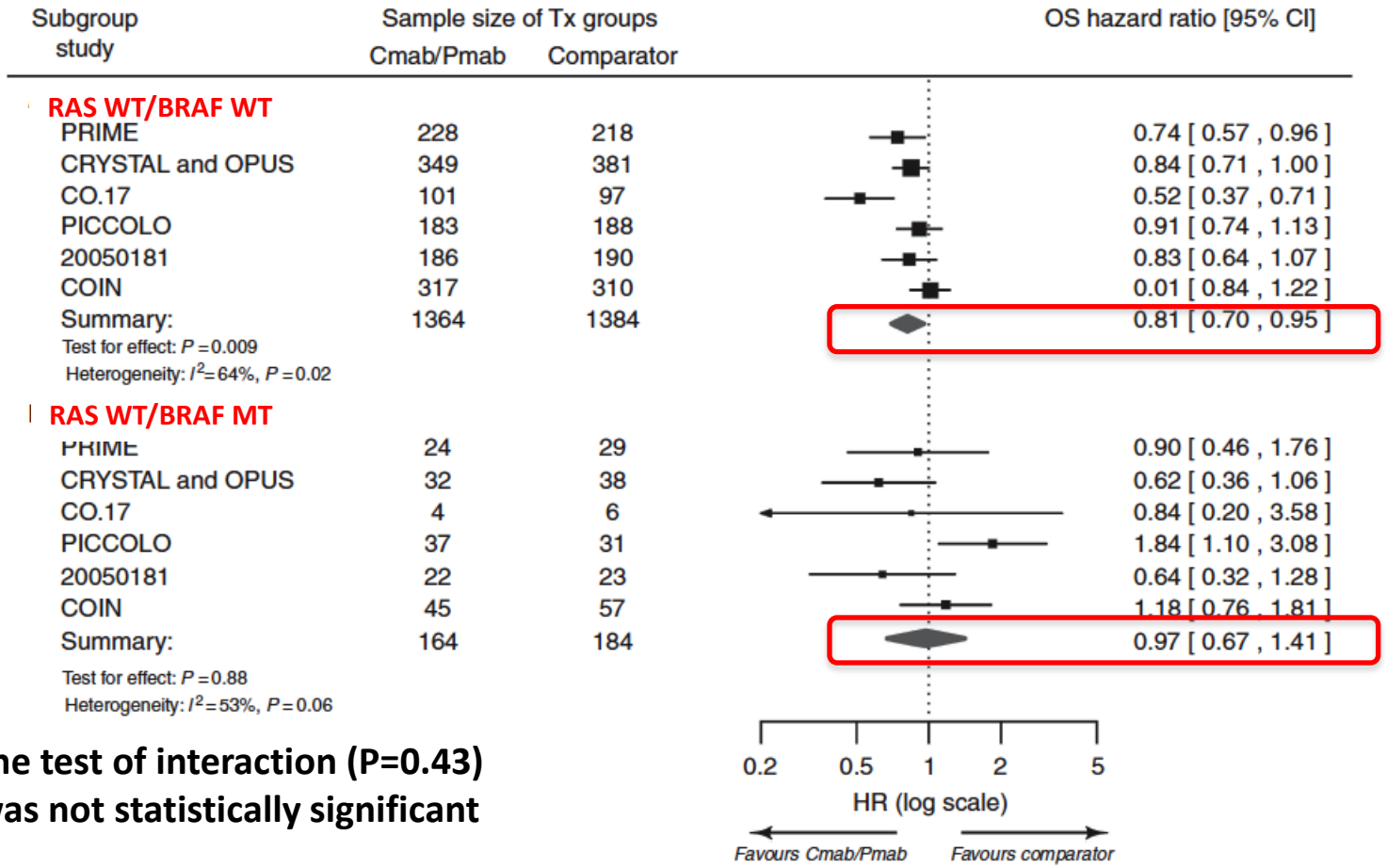
In a front-line, the effect was not significant in favour of anti-EGFR (HR, 0.76; 95% CI, 0.54–1.08; p= 0.13)

Pietrantonio F et al. Eur J Cancer 51: 587–594, 2015

Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR therapy

3168 participants with RAS WT tumours **/ 8 RCT**
2817 BRAF WT **/ 351 (11.1%) BRAF MT tumours**

Forest plot of the overall survival benefit with anti-EGFR mAb therapy



The test of interaction (P=0.43) was not statistically significant

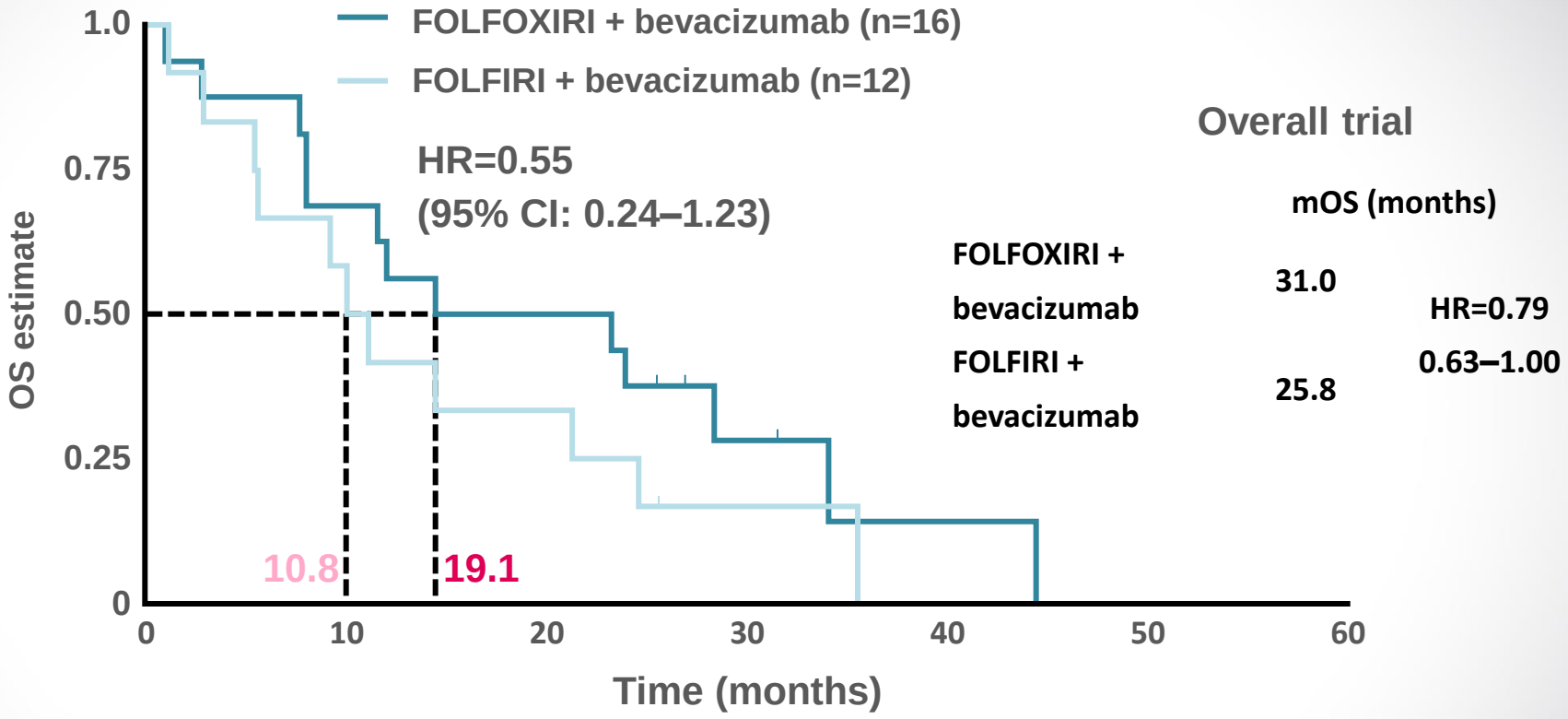
A Rowland et al. B J Cancer (2015) 112, 1888–1894

BRAF mt meta-analyses contrasting results

	Pietrantonio F et al.	***A Rowland et al***
Conclusion	Anti-EGFR therapy **did not increase the benefit**	**Insufficient evidence** to definitively consider BRAF MT a **negative predictive biomarker**
Statistical methods	Simply estimating anti-EGFR mAb efficacy in the BRAF MT subgroup	Assesed whether anti-EGFR mAb efficacy differs based on BRAF mutation status
Inclusion criteria	Included trials comparing anti-EGFR mAb therapy with bevacizumab	Excluded trials comparing anti-EGFR mAb therapy with bevacizumab

Pietrantonio F et al. Eur J Cancer 51: 587–594, 2015
A Rowland et al. B J Cancer 112, 1888–1894, 2015

TRIBE: benefit of more intensive treatment for patients with *BRAF*-mutated mCRC



Loupakis, et al. ASCO 2014. Abstract 3519

TREATING *BRAFV600E* MUTATIONS IN mCRC

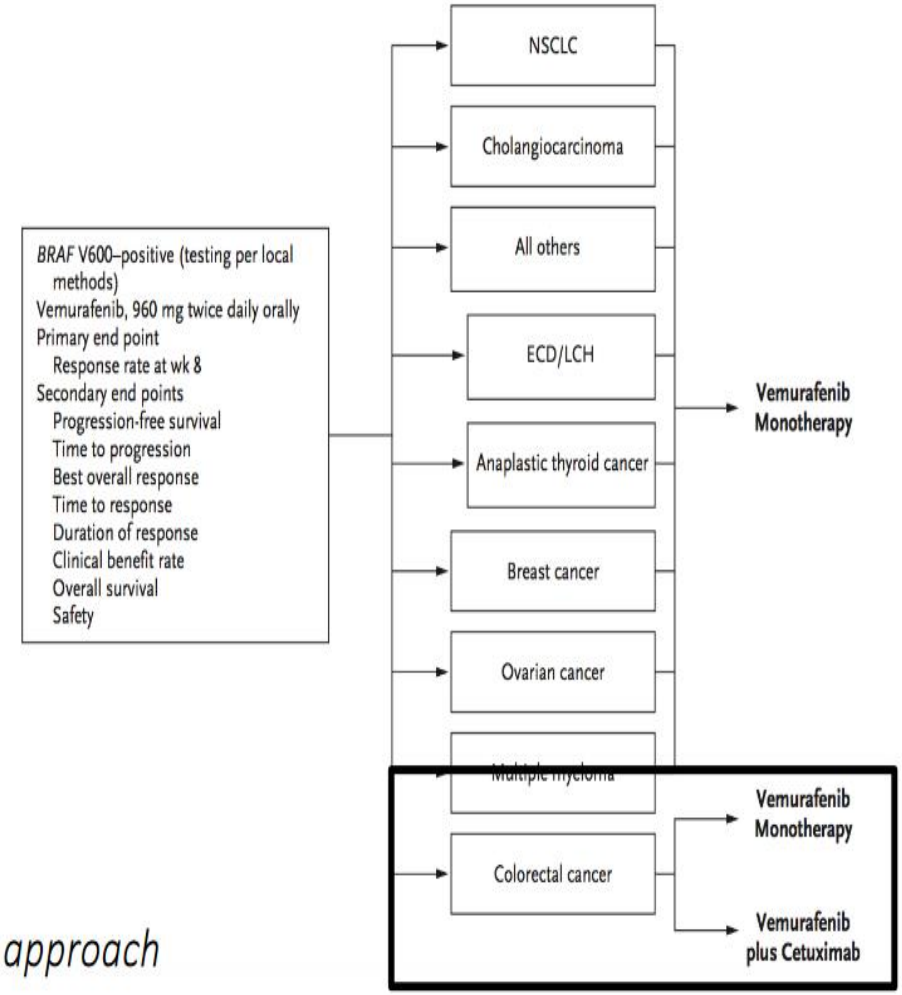
Basket trials include patients with a wide variety of histologies as long as they all harbor a cognate aberration. Often perceived as signal finding.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Vemurafenib in Multiple Nonmelanoma Cancers with *BRAF* V600 Mutations

David M. Hyman, M.D., Igor Puzanov, M.D., Vivek Subbiah, M.D., Jason E. Faris, M.D., Ian Chau, M.D., Jean-Yves Blay, M.D., Ph.D., Jürgen Wolf, M.D., Ph.D., Noopur S. Raje, M.D., Eli L. Diamond, M.D., Antoine Hollebecque, M.D., Radj Gervais, M.D., Maria Elena Elez-Fernandez, M.D., Antoine Italiano, M.D., Ph.D., Ralf-Dieter Hofheinz, M.D., Manuel Hidalgo, M.D., Ph.D., Emily Chan, M.D., Ph.D., Martin Schuler, M.D., Susan Frances Lasserre, M.Sc., Martina Makrutzki, M.D., Florin Sirzen, M.D., Ph.D., Maria Luisa Veronese, M.D., Josep Tabernero, M.D., Ph.D., and José Baselga, M.D., Ph.D.



The "Basket trial" is the paradigm of this approach

Hymann et al. New Engl J Med 2015

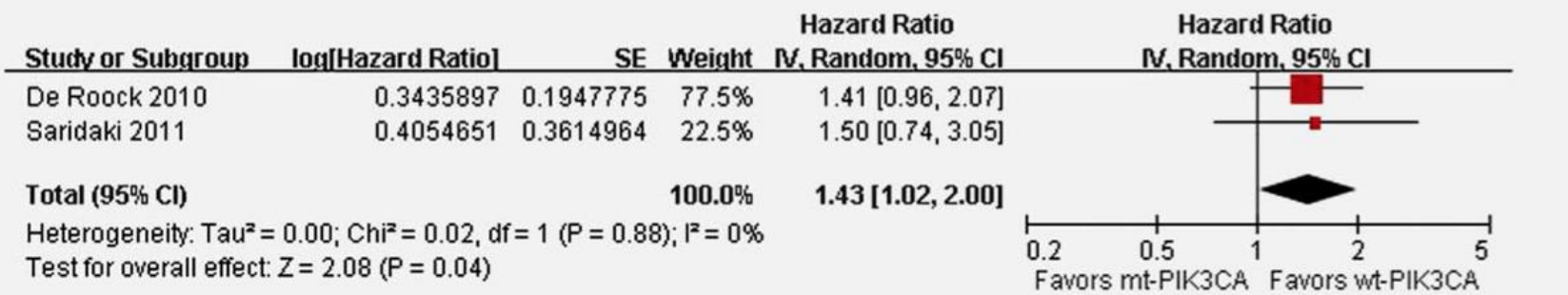
Targeting *BRAF*^{V600E}: studies to date

Regimen		Response rate*	PFS (months)	Citation
Single/doublet RAF/MEK	vemurafenib	5%	2.1	Kopetz, J Clin Oncol 2015
	dabrafenib	11%	NR	Falchook, Lancet 2008
	encorafenib	16%	NR	Gomez-Roca, ESMO 2014
	dabrafenib + trametinib	12%	3.5	Corcoran, J Clin Oncol 2015
Doublet with EGFR	vemurafenib + panitumumab	13%	3.2	Yeager et al., Clin Cancer Res 2015
	vemurafenib + cetuximab	4%	3.7	Hyman et al., New Engl J Med 2015
	encorafenib + cetuximab	19%	3.7	Van Geel et al., Cancer Discov 2017
	dabrafenib + panitumumab	10%	3.4	Atreya, ASCO 2015
Triplet with EGFR	vemurafenib + cetuximab + irinotecan	16%	4.4	Kopetz et al., ASCO 2017
	dabrafenib + trametinib + panitumumab	32%	4.2	Corcoran, ESMO 2016
	encorafenib + cetuximab + alpelisib	18%	4.2	van Geel et al., Can Disc 2017
	encorafenib + binimetinib + cetuximab	48%	8.0	Van Cutsem et al., GI ASCO 2018

ADDITIONAL PERSPECTIVES

- ***PIK3CA*** *is* the gene that encodes for p100α catalytic subunit of PI3K, a phosphoinositide kinase important in the PI3K/mTOR signalling pathway
  - Activation of this pathway leads to enhanced protein synthesis, cell cycle progression, cell growth and survival
  - Mutations in *PIK3CA* are found in about 20% of colorectal cancers (gene analysis included in most NGS panel investigations), with 48% of those occurring in the kinase domain and 43% occurring in the helical domain
- ***HER2*** amplification
  - Activation of human epidermal growth factor receptor 2 (HER2) is a rare event in colorectal cancers (3-5% of cases), leading to upregulation of RAS/RAF/MEK/ERK and PI3K/mTOR signaling pathways
  - Diagnostic: immunohistochemistry (as in gastric cancer), FISH

The associations of PIK3CA mutations and PTEN loss with the overall survival of wild-type-KRAS



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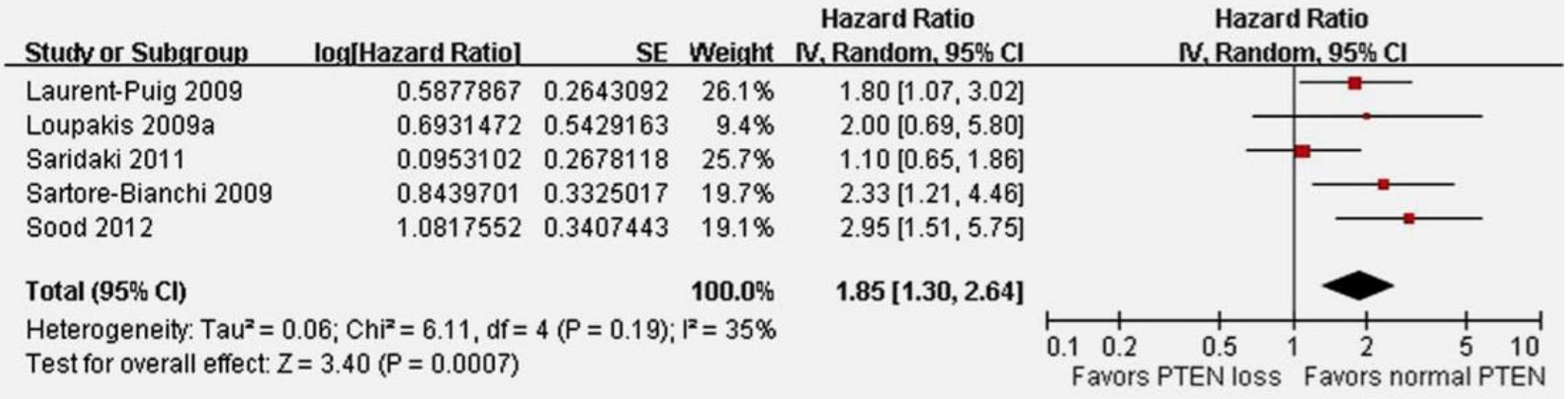
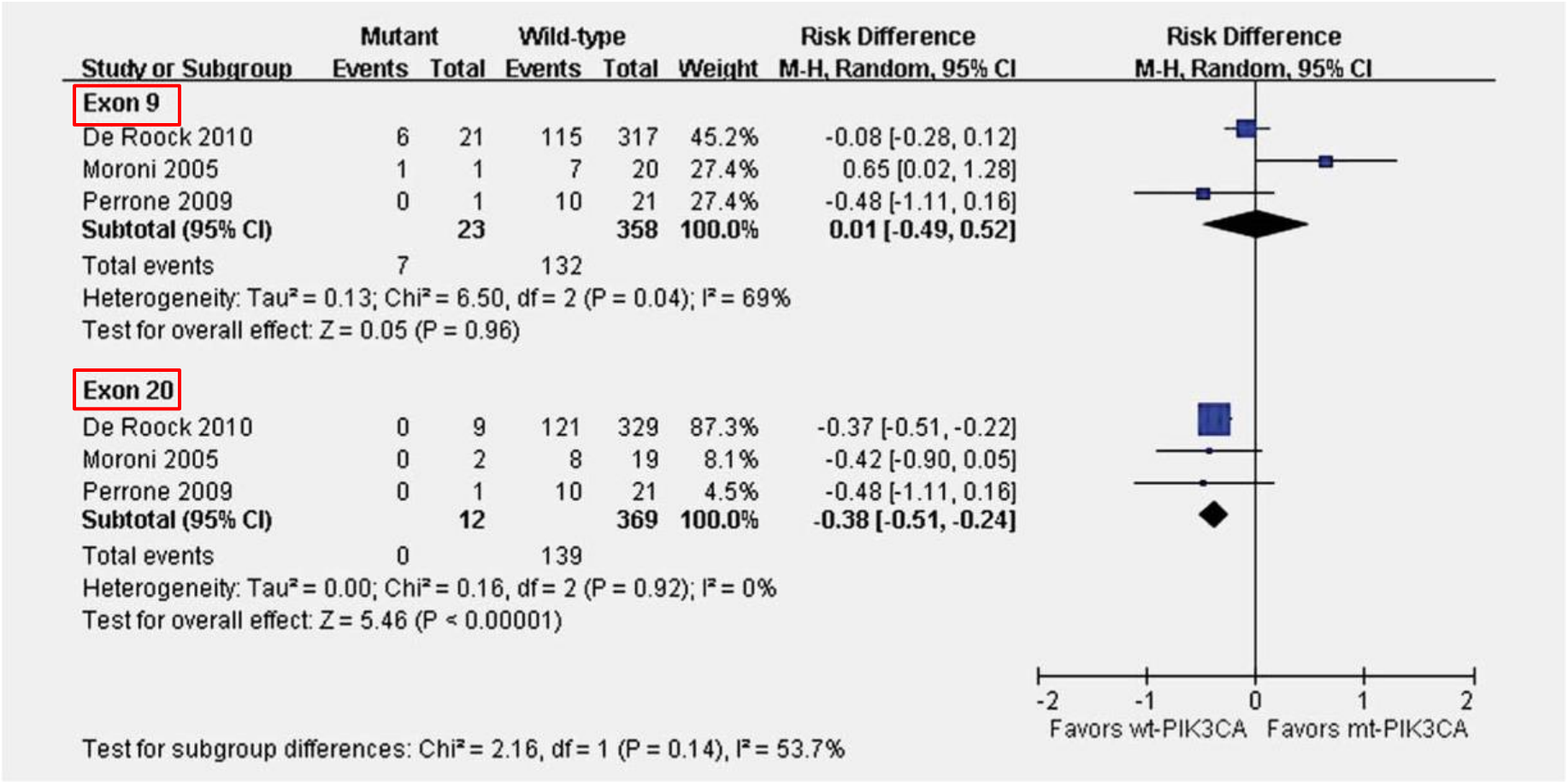


Int. J. Cancer: 133, 1914–1925 (2013)

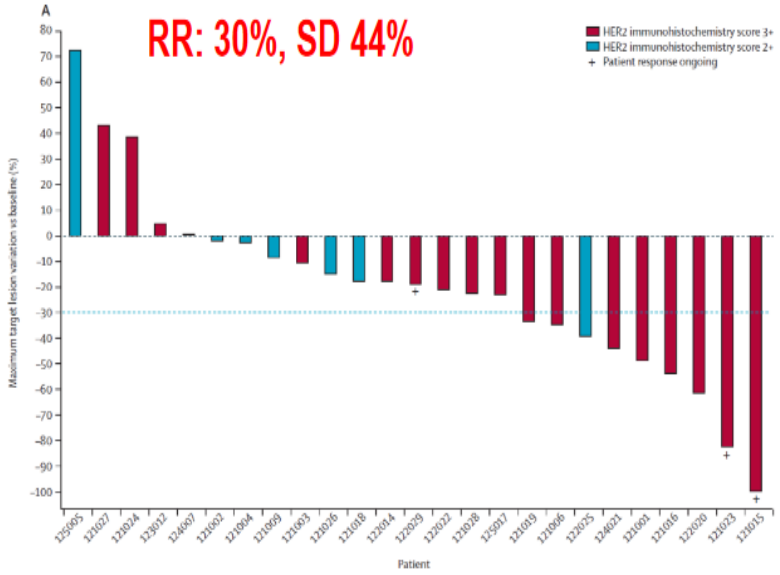
Comparison of PIK3CA exon 9 and exon 20 mutations with the objective response of wild-type-KRAS

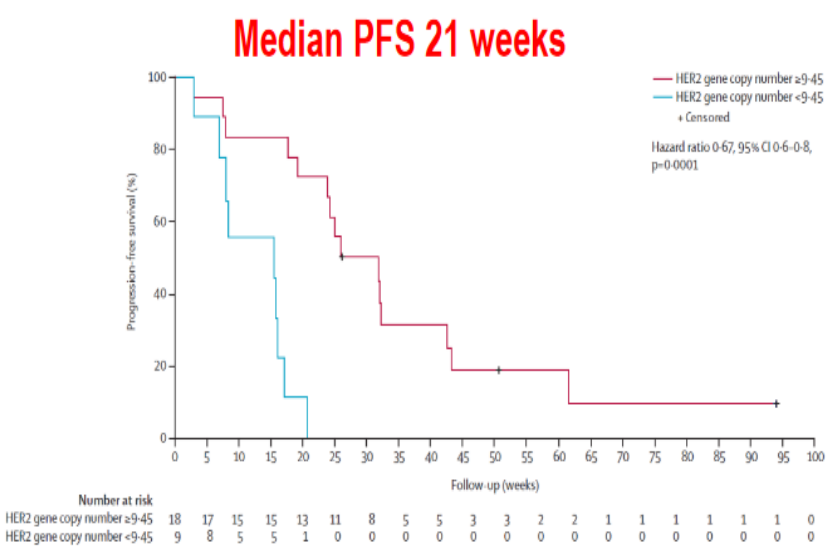


Int. J. Cancer: 133, 1914–1925 (2013)

Trastuzumab plus lapatinib in HER2-positive mCRC (HERACLES)

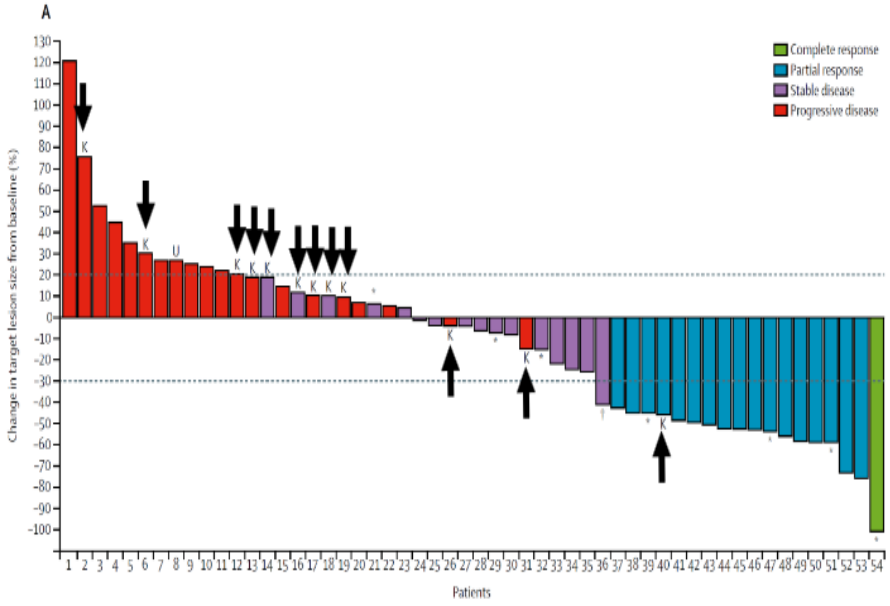
- 914 patients with *KRAS* exon 2 (codons 12 and 13) wild-type mCRC were screened
- 48 (5%) had HER2-positive tumors

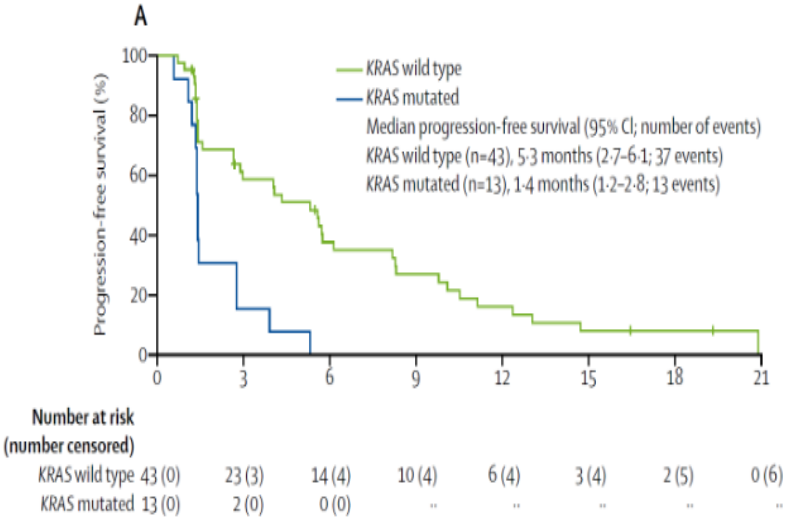


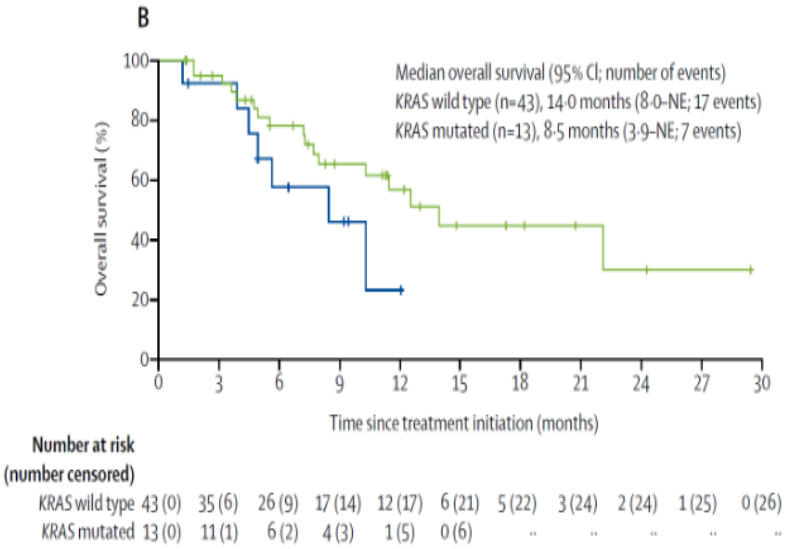


Sartore-Bianchi Lancet Oncol 2016

Pertuzumab plus trastuzumab for HER2-amplified mCRC (MyPathway)



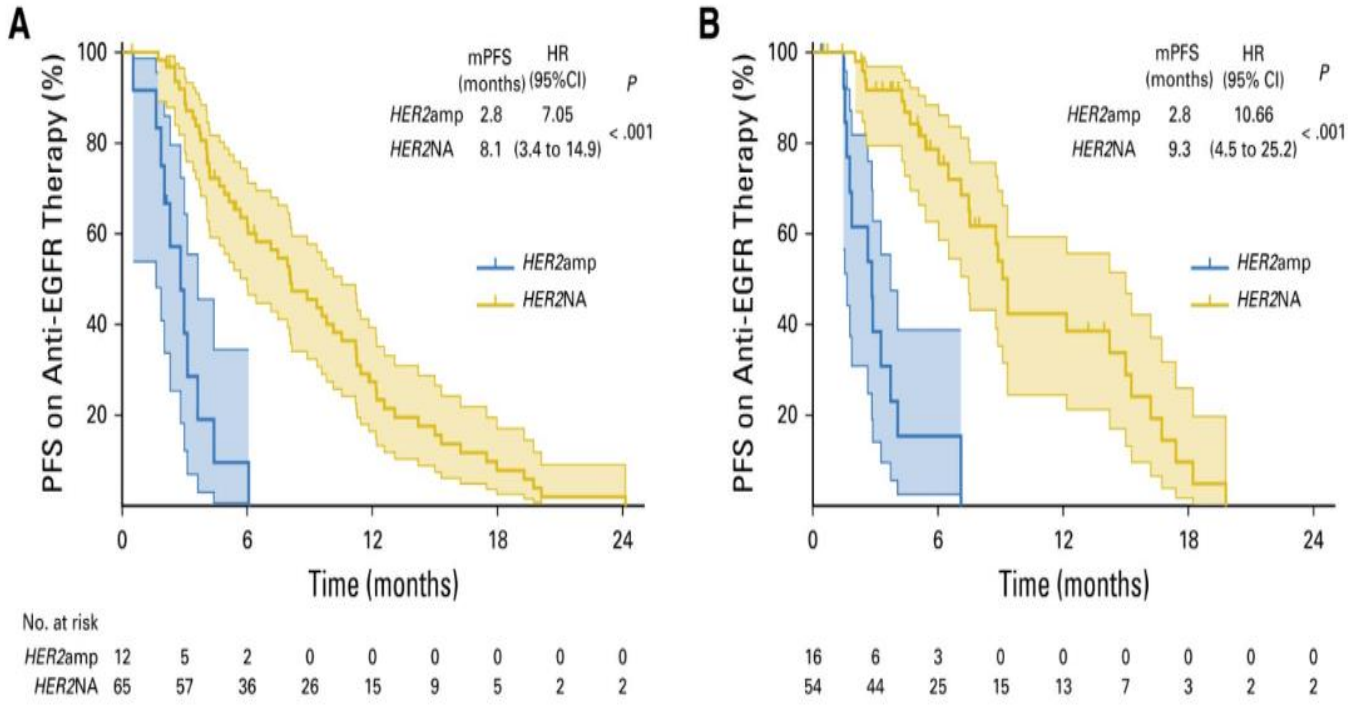




Meric-Bernstam Lancet Oncol 2019

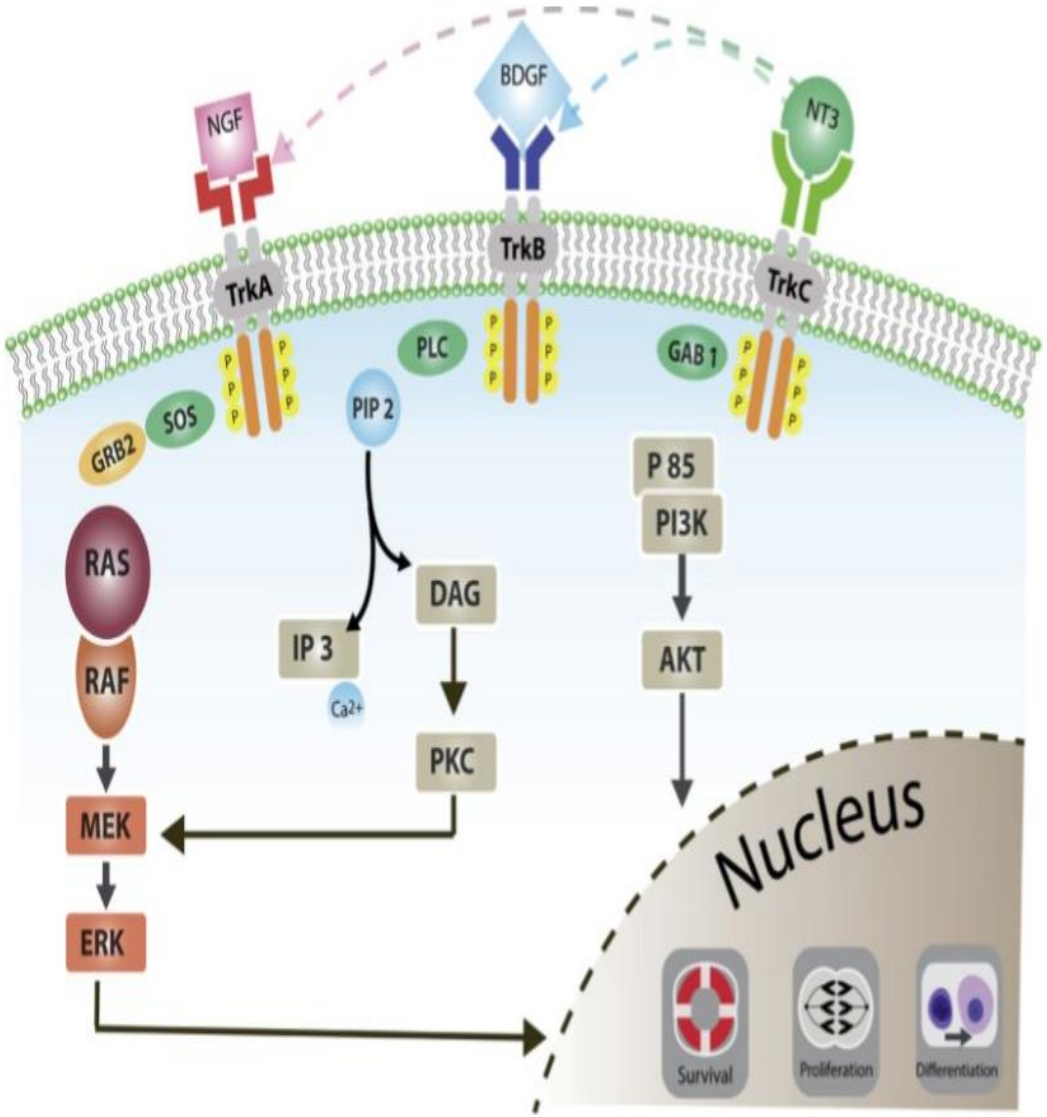
HER2 amplification in mCRC

- Found in about 2% of CRC
- More frequent in patients with KRAS/NRAS/BRAF wild type tumors (5%)
- Associated with resistance to anti-EGFR moAbs in retrospective analyses (2nd/3rd line treatment)



Raghav JCO Precis Oncol 2019

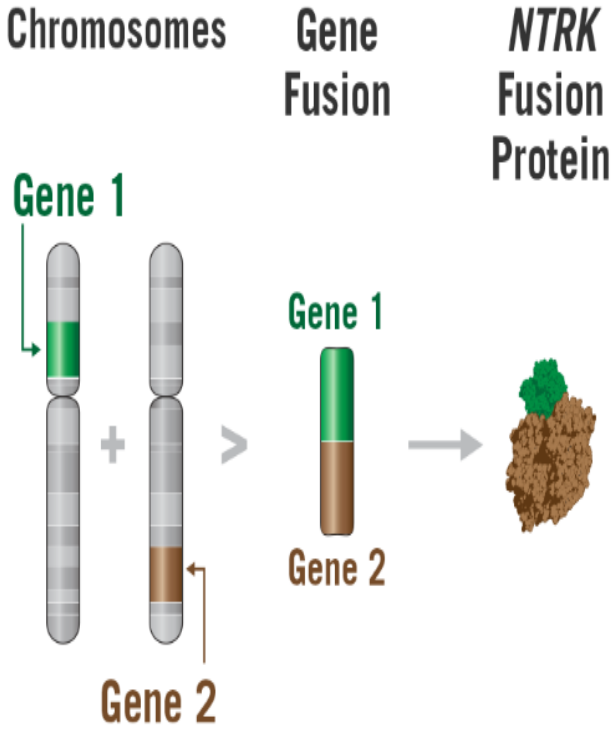
NTRK FUSIONS

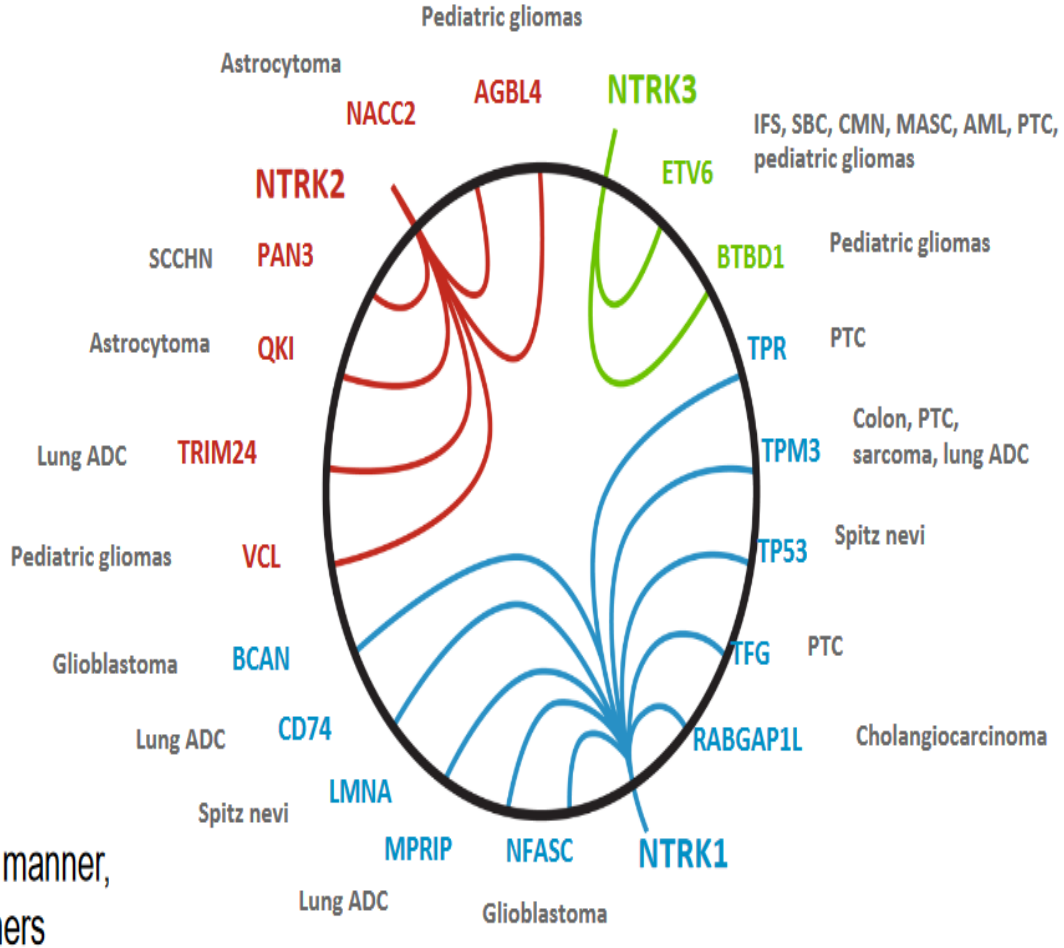


- The tropomyosin receptor kinase family comprise three transmembrane proteins referred to as TrkA, B and C receptors that are encoded by the NTRK1, NTRK2 and NTRK3 genes
- Gene fusions (intra- / interchromosomal rearrangement) involving NTRK genes lead to transcription of chimeric Trk proteins with constitutively activated or overexpressed kinas function conferring oncogenic potential
- These genetic abnormalities have recently emerged as targets for cancer therapy (entrectinib, larotrectinib)

Asmatu et al. ESMO Open 2016; Cocco et al. Nat Rev Clin Oncol 2019; Märkl et al. Pathol Res Pract 2019

MULTIPLE BREAKPOINTS AND FUSION PARTNERS



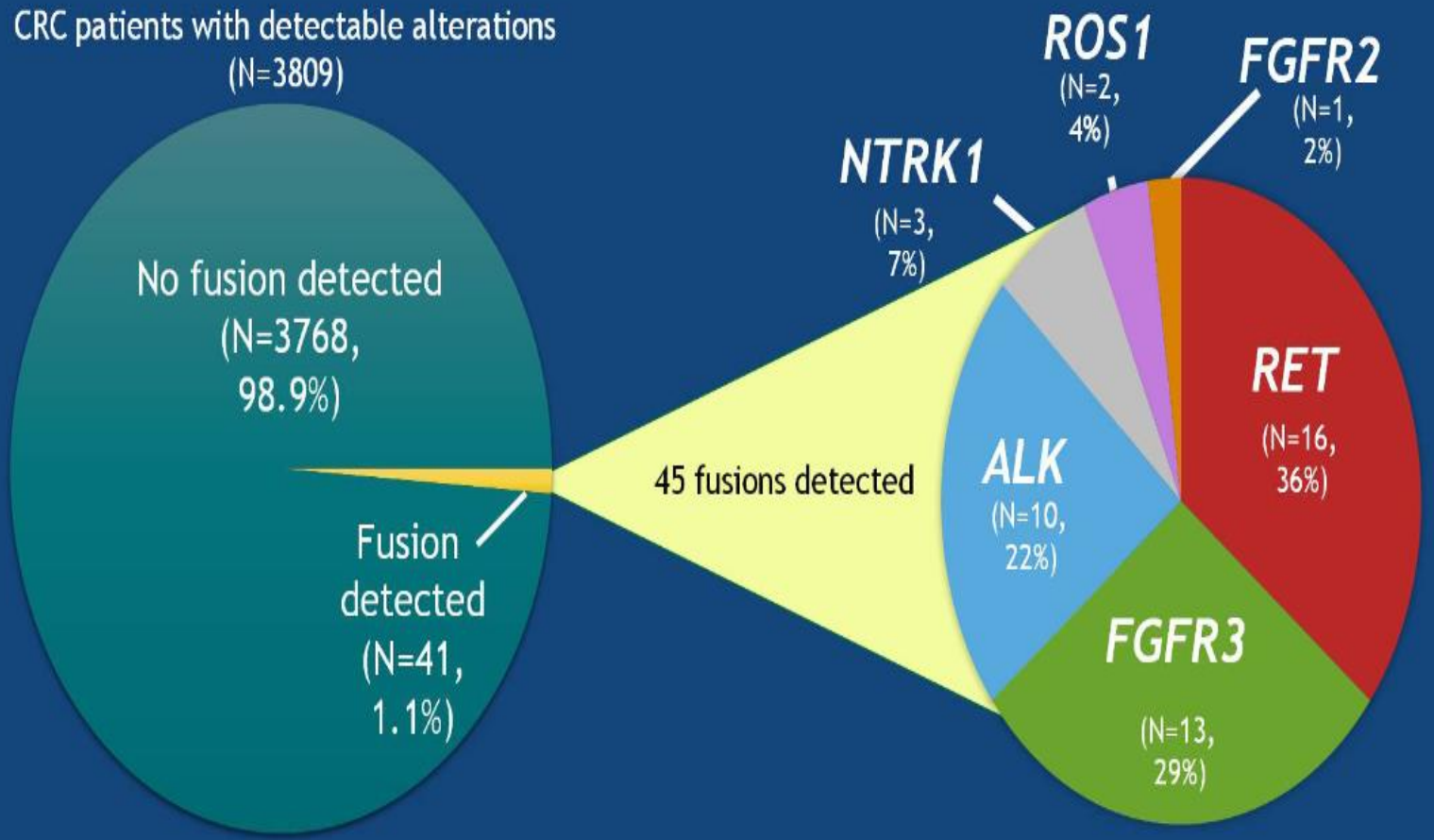


- NTRK gene fusions occur in a tumor-agnostic manner, with inconsistent break points and fusion partners
- The optimal detection method should not require knowledge of fusion break points and/or fusion partners

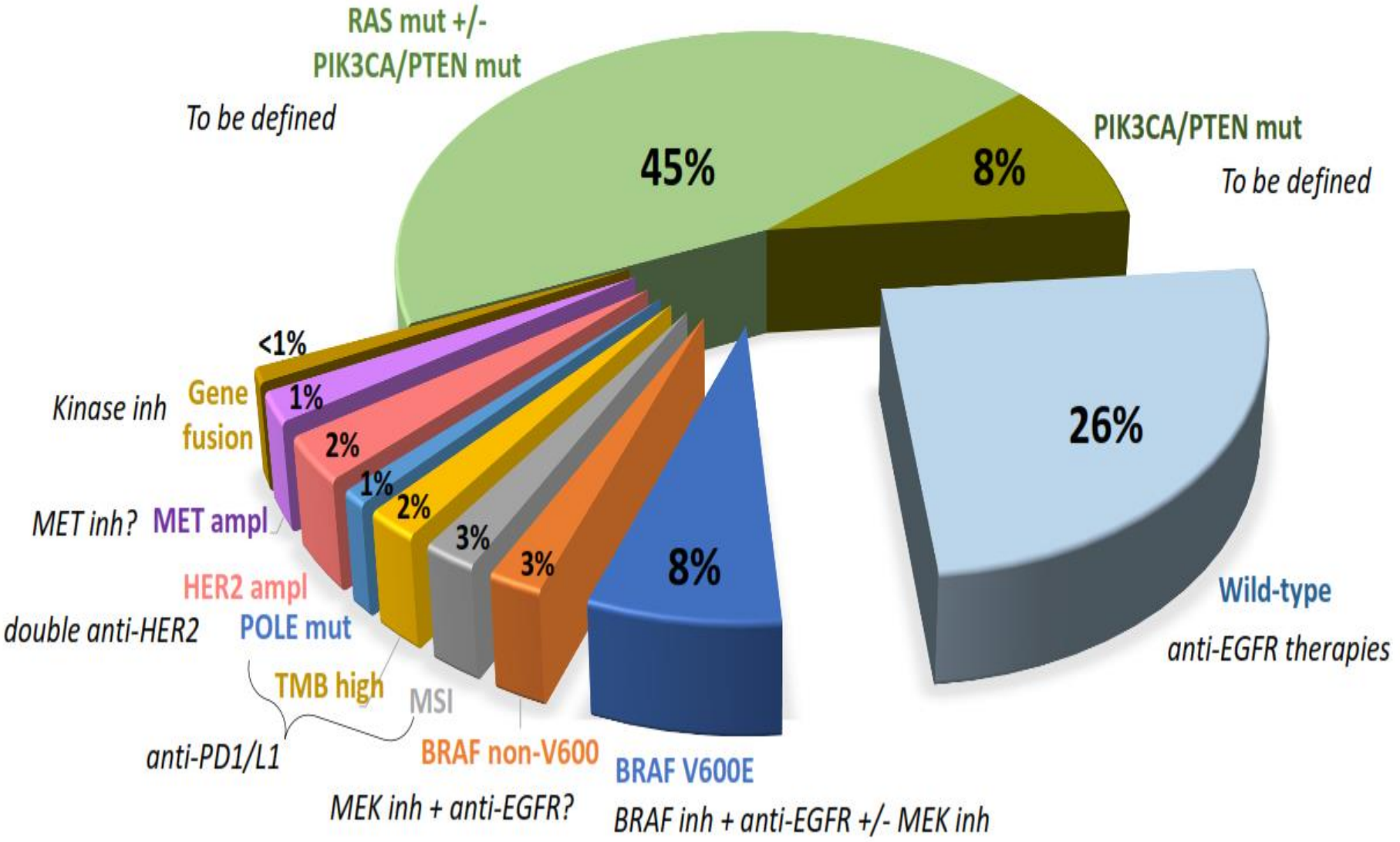
Gene fusions predictive value in metastatic CRC

Alteration	Prevalence	Targetability evidence	Enrichment
NTRK1-3 fusion	< 1%	Case reports	(> if right colon, RAS/*BRAF* wt, MSI)[1]
ALK fusion	< 1%	Case reports	(> if right colon, RAS/*BRAF* wt, MSI colitis-associated)[2]
ROS1 fusion	< 1%	Other tumors	(> if right colon, RAS/*BRAF* wt)[3]
RET fusion	< 1%	Other tumors	(> if right colon, RAS/*BRAF* wt)[3]
FGFR2-3 fusion	<1%	Other tumors	(> if RAS mut)[4]

Overall Fusion Prevalence in CRC with a ctDNA Assay

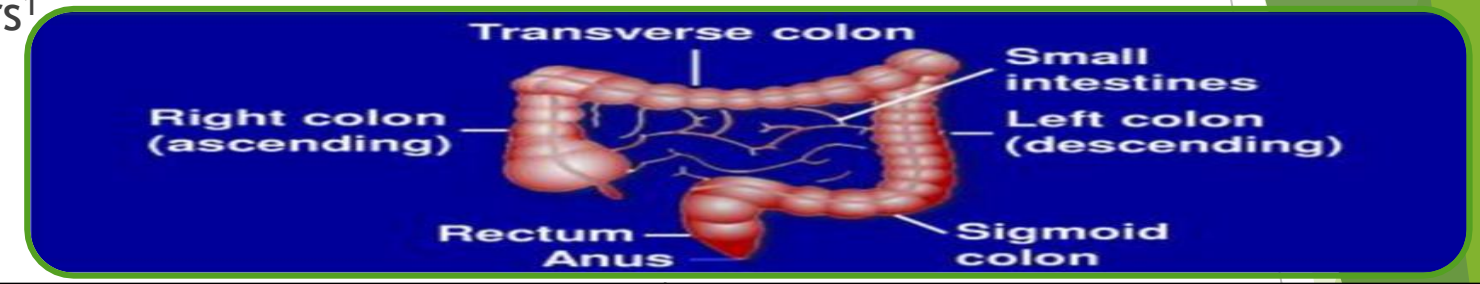


Genomic markers



The Colorectal Tract Is Highly Heterogeneous

- Developmental, genetic, and biologic differences in the proximal (right-side) and distal (left-side) segments of the colon have been documented for over 20 years, and may account for differences in left- vs right-side CRC tumours[1]



Right-side tumours	Left-side tumours
• Older patients • Higher incidence (40% increasing) • More common in female patients • Mucinous, signet ring histology • Poorly differentiated • **Microsatellite instability** • **Hypermethylation, higher mutation rates** • ***PI3KCA* mutation** • ***KRAS* mutations** • ***BRAF* mutations** • Carbs / Fat	• Younger patients • Incidence 60% • Better prognosis • Predominately WT • Chromosomal aberrations; 18q loss and 20q gain • Aneuploidy • ***p53* mutation** / COX2 expression • **EGFR gain** • **HER2 gain** • **High EGFR ligand expression (EREG and AREG expression)** - High *VEGF-1* mRNA expression • Protein / Meat / lose Calcium

1. Bufill. 1990; 2. Missiaglia. 2013; 3. Brule. 2013;
4. The Cancer Genome Atlas Network. 2012; 5. Bendardaf. 2008.

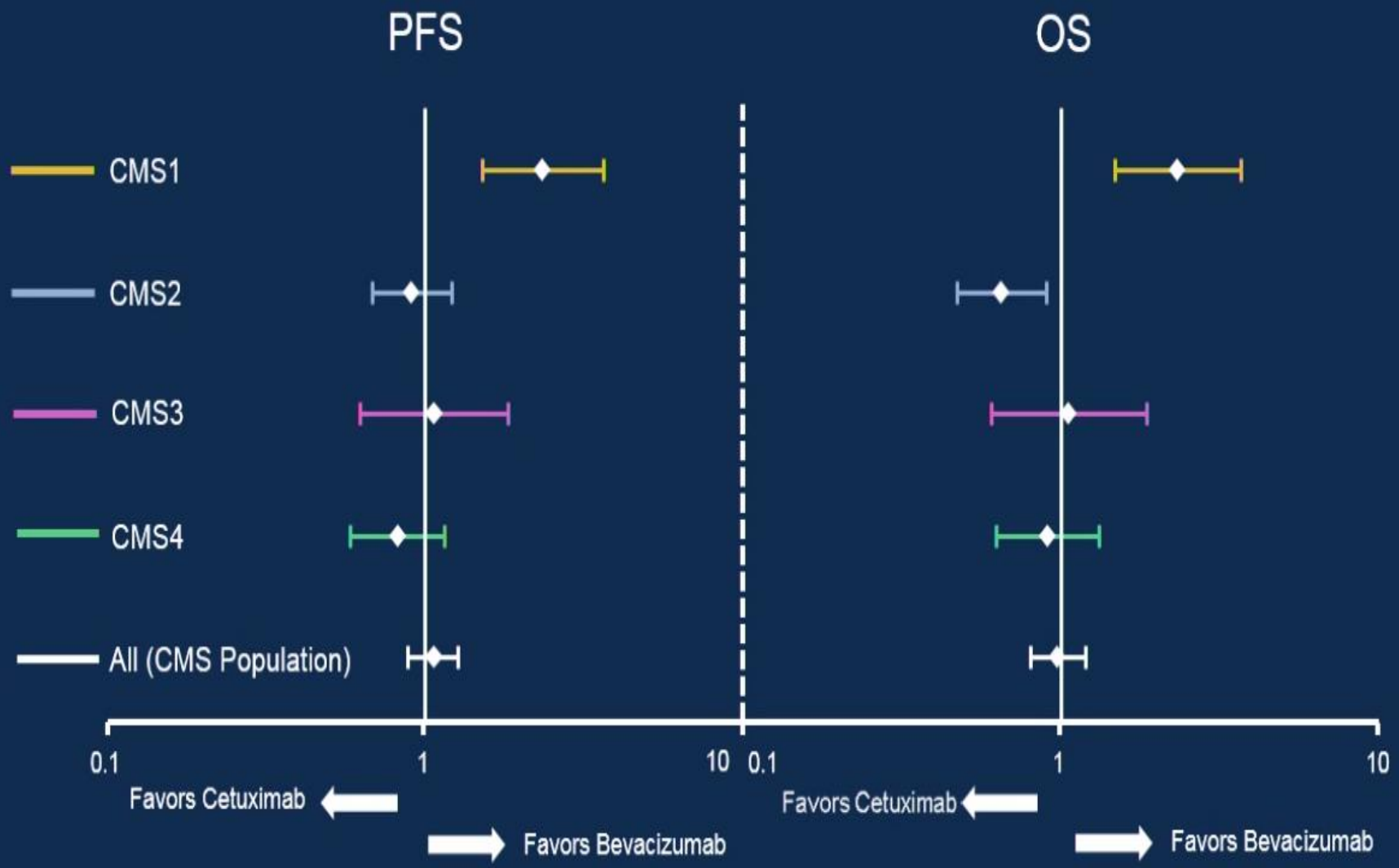
CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

Mixed features samples (13%): possibly are a transition phenotype

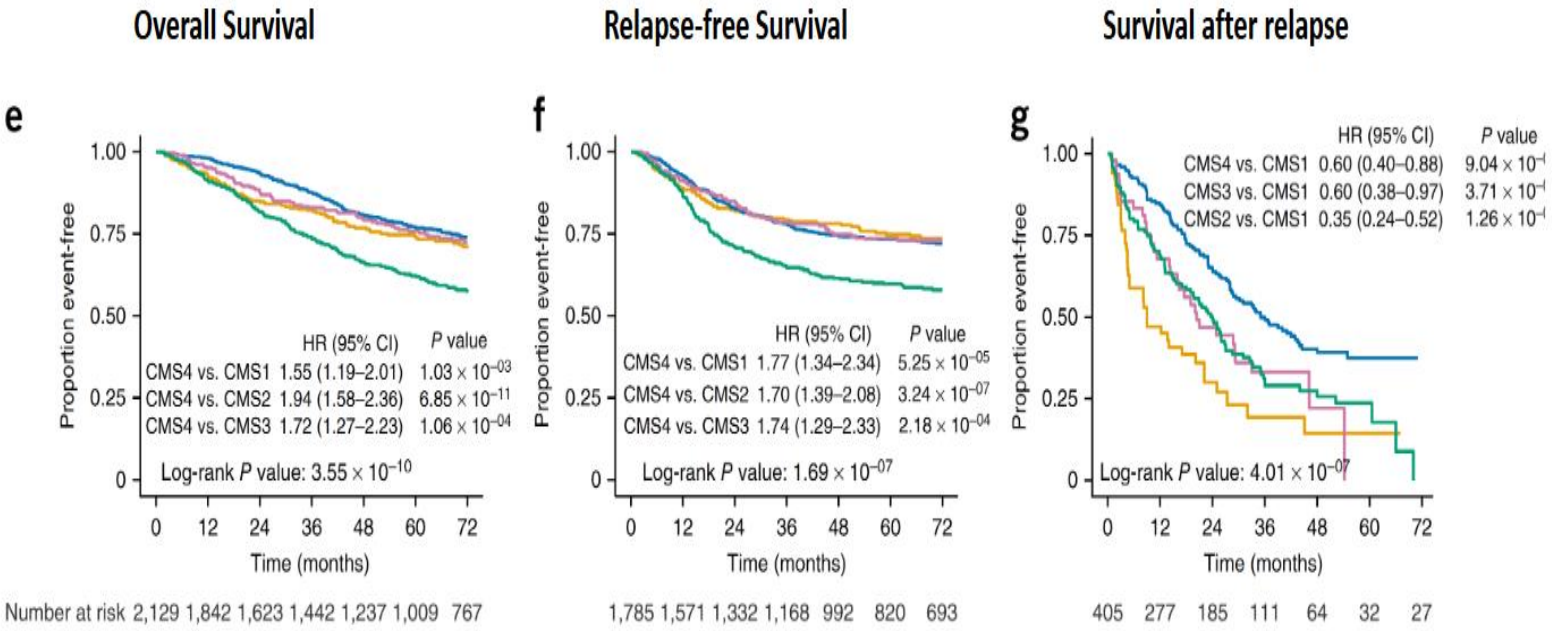
the CMS groups the most robust classification system currently available for CRC—with clear biological interpretability—and the basis for future clinical stratification and subtype-based targeted interventions

Nat Med. 2015 Nov;21(11):1350-6. doi: 10.1038/nm.3967.

Cetuximab vs Bevacizumab



Prognostic value of the CMS classification

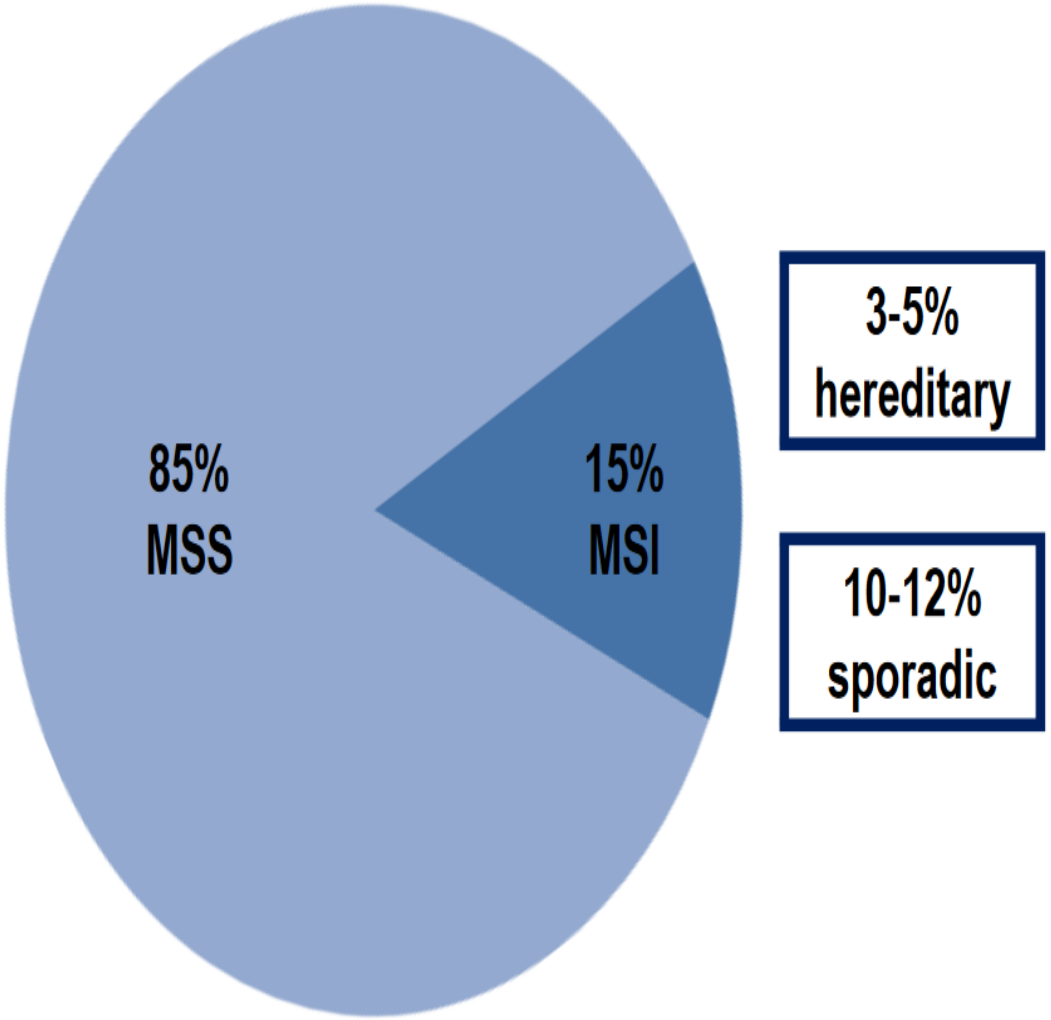


CMS1; CMS2; CMS3; CMS4

ESMO and NCCN Guidelines and Sidedness

RAS/RAF WT	Treatment Recommendations	
Primary Location	ESMO	NCCN
Left	"Cytotoxic doublet plus an EGFR inhibitor is the treatment of choice"	No clear preference for EGFR mAbs or BEV in first-line
Right	Cytotoxic triplet plus BEV or a cytotoxic doublet plus an EGFR antibody	No EGFR mAbs in first-line and potentially not in any line

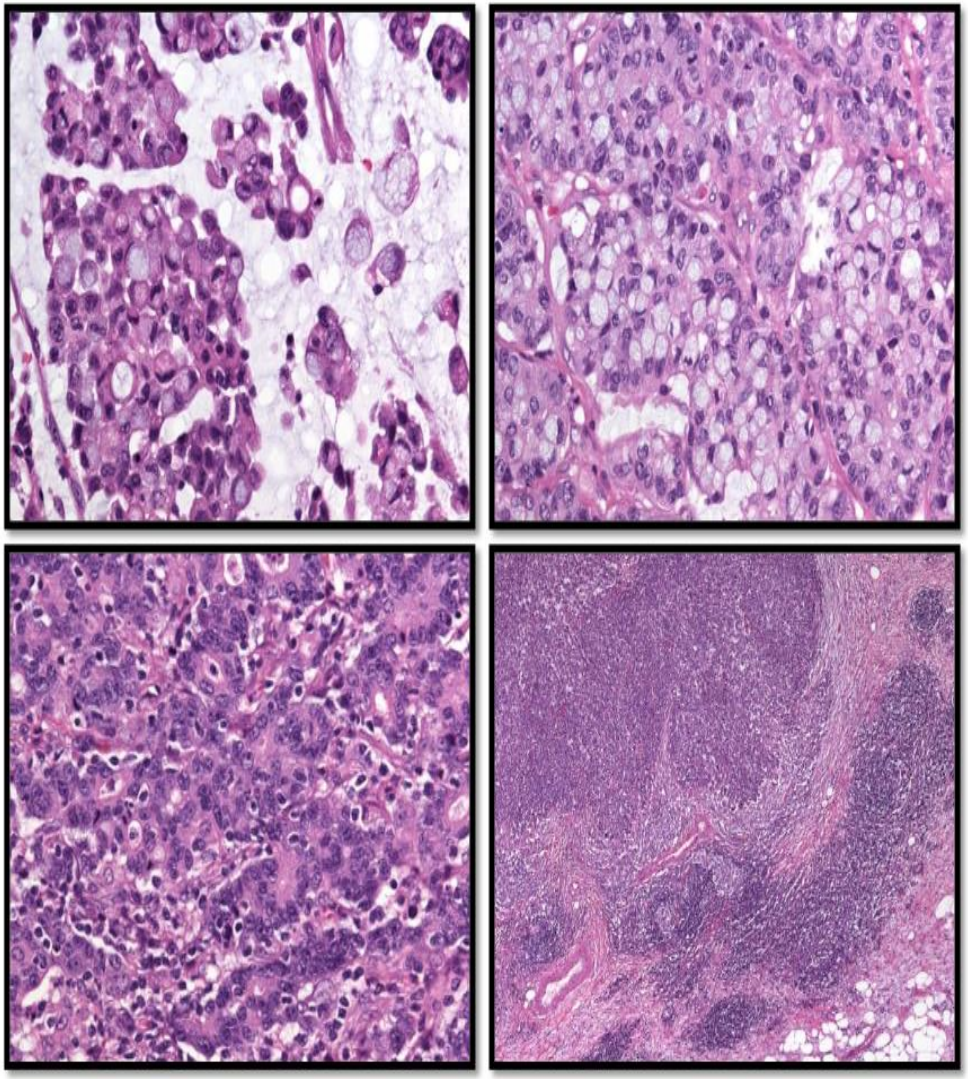
MICROSATELLITE INSTABILITY (MSI)



- Microsatellites are short repetitive sequences (e.g. tandem repeats) of DNA distributed throughout the genome that are commonly shortened (and display length variation, microsatellite instability, MSI) in the setting of deficient mismatch repair (dMMR) protein activity
- The most commonly altered DNA MMR genes are MLH1, MSH2, MSH6 and PMS2, with >90% having alterations in MLH1 and MSH2
- Secondary to dMMR status these tumours develop 100 to 1000s of mutations (→ enhanced neoantigen load) leading to the potential for enhanced immune recognition (→ candidates for immunotherapy)

Setaffy and Langner. Pol J Pathol 2015; De Stefanis et al. Curr Colorectal Cancer Rep 2019

FEATURES THAT RAISE SUSPICION OF AN MSI TUMOUR

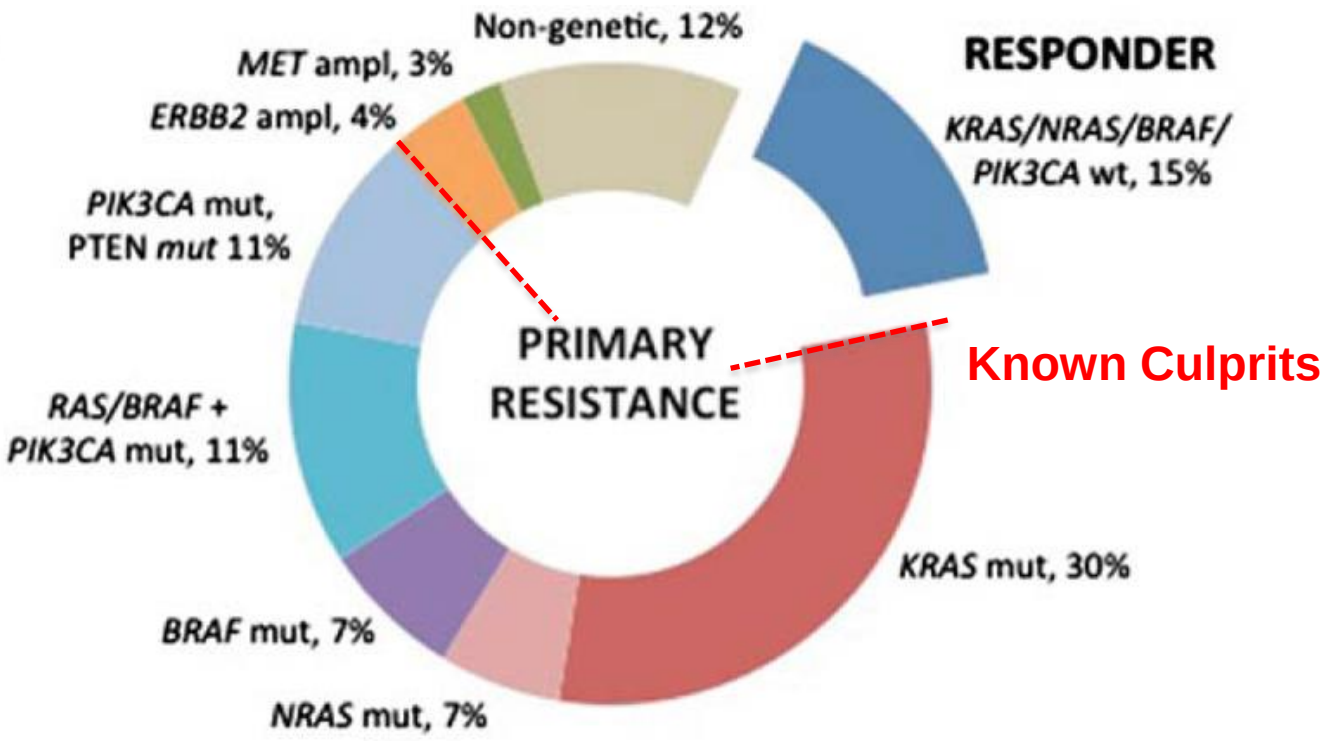


- **Clinical features**
  - Age < 60
  - Right-sided location
  - Multiple (synchronous or metachronous) CRCs
- **MSI-H histology**
  - Type: medullary, mucinous ("any mucin"), signet ring cell ("any signet ring cell")
  - Inflammation: tumour-infiltrating lymphocytes (TILs), peritumoural lymphocytes, lymph follicles ("Crohn-like reaction")
  - Histology: poor differentiation, expansive growth ("pushing border"), heterogeneity, no necrosis

Setaffy and Langner, Pol J Pathol 2015

Genes that, when mutated, drive primary resistance to anti-EGFR antibodies

Nearly 70% have heterogeneous genetic alterations in genes involved in EGFR signaling



ASCO. 2015 Educational Book

CHALLENGES IN cfDNA ANALYSIS

- The absolute levels are low: few nanongrams per ml of plasma
- The circulating cell-free DNA (cfDNA) contains both tumor-derived DNA (ctDNA) and normal DNA originating form dividing cells (blood cells, GI tract, skin)
- The ctDNA is only a fraction (<0.1% to 50%) of the cfDNA
- Levels are usually correlated with tumor burden and are higher in advanced cancer
- Highly fragmented, typically 50-200bp range (165bp peak)
- Short half-life (2 hours)

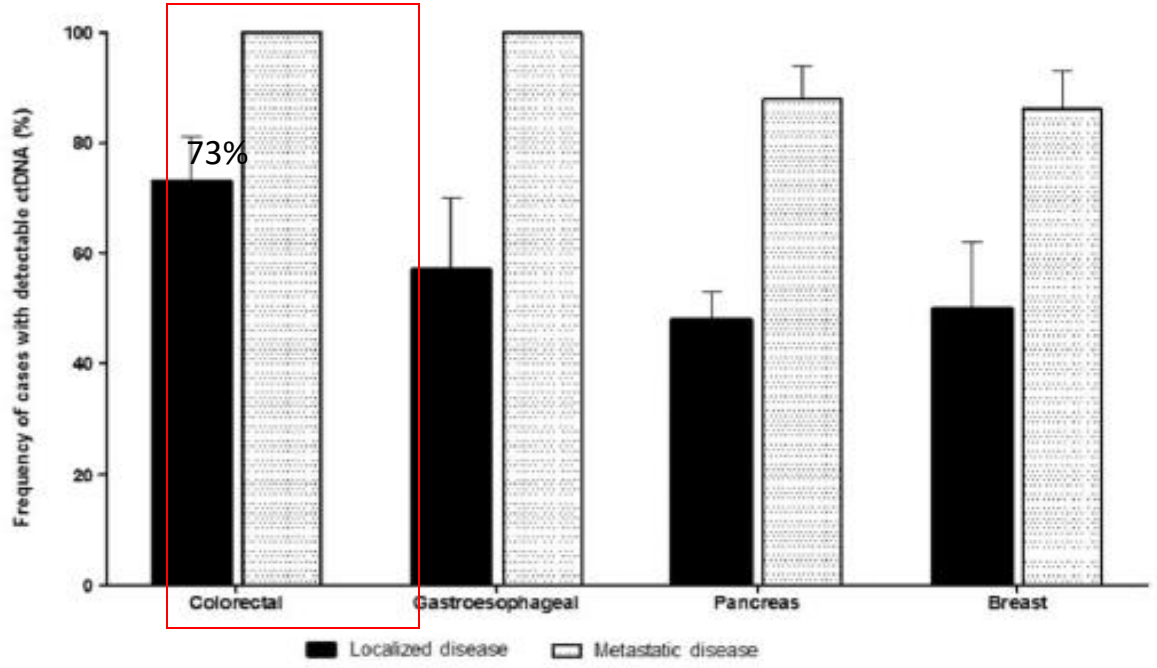
NGS assays: liquid biopsy

Test name*	FoundationOne® Liquid/bTMB	Guardant360/ GuardantOMNI™	MutatorDETECT	Unnamed	PredicineATLAS	Oncomine Pan-Cancer Cell-Free Assay	AVENIO ctDNA Kits
No. of genes measured	70/394	73/500+	64	508	600	52	17/77/197
Sequencing platform	Illumina HiSeq 4000	Guardant Health Digital Seq Platform	Illumina NGS‡	Illumina NGS	Not reported	Ion GeneStudio S5 series	Illumina NextSeq
Types of alterations	SNVs	SNVs, indels, fusions, CNAs	SNVs, indels, fusions, CNVs	SNVs, indels, CNVs	SNV, CNV, rearrangements§	SNVs, indels, fusions, CNVs	SNVs, indels, fusions, CNVs
Sample requirement	(20 ng cfDNA)	1–2 mL plasma (5–30 ng cfDNA)	Two 10 mL tubes of peripheral whole blood or 6–10 mL plasma‡	Plasma (single blood draw)	Plasma (5 mL)§	20 ng cfNA	10-50 ng cfDNA

APPLICATIONS OF cfDNA ANALYSIS IN THE MANAGEMENT OF COLORECTAL CARCINOMA

- Early diagnosis of cancer
- Detection of minimal residual disease
- Molecular profiling (identification of prognostic and predictive markers)
- Monitoring response to therapy and clonal evolution

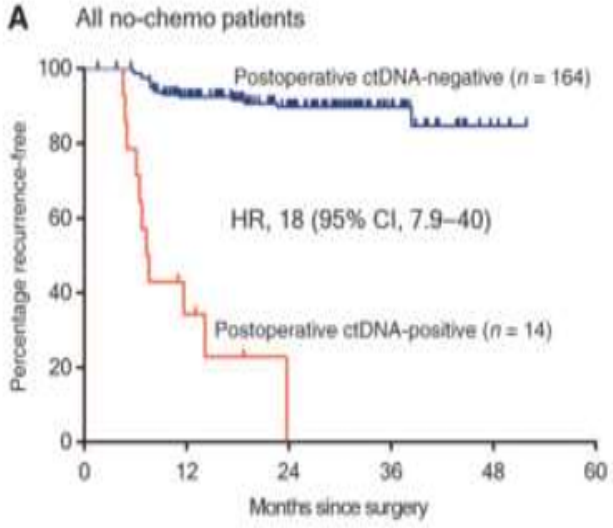
Circulating tumor DNA (ctDNA) to detect tumors in 640 patients with various cancer types



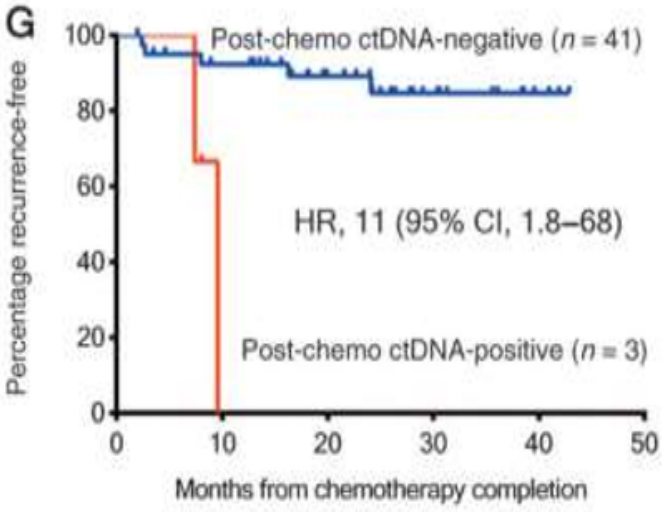
Fraction of patients with detectable ctDNA in localized (stages I to III) and metastatic colorectal, gastroesophageal, pancreatic, and breast cancers

Sci Transl Med. 2014 February 19; 6(224): 224ra24

ctDNA ANALYSIS PREDICTS RECURRENCE IN PATIENTS WITH STAGE II COLON CANCER



HR 28, CI 11-68, p<0.001 at multivariate analysis



HR 14, CI 6.8-28, p<0.001 at multivariate analysis

OncoBEAM RAS CRC ASSAY

OncoBEAM RAS CRC is the first liquid biopsy test to achieve CE-Mark status for RAS testing in metastatic colorectal cancer

Expanded RAS mutation analysis using BEAMing has been validated in anti-EGFR therapy clinical trials

- Over 1,200 patients have been tested using the BEAMing platform in the OPUS, CRYSTAL, and CALGN/SWOG 80405 clinical trials
- Expanded RAS testing was shown to improve the identification of mCRC patients eligible for anti-EGFR therapy

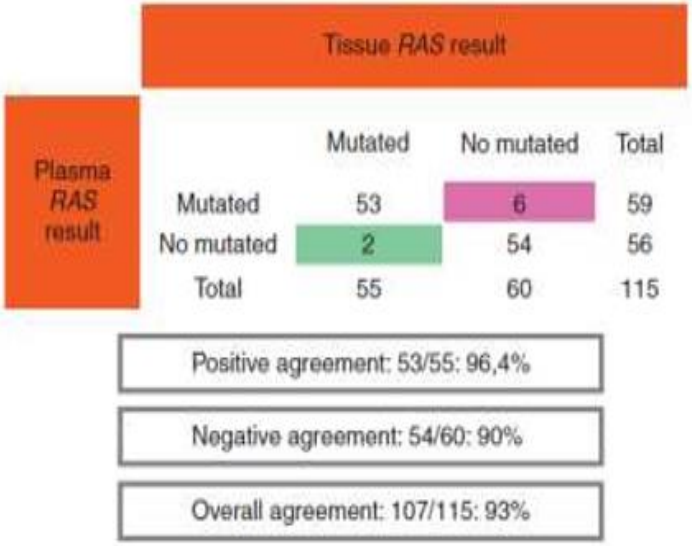
- Mutations Tested by OncoBEAM RAS CRC Kit:

Gene:	Exon 2	Exon 3	Exon 4
KRAS	G12S, G12R, G12C, G12D, G12A, G12V, G13D	A59T, Q61L, Q61R, Q61H, Q61H	K117N, K117N, A146T, A146V
NRAS	G12S, G12R, G12C, G12D, G12A, G12V, G13R, G13D, G13V	A59T, Q61K, Q61R, Q61L, Q61H, Q61H	K117N, K117N, A146T

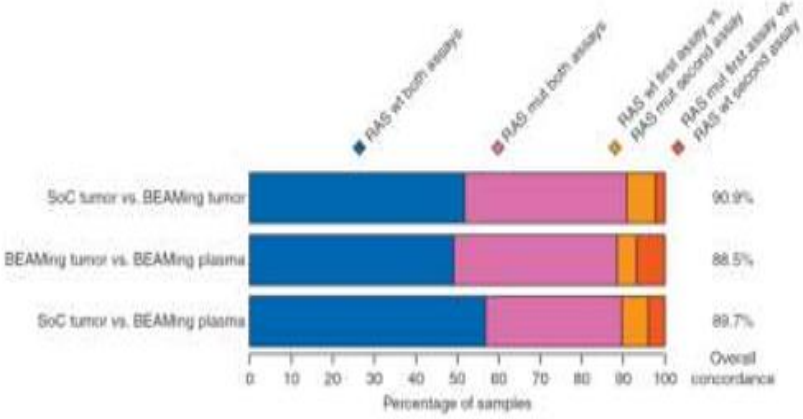
CONCORDANCE OF PLASMA AND TISSUE RAS MUTATION RESULTS

	Tumor tissue RAS result						
	RAS	Mutant	WT	Total	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
Plasma ctDNA RAS result	Mutant	47	3	50	100 × 47/52 = 90.4% (79%, 96%)	100 × 43/46 = 93.5% (82%, 98%)	100 × 90/98 = 91.8% (85%, 96%)
	WT	5	43	48			
	Total	52	46	98			

Schmiegel Mol Oncol 2017



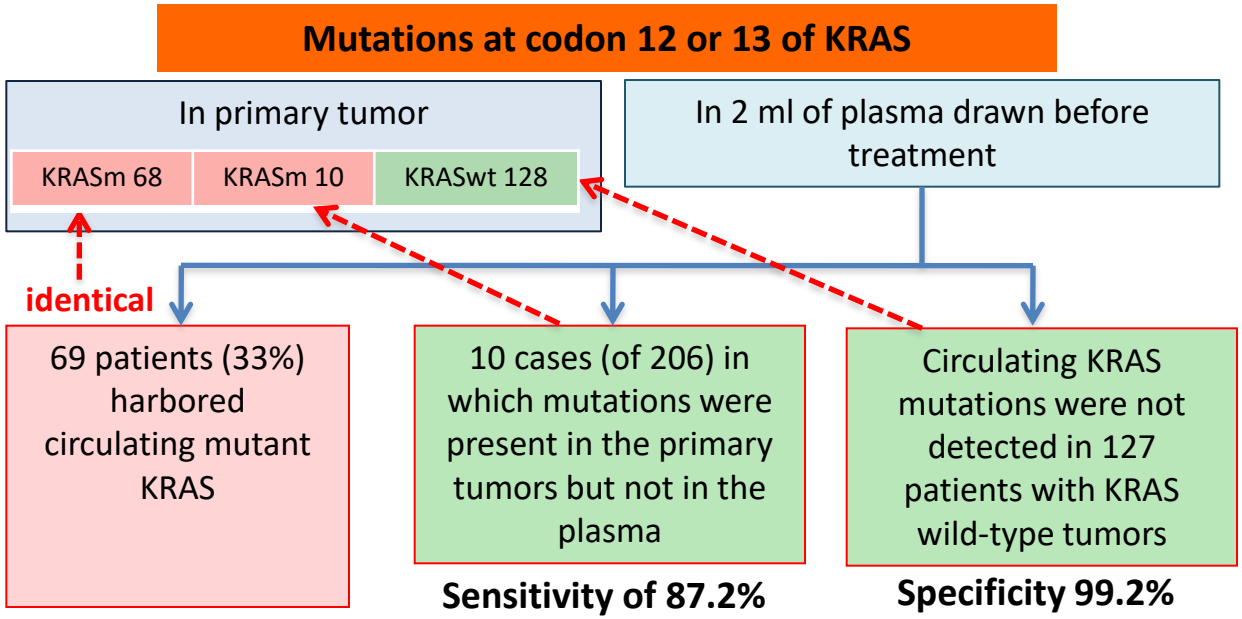
Vidal Ann Oncol 2017



Grasselli Ann Oncol 2017

The sensitivity of the liquid biopsy

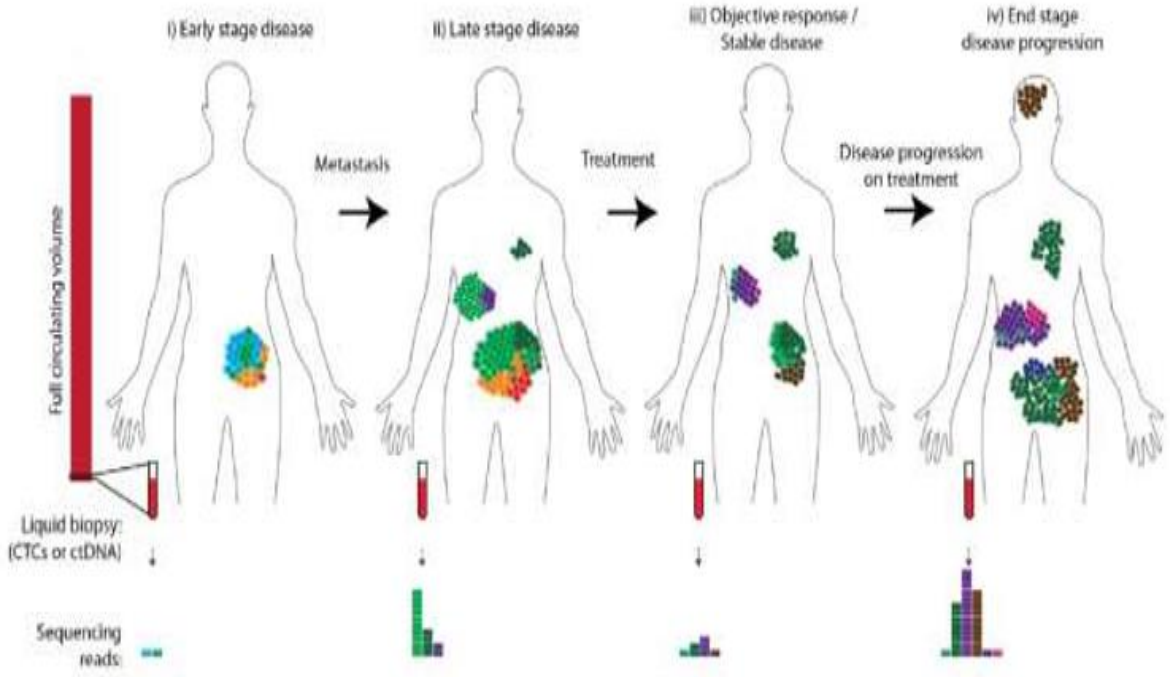
206 patients with metastatic CRC in a blinded fashion



Concordance between KRAS mutation status in the plasma and tumor tissue 95%
Agreement highly significant (κ statistic = 0.88, $P < 0.0001$)

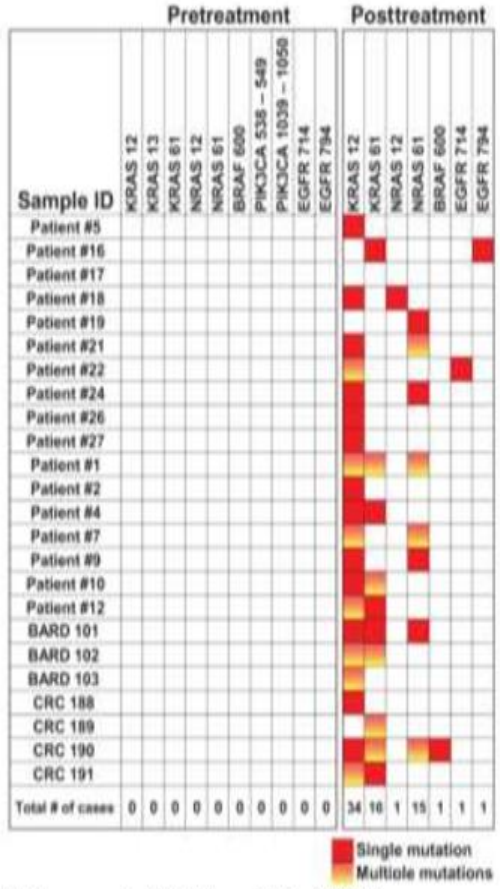
Sci Transl Med. 2014 February 19; 6(224): 224ra24

LIQUID BIOPSY CAN REPRESENT TEMPORAL AND SPATIAL HETEROGENEITY IN CANCER PROGRESSION



Burrell & Swanton Mol Oncol 2014

DETECTION OF DIFFERENT MECHANISMS OF RESISTANCE TO ANTI-EGFR MoAbs IN PLASMA OF CRC PATIENTS



Bettegowda Sci Transl Med 2014

Table 1 Identification of genetic alterations associated with resistance to anti-EGFR antibodies in plasma samples

Patient ID	Therapy	Resistance	Plausible genetic mechanism	Oncogenic alteration in COSMIC database
MOLI-CRC02	Cetux + Irino	Primary	*NRAS* p.Q61L	YES
ONCGH-CRC01	Cetux + Irino	Primary	*ERBB2* amplification*	YES
MOLI-CRC16	Cetux + Folfiri	Primary	*FLT3* amplification*	YES
MOLI-CRC07	Cetux + Folfiri	Primary	N.I.	-
ONCGH-CRC11	Cetux + Folfiri	Primary	*ERBB2* amplification*	YES
MOLI-CRC06	Panit	Primary	*NRAS* p.G12D	YES
MOLI-CRC15	Panit + Folfox4	Primary	*ERBB2* amplification*	YES
ONCG-CRC13	Panit	Primary	*MAP2K1* p.K57N*	YES
ONCG-CRC41	Panit	Primary	N.I.	-
ONCGH-CRC06	Cetux + Irino	Primary	*ERBB2* amplification*	YES
			FLT3 amplification*	
ONCG-CRC57	Panit	Acquired	*MET* amplification*	YES
ONCG-CRC57	Panit	Acquired	*KRAS* p.G12A	YES
			KRAS p.G12D	
			KRAS p.G13D	
AOJP-CRC04	Panit + Folfoxiri	Acquired	*KRAS* p.Q61H	YES
MOLI-CRC04	Cetux + Folfiri	Acquired	*KRAS* p.Q61H	YES
AOJP-CRC05	Panit + Folfoxiri	Acquired	*KRAS* p.G12D	YES
ONCG-CRC59	Cetux, then Panit	Acquired	*KRAS* p.G12Y	YES
			KRAS p.G13D	
AOJP-CRC01	Cetux + Folfoxiri	Acquired	*KRAS* p.Q61L	YES
MGH-CRC02	Cetux	Acquired	*KRAS* amplification	YES
AOJP-CRC06	Cetux + Folfoxiri	Acquired	*KRAS* p.Q61L	YES
AOJP-CRC03	Panit + Folfoxiri	Acquired	*KRAS* p.Q61L	YES
AOJP-CRC02	Panit + Folfoxiri	Acquired	*KRAS* p.Q61H	YES
ONCG-CRC70	Panit + Irino	Acquired	*KRAS* p.Q61H	YES
			EGFR p.S464L	
			EGFR p.G465R	
ONCG-CRC71	Panit	Acquired	*KRAS* p.Q61H	YES
ONCG-CRC72	Panit	Acquired	*MET* amplification*	YES
			EGFR p.G465R	
			EGFR p.G465E	
MOLI-CRC12	Cetux + Folfox4	Acquired	N.I.	-
ONCG-CRC73	Panit	Acquired	*MET* amplification*	YES

Siravegna Nat Med 2015

CONCLUSIONS 1

•*RAS* mutational status is a negative predictive biomarker for therapeutic choices involving EGFR antibody therapies in the metastatic disease setting [I, A].

- *RAS* testing should be carried out on all patients at the time of diagnosis of mCRC [I, A].

•*RAS* testing is mandatory before treatment with the EGFR-targeted monoclonal antibodies cetuximab and panitumumab [I, A].

•Primary or metastatic colorectal tumour tissue can be used for *RAS* testing *RAS* analysis should include at least *KRAS* exons 2, 3 and 4 (codons 12, 13, 59, 61, 117 and 146) and *NRAS* exons 2, 3 and 4 (codons 12, 13, 59, 61 and 117).

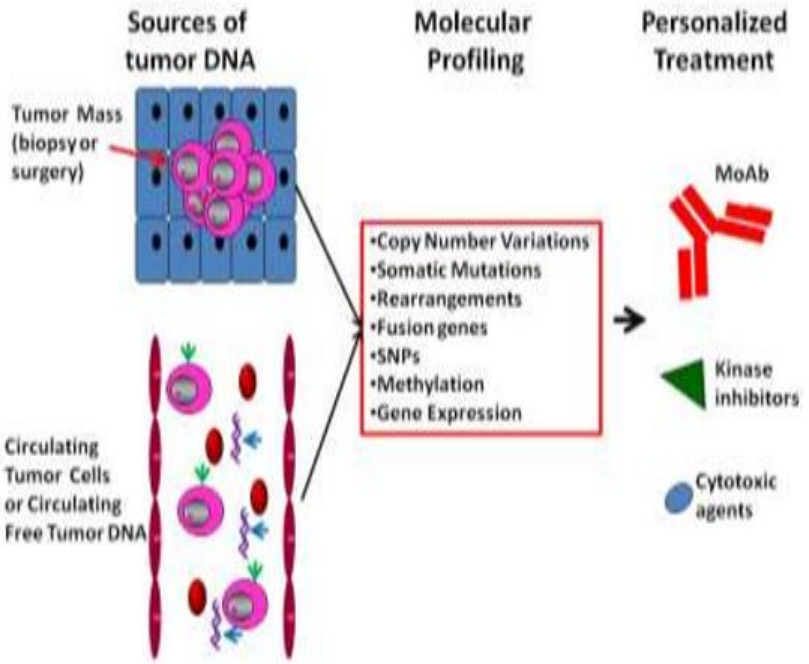
•Laboratories providing *RAS* testing of colorectal tumours should demonstrate their successful participation in a relevant external quality assessment scheme, and be appropriately accredited.

CONCLUSIONS 1

- BRAF mutation as a prognostic biomarker of poor prognosis
- Emerging biomarkers(PI3K,HER2,) not recommended for routine patient management outside of a clinical trial setting
- prevalence of MSI and *BRAF* mutations in the tumours of patients with mCRC is low.
- MSI testing has strong predictive value for the use of immune check-point inhibitors in the treatment of patients with mCRC

CONCLUSIONS

- Highly sensitive methods are required for the detection of biomarkers in the cfDNA
- NGS-based techniques can provide a comprehensive molecular portrait of the tumor starting from cfDNA
- Liquid biopsy might have a relevant role in the management of early and advanced CRC patients
- Analysis of cfDNA reveals high levels of tumor heterogeneity and clinical trials are needed to translate this information in therapeutic strategies
- The quality of liquid biopsy testing in the real life must be adequately monitored through EQA programs



Normanno J Cell Biochem 2013