PROFILI MOLECOLARI E TRATTAMENTO

Associazione Italiana di Oncologia Medica

Modena, 23 Novembre 2018

Dr Fabio Gelsomino

Outline

- **Current biomarkers**
  - **RAS**
  - **BRAF**
  - **MSI**
- **Emergent biomarkers**
- **Monitoring the clonal evolution of CRC**

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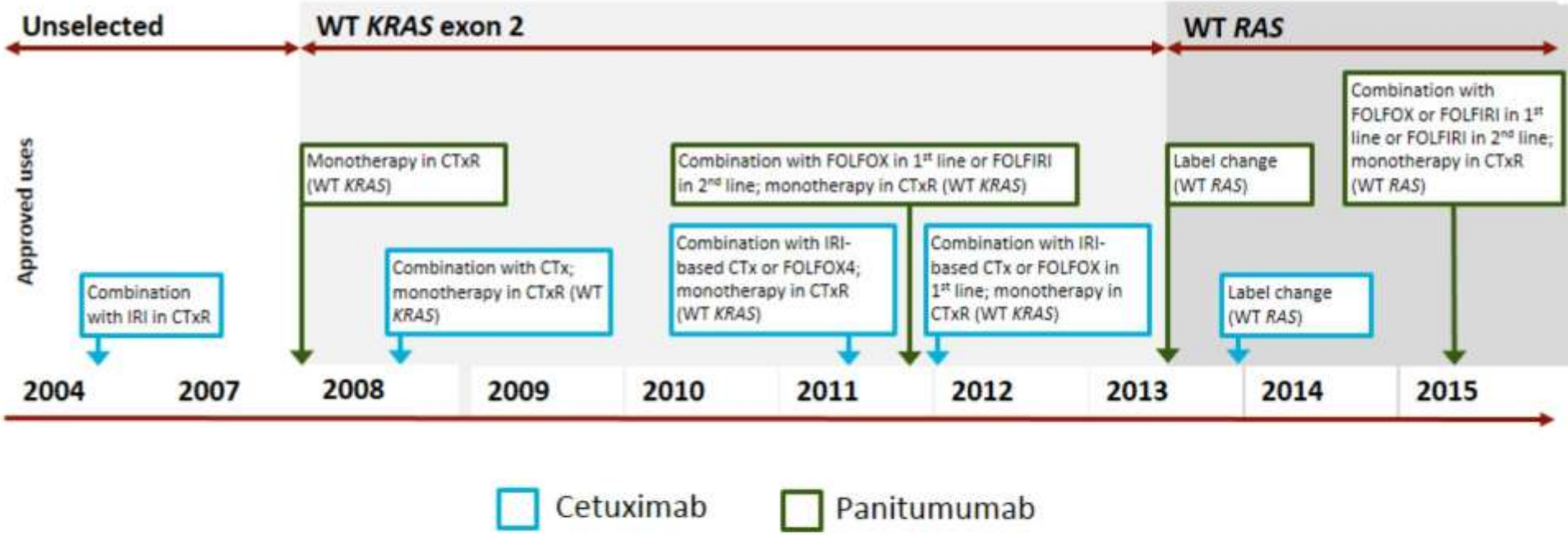
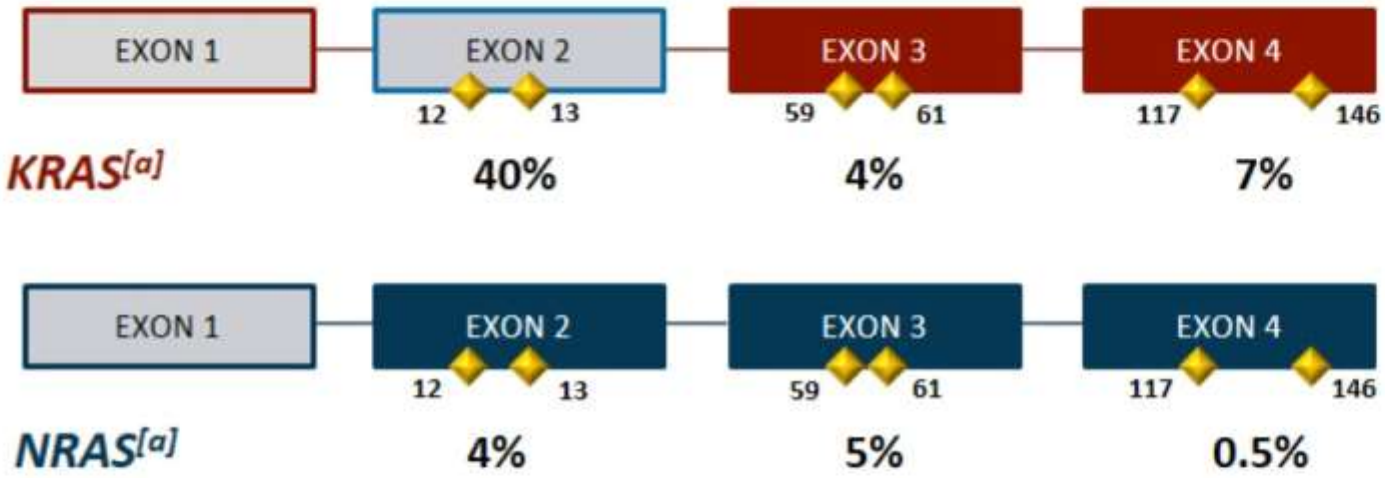
RAS Testing and Use of EGFR Antibody Therapy



All → ***KRAS*** → ***RAS***

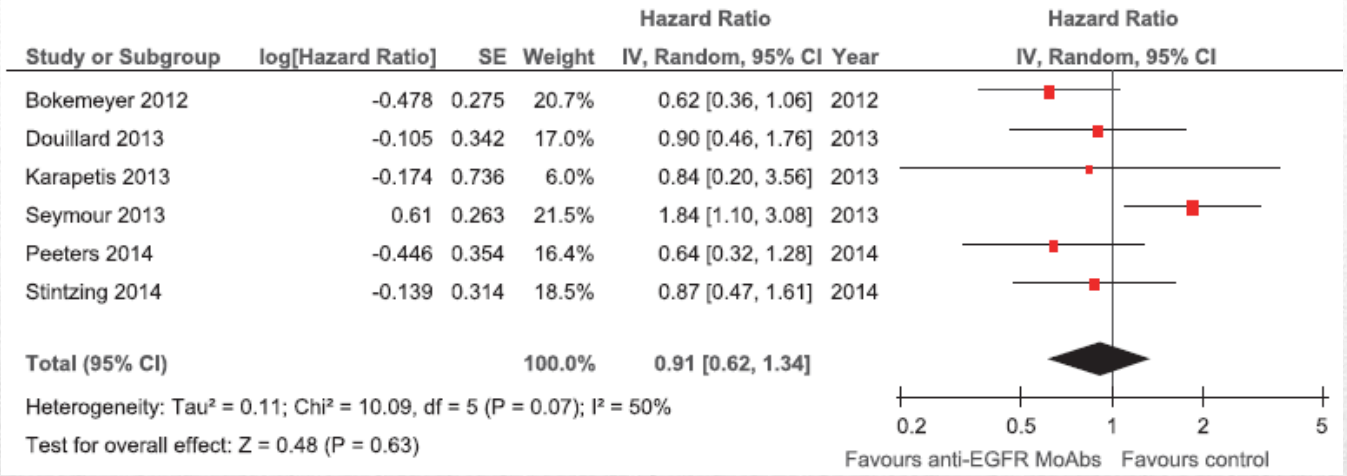
KRAS and *NRAS* Mutation Hotspots



- 17% of WT *KRAS* exon 2 tumors have mutations in other *RAS* exons[b]

a. Sorich MJ, et al. *Ann Oncol*. 2015;26:13-21.
b. Douillard JY, et al. *N Engl J Med*. 2013;369:1023-1034.

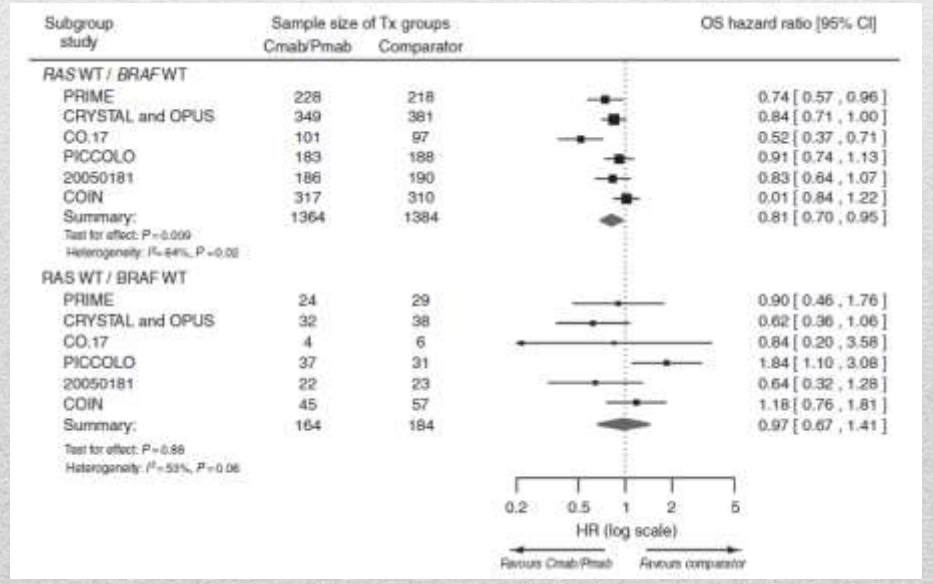
Current Biomarkers: BRAF



Study or Subgroup	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI	Year
Bokemeyer 2012	-0.478	0.275	20.7%	0.62 [0.36, 1.06]	2012
Douillard 2013	-0.105	0.342	17.0%	0.90 [0.46, 1.76]	2013
Karapetis 2013	-0.174	0.736	6.0%	0.84 [0.20, 3.56]	2013
Seymour 2013	0.61	0.263	21.5%	1.84 [1.10, 3.08]	2013
Peeters 2014	-0.446	0.354	16.4%	0.64 [0.32, 1.28]	2014
Stintzing 2014	-0.139	0.314	18.5%	0.87 [0.47, 1.61]	2014
Total (95% CI)			**100.0%**	**0.91 [0.62, 1.34]**	

Heterogeneity: Tau² = 0.11; Chi² = 10.09, df = 5 (P = 0.07); I² = 50%

Test for overall effect: Z = 0.48 (P = 0.63)



Subgroup study	Sample size of Tx groups Cmab/Pmab	Comparator	OS hazard ratio [95% CI]
RAS WT / *BRAF* WT			
PRIME	228	218	0.74 [0.57 , 0.96]
CRYSTAL and OPUS	349	381	0.84 [0.71 , 1.00]
CO.17	101	97	0.52 [0.37 , 0.71]
PICCOLO	183	188	0.91 [0.74 , 1.13]
20050181	186	190	0.83 [0.64 , 1.07]
COIN	317	310	0.01 [0.84 , 1.22]
Summary:	1364	1384	0.81 [0.70 , 0.95]
Test for effect: P = 0.009			
Heterogeneity: I^2 = 64%, P = 0.02			
RAS WT / BRAF WT			
PRIME	24	29	0.90 [0.46 , 1.76]
CRYSTAL and OPUS	32	38	0.62 [0.36 , 1.06]
CO.17	4	6	0.84 [0.20 , 3.58]
PICCOLO	37	31	1.84 [1.10 , 3.08]
20050181	22	23	0.64 [0.32 , 1.28]
COIN	45	57	1.18 [0.76 , 1.81]
Summary:	164	184	0.97 [0.67 , 1.41]
Test for effect: P = 0.88			
Heterogeneity: I^2 = 53%, P = 0.06			

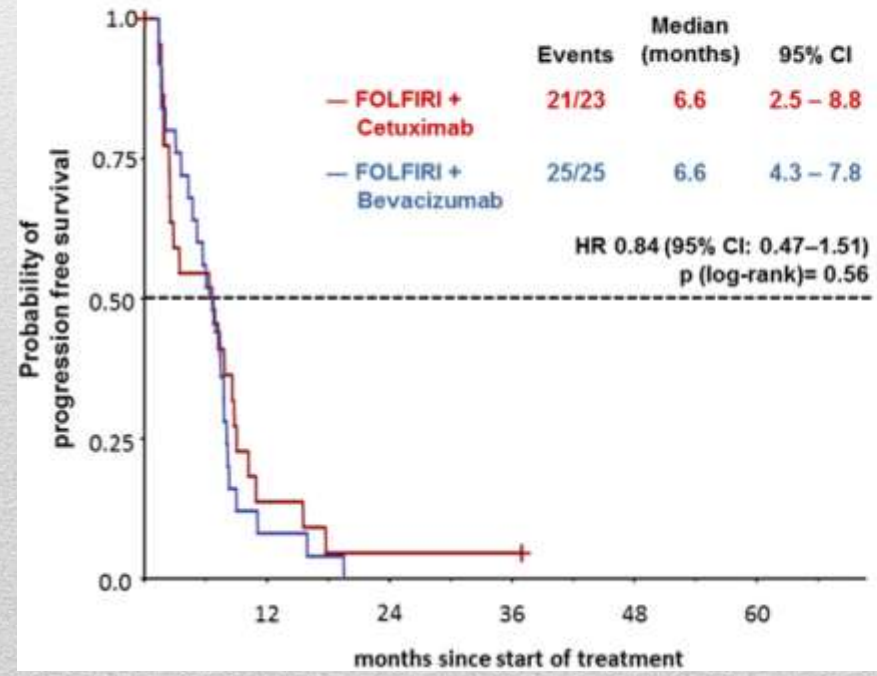
Pietrantonio F et al, 2015

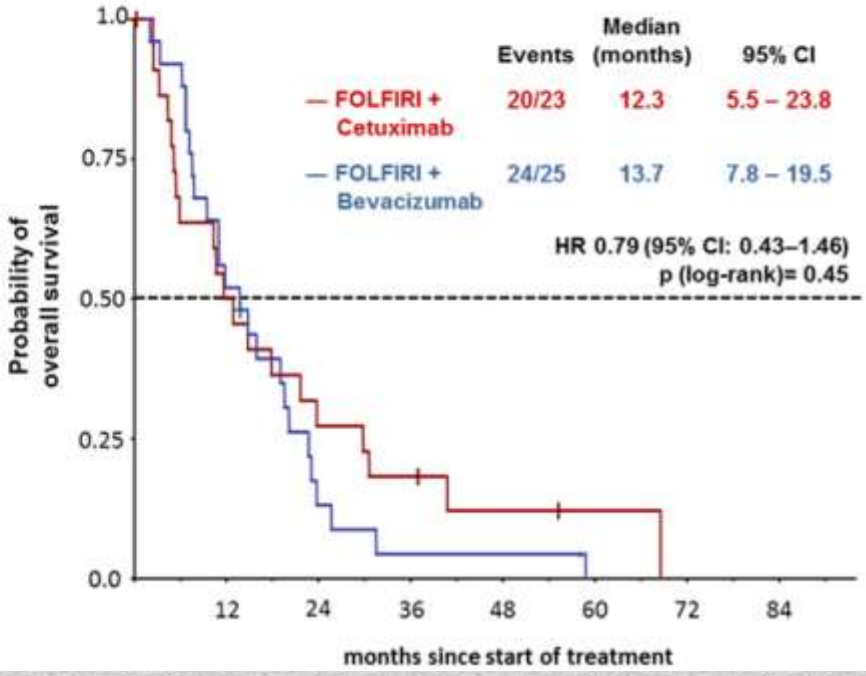
Rowland A et al, 2016

Current Biomarkers: BRAF

Impact of BRAF and RAS mutations on first-line efficacy of FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab: analysis of the FIRE-3 (AIO KRK-0306) study

(B) BRAF mutant population

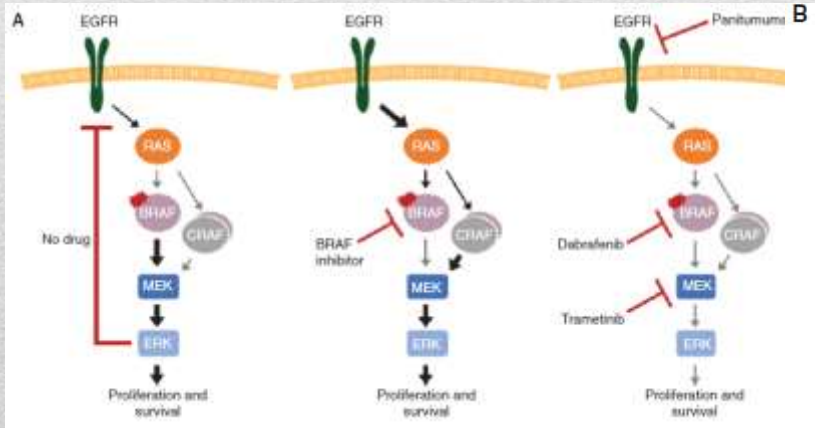




Stintzing S et al, 2017

Current Biomarkers: BRAF

Combined BRAF, EGFR, and MEK Inhibition in Patients with *BRAF*^{V600E}-Mutant Colorectal Cancer



B

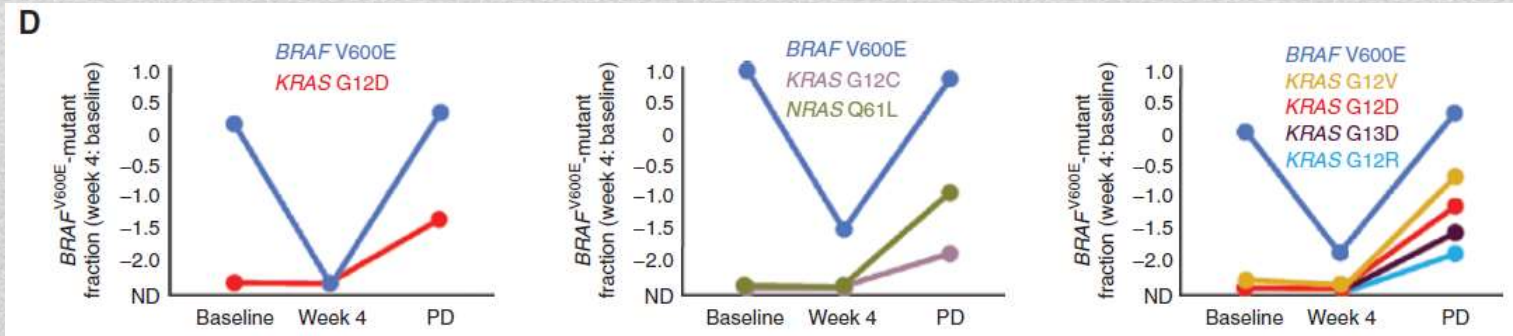
Dabrafenib + panitumumab (D + P) *n* = 20	D: 150 mg b.i.d. P: 6 mg/kg Q2W *n* = 20			
Dabrafenib + trametinib + panitumumab (D + T + P) *n* = 91	D: 150 mg b.i.d. T: 1.5 mg q.d. P: 4.8 mg/kg Q2W *n* = 3	D: 150 mg b.i.d. T: 1.5 mg q.d. P: 6 mg/kg Q2W *n* = 4	D: 150 mg b.i.d. T: 2 mg q.d. P: 6 mg/kg Q2W *n* = 48	D: 150 mg b.i.d. T: 2 mg q.d. P: 4.8 mg/kg Q2W *n* = 36
Trametinib + panitumumab (T + P) *n* = 31	T: 2 mg q.d. P: 6 mg/kg Q2W *n* = 11	T: 1.5 mg q.d. P: 6 mg/kg Q2W *n* = 10	T: 2 mg q.d. P: 4.8 mg/kg Q2W *n* = 10	

Corcoran R.B et al, 2018

Current Biomarkers: BRAF

Table 3. Summary of efficacy by treatment cohort (investigator review)

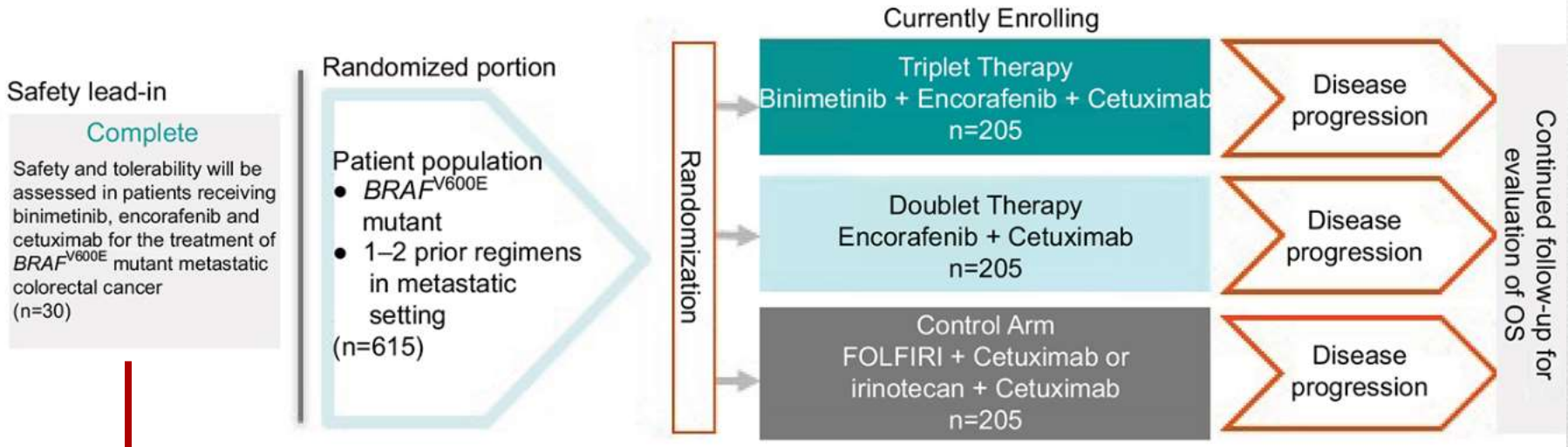
Assessment	D+T+P (*n* = 91)	T+P (*n* = 31)	D+P (*n* = 20)	D+T (*n* = 43)[a]
Best confirmed response, *n* (%)				
CR	1 (1)	0	1 (5)	1 (2)
PR	18 (20)	0	1 (5)	2 (5)
SD	59 (65)	17 (55)	16 (80)	24 (56)
PD	8 (9)	12 (39)	2 (10)	10 (23)
NE	5 (5)	2 (6)	0	6 (14)
ORR (CR + PR), *n* (%) (95% CI)	19 (21) (13.1–30.7)	0 (0–11.2)	2 (10) (1.2–31.7)	3 (7)
DOR (95% CI), months	7.6 (2.9–NR)	0	6.9 (5.9–8.0)	–
DCR (CR + PR + SD), %	86	55	90	68
Median PFS, months	4.2	2.6	3.5	3.5
Unconfirmed CR + PR, *n* (%)	29 (32)	1 (3)	3 (15)	5 (12)



Corcoran R.B et al, 2018

Current Biomarkers: BRAF

Figure 2. Schematic representation of the Phase III study of encorafenib + cetuximab plus or minus binimetinib vs irinotecan/cetuximab or infusional 5-FU/FA/irinotecan (FOLFIRI)/cetuximab with a safety lead-in of encorafenib + binimetinib + cetuximab in patients with BRAFV600E-mutant metastatic CRC (BEACON CRC).



Abbreviations: CRC, colorectal cancer; FA, folinic acid; 5-FU, 5-fluorouracil; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan.

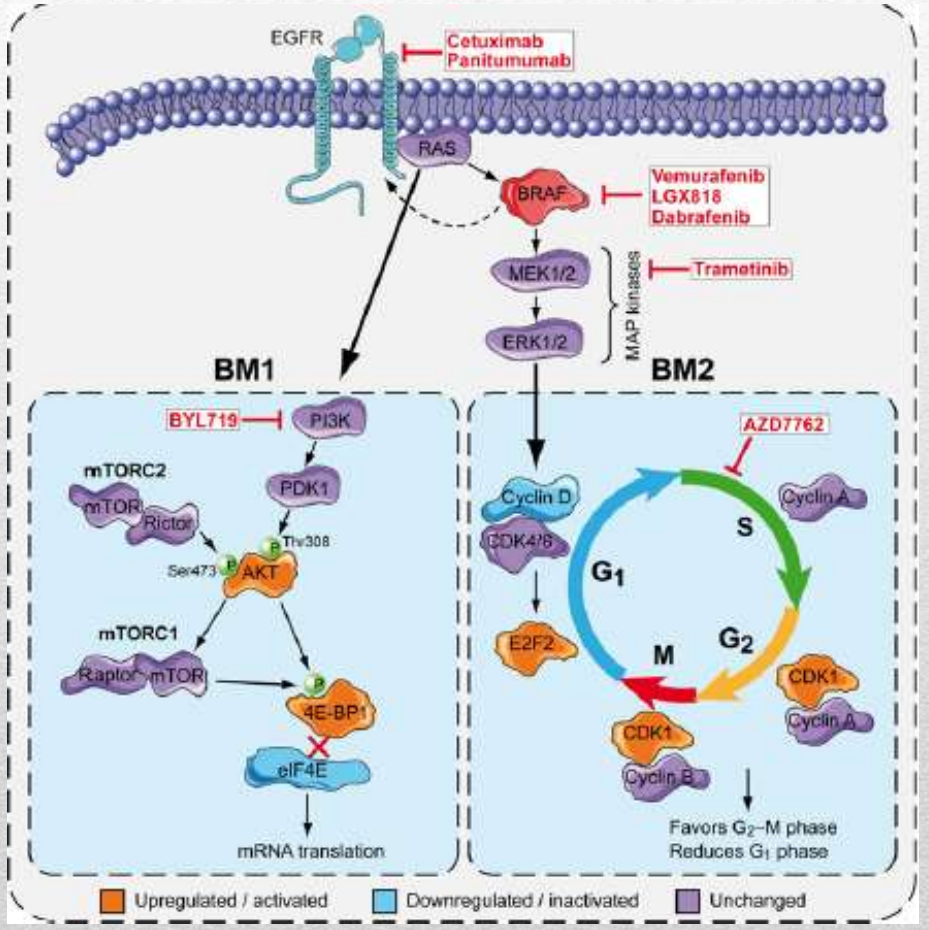
Confirmed ORR 41%

Van Cutsem E et al, 2018

Current Biomarkers: BRAF

Clinical Cancer Research

BRAF V600E **Mutant Colorectal Cancer Subtypes Based on Gene Expression**



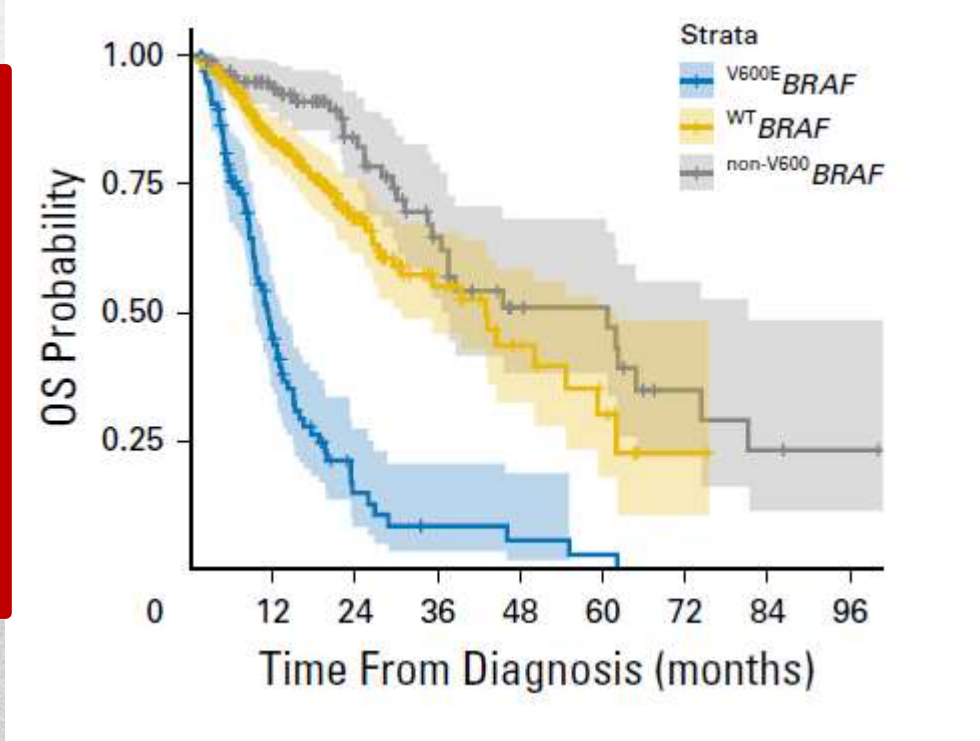
Barras D et al, 2017

Current Biomarkers: BRAF

Non-V600 *BRAF* Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer

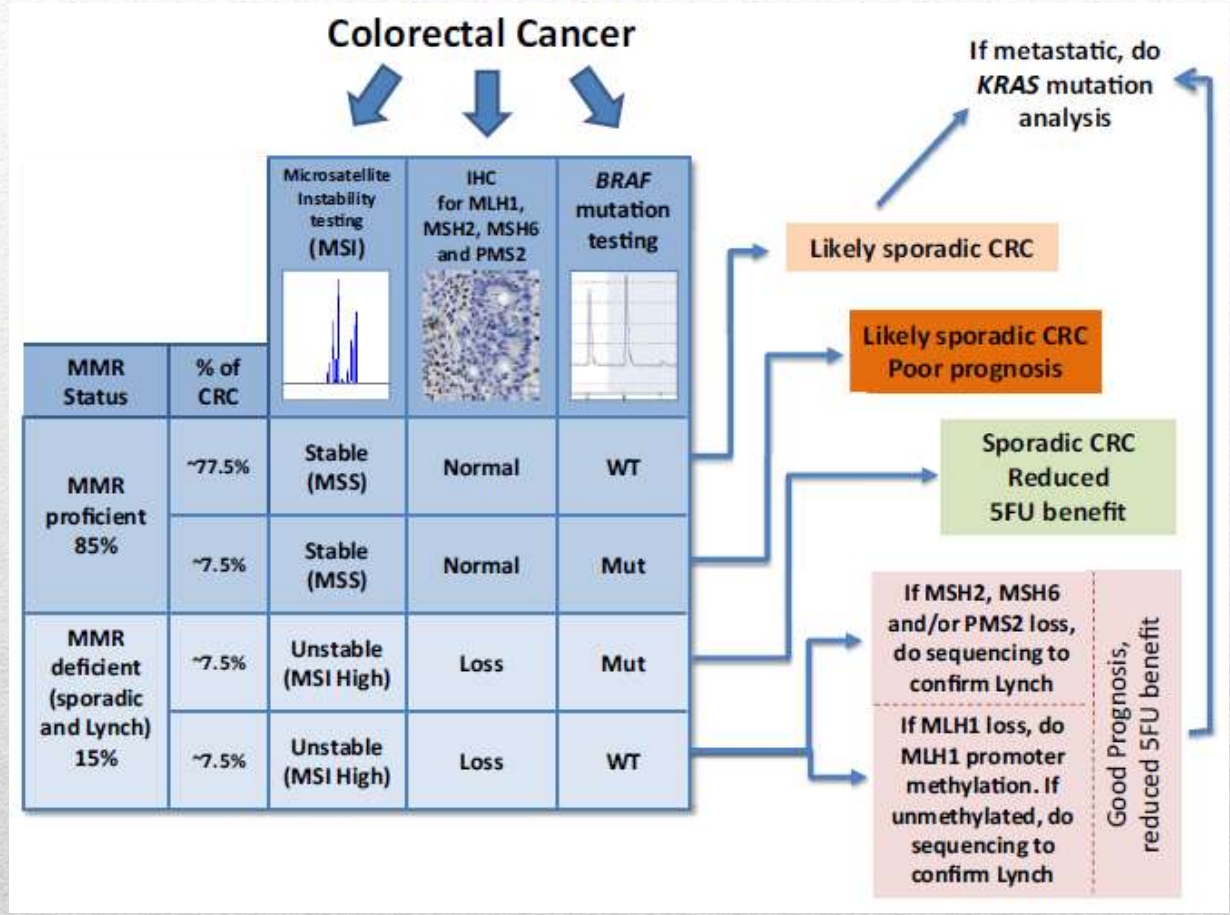
- 2,2% of pts
- 22% of all BRAF mutations
- Compared to BRAF V600:

- Younger
- M > F
- Left-sided
- MSS
- > OS
- possible coexisting RAS mutations



Jones JC et al, 2017

Current Biomarkers: MSI



MMR Status	% of CRC	Microsatellite Instability testing (MSI)	IHC for MLH1, MSH2, MSH6 and PMS2	*BRAF* mutation testing
MMR proficient 85%	~77.5%	Stable (MSS)	Normal	WT
	~7.5%	Stable (MSS)	Normal	Mut
MMR deficient (sporadic and Lynch) 15%	~7.5%	Unstable (MSI High)	Loss	Mut
	~7.5%	Unstable (MSI High)	Loss	WT

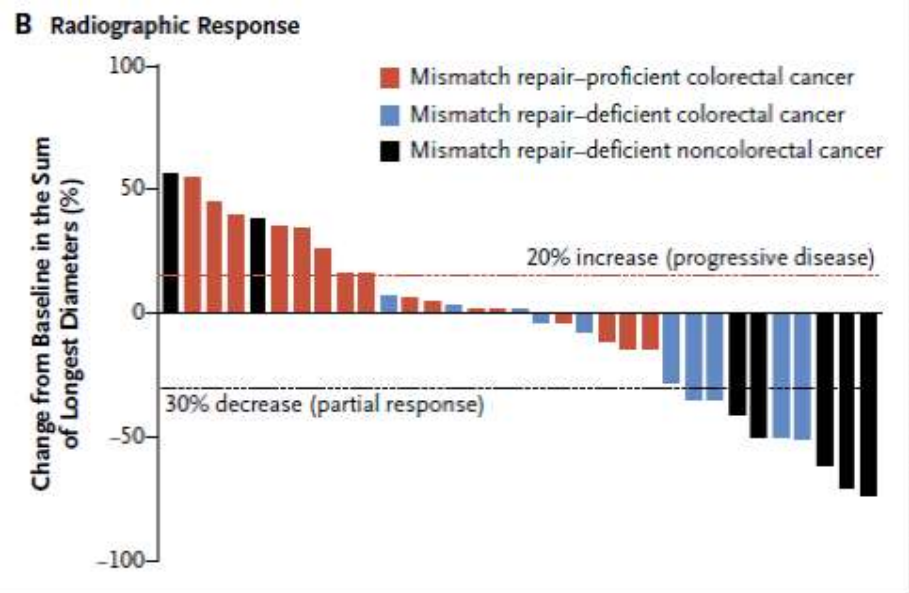
15% all stages → 4% in stage IV

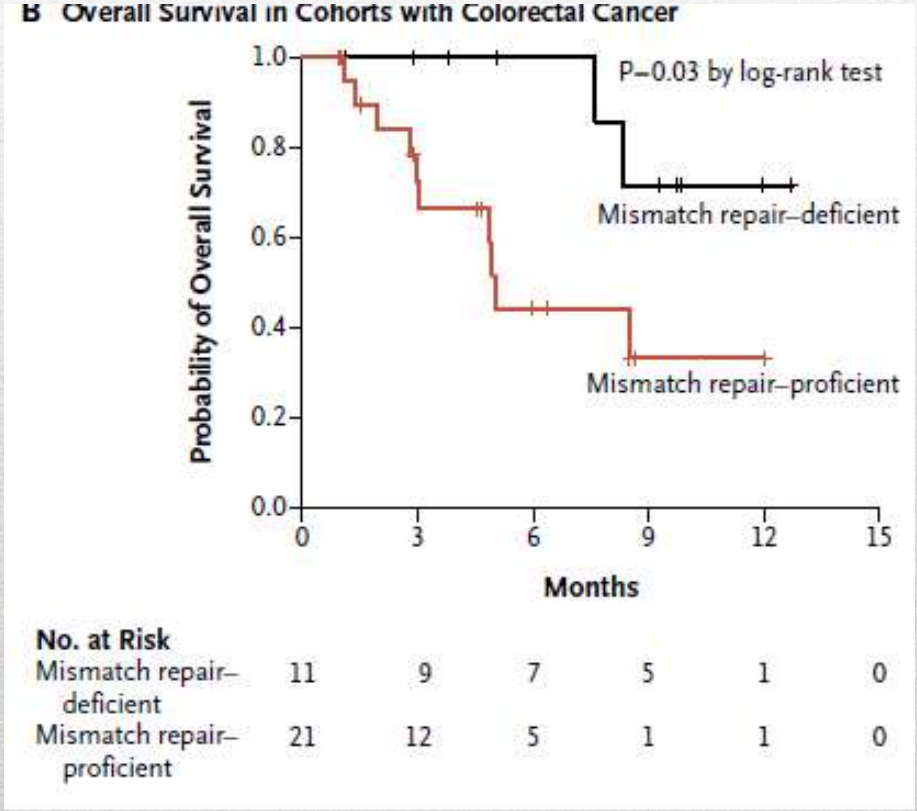
Funkhouser et al, 2012

Current Biomarkers: MSI

The NEW ENGLAND JOURNAL of MEDICINE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

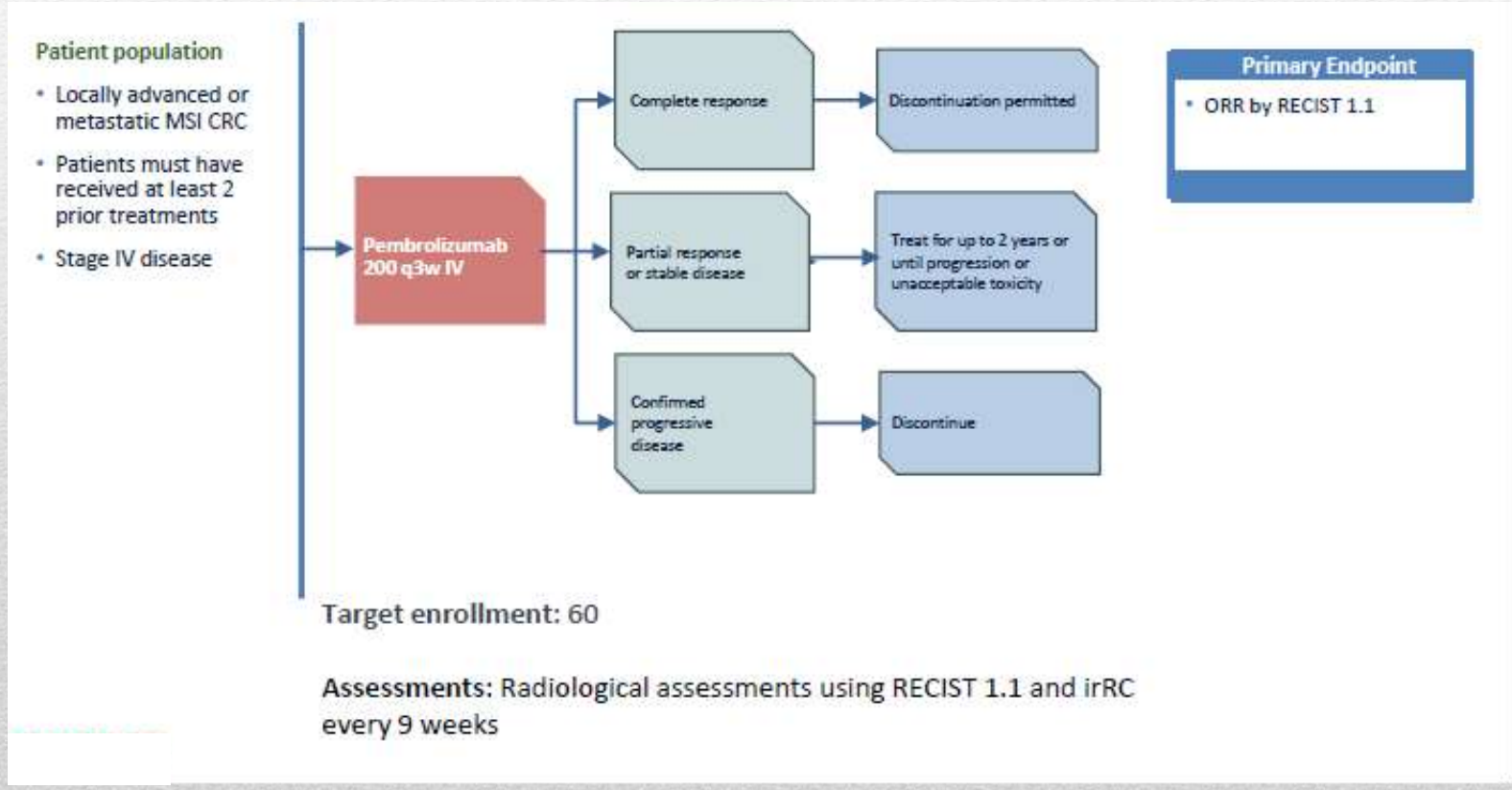




Le DT et al, 2015

Current Biomarkers: MSI

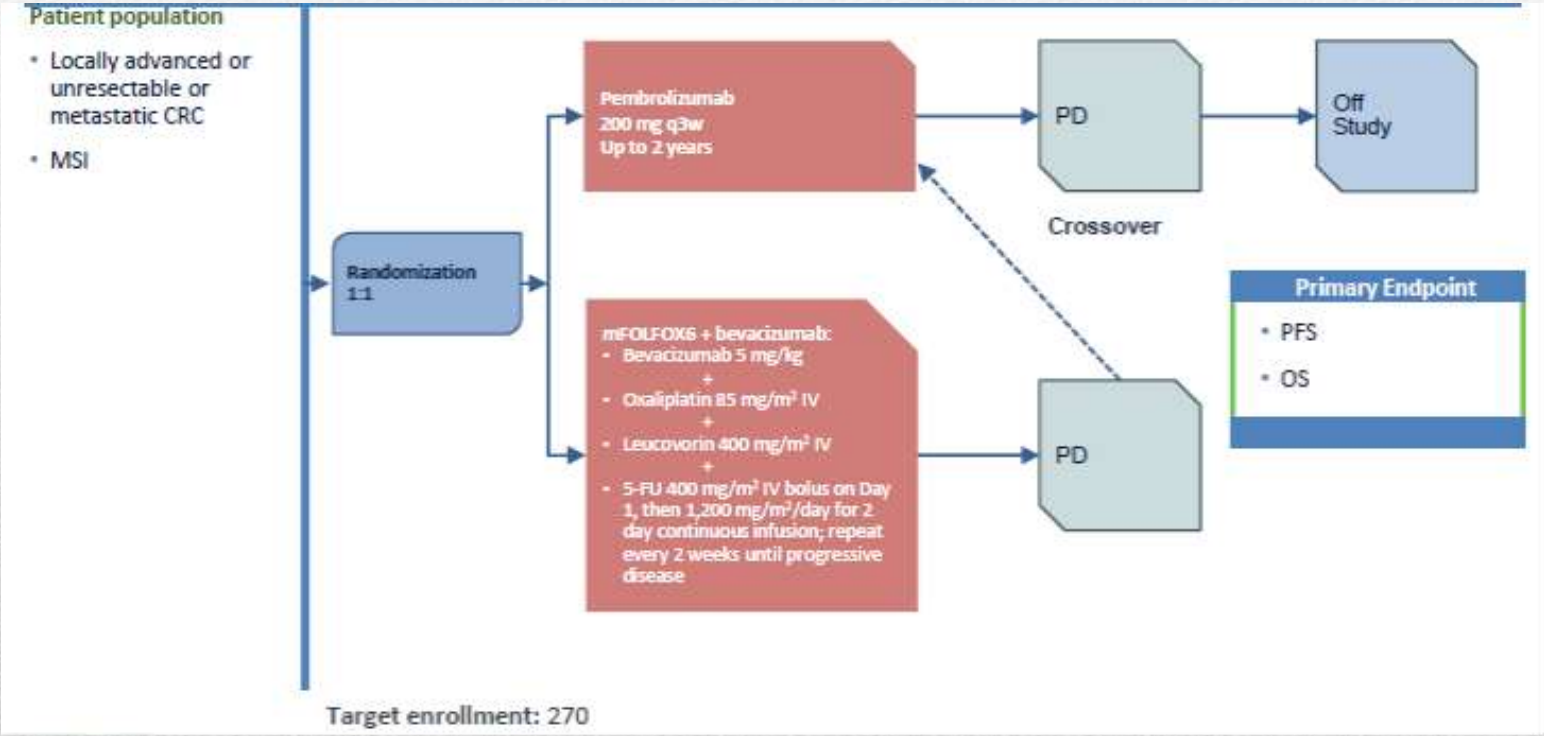
A phase 2, Single-arm Study of Pembrolizumab in Pretreated patients to Address Significant Patient Unmet Needs



KEYNOTE-164-3rd line (refractory)

Current Biomarkers: MSI

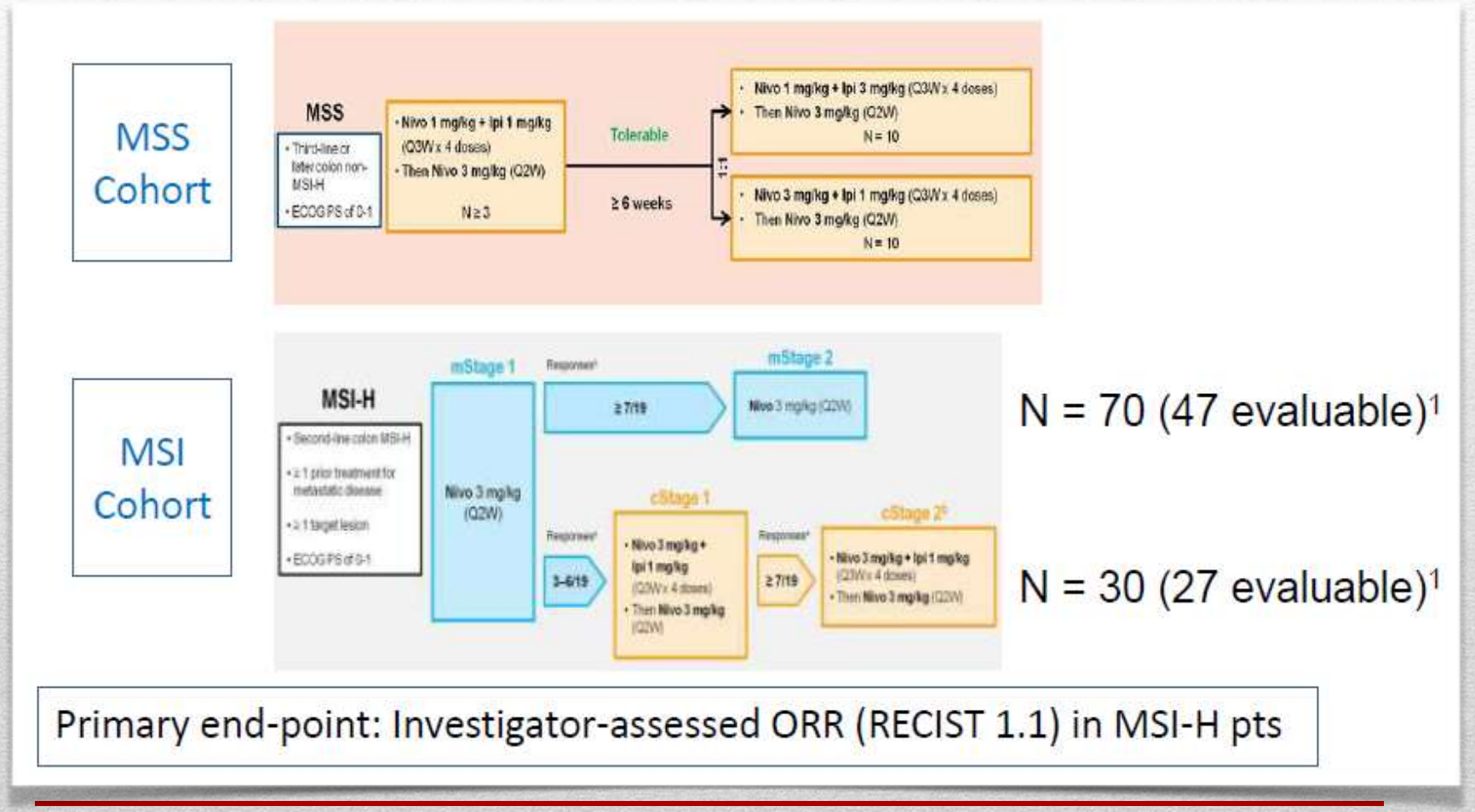
A phase 3 Study of Pembrolizumab Monotherapy vs Standard Chemotherapy in 1L MSI CRC



KEYNOTE-177-1st line

Current Biomarkers: MSI

Nivolumab +/- Ipilimumab (Checkmate 142)

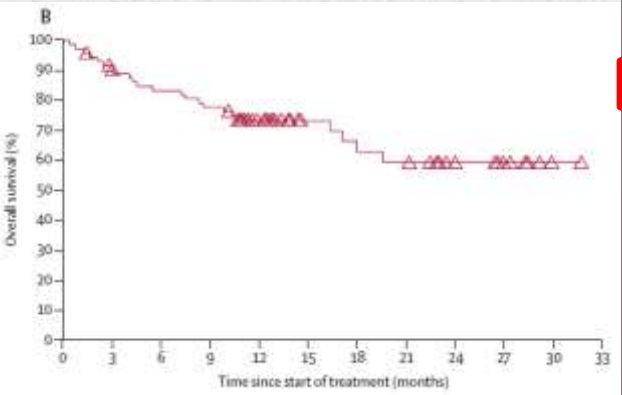


Primary end-point: Investigator-assessed ORR (RECIST 1.1) in MSI-H pts

Overman MJ et al, 2016; Overman MJ et al, 2017

Current Biomarkers: MSI

Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study



	dMMR/MSI-H per local assessment (n=74)		dMMR/MSI-H per central assessment (n=53)	
	Investigator	Blinded independent central review	Investigator	Blinded independent central review
Objective response	23 (31·1%, 20·8–42·9)	24 (32%, 22–44)	19 (36%, 23–50)	19 (36%, 23–50)
Best overall response				
Complete response	0	2 (3%)	0	1 (2%)
Partial response	23 (31%)	22 (30%)	19 (36%)	18 (34%)
Stable disease	28 (38%)	25 (34%)	20 (37%)	19 (36%)
Progressive disease	19 (26%)	21 (28%)	11 (21%)	12 (23%)
Not determined	4 (5%)	4 (5%)	3 (6%)	3 (6%)
Disease control for ≥12 weeks	51 (69%, 57–79)	47 (64%, 52–74)	39 (74%, 60–85)	37 (70%, 56–82)

Data are n (%, 95% CI) or n (%). dMMR/MSI-H=DNA mismatch repair deficient/microsatellite instability-high.

Table 2: Objective response, best overall response, and disease control per investigator and masked independent central review assessments

Overman MJ et al, 2017

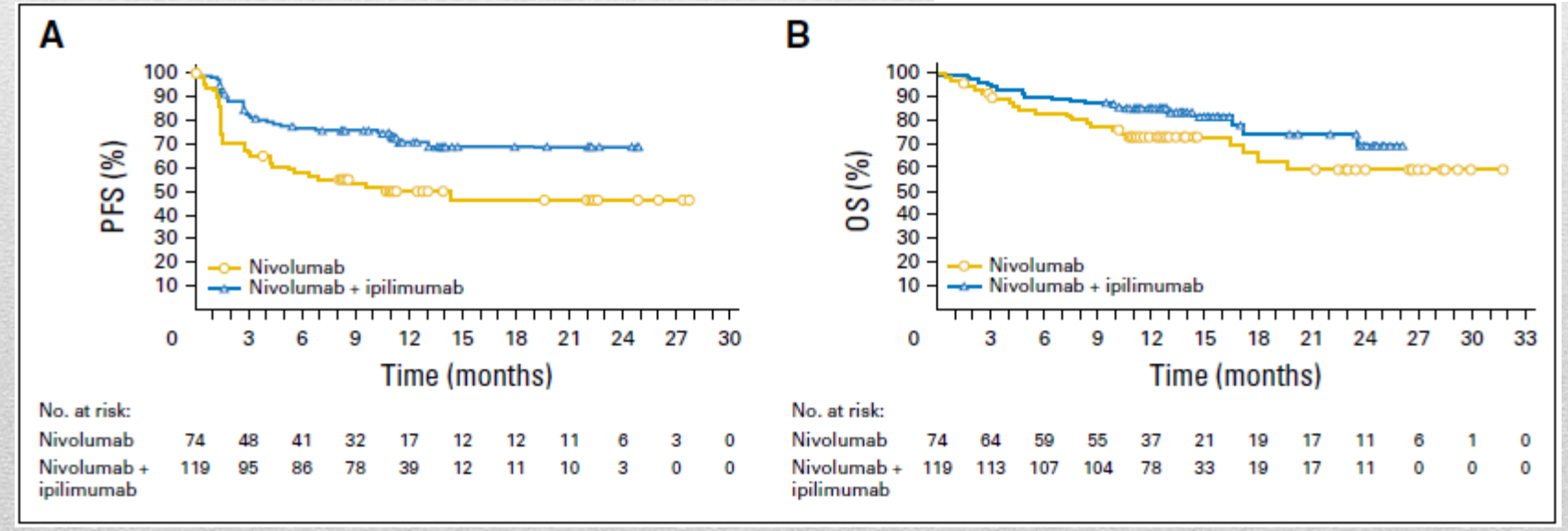
Current Biomarkers: MSI

Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair–Deficient/Microsatellite Instability–High Metastatic Colorectal Cancer

Table 2. ORR, Best Overall Response, and DCR per Investigator Assessment (N = 119)

Response	No. (%)	95% CI
ORR	65 (55)	45.2 to 63.8
Best overall response		
Complete response	4 (3)	
Partial response	61 (51)	
Stable disease	37 (31)	
Progressive disease	14 (12)	
Not determined	3 (3)	
Disease control for ≥ 12 weeks	95 (80)	71.5 to 86.6

Abbreviations: DCR, disease control rate; ORR, objective response rate.



Overman JM et al, 2018

Current Biomarkers: MSI

Web Exclusives >

FDA Approves Nivolumab for MSI-H or dMMR Colorectal Cancer

Jason M. Broderick @jasoncology
Published Online: Tuesday, Aug 01, 2017

The FDA has granted an accelerated approval to nivolumab (Opdivo) for the treatment of adult and pediatric patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer (mCRC) that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.

The approval is based on results from the phase II CheckMate-142 trial, in which the overall response rate (ORR) was 28% in mCRC patients who received prior fluoropyrimidine, oxaliplatin, and irinotecan, including 1 complete response (CR) and 14 partial responses (PRs).

The FDA-recommended dose for the PD-1 inhibitor in this setting is 240 mg IV every 2 weeks until disease progression or unacceptable toxicity. The accelerated approval of nivolumab for

FDA Approves Pembrolizumab for Microsatellite Instability-High and Mismatch Repair Deficient Cancers

FDA Approves Immunotherapy Combination for Metastatic Colorectal Cancer

The FDA approved Opdivo plus Yervoy to treat a certain subset of patients with metastatic colorectal cancer.

BY BRIELLE URCIUOLI
PUBLISHED JULY 11, 2018

The Food and Drug Administration (FDA) approved the combination use of intravenous Opdivo (nivolumab) plus Yervoy (ipilimumab) for patients with previously treated microsatellite instability-high (MSI-H) or DNA mismatch repair deficient (dMMR) metastatic colorectal cancer (CRC), according to Bristol-Myers Squibb, the manufacturer of the drugs.

Current Biomarkers: MSI

Istituzionali

Nivolumab e pembrolizumab nel CRCm e MSI-H pretrattato: AIFA nega l'inserimento in 648

16 Mag 2018

Si comunica che il CTS dell'AIFA, nella seduta del 9-10-11 aprile u.s., ha espresso parere non favorevole alla richiesta AIOM di inserimento di nivolumab e pembrolizumab nell'elenco della legge n. 648/96 per il trattamento del carcinoma del colon metastatico con elevata instabilità micro satellitare (MSI-H) pretrattato.

CRITICAL REVIEWS IN Oncology Hematology

Current Biomarkers: MSI

Polymerase proofreading domain mutations: new opportunities for immunotherapy in hypermutatad colorectal cancer beyond MMR deficiency

Analysis of The Cancer Genome Atlas colorectal adenocarcinomas samples. Three types of tumors are identified: hypermutated MSI and MSS and non-hypermutated MSS. All hypermutated MSS tumors harbor POLE mutation.

	Hypermutated (n = 35; 16%)		Non-hypermutated (n = 189; 84%)	
	MSI(n = 28; 80%)% (No.)	MSS(n = 7; 20%)% (No.)	MSI(n = 0 0%)% (No.)	MSS(n = 189%)% (No.)
MLH1	14.3% (4)	14.3% (1/7)	NA	0.5% (1)
MSH2	0.0% (0)	71.4% (5)	NA	0.0% (0)
MSH3	42.9% (12)	28.6% (2)	NA	0.5% (1)
MSH6	32.1% (9)	71.4% (5)	NA	0.5% (1)
PMS2	7.1% (2)	14.3% (1)	NA	1.1% (2)
POLE	21.4% (6)	**100% (7)**	NA	1.1% (2)

MSI: microsatellite instability; MSS: microsatellite stable; NA: not annotated.

POLE mut 0,5-2% of mCRC
Somatic > germline
Germline mut associated with multiple adenoma and CRC (PPAP)
Younger, male, right-sided, BRAF mut (32%), earlier-stage and better prognosis in early-stage
Mutations in exonuclease domain of POLE associated with high mutation rate, multiple tumoral neo-epitopes and T-Lymphocytes infiltration → STRONG RATIONALE FOR IMMUNOTHERAPY

Bourdais R et al, 2017

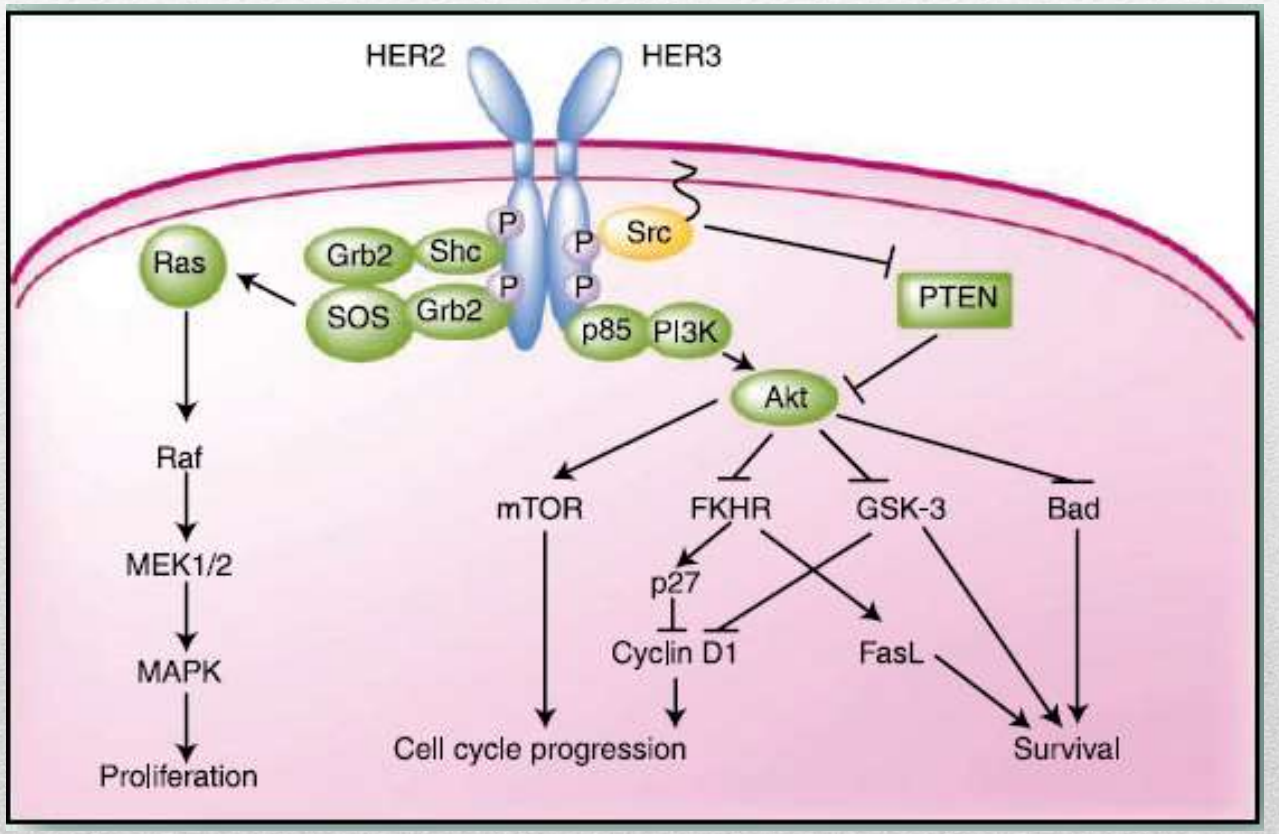
Outline

- Current biomarkers
  - RAS
  - BRAF
  - MSI
- **Emergent biomarkers**
- Monitoring the clonal evolution of CRC

Emergent Biomarkers: HER-2

VIEWPOINT

Human Epidermal Growth Factor Receptor 2 as a Molecular Biomarker for Metastatic Colorectal Cancer



3-5% in KRAS exon 2 WT stage IV CRC; distal > proximal

Lin Nu et al, 2007
Sartore-Bianchi A et al, 2018

Emergent Biomarkers: HER-2

Table 1. Studies investigating *HER2* amplification as a predictor of resistance to anti-EGFR agents.

Reference	Study Design	Population	Main Results
Bertotti et al. [38]	Preclinical	85 xenopatients [a], expanded in two molecularly unselected cohorts; randomized to receive or not cetuximab	*HER2* amplification or overexpression in 6 cases out of 44 *KRAS* wild-type patients resistant to anti-EGFR vs. 0 out of 45 *KRAS* wild-type patients with objective response to anti-EGFR ($p < 0.05$)
Yonesaka et al. [39]	Retrospective	182 *KRAS* wild-type patients treated with cetuximab-based therapy	Worse outcome (PFS and OS) for patients with *HER2*-amplified vs. *HER2*-nonamplified tumors
Martin et al. [40]	Retrospective	162 *KRAS* wild-type patients treated with anti-EGFR	Worse outcome (RR, PFS and OS) for patients with *HER2* FISH$^+$ vs. *HER2* FISH$^-$ tumors
Raghav et al. [41]	Retrospective	196 *RAS* and *BRAF* wild-type mCRC patients treated with anti-EGFR therapy	Worse outcome (PFS) for patients with *HER2*-amplified vs. *HER2*-nonamplified tumors
Sartore-Bianchi et al. [42]	Retrospective	80 patients with *HER2*-amplified and *KRAS* wild-type tumors	Worse outcome (RR and PFS) for patients treated with anti-EGFR vs. patient not treated with anti-EGFR
Sawada et al. [43]	Retrospective	11 patients with *HER2*-amplified and *RAS* and *BRAF* wild-type tumors	Worse outcome (RR, PFS and OS) for patients with *HER2*-amplified and *RAS/BRAF* wild-type vs. *HER2*-nonamplified and *RAS/BRAF* wild-type tumors
Cremolini et al. [44]	Prospectivecase-control	94 *RAS/BRAF* wild-type patients: 47 patients resistant and 47 patients sensitive to anti-EGFR-based therapy	*HER* amplification in 7 cases out of 47 resistant patients vs. 0 out 47 sensitive patients ($p = 0.01$)

[a] human cancer specimens directly transplanted into mice. FISH: Fluorescent in situ hybridization. RR: Response Rate; PFS: Progression-free survival; OS: Overall survival.

Antoniotti C et al, 2018

Emergent Biomarkers: HER-2

Potential role of HER2 blockade in overcoming resistance to anti-EGFR therapy

Trastuzumab + lapatinib in patients with HER2 amplified, KRAS exon 2 wt **mCRC refractory to standard of care** (including anti-EGFR therapy)[1]
N=27*

Tumor response	N	% (95% CI)
ORR	8	30% (14–50)
SD	12	44% (25–63)

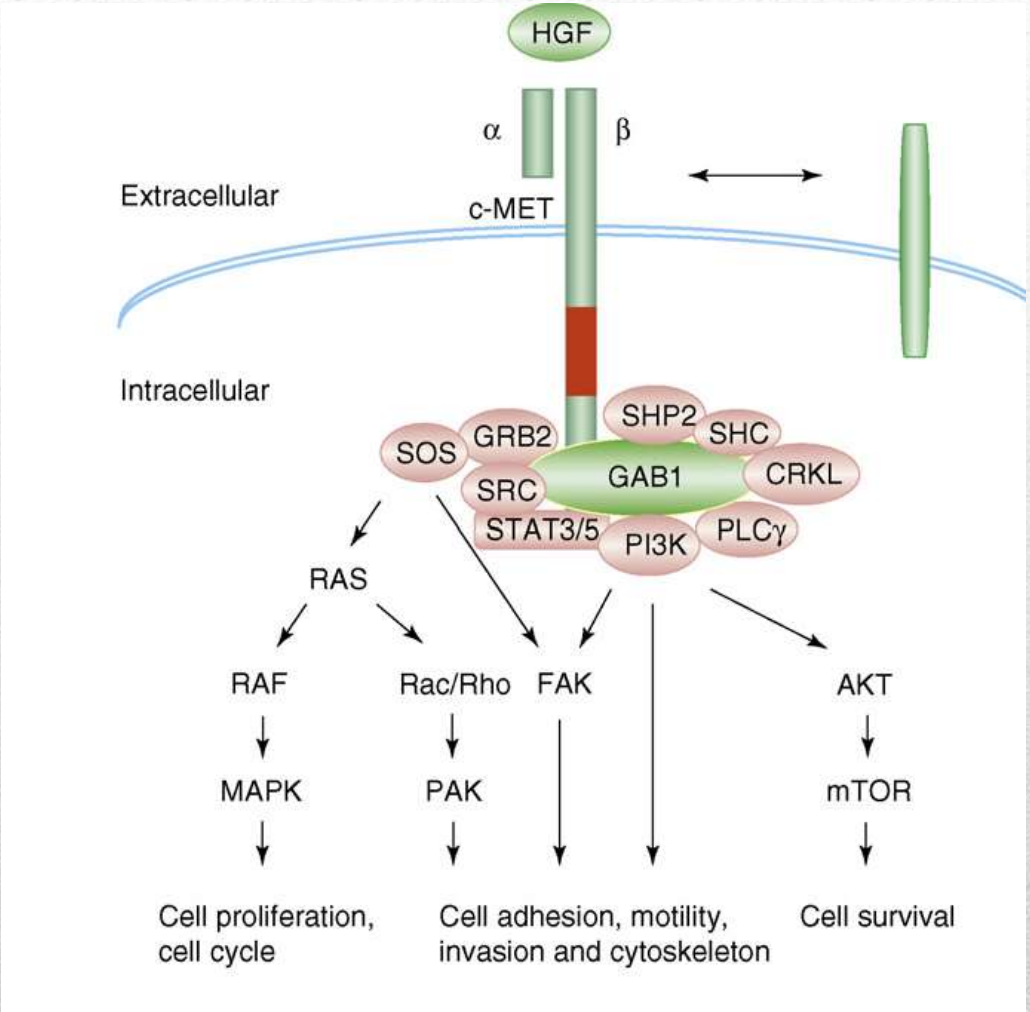
Pertuzumab + trastuzumab in patients with HER2 amplified, unselected **mCRC refractory to treatment** (including anti-EGFR therapy)[2]
N=34

Tumor response	N	%
ORR	13	38.2
SD	4	11.8

*The aim to enroll 27 patients was achieved at data cut-off 15 October 2015

Sartore-Bianchi A et al, 2016
Hurwitz W et al, 2017

Emergent Biomarkers: c-MET

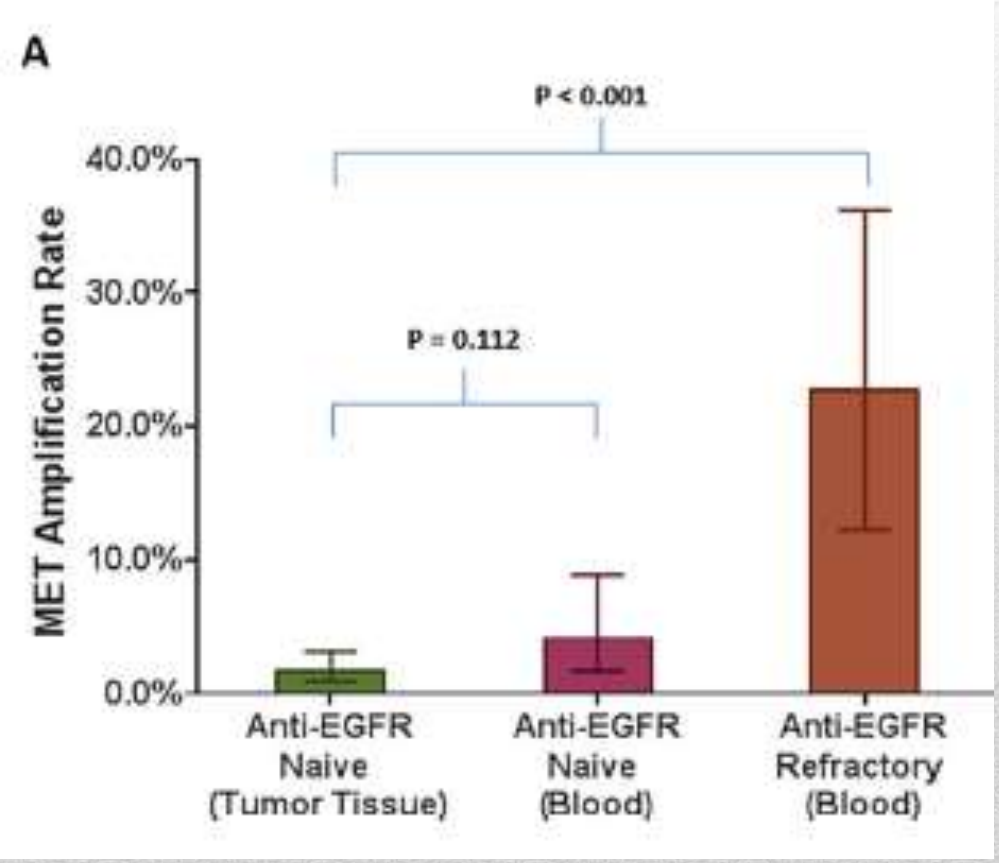


1-2% of de novo mCRC

Liu X et al, 2010

Emergent Biomarkers: c-MET

MET amplification in metastatic colorectal cancer: an acquired response to EGFR inhibition, not a *de novo* phenomenon



Raghav K et al, 2016

Emergent Biomarkers: c-MET

Clinical Cancer Research

Randomized Phase Ib/II Trial of Rilotumumab or Ganitumab with Panitumumab versus Panitumumab Alone in Patients with Wild-type *KRAS* Metastatic Colorectal Cancer

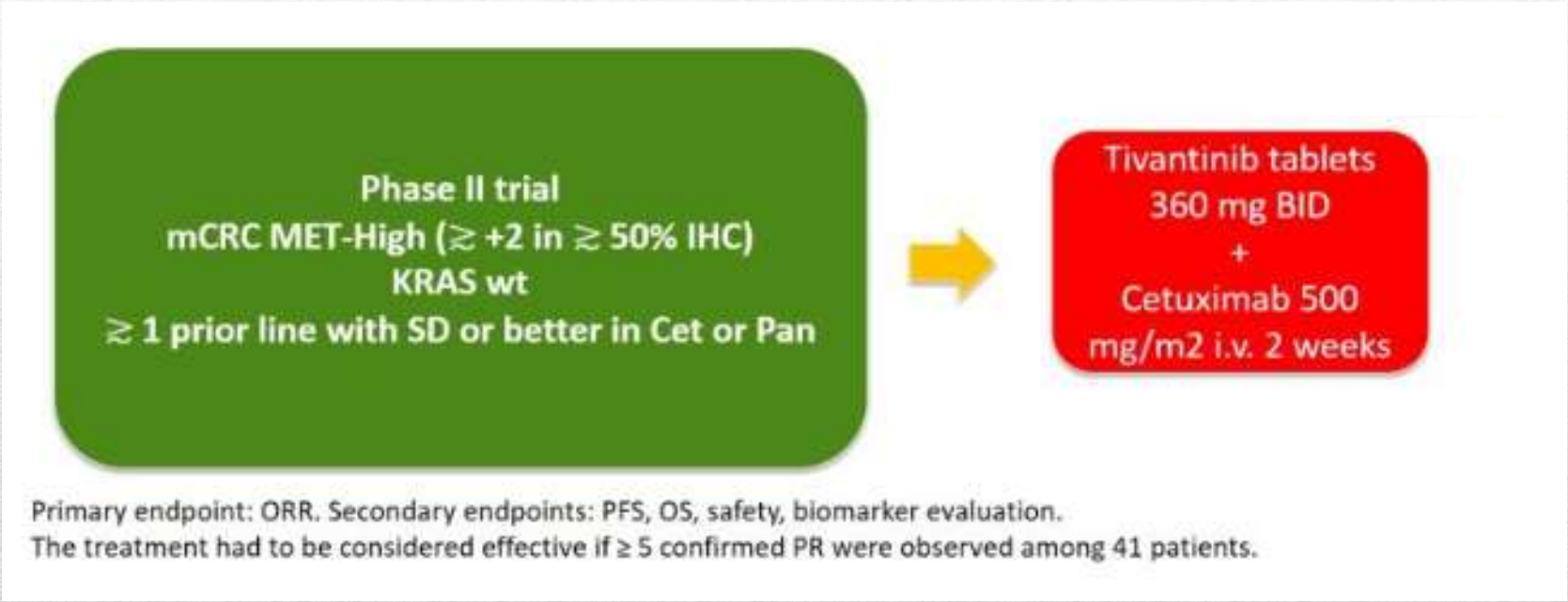
Table 2. Primary endpoint: ORR

	Panitumumab + rilotumumab (AMG 102) ($n = 48$)	Panitumumab + ganitumab (AMG 479) ($n = 46$)	Panitumumab + placebo ($n = 48$)
Patients with baseline measurable disease, *n* (%)	48 (100)	46 (100)	48 (100)
Objective response, *n* (%)	15 (31)	10 (22)	10 (21)
Complete response	0 (0)	0 (0)	0 (0)
Partial response	15 (31)	10 (22)	10 (21)
Stable disease	19 (40)	18 (39)	17 (35)
Progressive disease	11 (23)	15 (33)	16 (33)
Unevaluable/not done	3 (6)	3 (6)	5 (10)
Disease control rate[a], % (95% CI)	71 (56–83)	61 (45–75)	56 (41–71)
Duration of response, median months (95% CI)	5.1 (3.7–5.6)	3.7 (3.6–5.8)	3.7 (3.6–NE)
Posterior probability of odds ratio > 1[b]	0.93	0.63	

Abbreviation: NE, not estimable.

Van Cutsem E et al, 2014

Emergent Biomarkers: c-MET



Primary endpoint: ORR. Secondary endpoints: PFS, OS, safety, biomarker evaluation.
The treatment had to be considered effective if ≥ 5 confirmed PR were observed among 41 patients.

Rimassa L et al, 2017

Emergent Biomarkers: c-MET

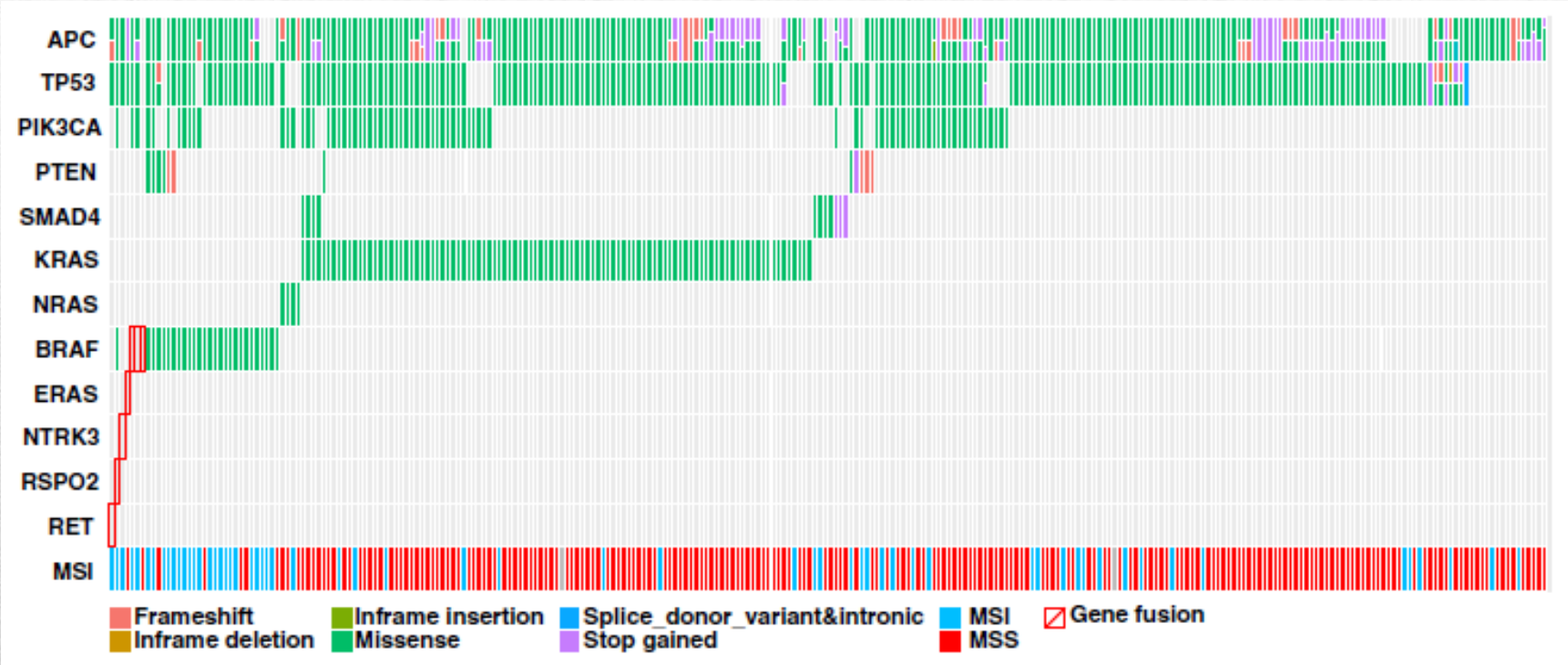
Endpoint	N= 42 (%)	Median duration months (range)
Best Responce		
Complete Response	1 (2.4)	16.6
Partial Response	3 (7.3)	5.5 (1.6-17.8)
Stable Disease	14 (34.1)	3.3 (1.2-7.5)
Progressive Disease	21 (51.2)	-
Not Evaluable	2 (4.9)	-
Overall Response Rate (CR+PR)	4 (9.8)	11 (1.6-17.8)
Disease Control Rate (CR + PR + SD)	18 (43.9)	3.6 (1.2-17.8)

Rimassa L et al, 2017

Emergent Biomarkers: gene fusions

Cancer Research

A Systematic Analysis of Oncogenic Gene Fusions in Primary Colon Cancer

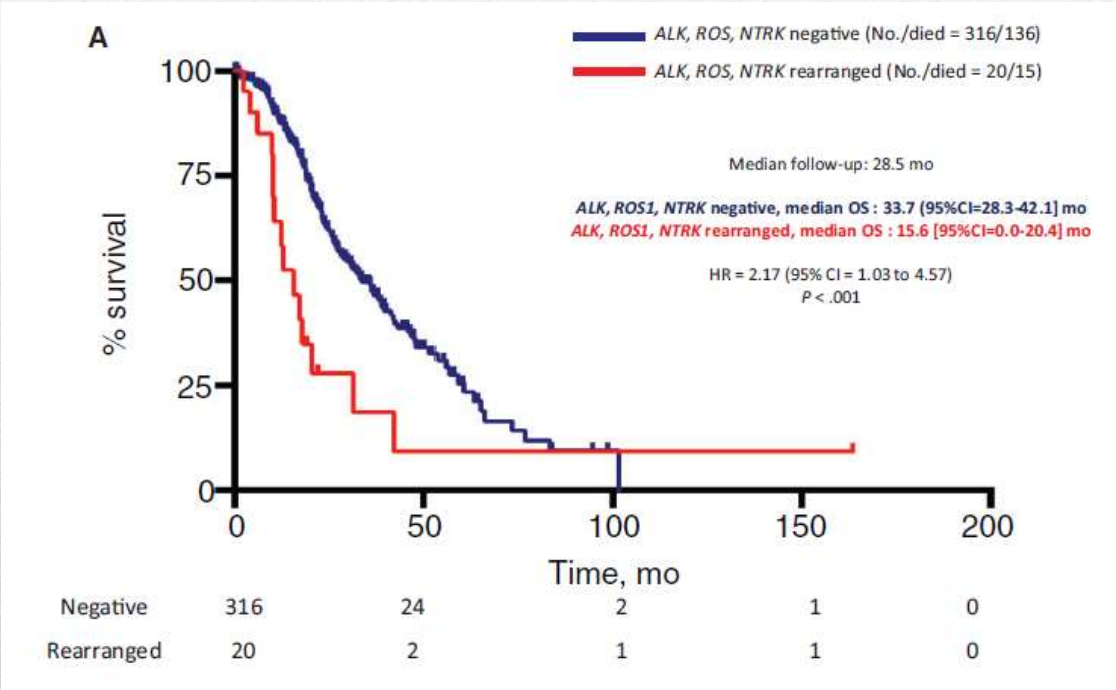


2.5% of CRC

Kloosterman WP et al, 2017

Emergent Biomarkers: gene fusions

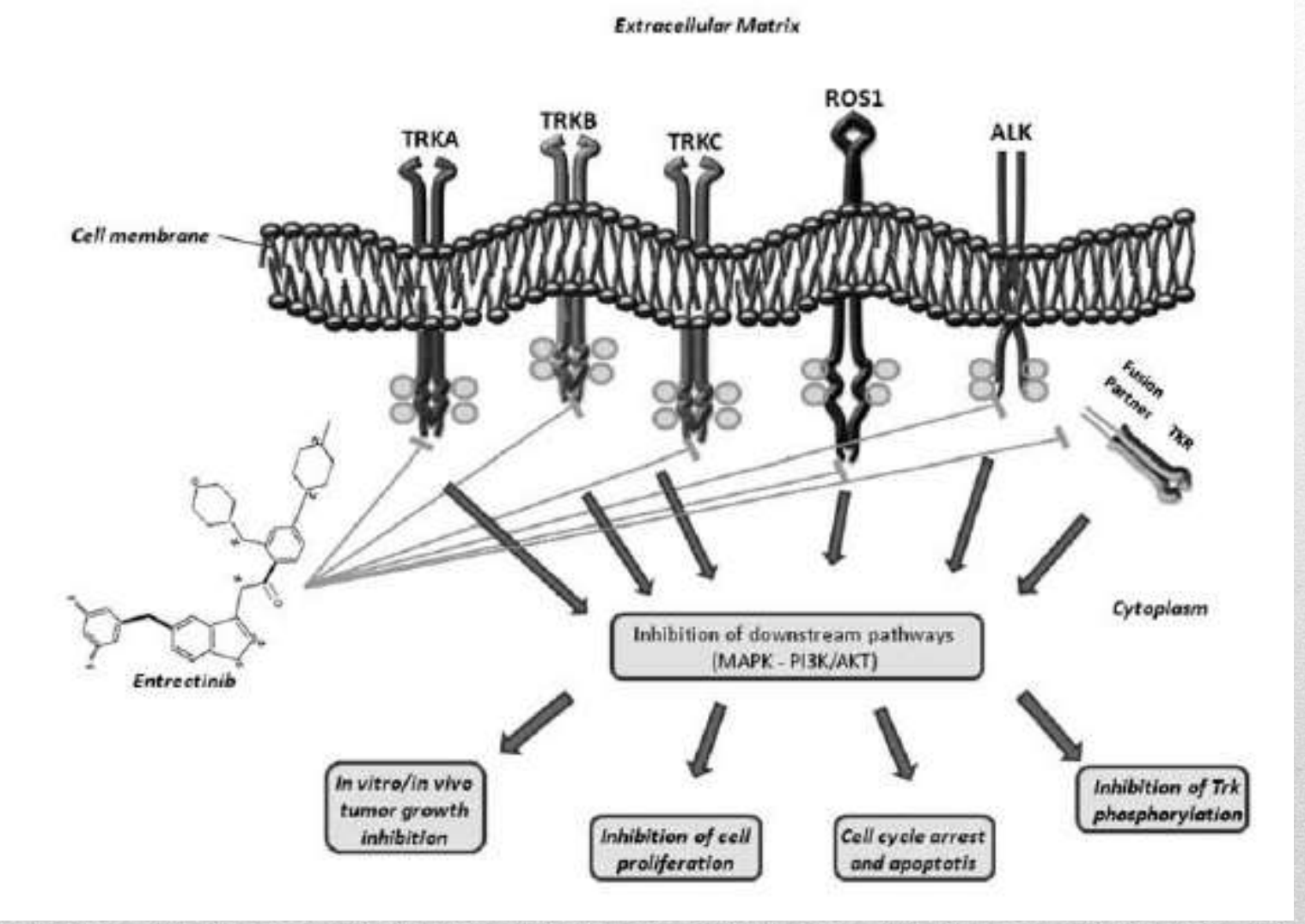
ALK, *ROS1*, and *NTRK* Rearrangements in Metastatic Colorectal Cancer



- 0.2-2.4% of CRC
- Elderly
- Right-sided
- Node-spreading
- RAS WT
- MSI-H
- Poor prognosis
- Resistance to anti-EGFR

Pietrantonio F et al, 2017

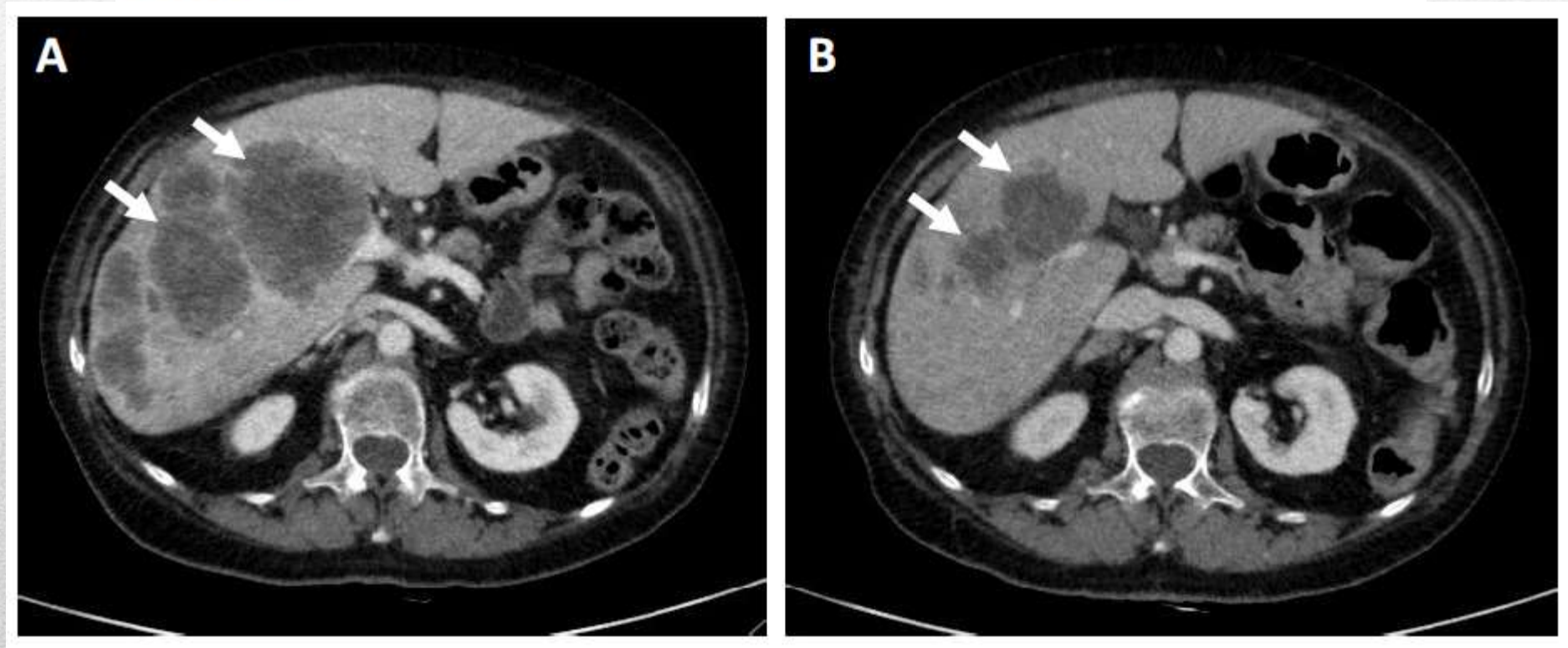
Emergent Biomarkers: gene fusions



Rolfo C et al, 2015

Emergent Biomarkers: gene fusions

Sensitivity to Entrectinib Associated With a Novel LMNA-NTRK1 Gene Fusion in Metastatic Colorectal Cancer



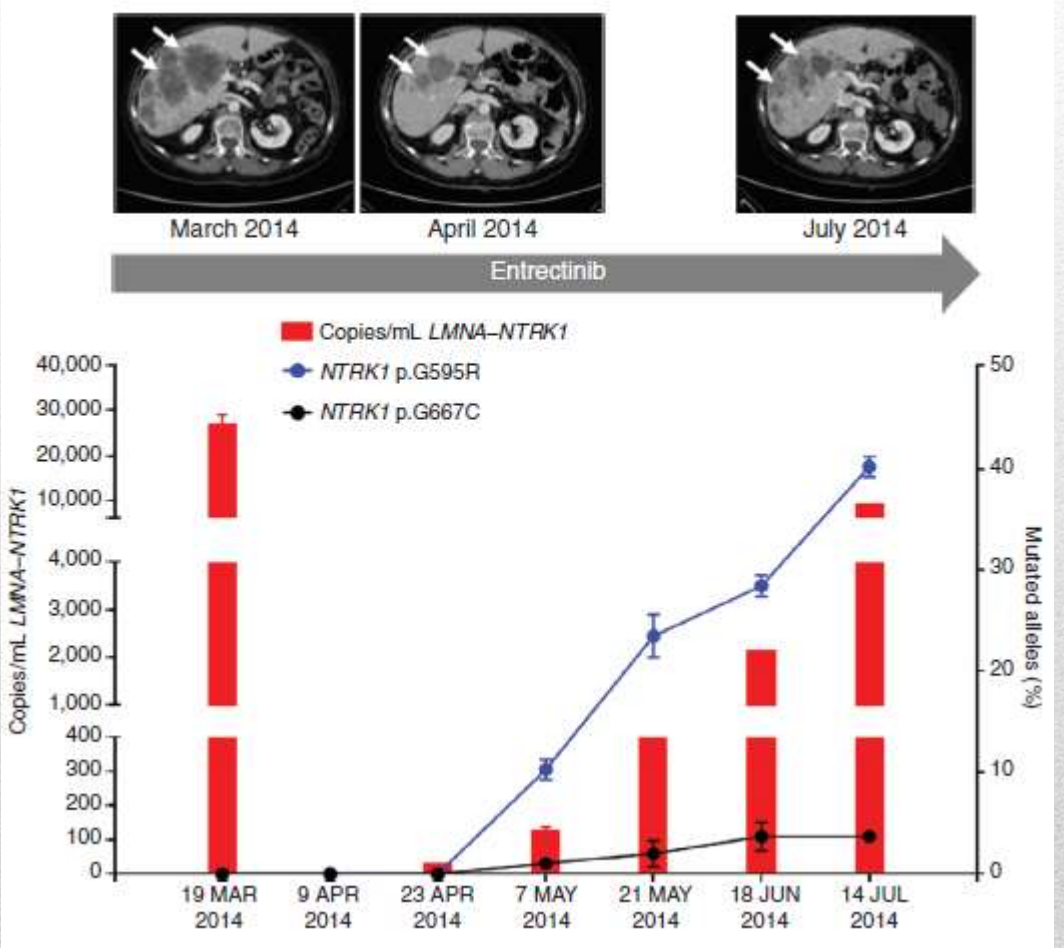
Sartore-Bianchi A et al, 2016

Emergent Biomarkers: gene fusions

Emergent Biomarkers: gene fusions

Emergent Biomarkers: gene fusions

Acquired Resistance to the TRK Inhibitor Entrectinib in Colorectal Cancer

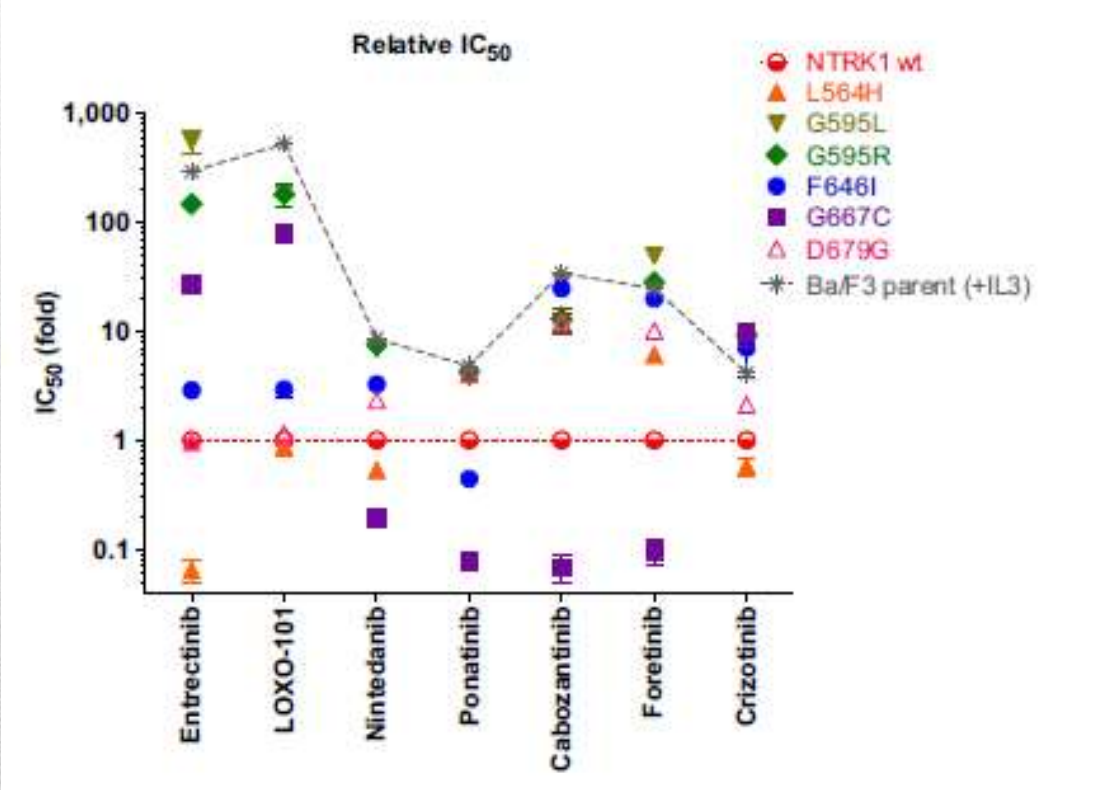


Russo M et al, 2015

Emergent Biomarkers: gene fusions

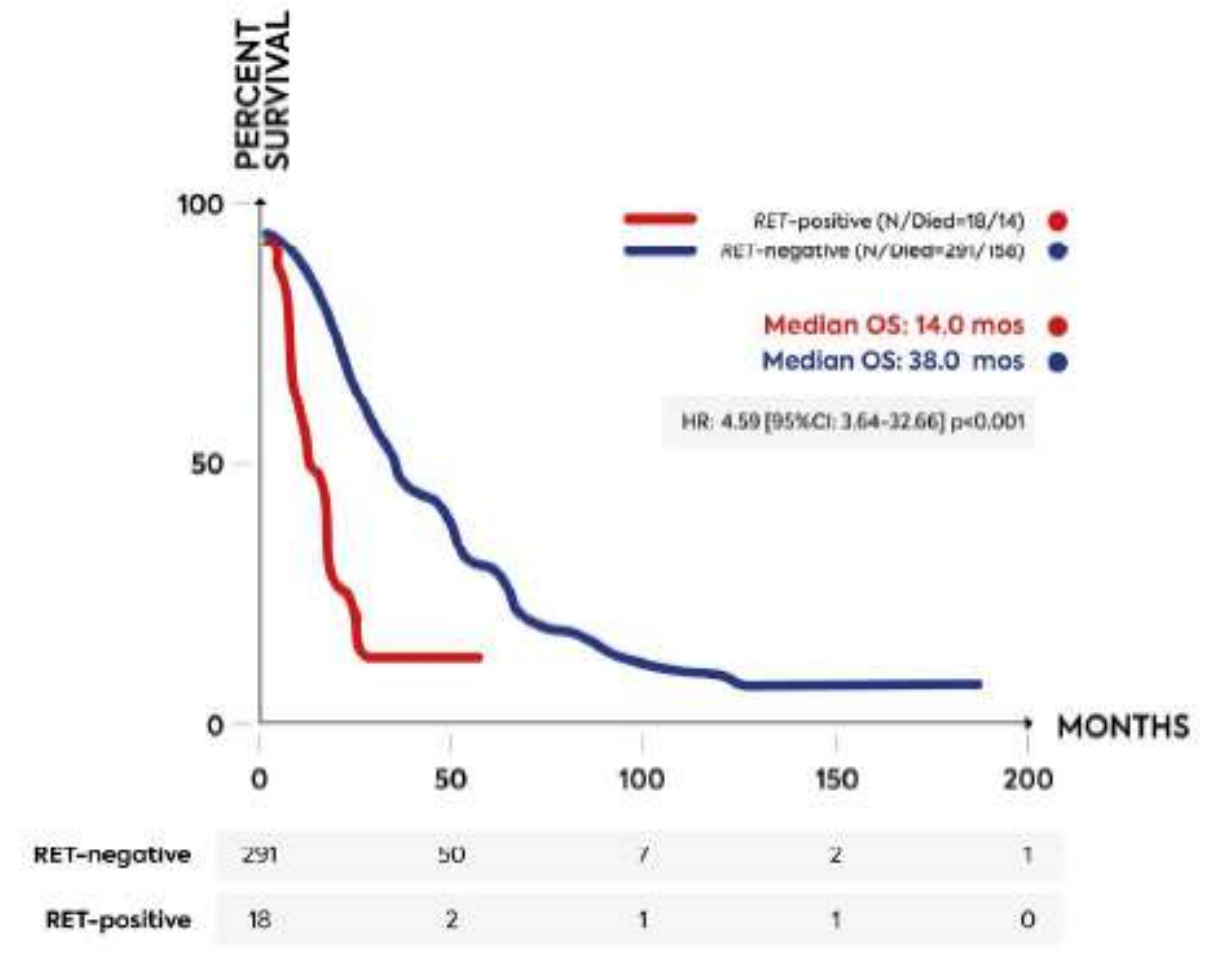
Molecular Cancer Therapeutics

Mechanisms of Resistance to NTRK Inhibitors and Therapeutic Strategies in NTRK1-Rearranged Cancers



Fuse MJ et al, 2017

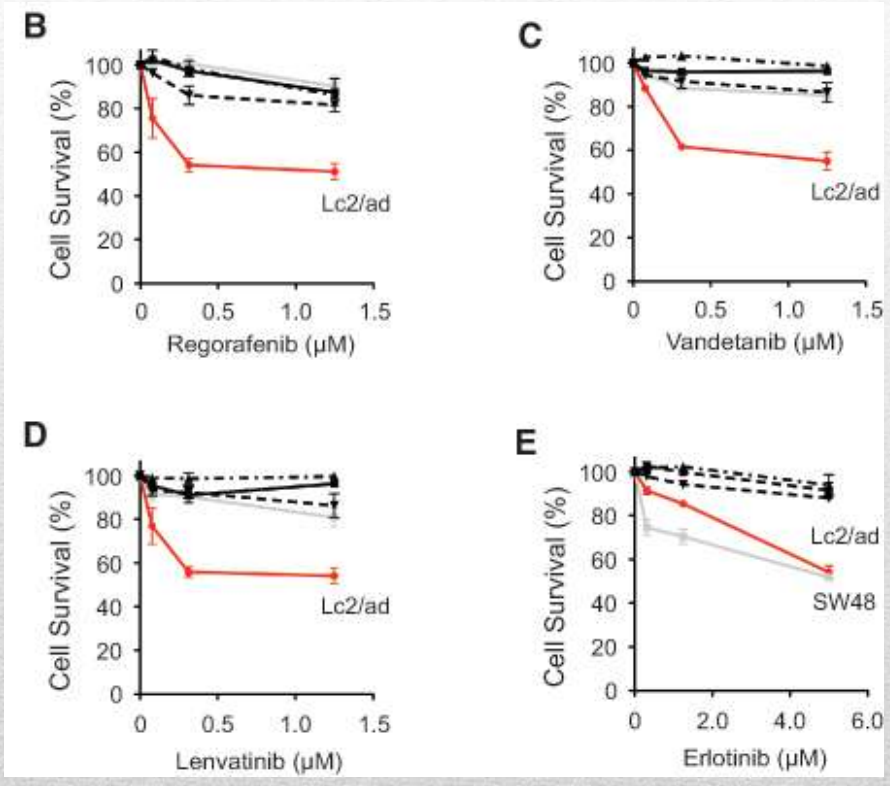
Emergent Biomarkers: gene fusions

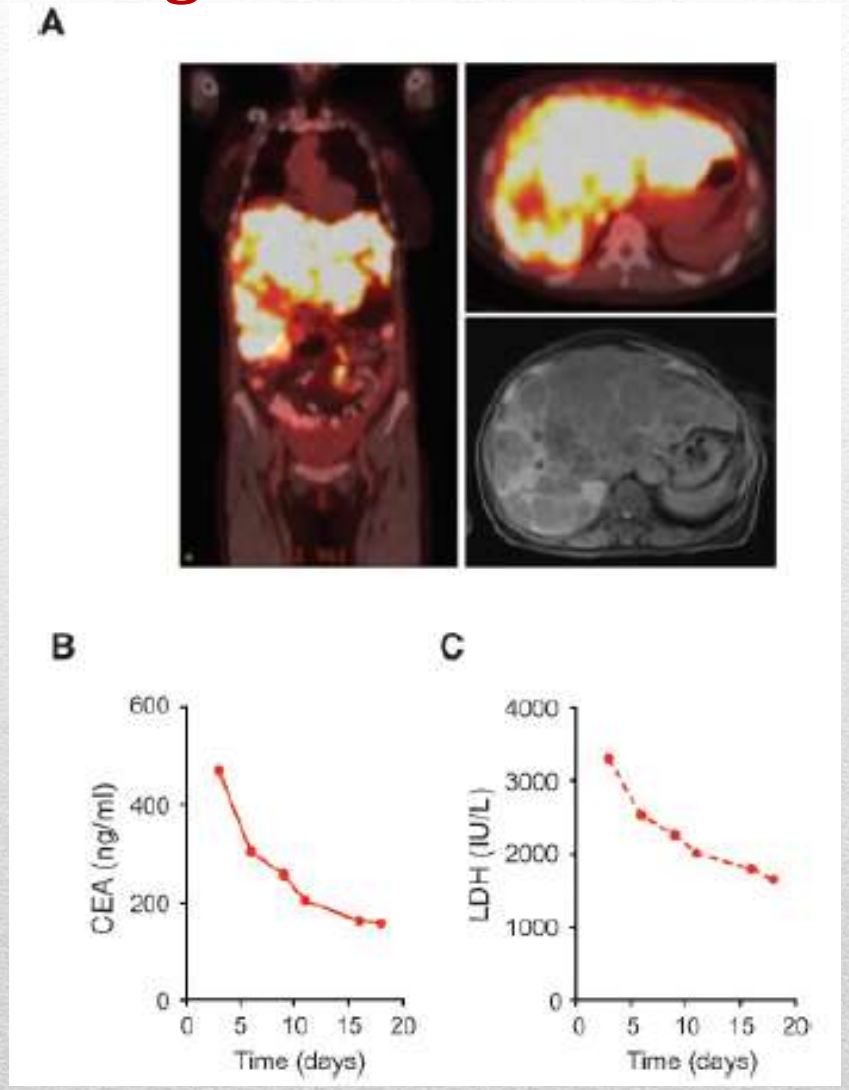


0,2%
Older pts
Right-sided
MSI-H
RAS and BRAF WT

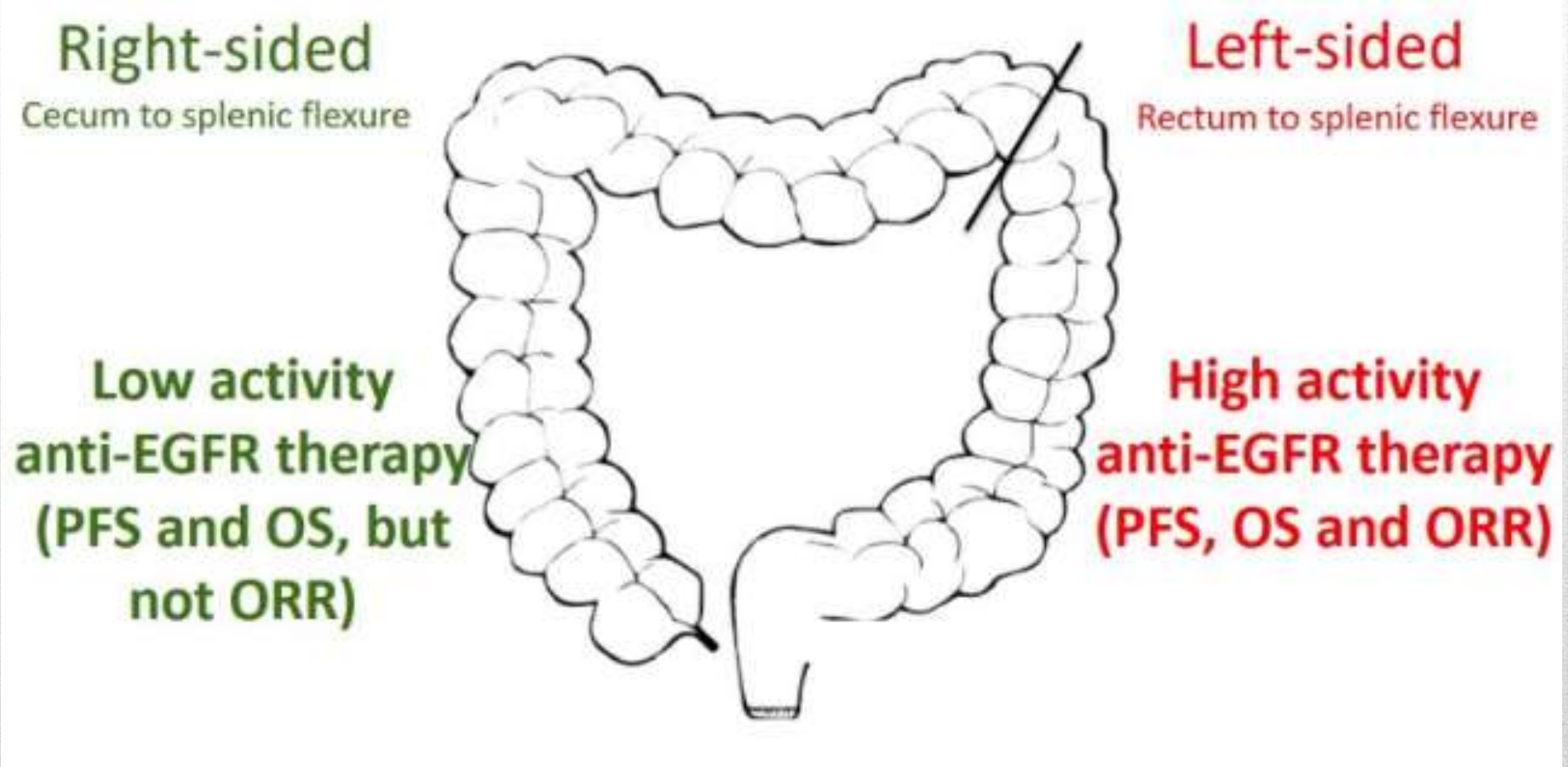
Pietrantonio F et al, 2018

Emergent Biomarkers: gene fusions

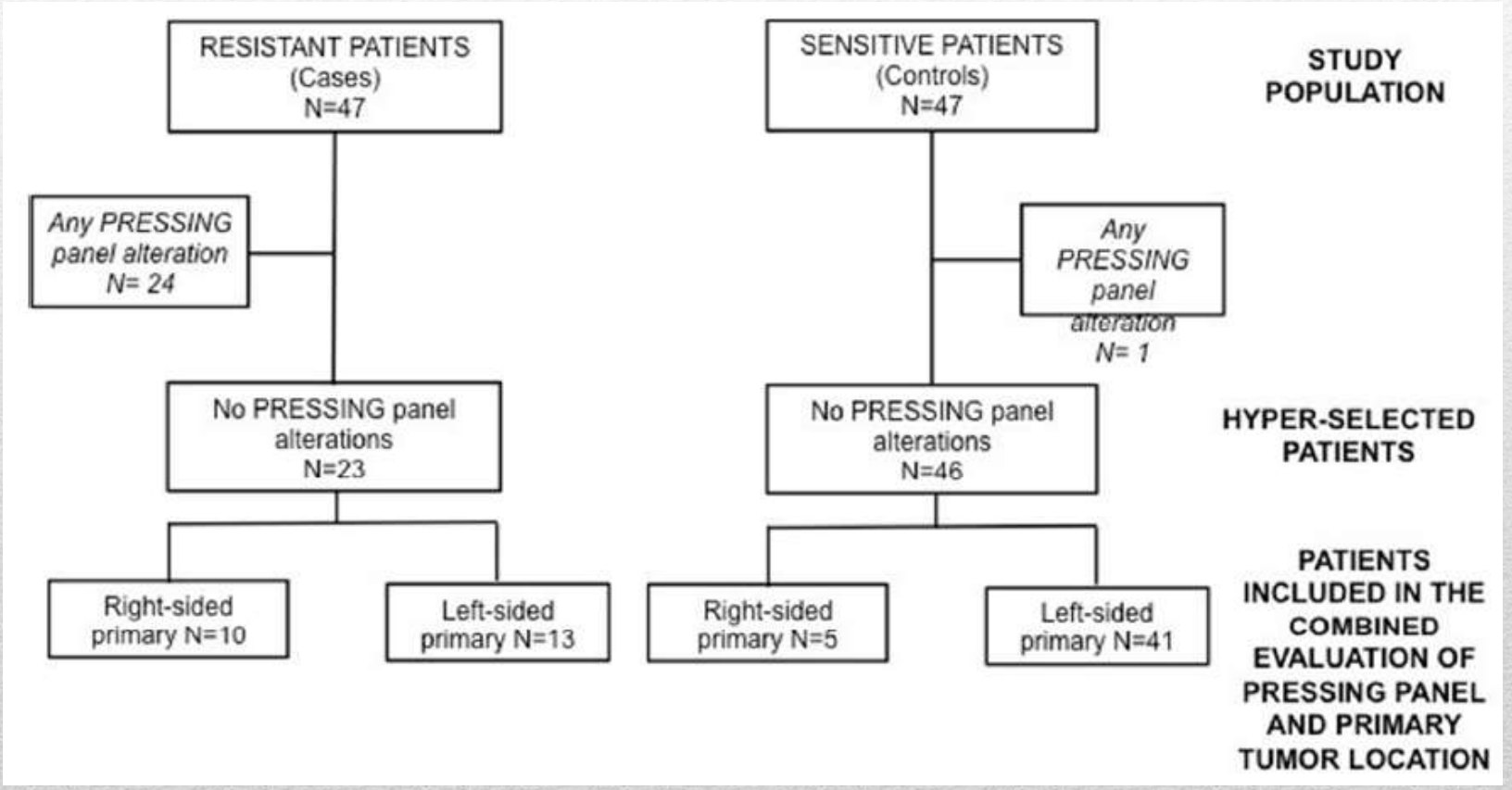




Le Rolle AF et al, 2015

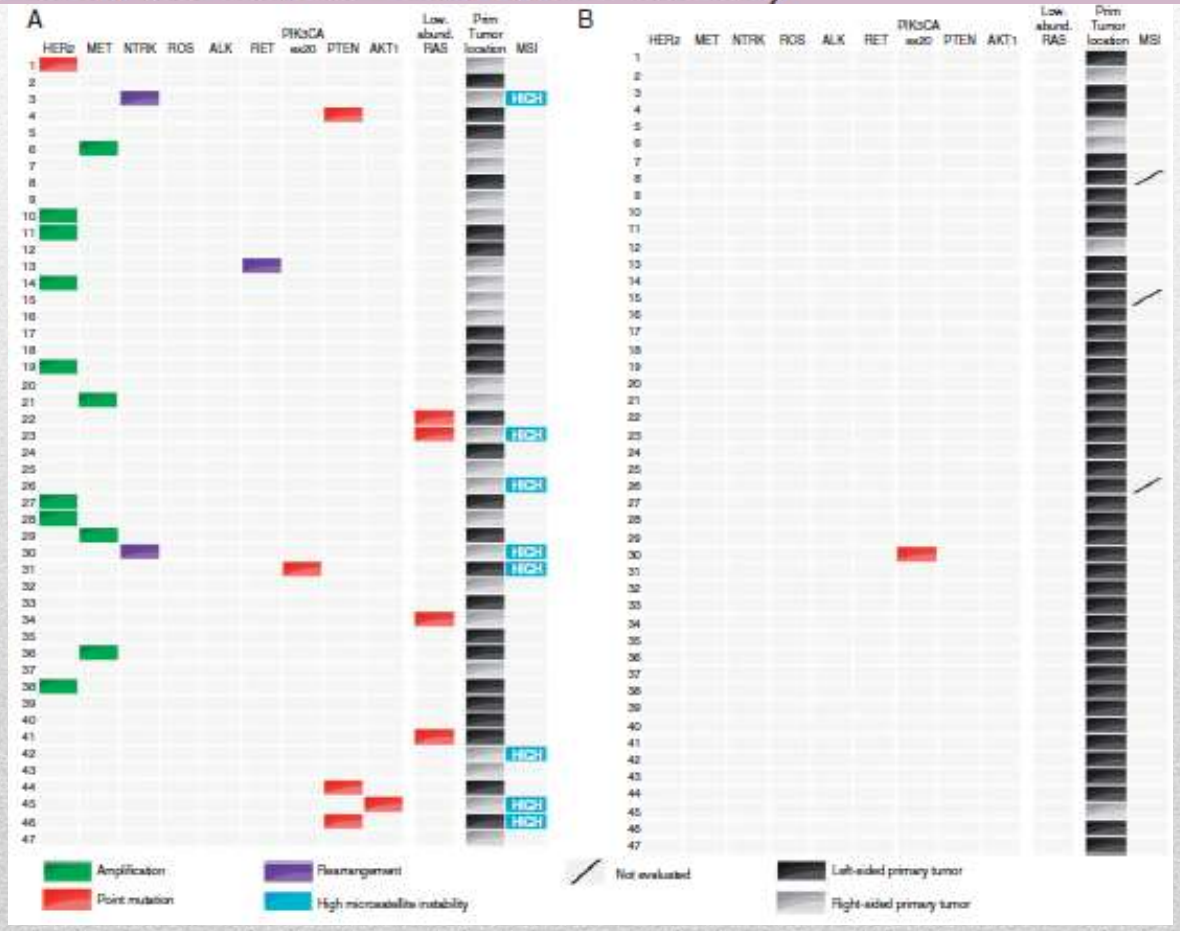

Right-sided
Cecum to splenic flexure
Left-sided
Rectum to splenic flexure
Low activity anti-EGFR therapy (PFS and OS, but not ORR)
High activity anti-EGFR therapy (PFS, OS and ORR)

Negative hyper-selection of metastatic colorectal cancer patients for anti-EGFR monoclonal antibodies: the PRESSING case–control study



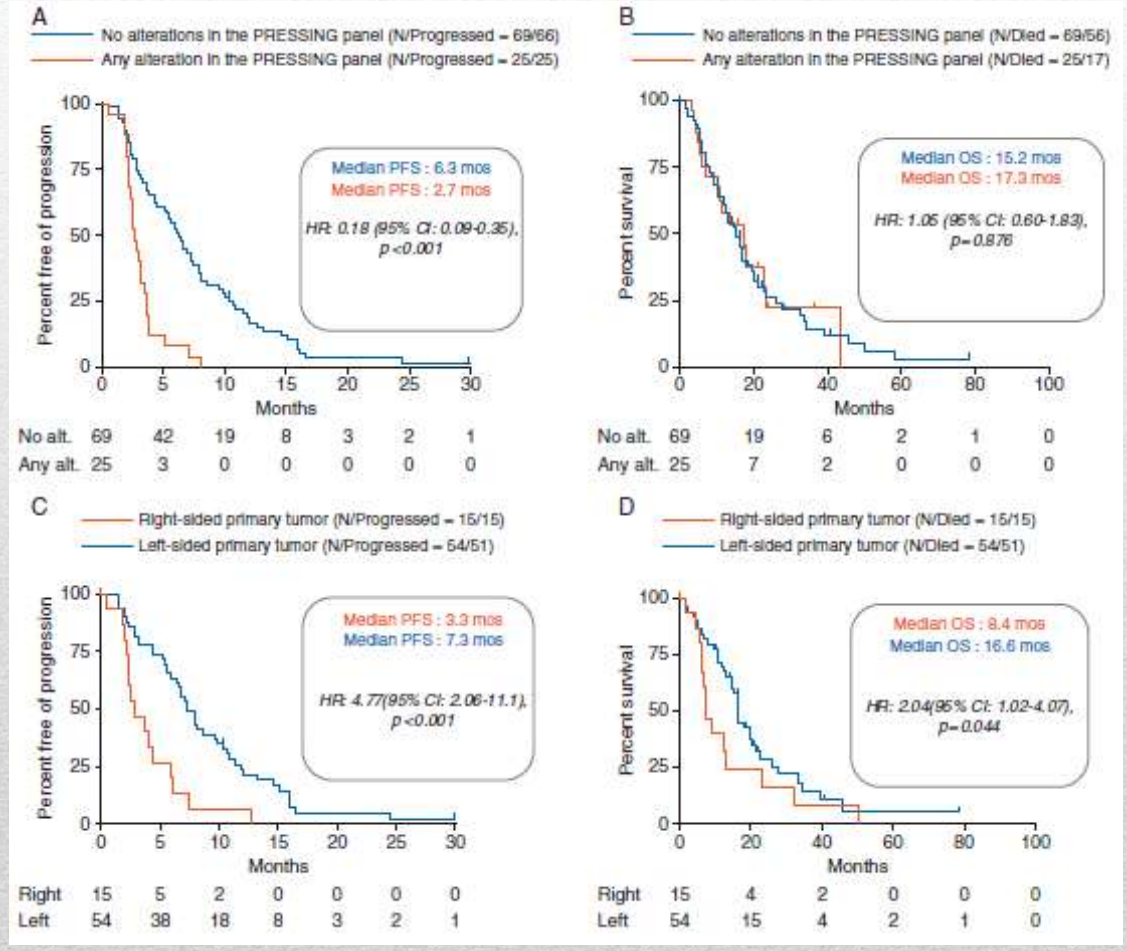
Cremolini C et al, 2017

Negative hyper-selection of metastatic colorectal cancer patients for anti-EGFR monoclonal antibodies: the PRESSING case–control study



Cremolini C et al, 2017

Negative hyper-selection of metastatic colorectal cancer patients for anti-EGFR monoclonal antibodies: the PRESSING case–control study

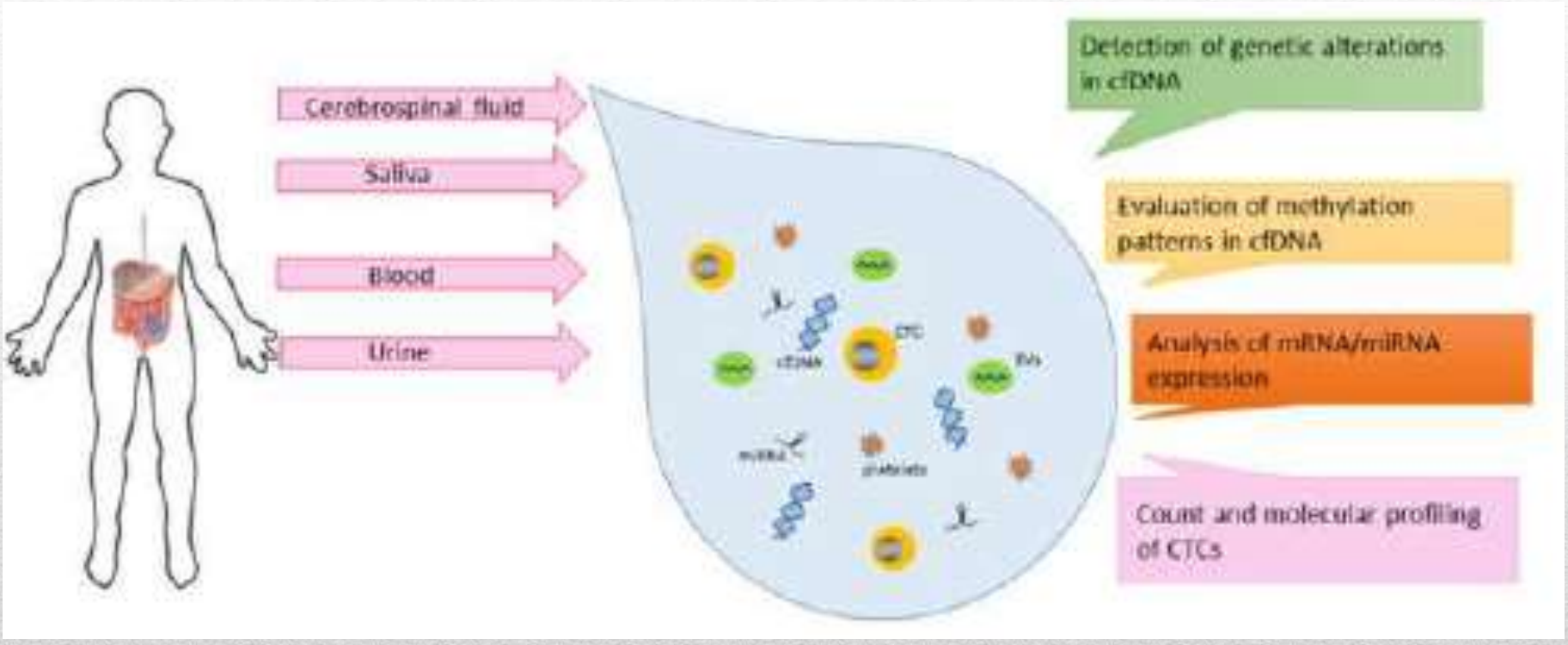


Cremolini C et al, 2017

Outline

- Current biomarkers
  - RAS
  - BRAF
  - MSI
- Emergent biomarkers
- **Monitoring the clonal evolution of CRC**

Monitoring the clonal evolution of CRC



Normanno N et al, 2018

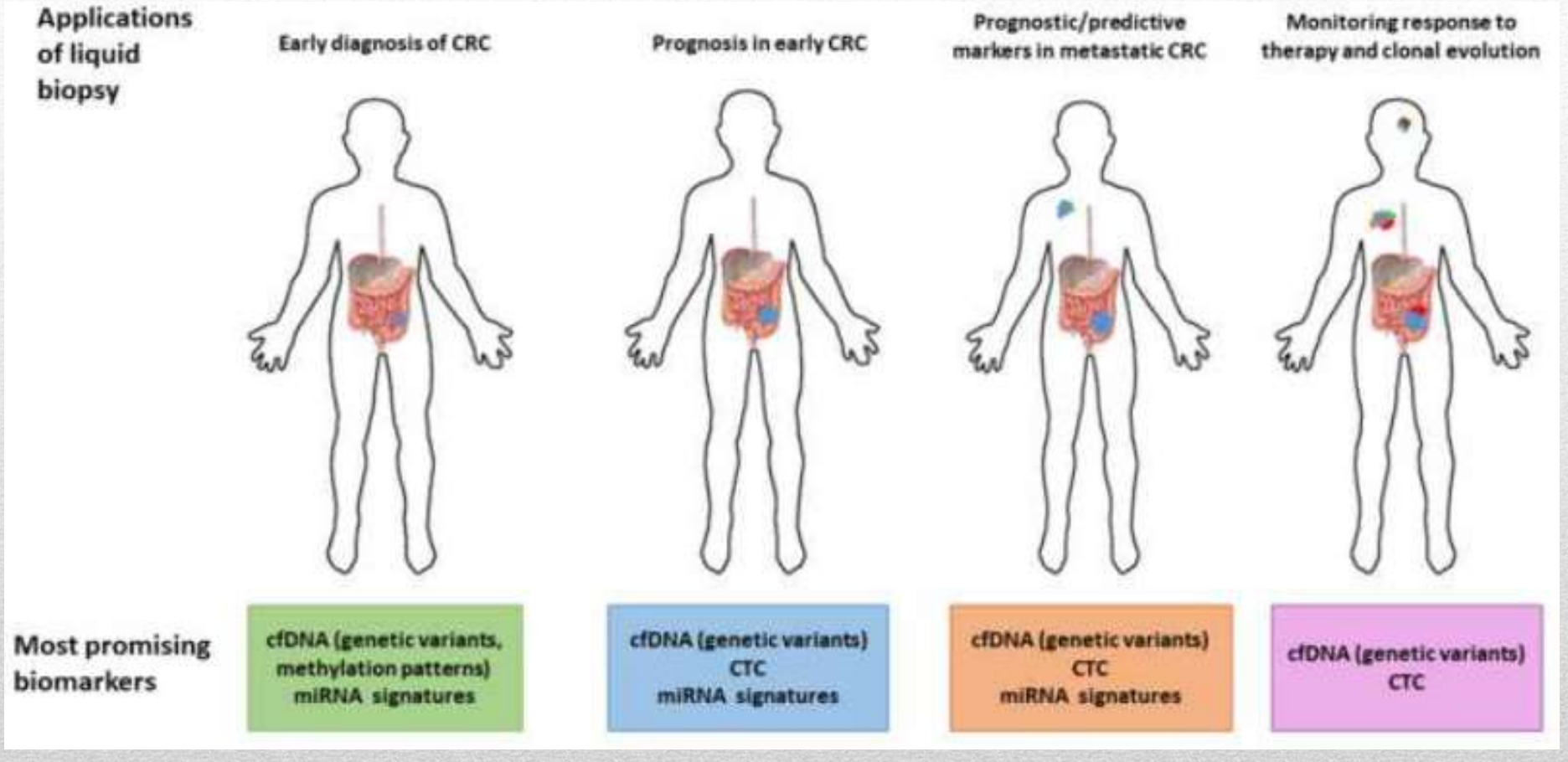
Monitoring the clonal evolution of CRC

Tissue-based vs Liquid Biopsy *RAS* Analysis: Pros and Cons

Tissue biopsy	Liquid biopsy
• Available for primary tumor • Well-established and validated techniques • Proven as biomarker • Morphological control • Only one site and time	• Less invasive • More complete assessment of tumor heterogeneity and low-level subclones[a] • Techniques not standardized • Not proven as biomarker

Normanno N et al, 2015

Monitoring the clonal evolution of CRC



Normanno N et al, 2018

Monitoring the clonal evolution of CRC

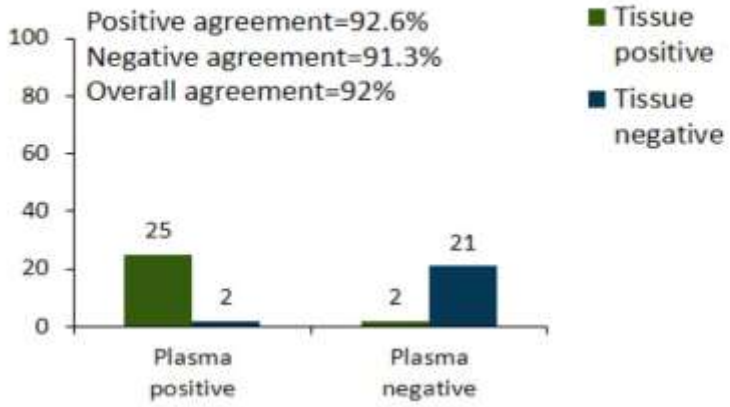
RAS Mutation Testing Using BEAMing PCR

Mutation Panel[a,b]

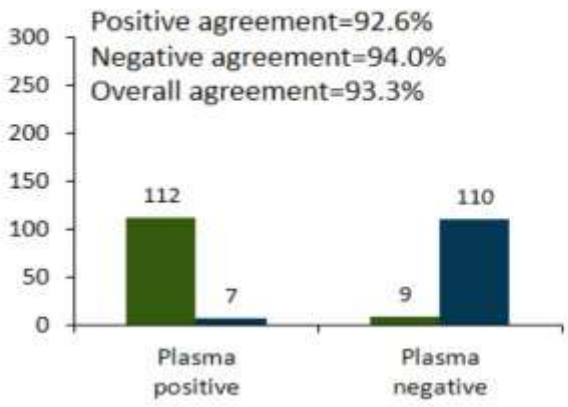
	Exon 2	Exon 3	Exon 4
KRAS	G12S, G12R, G12C, G12D, G12A, G12V, G13D	A59T, Q61L, Q61R, Q61H, Q61H	K117N, K117N, A146T, A146V
NRAS	G12S, G12R, G12C, G12D, G12A, G12V, G13R, G13D, G13V	A59T, Q61K, Q61R, Q61L, Q61H, Q61H	K117N, K117N, A146T

Concordance of Plasma and Tissue Based Testing

N=50 treatment-naïve patients[a]

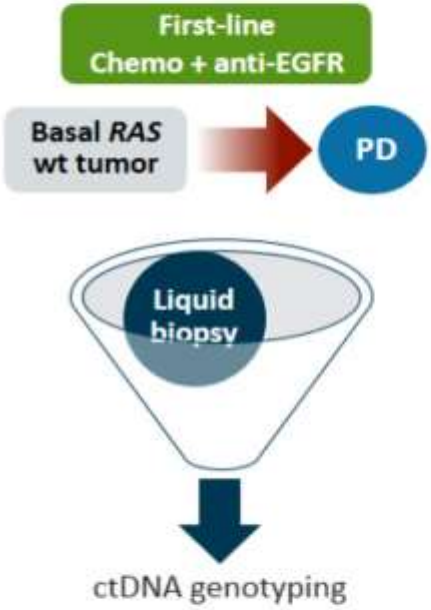


N=238 treatment-naïve and recurrent patients[b]



Hahn S et al, 2015; Jones F et al, 2016

How Can Liquid Biopsy-Based Biomarker Testing Potentially Guide Treatment Decisions After Anti-EGFR Therapy? Ongoing Trials

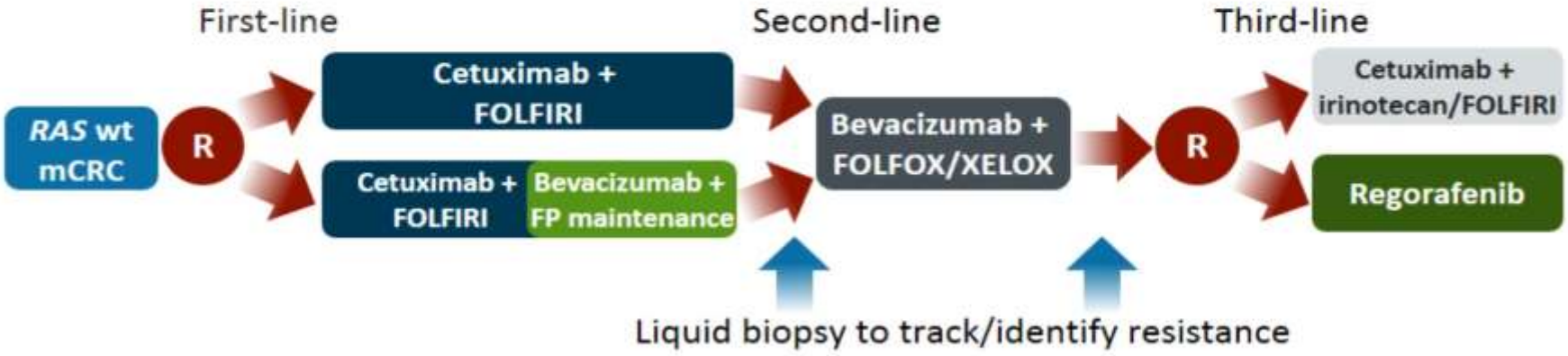


Mechanism of Resistance	Therapeutic Strategy	Example	Trial
***HER2* amplification**	Pan-HER TKI+ anti-HER2	Trastuzumab + lapatinib	HERACLES trial (phase II)
***MET* amplification**	Anti-EGFR + MET inh	Cetuximab + ARQ197	NCT01892527 (phase II)
***RAS* mutation**	Anti-EGFR + MEK inh Rechallenge	Panitumumab + MEK162	NCT01927341 (phase II) CHRONOS; FIRE-4
***EGFR S492R* mutation** ***EGFR* ECD mutations**	Panitumumab Second-generation anti-EGFR	Panitumumab Sym004 PAN-HER MM151	NCT02083653 (phase II) NCT01520389 (phase I)
***BRAF* mutation**	Anti-EGFR mAbs + BRAF inhibitor	BRAFi + anti-EGFR + PI3Ki/ MEKi	ARRAY, BEACON study
***MEK* mutation**	Anti-EGFR + MEKi	Cetuximab + trametinib	*MEK* mutation

Rechallenge With Anti-EGFR Therapy: FIRE-4 Study

FIRE-4 (phase 3, N=550)

- Primary endpoint: OS after randomization 2
- Results expected: January 2022



ClinicalTrials.gov Web site. NCT02934529. Accessed March 16, 2017.

Conclusions

- Only 50% of patients with RAS and BRAF WT CRC achieve a response to anti-EGFR
- A panel uncommon genetic alterations may help to «negatively hyperselect» patients who benefit from anti-EGFR (PRESSING panel)
- HER-2,c-MET, NTRK and RET are not only predictor of resistance to anti-EGFR but druggable targets
- Liquid biopsy is the most intriguing tool to monitor the molecular evolution of the disease, but deserves further validations

Thanks!!!