CCA - COLORECTAL CANCER ACADEMY: COSTRUIRE IL SAPERE

2ª Edizione

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L'importanza dei biomarcatori emergenti

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Biomarkers in colorectal cancer

Tissue-based

- Microsatellite instability
- *POLE* mutation
- TMB
- *RAS* (*KRAS-G12C*) mutation
- *BRAF* (*V600E*) mutation
- HER2 iperexpression/amplification
- Gene fusions
- *MGMT* deficiency
- CMS
- Other gene alterations

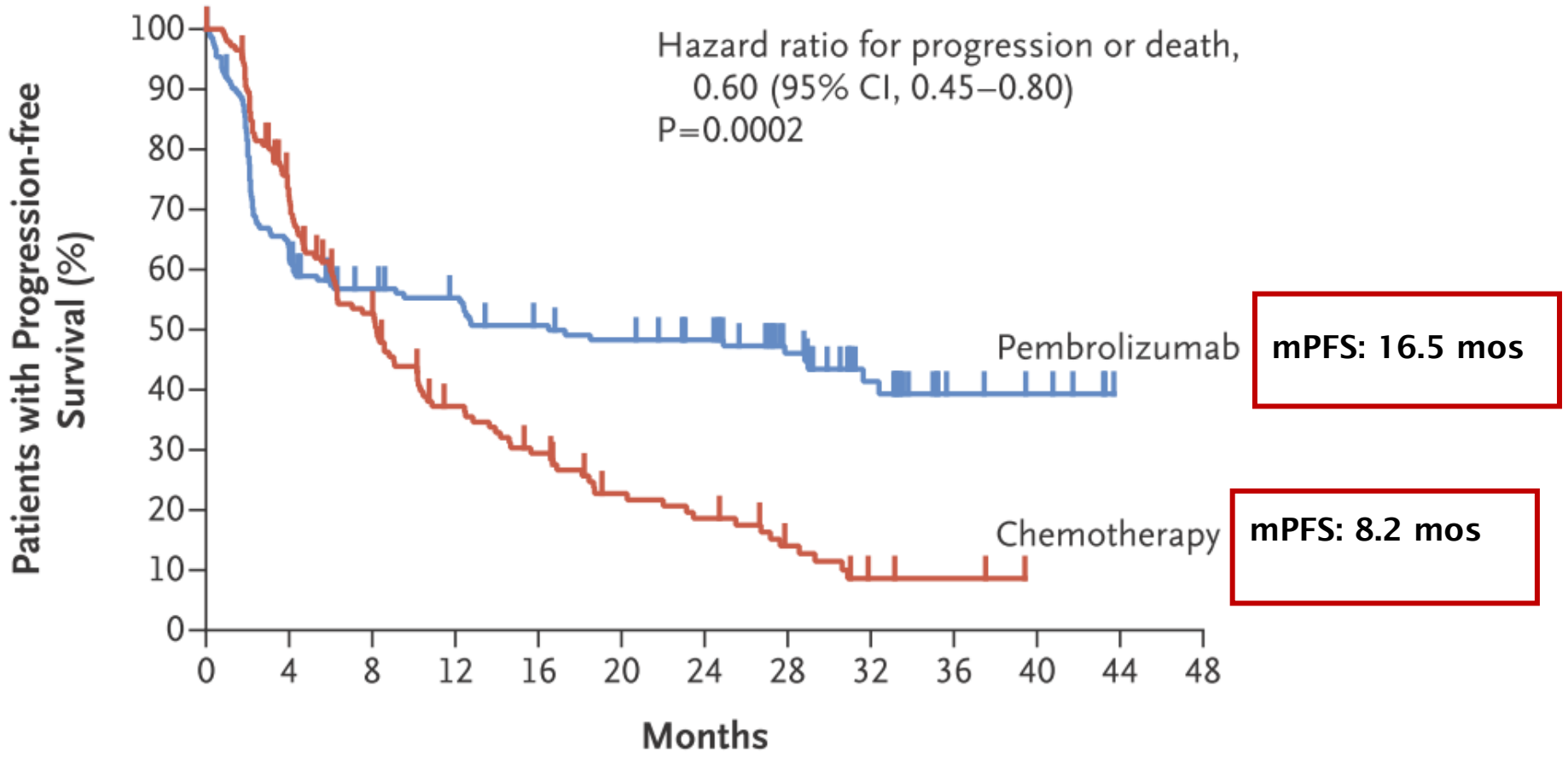
Plasma-based

- CEA and CA19.9
- ctDNA
- Circulating Tumor Cells
- Exosoms
- Circulating RNA
- Proteins

Tissue biomarkes in colorectal cancer

Alterations	Prevalence	Targetability evidence	Enrichment
RAS mutations	55-60%	NO	-
KRAS G12C mutation	3%	YES	-
BRAF V600E mutation	8-10%	YES	(> if right colon, *RAS* wt, MSI)
PI3K mutations	8%	Probably YES	-
Microsatellite instability	5%	YES	(> if right colon, *BRAF* mut)
BRAF non-V600E mutations	2%	NO	(> if left/rectum colon, *RAS* mut, MSS)
HER2 amplification	2%	YES	(> if left/rectum colon, *RAS/BRAF* wt)
MET amplification	2%	Case report	-
POLE mutations	1%	YES	(> if right colon, MSS)
TRK1-3, *ALK*, *ROS1* translocations	<1%	YES	(> if right colon, *RAS/BRAF* wt, MSI)
RET translocations	<1%	Case report	(> if right colon, *RAS/BRAF* wt, MSI)
MGMT silencing	40%	YES	(> if right colon, *RAS* mut, MSS)

Anti-PD1 in MSI-H mCRC - Keynote-177 – Primary endpoint met: PFS superiority



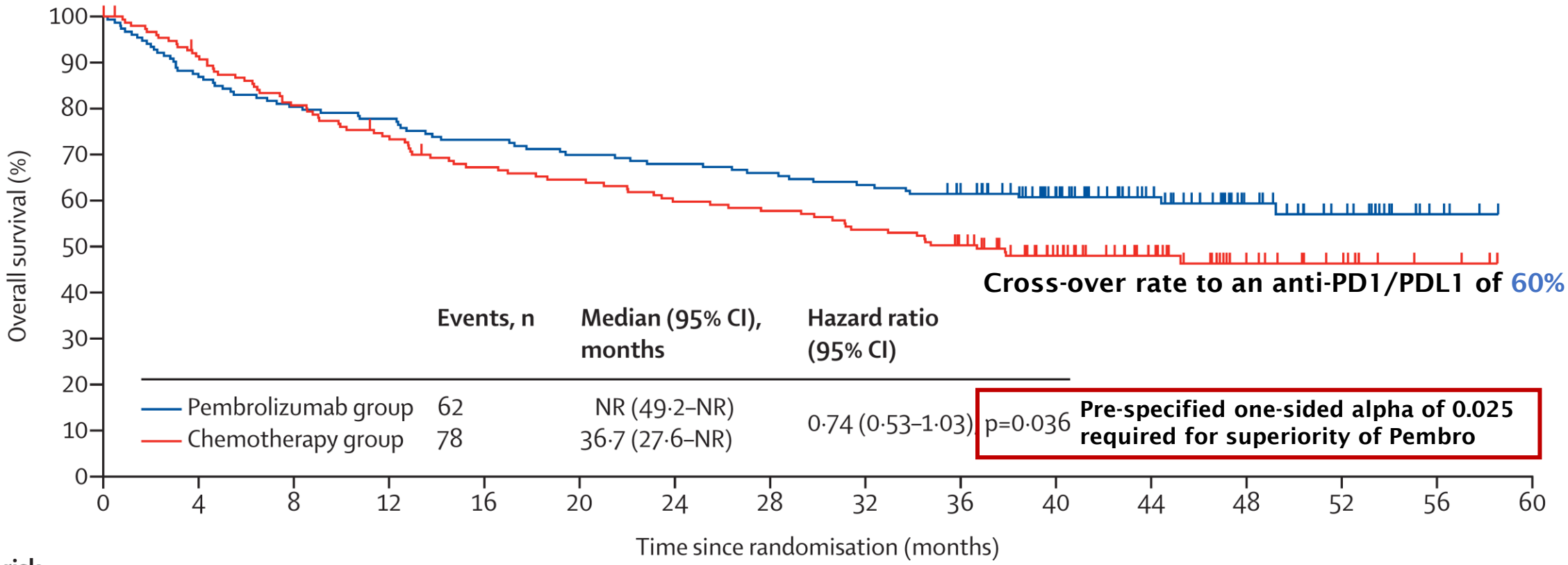
mPFS: 16.5 mos

mPFS: 8.2 mos

Objective response rate
45.1% *versus* 33.1%

André et al, N Eng J Med 2020
Diaz et al, Lancet Oncol 2022

Anti-PD1 in MSI-H mCRC - Keynote-177 – Primary endpoint was not met: OS superiority



Number at risk (number censored)															
Pembrolizumab group	153 (0)	134 (0)	123 (0)	119 (0)	112 (0)	107 (0)	104 (0)	101 (0)	97 (2)	92 (23)	70 (45)	48 (64)	28 (75)	16 (78)	4 (91)	0 (91)
Chemotherapy group	154 (4)	137 (4)	121 (5)	110 (6)	99 (6)	95 (6)	88 (6)	85 (6)	79 (9)	71 (24)	53 (41)	36 (58)	18 (65)	11 (73)	3 (76)	0 (76)

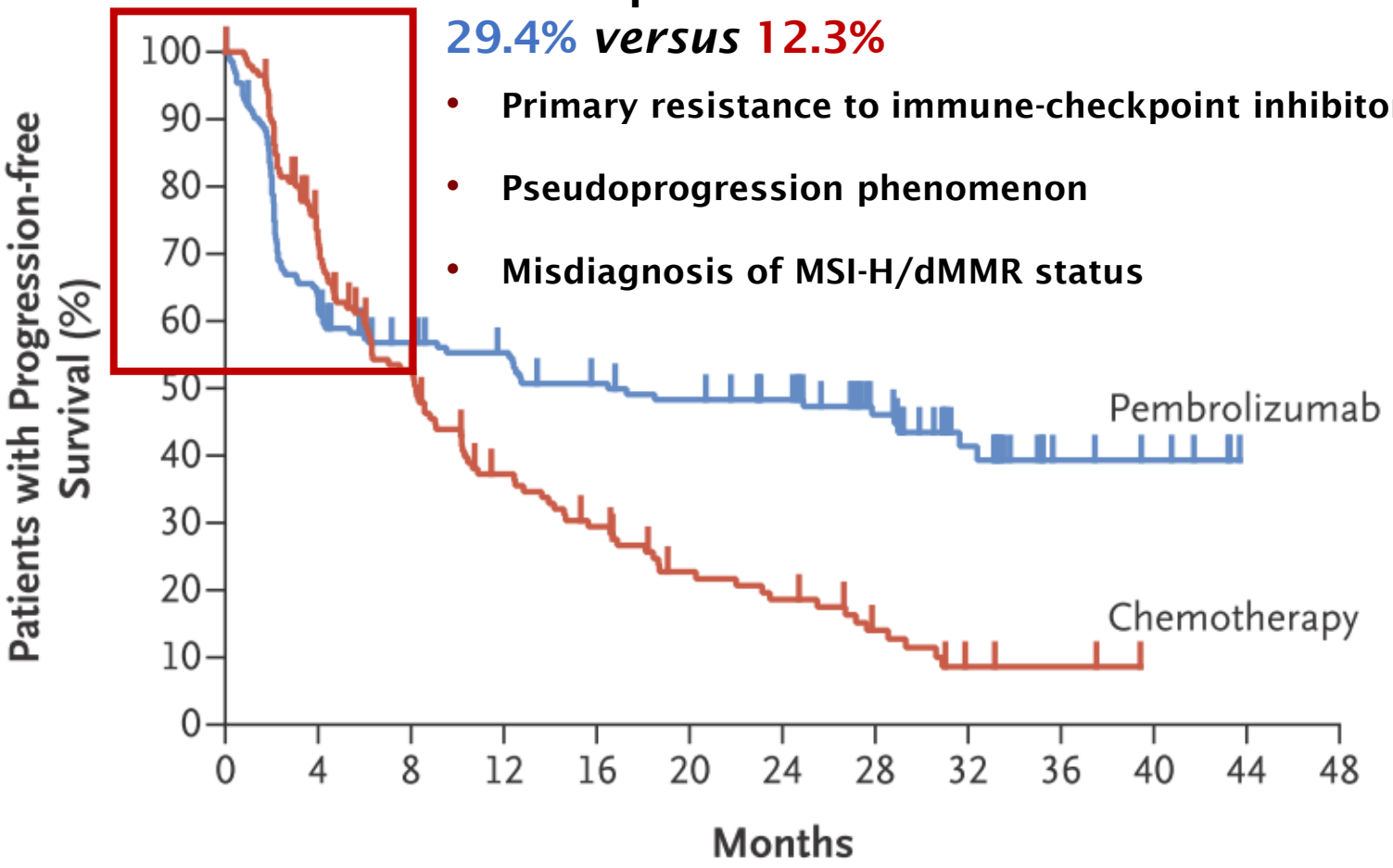
Diaz et al, Lancet Oncol 2022

Primary resistance to pembrolizumab

Best response PD

29.4% *versus* 12.3%

- **Primary resistance to immune-checkpoint inhibitors**
- **Pseudoprogression phenomenon**
- **Misdiagnosis of MSI-H/dMMR status**

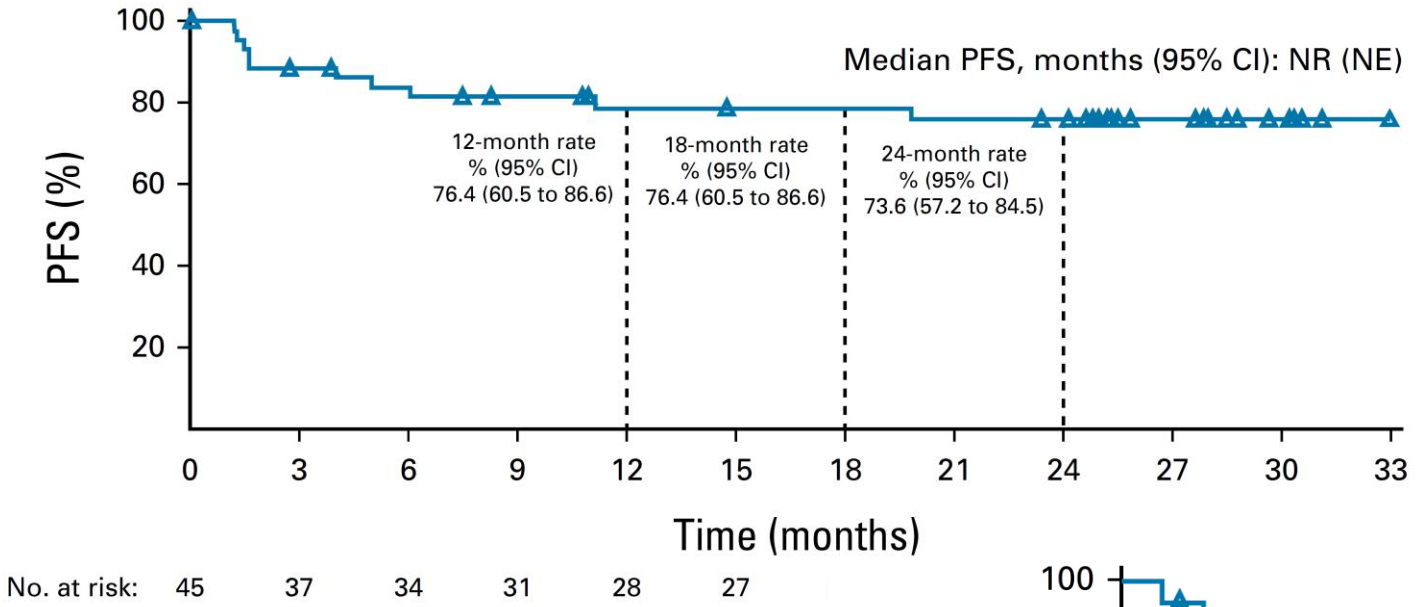


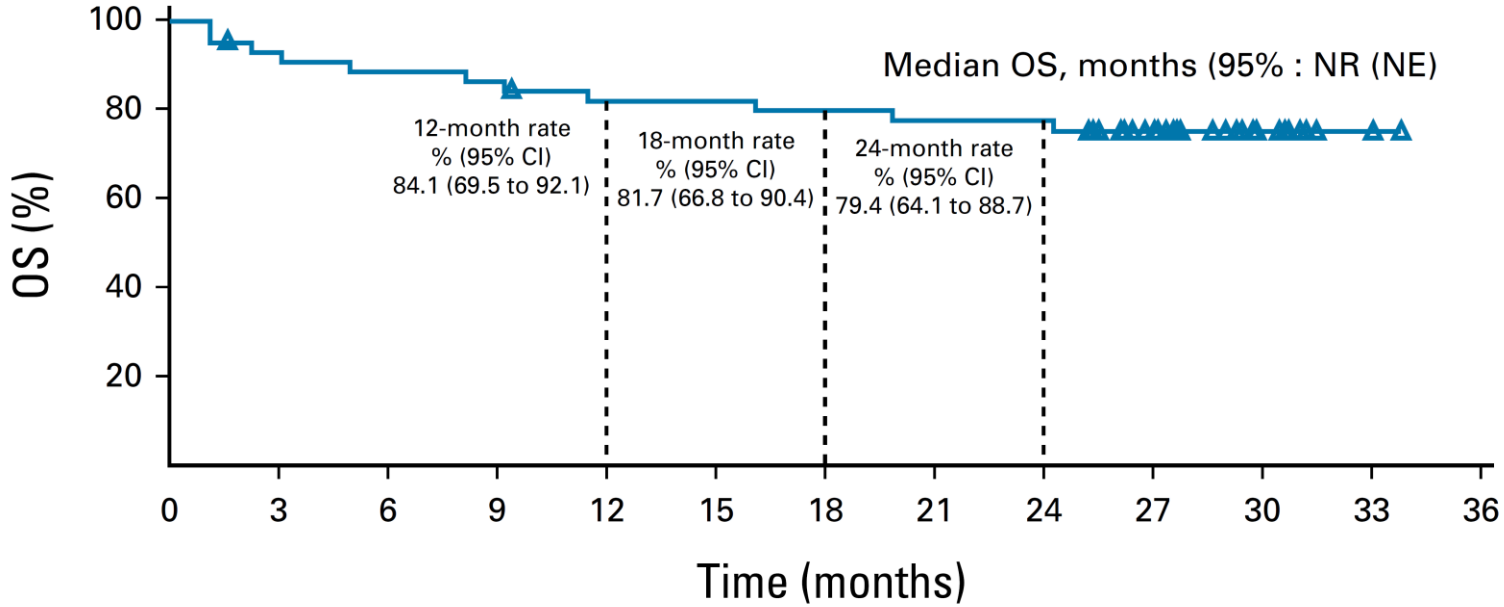
No. at Risk

Pembrolizumab	153	96	77	72	64	60	55	37	20	7	5	0	0
Chemotherapy	154	100	68	43	33	22	18	11	4	3	0	0	0

André et al, N Eng J Med 2020

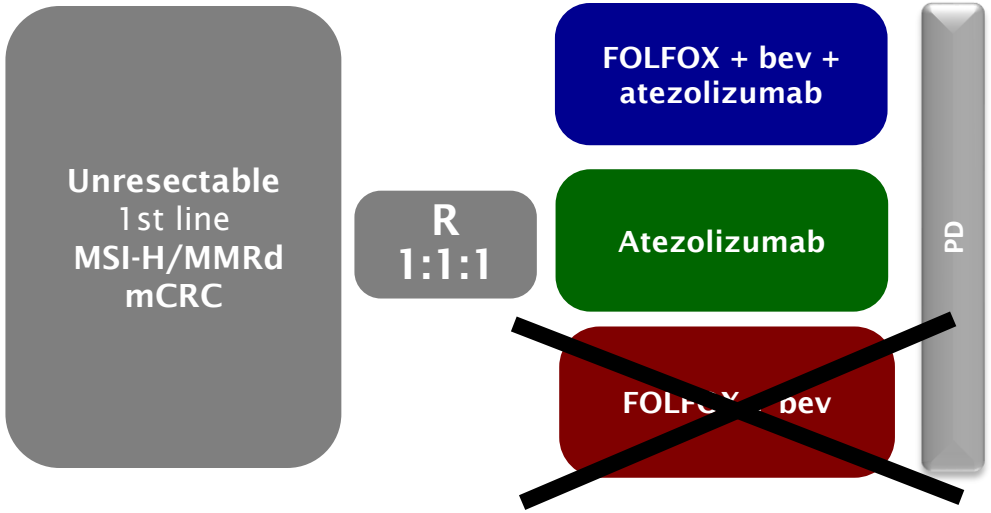
Combination strategies: Nivo + Ipi in first line





Lenz et al, J Clin Oncol 2021

Combination strategies: Ongoing studies in first line

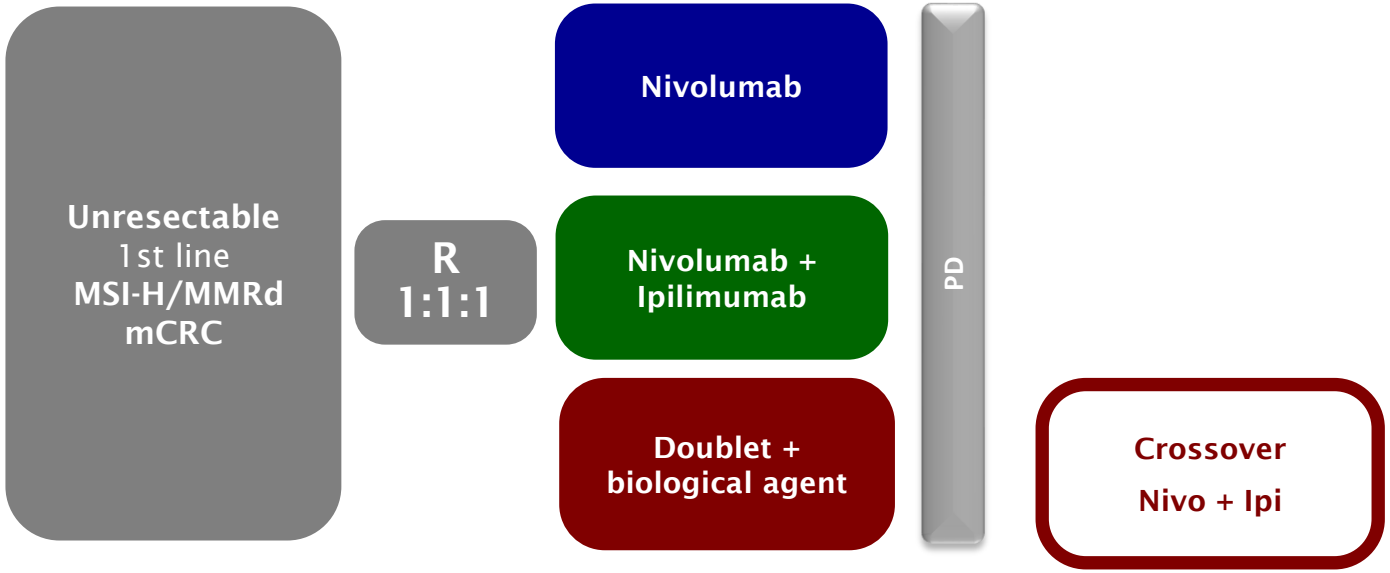


COMMIT

Phase III trial

Primary endpoint:
- **PFS**

ClinicalTrials.gov Identifier: NCT02997228



CheckMate 8HW

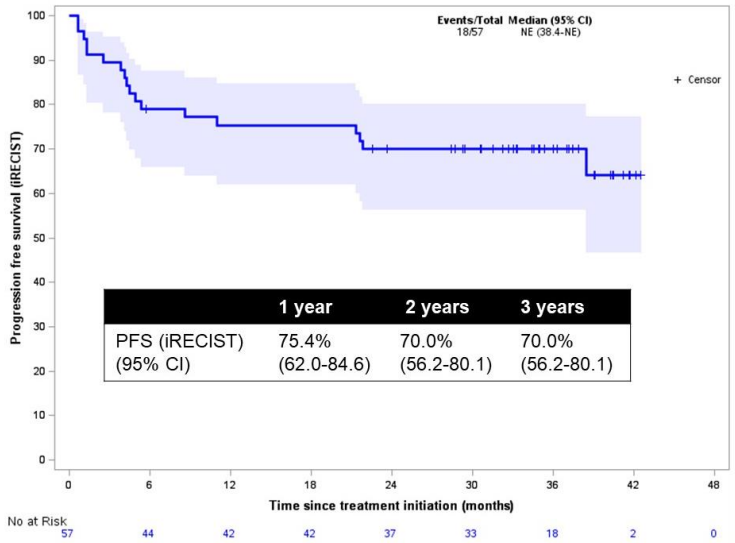
Phase III trial

Primary endpoint:
- **PFS**

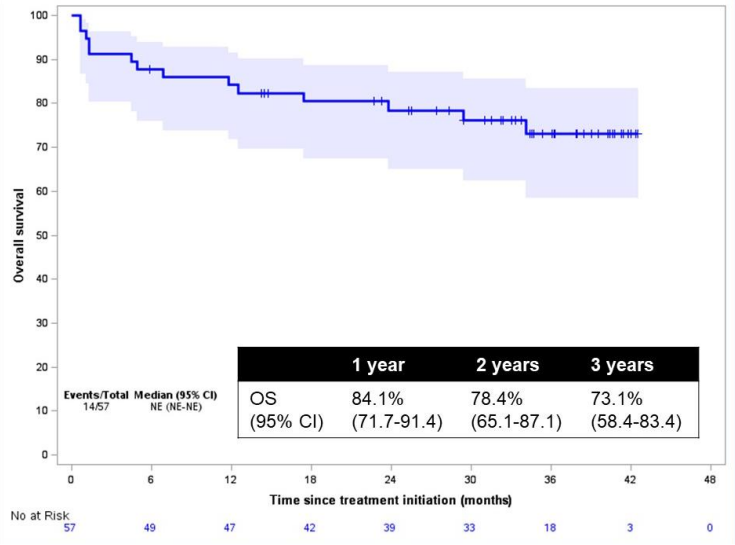
ClinicalTrials.gov Identifier: NCT04008030

Duration of immunotherapy in chemorefractory MSI-H/dMMR mCRC

Immunoterapy with Nivo+Ipi until PD or maximum 1 year
Median follow-up: 35.5 months



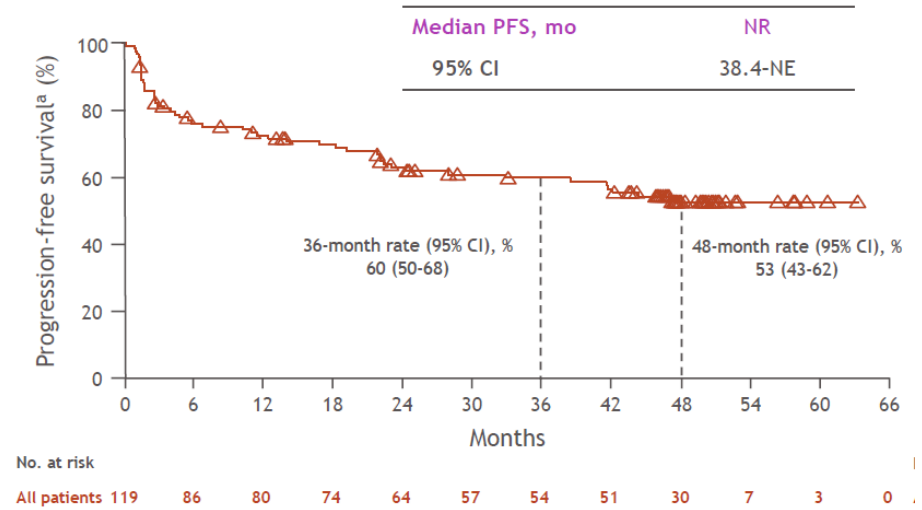
	1 year	2 years	3 years
PFS (iRECIST) (95% CI)	75.4% (62.0-84.6)	70.0% (56.2-80.1)	70.0% (56.2-80.1)

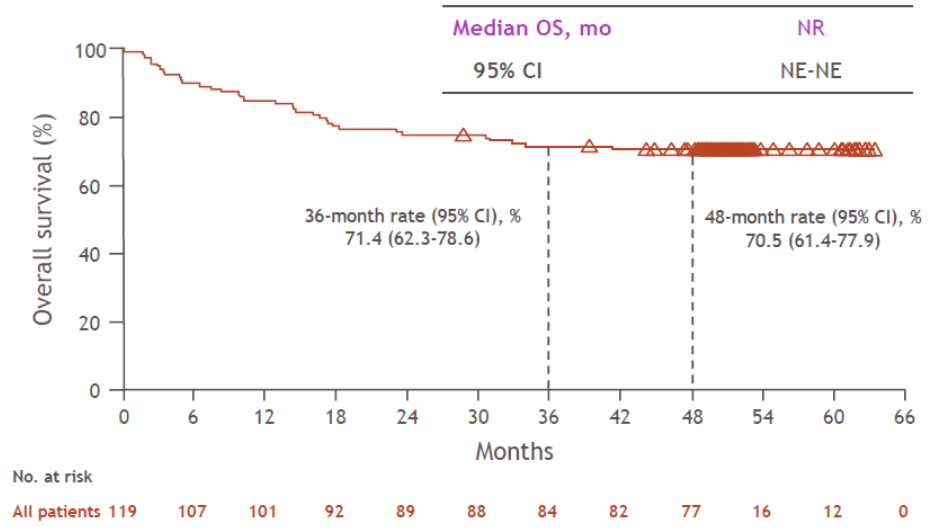


	1 year	2 years	3 years
OS (95% CI)	84.1% (71.7-91.4)	78.4% (65.1-87.1)	73.1% (58.4-83.4)

Cohen et al, ASCO GI 2022

Immunoterapy with Nivo+Ipi until PD (median duration of therapy was 24.9 months)
Median follow-up: 50.9 months

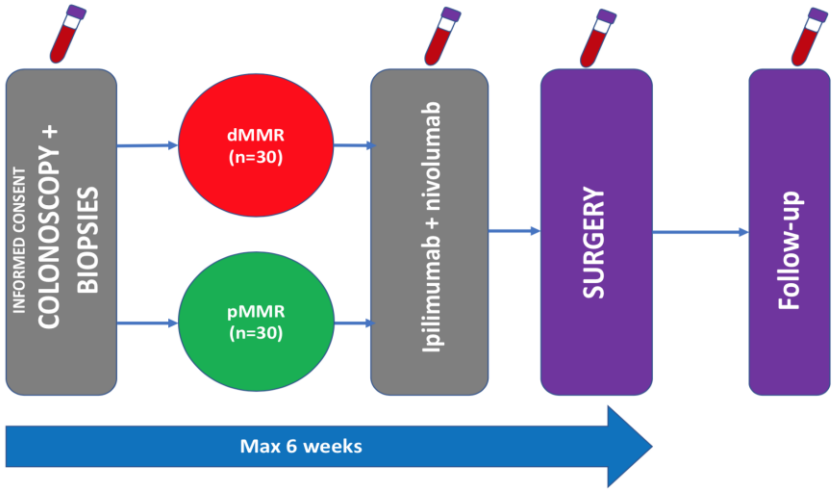


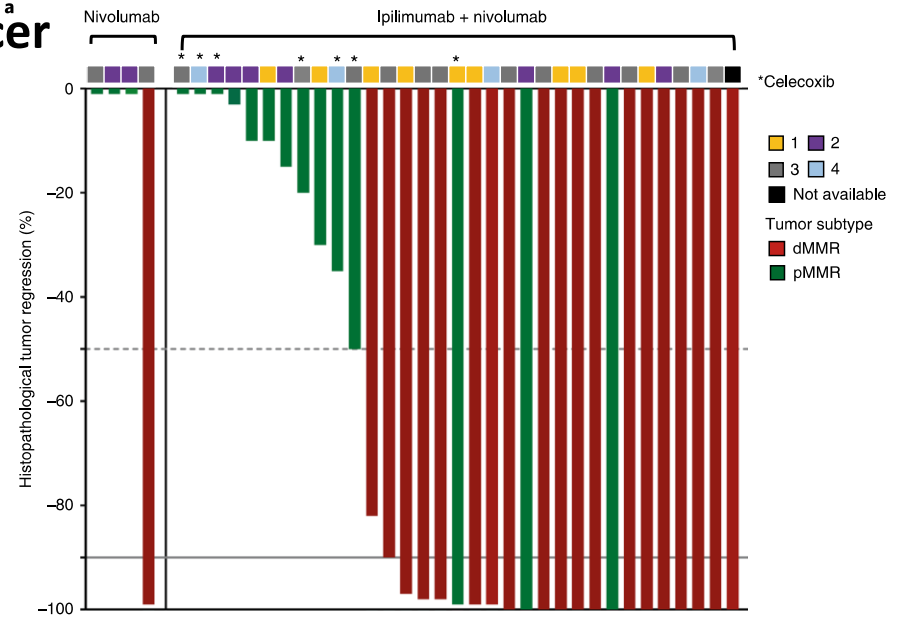


Andrè et al, WCGI 2021

Bringing immunotherapy to early stages colon cancer

Preop Nivo +/- Ipi in MSI-high early colon cancer[a]





Chalabi et al, Nat Med 2020

Preop anti-PD1 in MSI-high early colon cancer

Patient	Pathological response		Radiographic response		
	Primary tumour	Lymph Nodes	Extraluminal mucin after treatment	Progressive homogeneity of mucin after treatment	RECIST 1.1
1	Near -complete	0 of 81	Yes	Yes	Stable
2	Complete	0 of 43	Yes	Yes	Partial response
3	Complete	0 of 18	Yes	Yes	Partial response
4	Complete	0 of 22	Yes	Yes	Partial response
5	Complete	0 of 59	No	No	Partial response
6	Complete	1 of 91	Yes	Yes	Partial response
7	Complete	0 of 19	–*	Yes	Stable
8	Complete	0 of 21	Yes	Yes	Stable
9	Complete	0 of 11	Yes	n.a.	Stable

Kothari et al, Br J Surg 2022

Bringing immunotherapy to early stages rectal cancer

Phase II, single arm study in dMMR/MSI-H LARC treated with neoadj dostarlimab for 6 months
Co-primary endpoints:

- **ORR**
- **cCR or pCR with or without CTRT at 12 months**

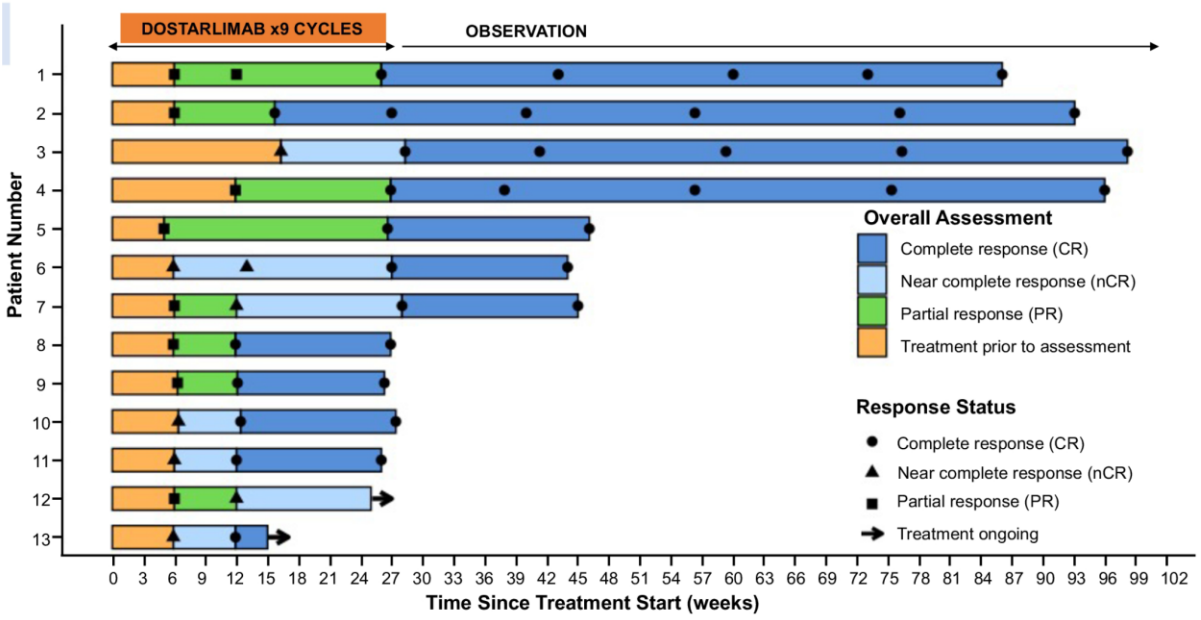


Figure 1. Endoscopic response from time of treatment initiation (n=13). Thirteen of 16 patients underwent minimum of two endoscopic assessments. Eleven (11) patients completed 6 months of dostarlimab and 100% (11/11) achieved endoscopic CR without chemoRT or surgery. Patient 13 achieved endoscopic CR prior to completing treatment.

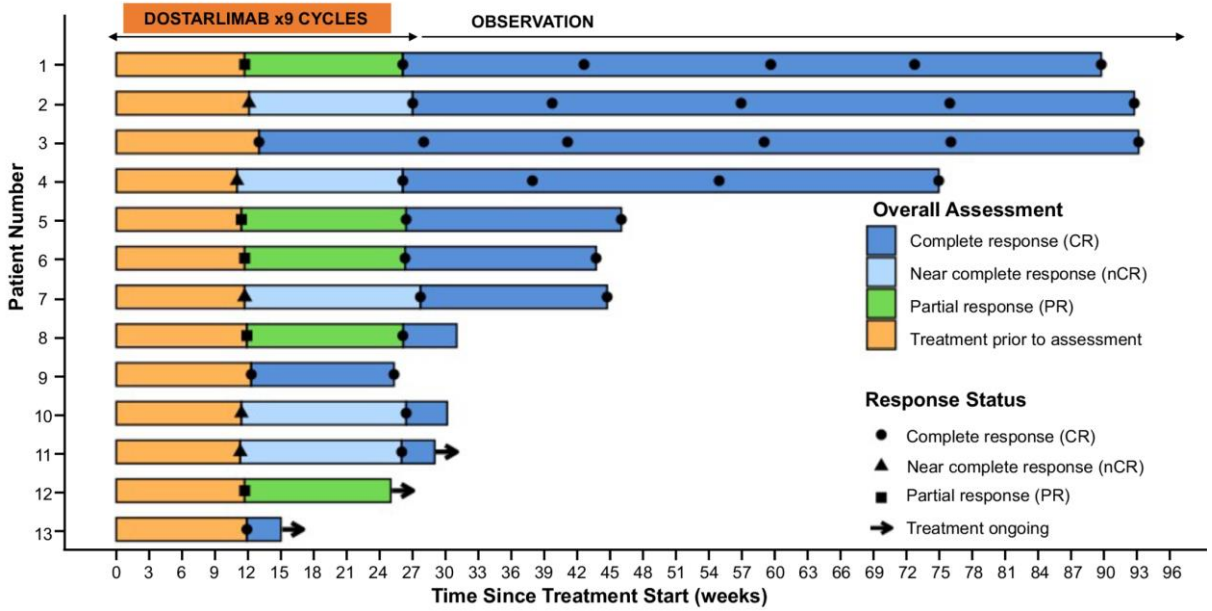
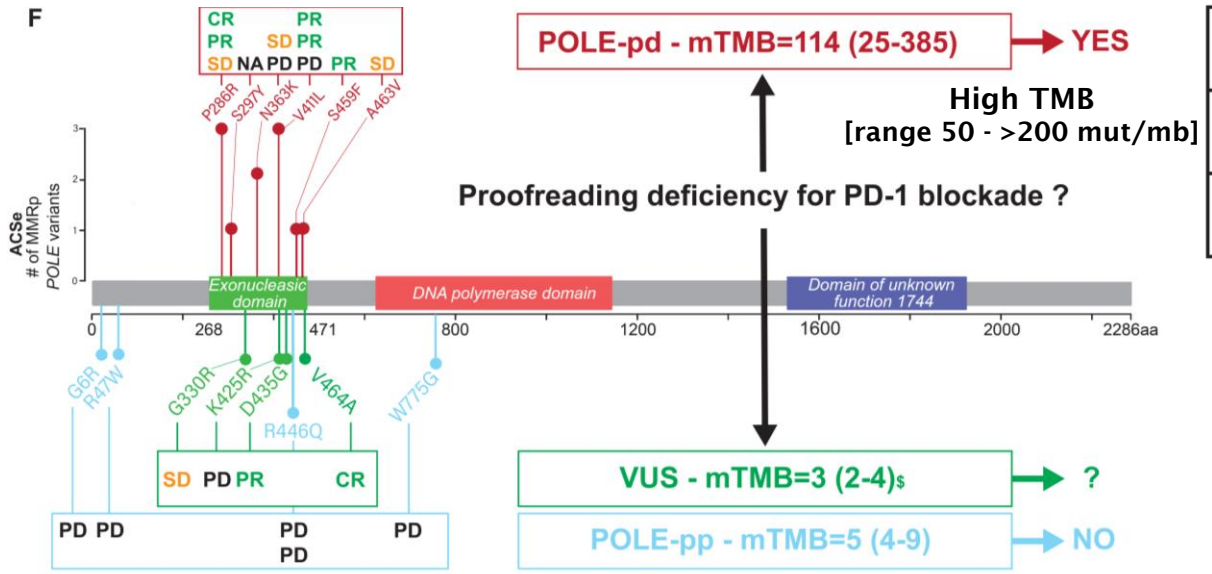


Figure 2. Radiographic response from time of treatment initiation (n=13). Thirteen of 16 patients underwent minimum of one radiographic assessment. Eleven (11) patients completed the full 6 months of dostarlimab and 100% (11/11) achieved radiographic CR. Patient 13 achieved radiographic CR prior to completing treatment.

Lumish et al., ASCO GI 2022

Ultramutated mCRC: *POLE* mutations

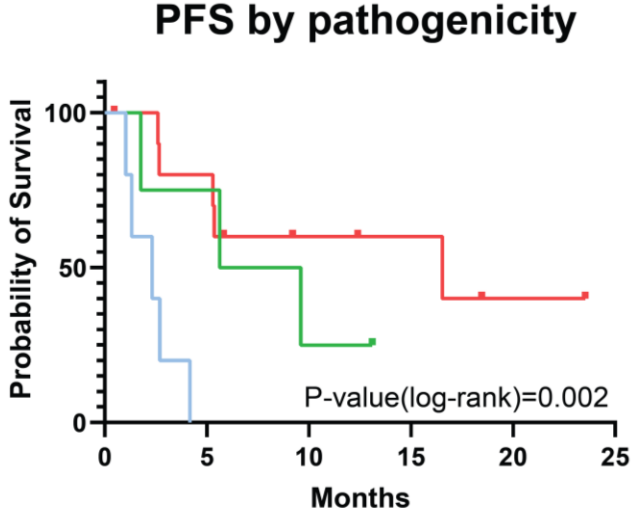


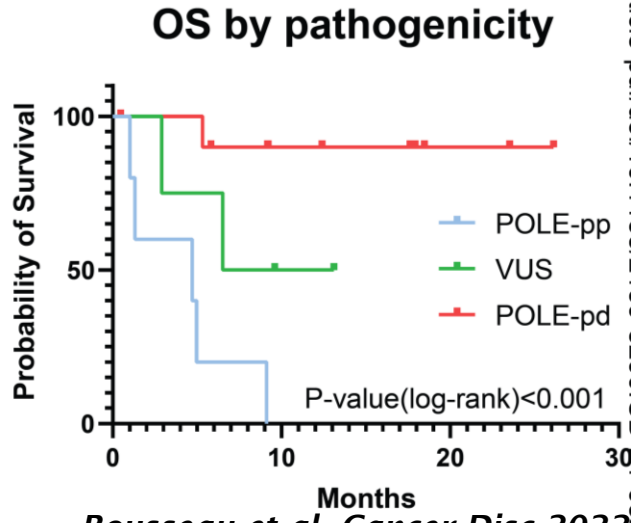
PanCancer IMPACT	# Samples	POLE alteration total	Pathogenic missense	Non-pathogenic or unknown
All Stage	43,680	1,469 (3.4%)	167 (0.4%)	1,302 (3.0%)
Metastasis	15,672	533 (3.4%)	13 (0.1%)	520 (3.3%)

Colorectal
0.6 to 1.7%

Nivolumab

Outcome	POLE-pp N=5	POLE-pd/VUS N=15*	HR (CI95%)
ORR at 12W	0%	50% (7/14)	-
DCR at 12W	0%	79% (11/14)	-
mPFS	2.3 months	9.6 months 16.5/7.6	0.2 (0.1-0.7)
mOS	4.7 months	Not reached NR/9.8	0.1 (0.02-0.7)

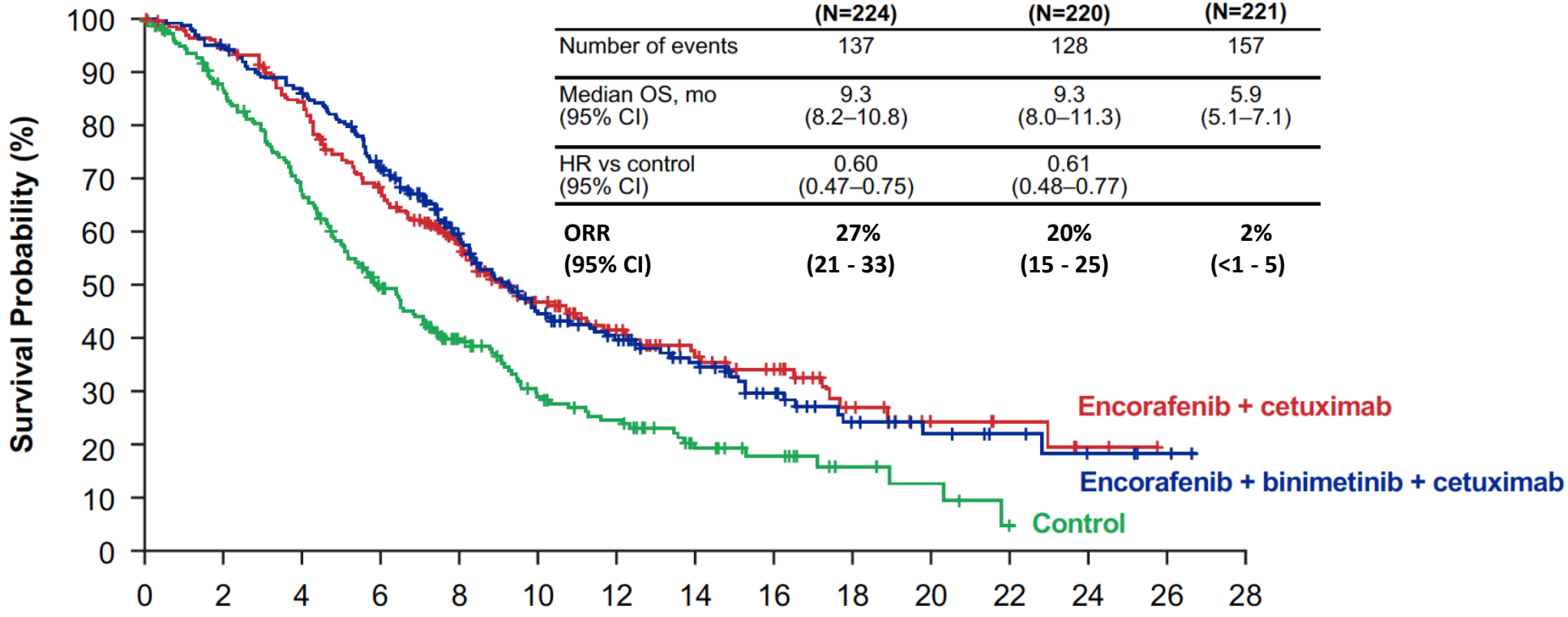




Rousseau et al, Cancer Disc 2022

Anti-BRAFV600E + anti-EGFR +/- anti-MEK in advanced mCRC: BEACON trial

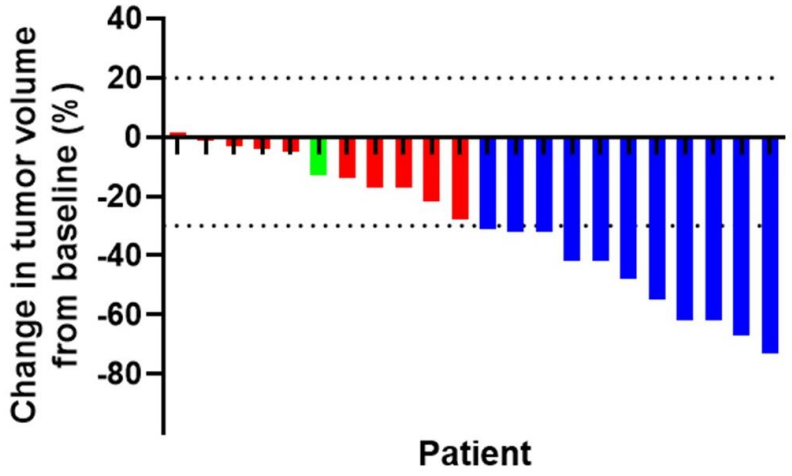
	Encorafenib + binimetinib + cetuximab (N=224)	Encorafenib + cetuximab (N=220)	Control (N=221)
Number of events	137	128	157
Median OS, mo (95% CI)	9.3 (8.2–10.8)	9.3 (8.0–11.3)	5.9 (5.1–7.1)
HR vs control (95% CI)	0.60 (0.47–0.75)	0.61 (0.48–0.77)	
ORR (95% CI)	**27% (21 - 33)**	**20% (15 - 25)**	**2% (<1 - 5)**



No. at risk															
ENCO+BINI+CETUX	224	211	191	157	109	71	56	40	27	15	10	7	4	2	0
ENCO+CETUX	220	206	181	143	105	70	47	33	26	13	7	5	2	0	0
Control	221	183	142	98	65	42	33	18	13	6	4	1	0	0	0

Tabernero et al, JCO 2021

Anti-BRAFV600E + anti-EGFR + anti-PD-1in refractory *BRAF* mut mCRC



- Partial Response
- Stable Disease
- Progressive Disease

22 evaluable patients:

ORR 50%(95%CI, 28-72)
DCR 96% (95% CI, 77-100)

SWOG 2107

Pts with MSS, *BRAF*^{V600E} metastatic CRC, AND
- 1-2 prior lines of systemic therapy
- ECOG PS 0-1
- No prior (1) BRAF, MEK, ERK; (2) anti-EGFR; or (3) immune checkpoint therapy

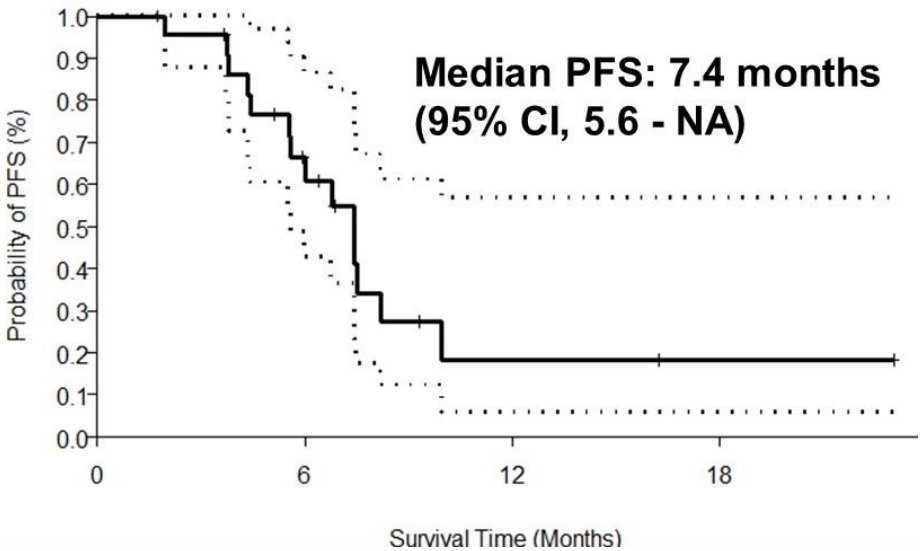
R

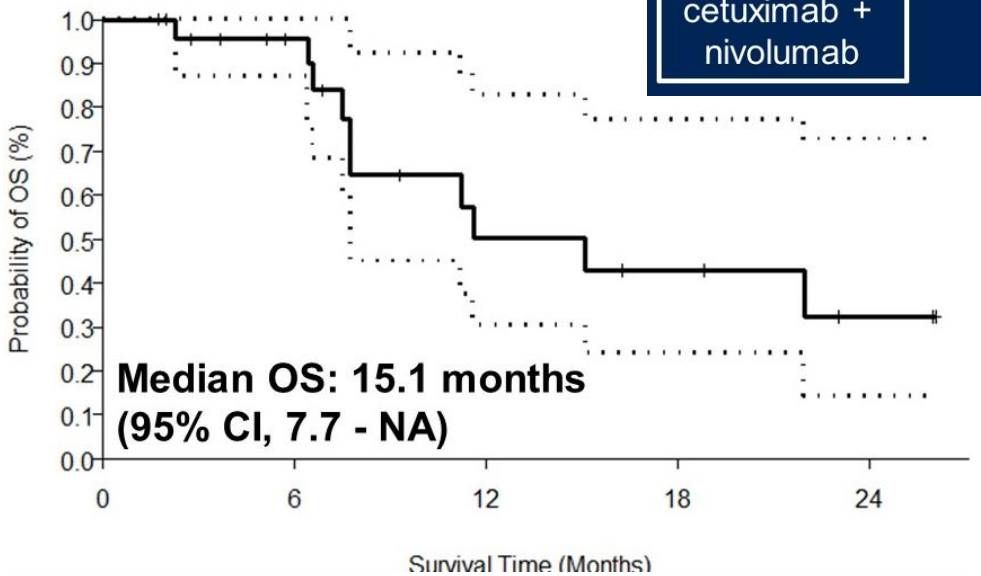
2:1

N=75

Encorafenib + cetuximab + nivolumab

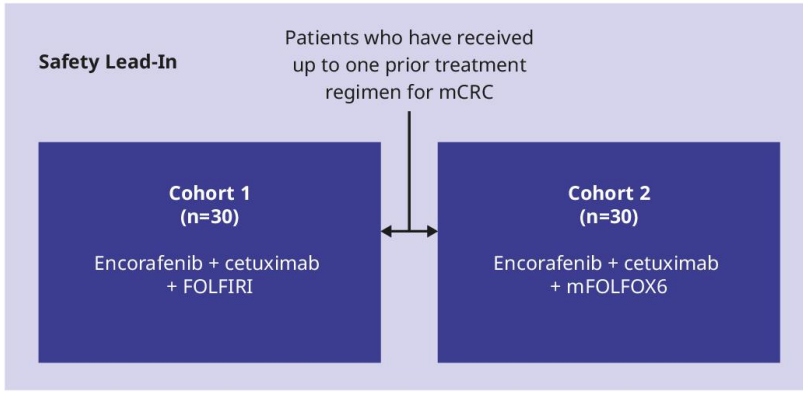
Encorafenib + cetuximab





Morris et al, ASCO-GI 2022

Anti-BRAFV600E + anti-EGFR +/- chemotherapy in first-line *BRAF* mut mCRC: BREAKWATER trial – safety lead-In results



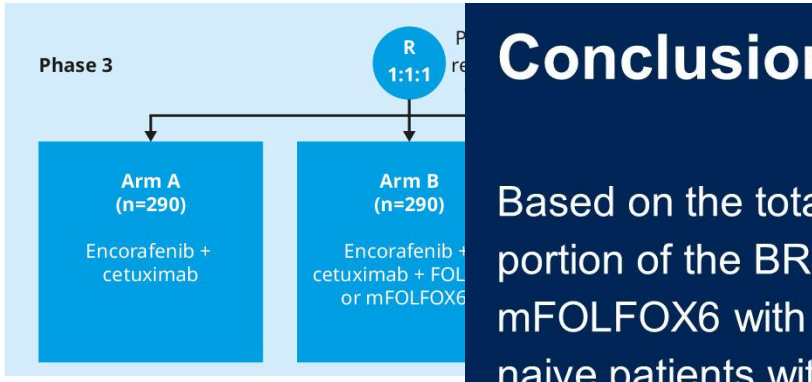


Table 3. Treatment-Emergent All-Causality AEs Occurring in ⩾20% of Patients in Either Cohort

AE (Preferred Term), n (%)	EC + mFOLFOX6 (n=27)		EC + FOLFIRI (n=30)	
	All Grades	Grade ⩾3	All Grades	Grade ⩾3
Nausea	19 (70.4)	0	13 (43.3)	0
Peripheral sensory neuropathy	10 (37.0)	0	2 (6.7)	0
Pyrexia	9 (33.3)	0	3 (10.0)	0
Vomiting	8 (29.6)	1 (3.7)	3 (10.0)	0
Alopecia	2 (7.4)	0	6 (20.0)	1 (3.3)

Conclusion

Based on the totality of the BREAKWATER Safety Lead-In data, the phase 3 portion of the BREAKWATER trial will compare encorafenib + cetuximab ± mFOLFOX6 with mFOLFOX6/FOLFOXIRI/CAPOX ± bevacizumab in treatment-naive patients with *BRAF* V600E-mutant mCRC

DRUG–DRUG INTERACTIONS

- In the EC + mFOLFOX6 cohort, steady-state encorafenib did not significantly alter oxaliplatin exposures compared with values after a single encorafenib dose (Table 4)
- In the EC + FOLFIRI cohort, AUC_{inf} of irinotecan and its active metabolite SN-38 significantly decreased by 24% and 38%, respectively, in the presence of steady-state encorafenib compared with values in the absence of encorafenib; changes in C_{max} were not significant (Table 5)

Table 4. Plasma PK Parameters of Platinum in Plasma and Plasma Ultrafiltrate in the EC + mFOLFOX6 Cohort

Analyte	Parameter	n	Geometric Least Squares Mean: Cycle 1 Day 1	Geometric Least Squares Mean: Cycle 1 Day 15	Ratio of Geometric Least Squares Mean: Estimate	Ratio of Geometric Least Squares Mean: 90% CI
Total platinum in plasma	C_{max}, ng/mL	10	2380	2500	1.05	0.955–1.15
	AUC_{last}, h*ng/mL	10	47,400	54,400	1.15	1.03–1.28
Platinum in plasma	C_{max}, ng/mL	10	656	717	1.09	0.925–1.29
		7	6740	6820	1.01	0.939–1.09
		10	5800	6050	1.04	0.975–1.12

…ncentration–time curve extrapolated to infinity; AUC_{last}=area …st measurable concentration; CI=confidence interval; …afenib + cetuximab; PK=pharmacokinetics.

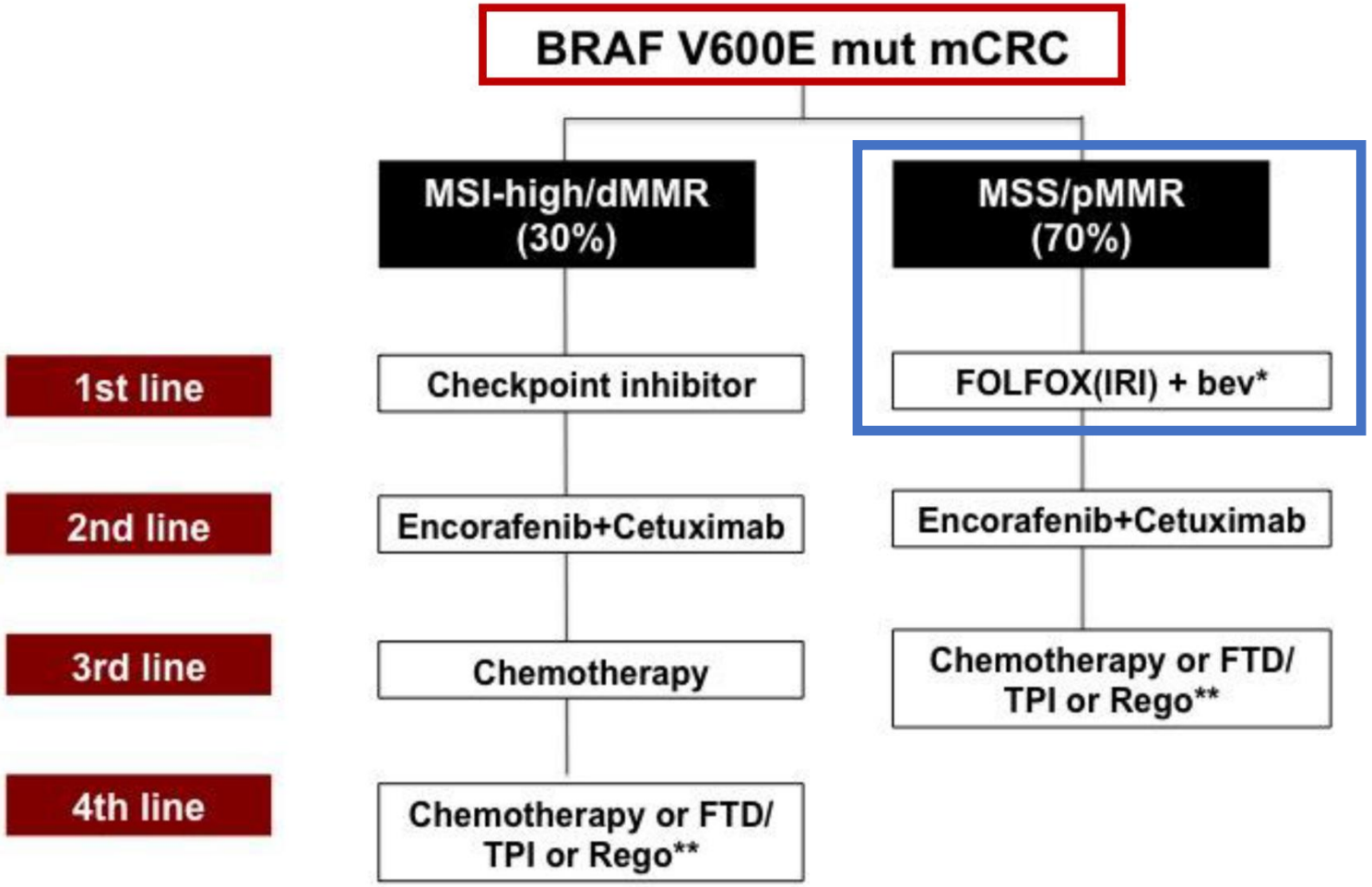
…arameters of Irinotecan and …LFIRI Cohort

		n	Geometric Least Squares Mean: Cycle 1 Day 1	Geometric Least Squares Mean: Cycle 1 Day 15	Ratio of Geometric Least Squares Mean: Estimate	Ratio of Geometric Least Squares Mean: 90% CI
	C_{max}, ng/mL	21	1790	1710	0.957	0.880–1.04
Irinotecan	AUC_{inf}, h*ng/mL	18	11,800	8910	0.756	0.697–0.820
	AUC_{last}, h*ng/mL	21	10,700	8070	0.752	0.655–0.865
	C_{max}, ng/mL	21	21.5	24.9	1.16	0.991–1.35
SN-38	AUC_{inf}, h*ng/mL	6	419	259	0.617	0.485–0.784
	AUC_{last}, h*ng/mL	21	194	142	0.730	0.552–0.967

Abbreviations: AUC_{inf}=area under the concentration–time curve extrapolated to infinity; AUC_{last}=area under the concentration–time curve to last measurable concentration; CI=confidence interval; C_{max}=maximum concentration; EC=encorafenib + cetuximab; PK=pharmacokinetics.

Kopetz et al, ASCO-GI 2022

Algorithm for *BRAFV600E* mut mCRC



* Consider reintroduction after PD in the case of very good duration of response (PFS > 12 mos);
**based on previous treatments and their outcome

Courtesy of Chiara Cremolini

Upfront chemo-intensity for *BRAFV600E* mut
Progression-free Survival – Subgroup analysis

original reports

Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer

Chiara Cremolini, MD, PhD[1]; Carlotta Antoniotti, MD[1]; Alexander Stein, MD[2]; Johanna Bendell, MD[3]; Thomas Gruenberger, MD[4]; Daniele Rossini, MD[1]; Gianluca Masi, MD[1]; Elena Ongaro, MD[1,5]; Herbert Hurwitz, MD[6]; Alfredo Falcone, MD[1]; Hans-Joachim Schmoll, MD, PhD[7]; and Massimo Di Maio, MD[8]

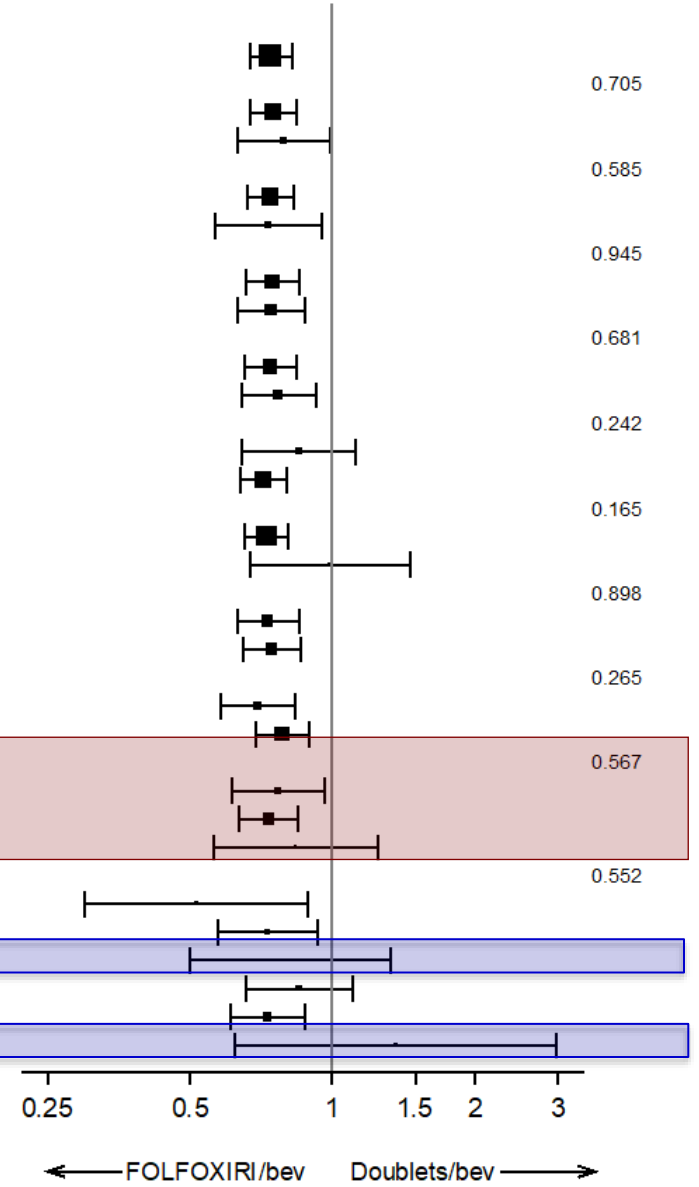
1697 mCRC pts
1st line mCRC

→ Doublet+ BV

→ FOLFOXIRI + BV

	Doublet + bev N = 851	FOLFOXIRI + bev N = 846
***BRAF* mut**	54 (8%)	61 (9%)

Subgroup	Doublets/bev Events/N (%)		FOLFOXIRI/bev Events/N (%)		HR (95% CI)	P Value
Intention to treat population	761/851	(89.4)	728/846	(86.1)	0.74 (0.67, 0.82)	
ECOG PS						0.705
0	584/656	(89.0)	571/667	(85.6)	0.75 (0.67, 0.84)	
1-2	175/192	(91.1)	153/175	(87.4)	0.79 (0.63, 0.99)	
Age						0.585
<70 years	645/722	(89.3)	605/707	(85.6)	0.74 (0.66, 0.83)	
>70 years	116/129	(89.9)	123/139	(88.5)	0.73 (0.57, 0.95)	
Gender						0.945
Male	470/518	(90.7)	424/489	(86.7)	0.75 (0.66, 0.85)	
Female	291/333	(87.4)	304/357	(85.2)	0.74 (0.63, 0.87)	
Liver only						0.681
No	538/596	(90.3)	475/543	(87.5)	0.74 (0.65, 0.84)	
Yes	223/254	(87.8)	252/300	(84.0)	0.77 (0.64, 0.92)	
Time to metastases						0.242
Metachronous	107/130	(82.3)	102/130	(78.5)	0.85 (0.64, 1.12)	
Synchronous	653/720	(90.7)	626/716	(87.4)	0.72 (0.64, 0.80)	
Previous adjuvant						0.165
No	707/790	(89.5)	674/782	(86.2)	0.73 (0.65, 0.81)	
Yes	54/61	(88.5)	54/63	(85.7)	0.99 (0.67, 1.47)	
Primary resection						0.898
No	356/386	(92.2)	351/400	(87.8)	0.73 (0.63, 0.85)	
Yes	405/465	(87.1)	377/445	(84.7)	0.75 (0.65, 0.86)	
Tumor site						0.265
Right	227/255	(89.0)	248/295	(84.1)	0.70 (0.58, 0.84)	
Left / rectum	479/535	(89.5)	442/496	(89.1)	0.78 (0.69, 0.89)	
RAS and BRAF status						0.567
RAS – BRAF wt	153/172	(89.0)	151/177	(85.3)	0.77 (0.61, 0.96)	
RAS mut	389/430	(90.5)	371/422	(87.9)	0.73 (0.64, 0.85)	
BRAF mut	48/54	(88.9)	57/61	(93.4)	0.84 (0.56, 1.25)	
Site – RAS/BRAF						0.552
Right - RAS/BRAF wt	28/31	(90.3)	35/44	(79.5)	0.52 (0.30, 0.89)	
Right - RAS mut	133/149	(89.3)	143/168	(85.1)	0.73 (0.57, 0.93)	
Right - BRAF mut	36/40	(90.0)	37/39	(94.9)	0.82 (0.50, 1.33)	
Left - RAS/BRAF wt	118/134	(88.1)	116/132	(87.9)	0.85 (0.66, 1.10)	
Left - RAS mut	249/273	(91.2)	224/250	(89.6)	0.73 (0.61, 0.88)	
Left - BRAF mut	11/13	(84.6)	20/22	(90.9)	1.36 (0.62, 2.99)	

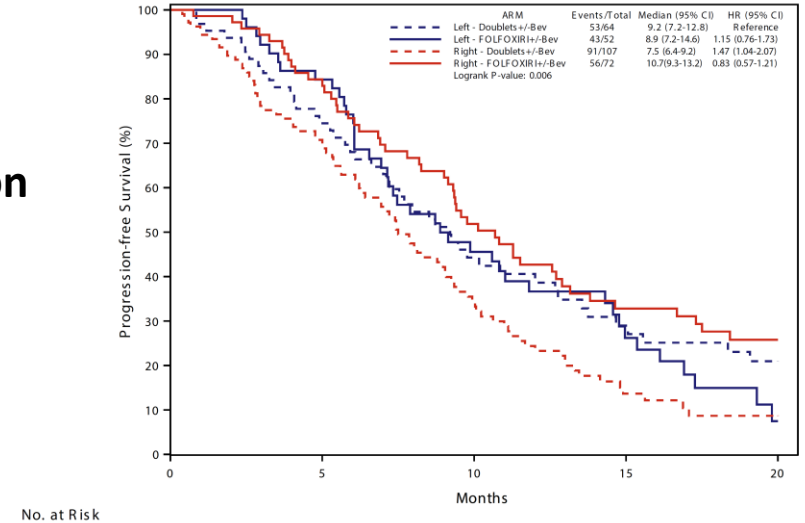


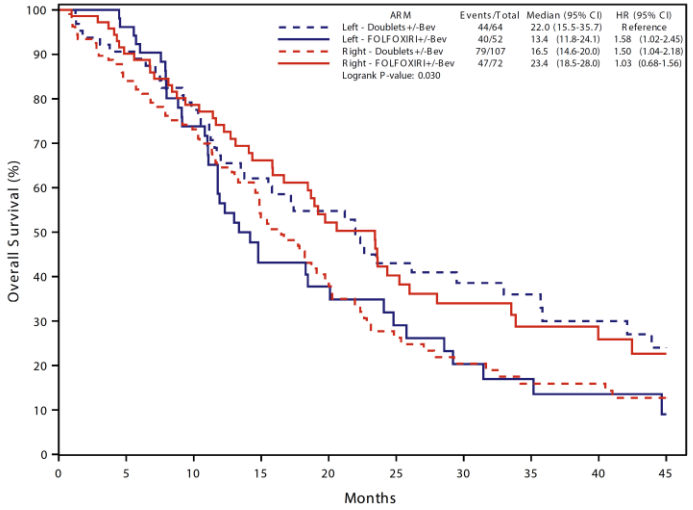
Cremolini et al, J Clin Oncol 2020

Upfront chemo-intensity for *BRAFV600E* mut based on PTL: Validation in a real-life setting – BRAF BeCool

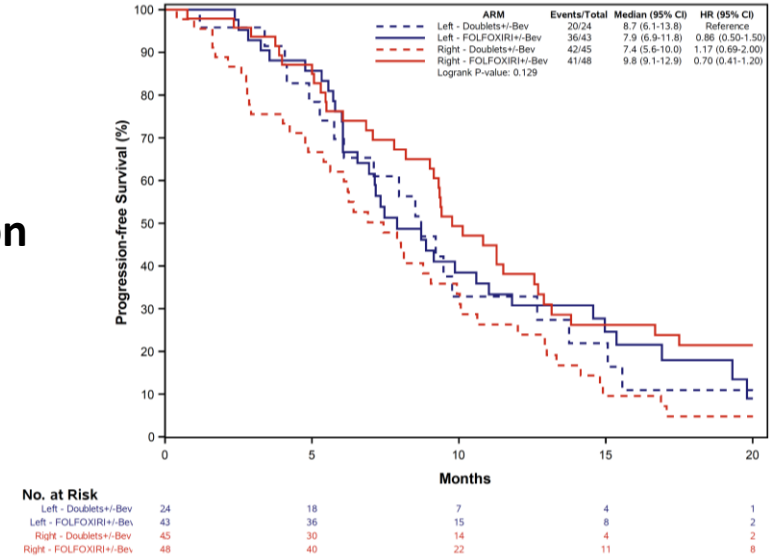
295 *BRAF* mut mCRC pts < 70 ys or 71-75 and ECOG PS 0

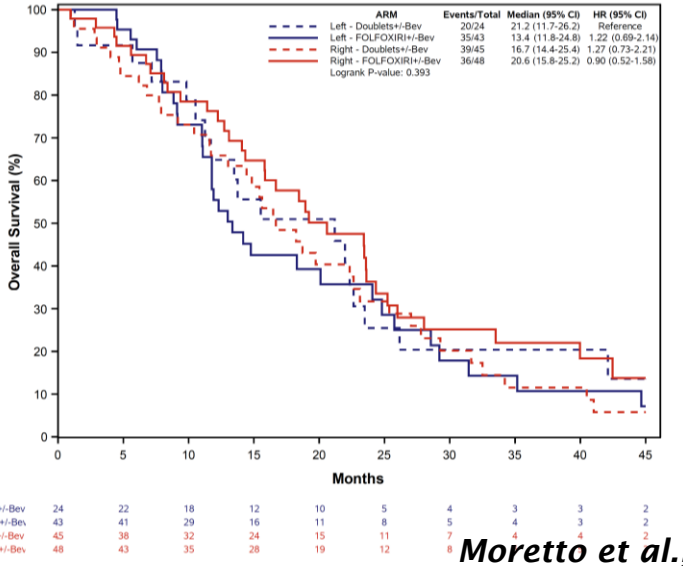
ITT population





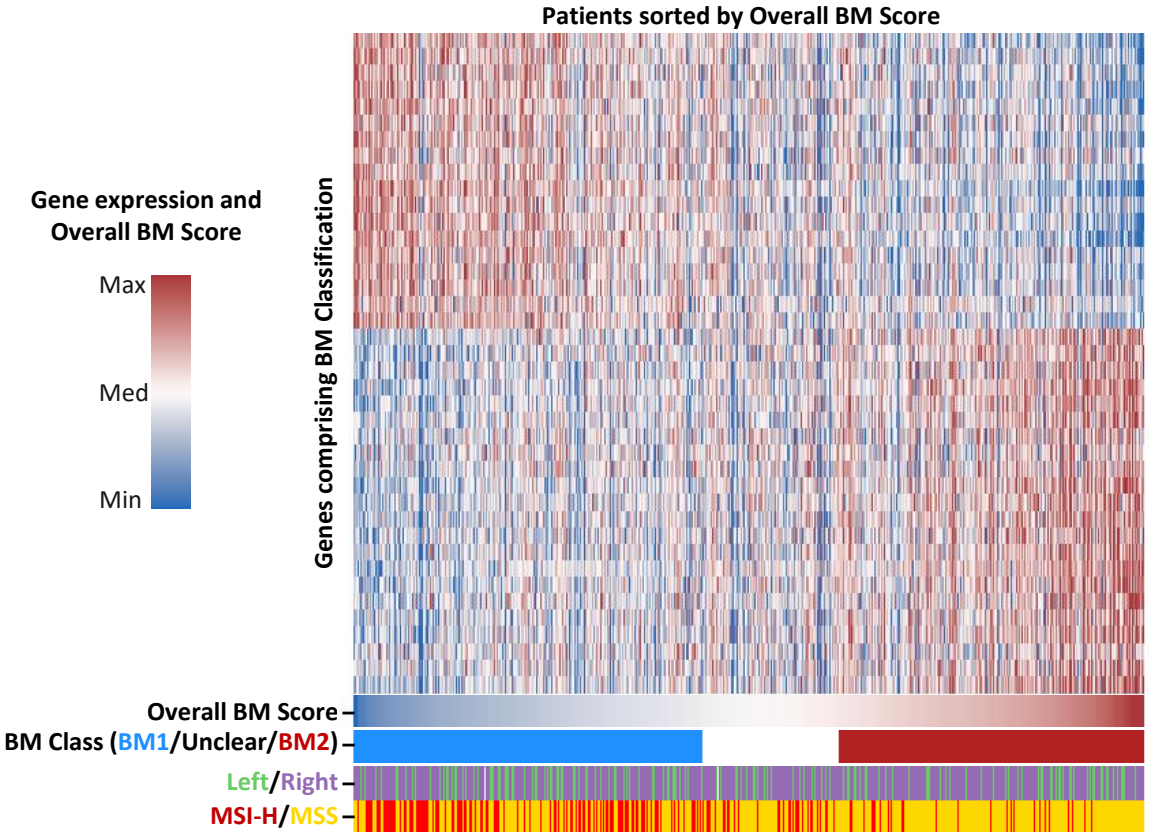
MSS population

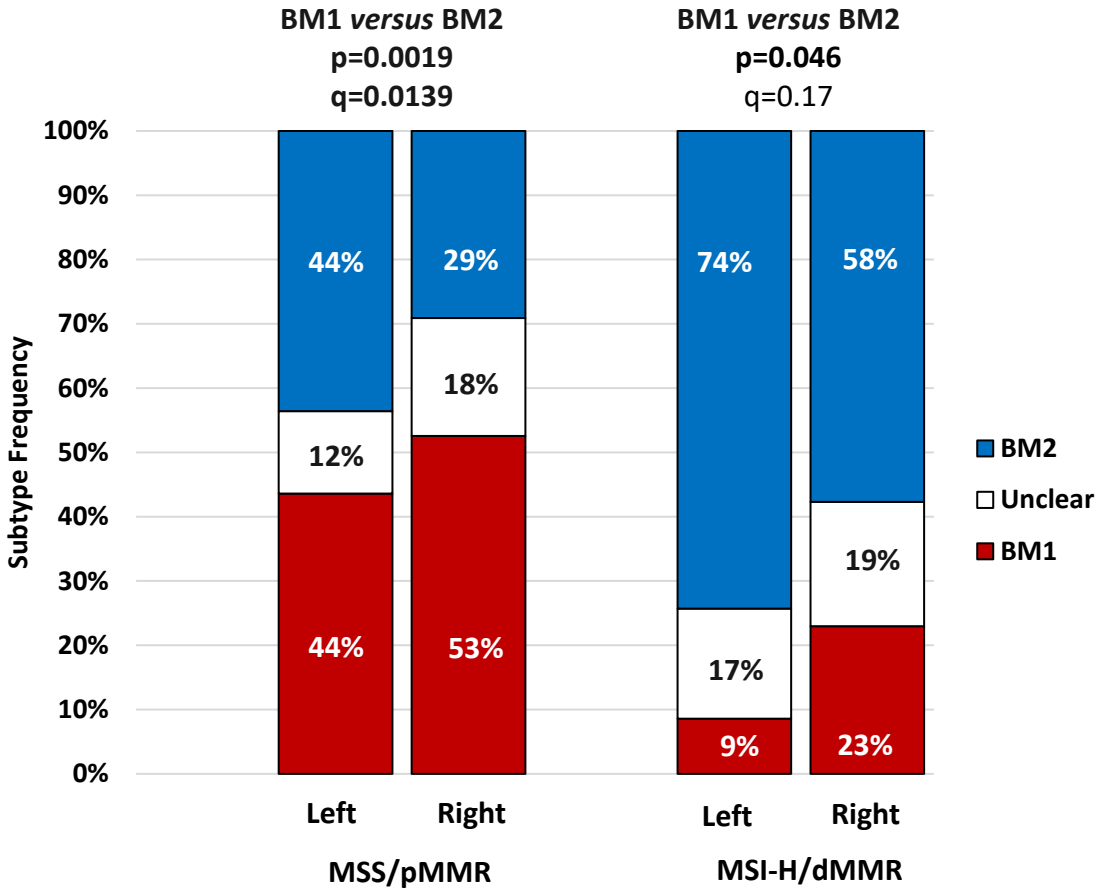




Moretto et al., Br J Cancer, under publication

Upfront chemo-intensity for *BRAFV600E* mut based on PTL: Validation in a real-life setting – BRAF BeCool





Moretto et al., Br J Cancer, under publication

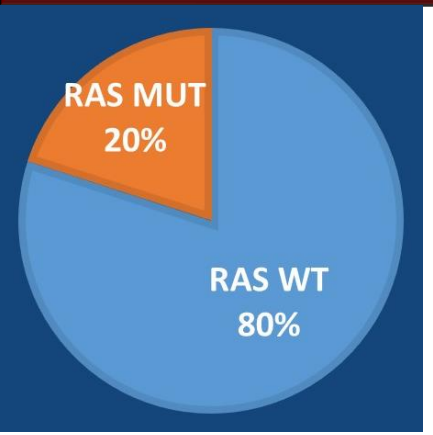
Anti-HER2 treatments: consistent efficacy results

	HERACLES-A[1] n=27	MyPathway[2] n=43*	TRIUMPH[3] n=27/25 tissue/ctDNA	TAPUR[4] n=28	MOUNTAINEER[5] n=26	DESTINY-CRC01[6] n=53**	Tsurutani[7] N=20	HERACLES-B[8] n=30***	Yuan[9] n=11	Meric-Bernstam[10] n=13
Regimen	**Trastuzumab + Lapatinib**	**Trastuzumab + Pertuzumab**	**Trastuzumab + Pertuzumab**	**Trastuzumab + Pertuzumab**	**Trastuzumab + Tucatinib**	**Trastuzumab-deruxtecan**	**Trastuzumab -deruxtecan**	**T-DM1 + Pertuzumab**	**Trastuzumab + Pyrotinib**	**Zanidatamab**
Response rate	30%	40%	30%/28%	25%	52%	45%	15%	10%	27%	31%
Median PFS, mos	4.8	5.3	4.0/3.1	4.0	8.1	6.9	4.1	4.8	NA	NA
Median OS, mos	10.6	14.0	10.1/8.8	25.0	18.7	15.5	NA	NA	NA	NA
Most common AEs	Fatigue (15% G3) **Rash (4%G3; 44% G1/2)** **Diarrhea (78% G1/2)**	Diarrhea (4% G3; 30% G1/2) Nausea (2% G3; 28% G1/2			Diarrhea (4% G3) Hypertension (4% G3)	Nausea (5% G3/4) Anemia (10-15% G3/4) **Neutropenia (26% G3/4)** Thrombocytopenia (10% G3/G4) Diarrhea (2% G3/4; 25% G1/2) **ILD (6% G2-5) 2 fatal cases**		Thrombocytopenia (7% G3)	**diarrhea (73% G3)**	diarrhea (49% G1/2) infusion related reaction (34% G1/2)

**KRAS* wt subgroup
** 30% prior anti-HER2 therapy
*** Did not met primary endpoint

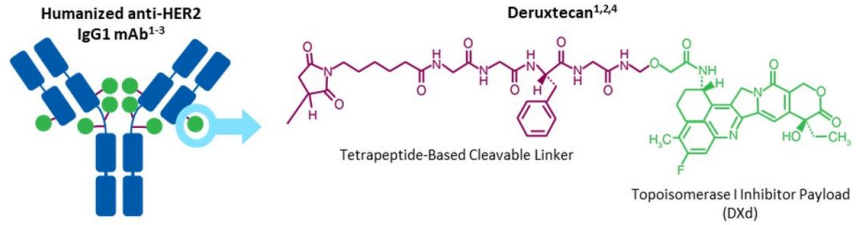
1. Sartore-Bianchi et al. Lancet Oncol 2016; 2. Meric-Bernstamet et al. Lancet Oncol 2019
3. Nakamura et al. Nature Med 2021; 4. Gupta et al. ASCO-GI Congress 2020
5. Strickler et al. ESMO Congress 2019; 6. Siena et al. Lancet Oncol 2021
7. Tsurutani J, et al. Cancer Disc 2020; 8. Sartore-Bianchi et al. ESMO Open 2020; 9. Yuan et al. ASCO-GI Congress 2021; 10. Meric-Bernstm et al. ESMO Congress 2019;

Efficacy of anti-HER2 therapy in (*K*)*RAS* mut tumours

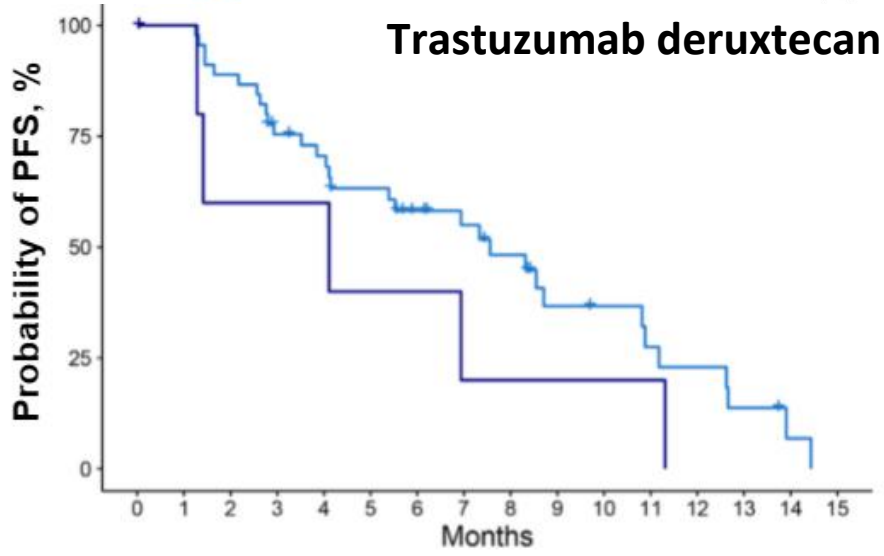


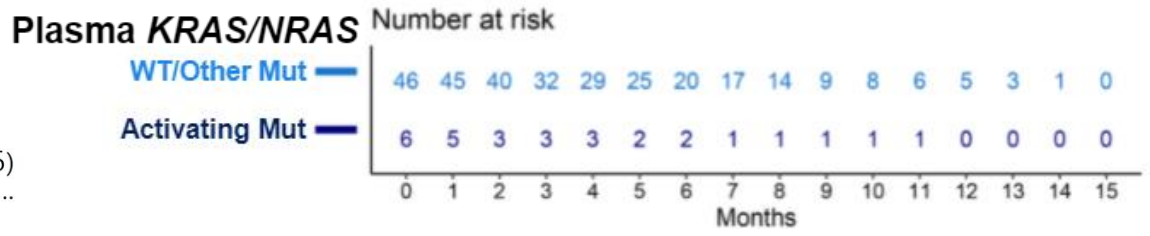
T-DXd is an ADC with 3 components:

- A humanized anti-HER2 IgG1 mAb with the same amino acid sequence as trastuzumab
- A topoisomerase I inhibitor payload, an exatecan derivative
- A tetrapeptide-based cleavable linker

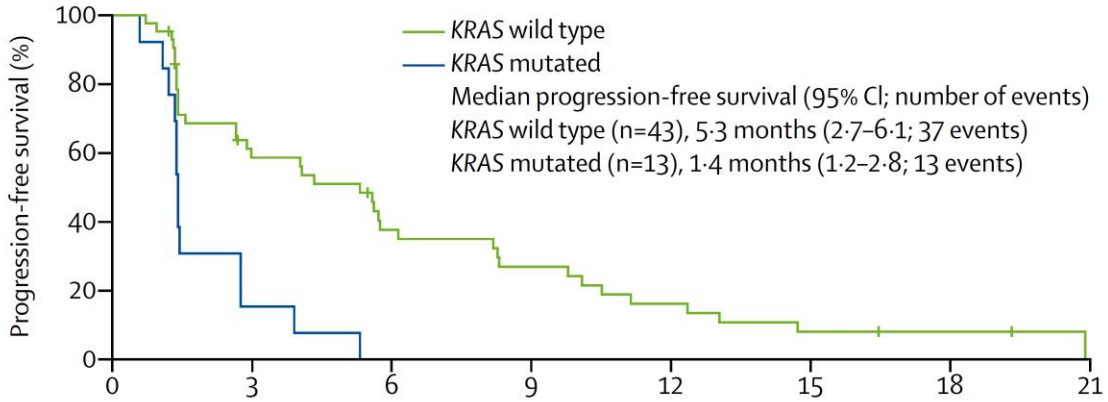


Trastuzumab deruxtecan





Trastuzumab + pertuzumab



Number at risk
(number censored)

KRAS wild type	43 (0)	23 (3)	14 (4)	10 (4)	6 (4)	3 (4)	2 (5)	0 (6)
KRAS mutated	13 (0)	2 (0)	0 (0)	..	..	..	..	..

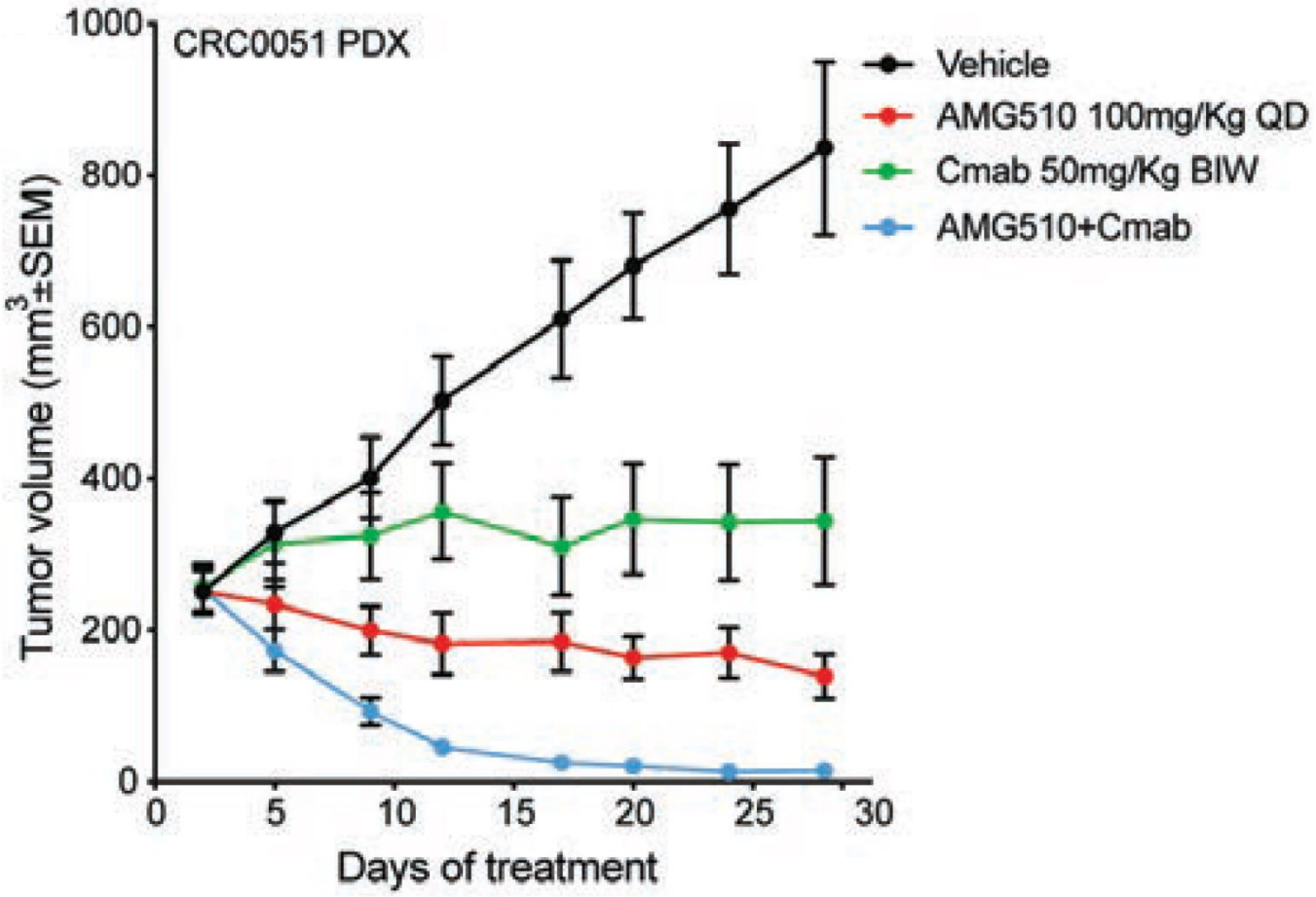
Yaeger et al. Cancer Cell 2018 ; Meric-Bernstamet et al, Lancet Oncology 2019; Siena et al. ESMO Congress 2021

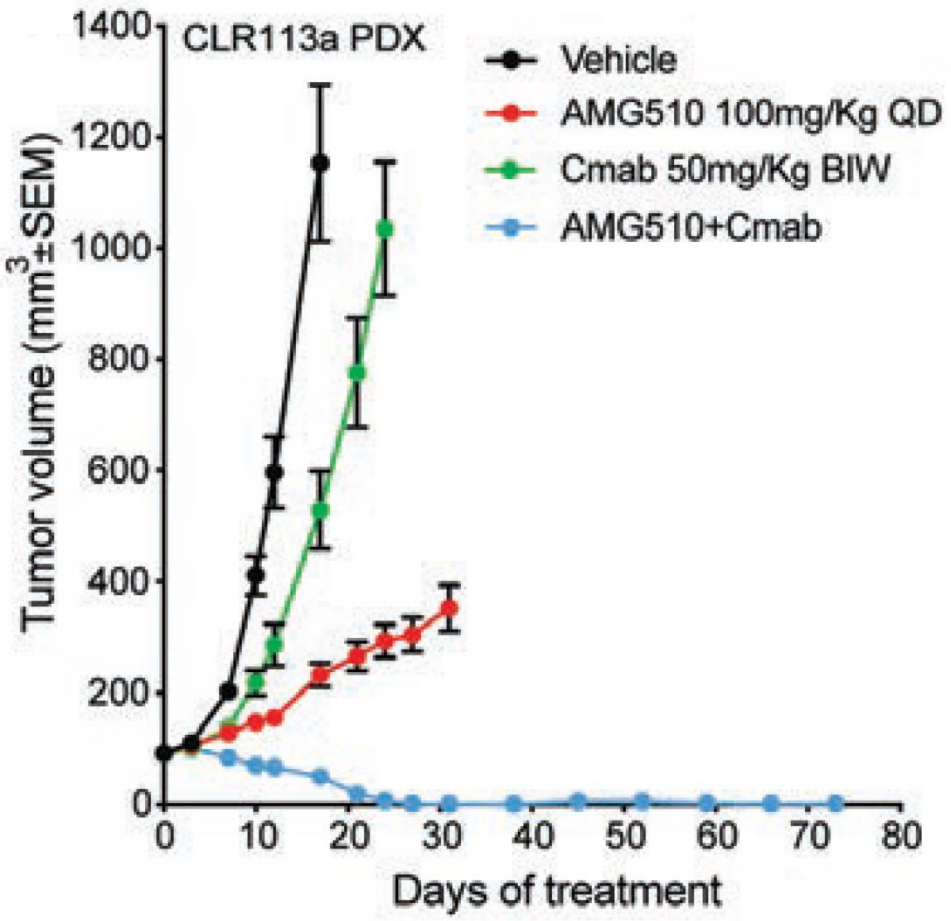
anti-HER2 strategies in HER2+ mCRC: ongoing trials

Study	Phase	N pts	Drugs	Primary endpoint	Country
HERACLES RESCUE	II	13	**T-DM1**	ORR	
MOUNTAINEER	II	115	**Tucatinib vs Tucatinib + Trastuzumab**	ORR	
NCT04430738	I/II	65	**Tucatinib + Trastuzumab + FOLFOX/CAPOX**	Safety/ORR	
NCT04380012	II	40	**Pyrotinib + Trastuzumab**	ORR	
MODUL - maintenance	II	-	**Trastuzumab + Pertuzumab + Capecitabine**	PFS	
NSABP FC-11	II	35	**Neratinib + Trastuzumab vs Neratinib + Cetuximab**	ORR	
DESTINY-CRC02	II	120 (including *RAS* mut)	**T-DXd 5.4 mg/kg vs 6.4 mg/kg**	ORR	
SWOG S1613	II	130	**Trastuzumab + Pertuzumab vs Cetuximab + Irinotecan**	PFS	

http://clinicaltrials.gov

KRAS G12C inhibitor +/- anti-EGFR: preclinical data
Similarity between *BRAF* V600E and *KRAS* G12C





Amodio et al. Cancer Discovery 2020

Anti *KRAS* G12C in chemorefractory mCRC

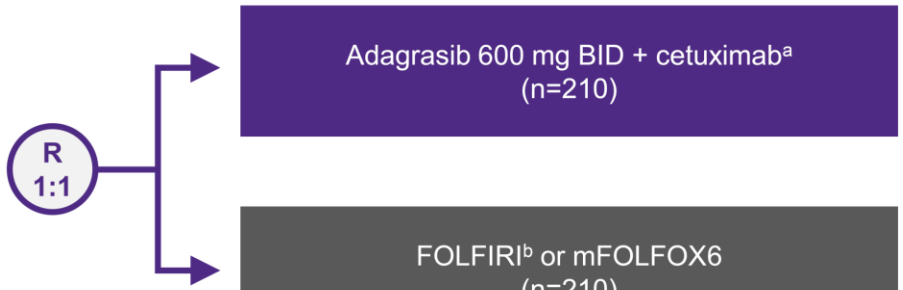
	CodeBreak100[1] n=62	CodeBreak101[2] n=31	KRISTAL-1[3] n=46	KRISTAL-1[3] n=28
Regimen	**Sotorasib**	**Sotorasib + Panitumumab**	**Adagrasib**	**Adagrasib + Cetuximab**
Response rate	10%	27%	22%	43%
Disease control rate	82%	81%	87%	100%
Median PFS, mos	4.0	NA	5.6	NA
Median OS, mos	10.6	NA	NA	NA
Most common AEs	Fatigue (9% G1/2) Nausea/Vomiting (5% G1/2) **Anemia (5% allG; 2%G3)** **Diarrhea (18% allG; 2% G3)**	**Rash acn. (59% allG; 6% G3)** Nausea (26% G1/2) **Diarrhea (23% allG; 3% G3)** **HypoK/Mg (16 allG; 3% G3)**	**Diarrhea (63% allG; G3/4 7%)** Nausea (57% G1/2) **Fatigue (46% allG; G3/4 4%)** Vomiting (46% G1/2) Decreased appetite (15% G1/2) Peripheral edema (15% G1/2) AST/ALT incr. (13% allG; G3/4 4%) **QT prolong. (13% allG; G1/2 2%)** Anemia (11% allG; 2% G3/4)	Nausea (63% G/2) **Diarrhea (56% allG; G3/4 3%)** Vomiting (50% G1/2) Fatigue (47% G1/2) **Rash acn (44% allG; 3% G3/4)** **Infusion react.(19% allG; G3/4 3%)** Peripheral edema (19% G/2) **Stomatitis (19% allG; 3% G3/4)** **QT prolong. (16% allG; 3% G3/4)** ALT incr. (13% G1/2)

1.Fakih et al., Lancet Oncol 2021
2. Fakih et al., ESMO Congress 2021
3. Weiss et al., ESMO Congress 2021

anti-KRASC12C strategies in advanced mCRC: ongoing phase III trials

Key Eligibility Criteria

- Histologically confirmed diagnosis of advanced or metastatic CRC
- Confirmed $KRAS^{G12C}$ mutation in tumor tissue
- Progression on 1L fluoropyrimidine-based regimen containing oxaliplatin or irinotecan

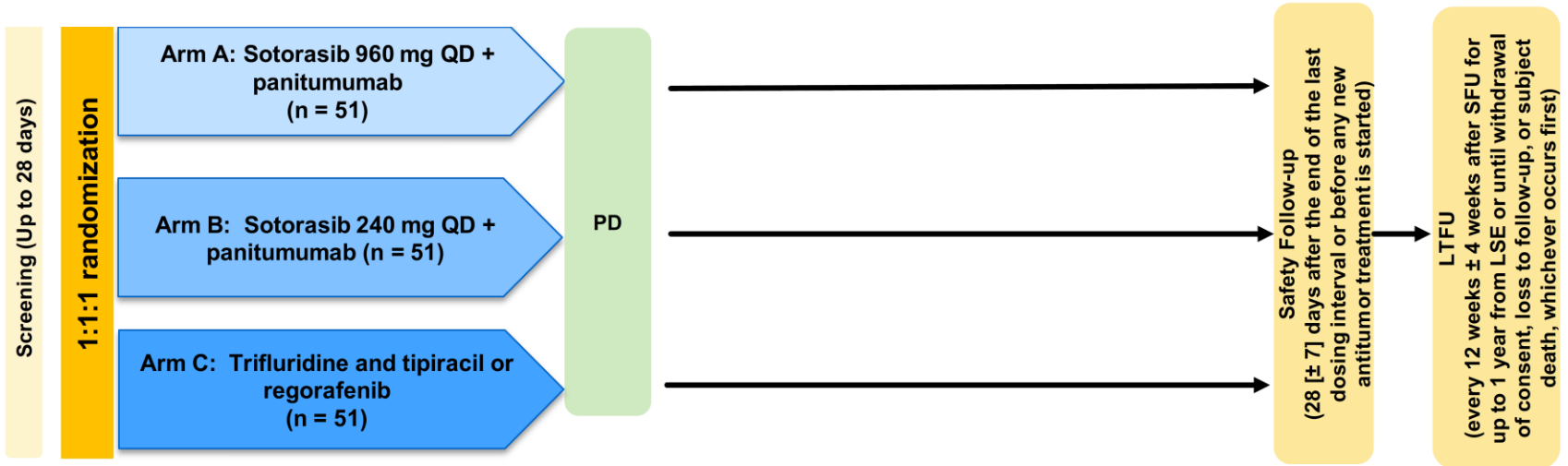


Anti-VEGF/VEGFR allowed per investigator discretion in comparator arm

Outcome Measures

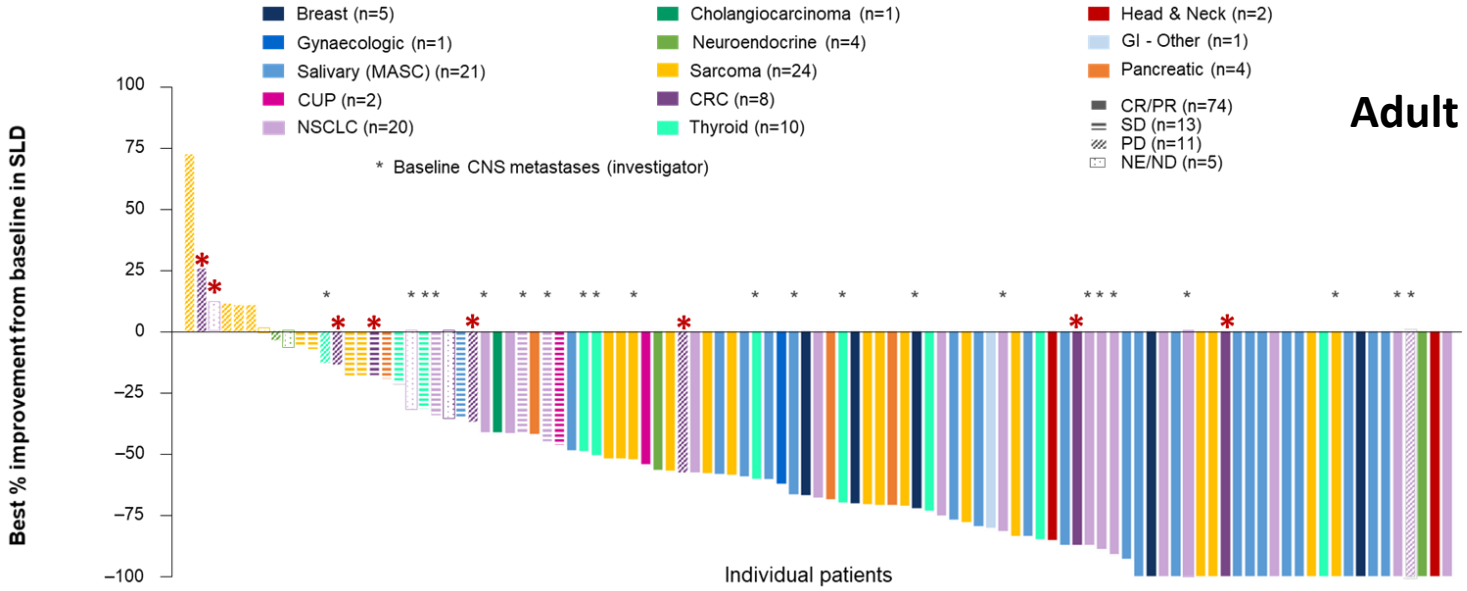
Primary: PFS, OS

Secondary: Safety, ORR (RECIST 1.1), 1-year OS, DOR, PK, PROs



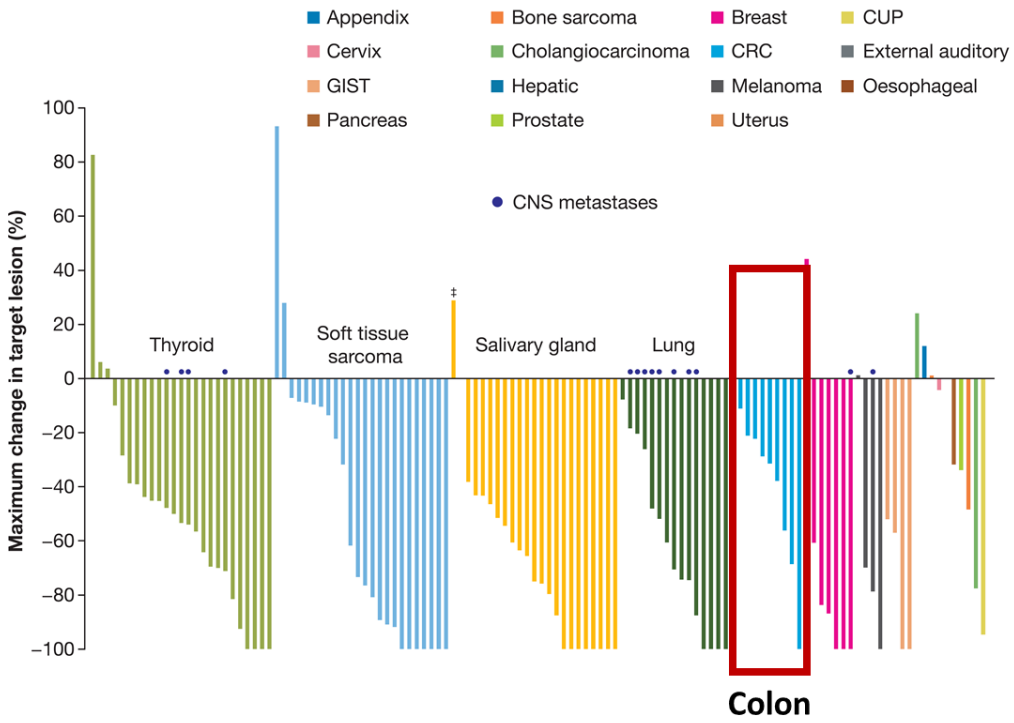
KRYSTAL-10, NCT04793958
CodeBreak-300, NCT05198934

Gene fusions as a target: entrectinib and larotrectinib



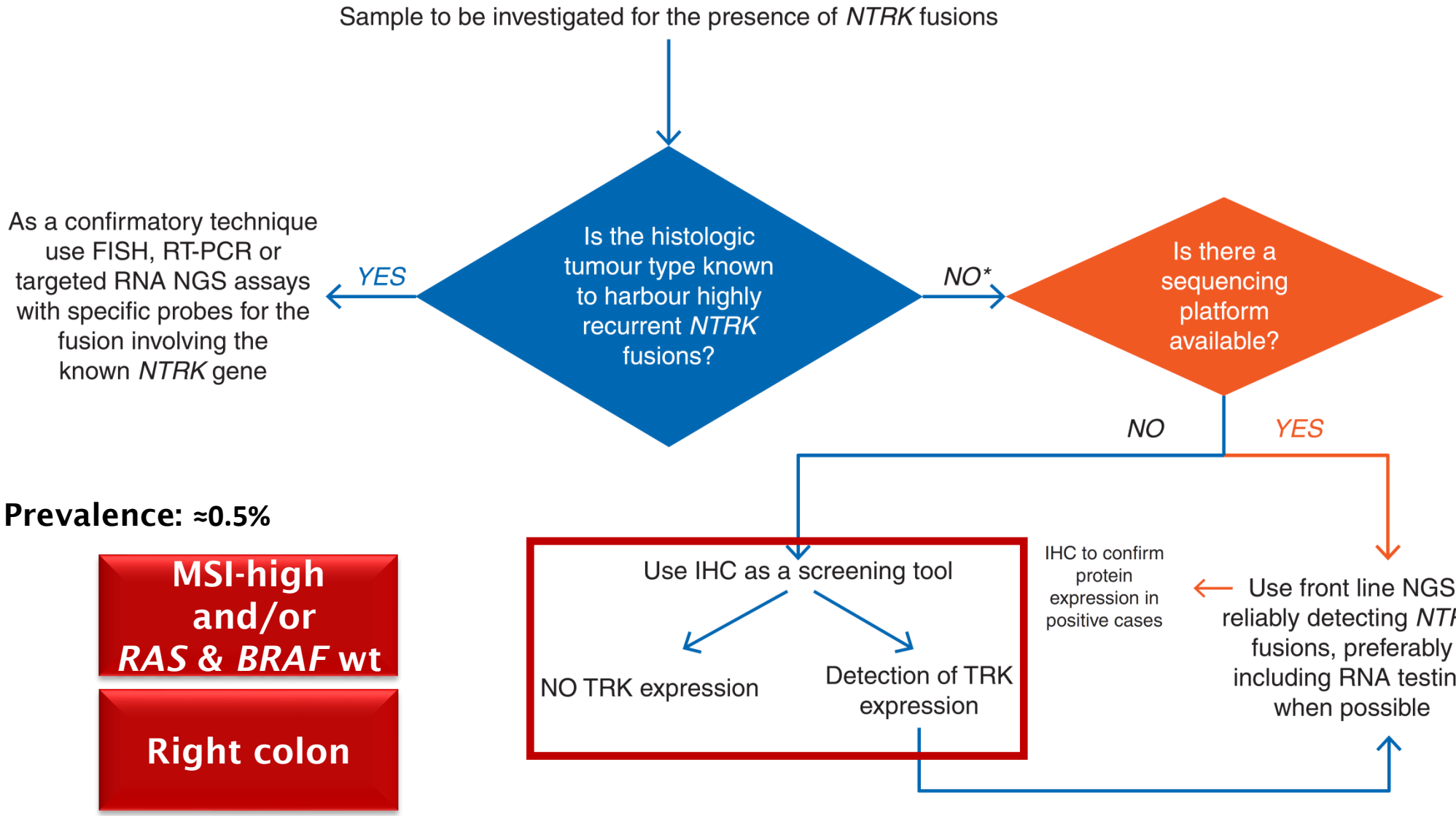
Entrectinib
Adult pts with *NTRK* rearranged tumors
N=121 (10 mCRC)
ORR in mCRC: 2/10 (20%)

Larotrectinib
Adult pts with *NTRK* rearranged tumors
N=140 (10 mCRC including 7 MSI-H)
ORR in mCRC: 5/10 (50%)



Demetri et al., Clin Cancer Res 2022
Brose et al., ESMO Congress 2021

Algorithm for NTRK gene fusion testing



Prevalence: ≈0.5%

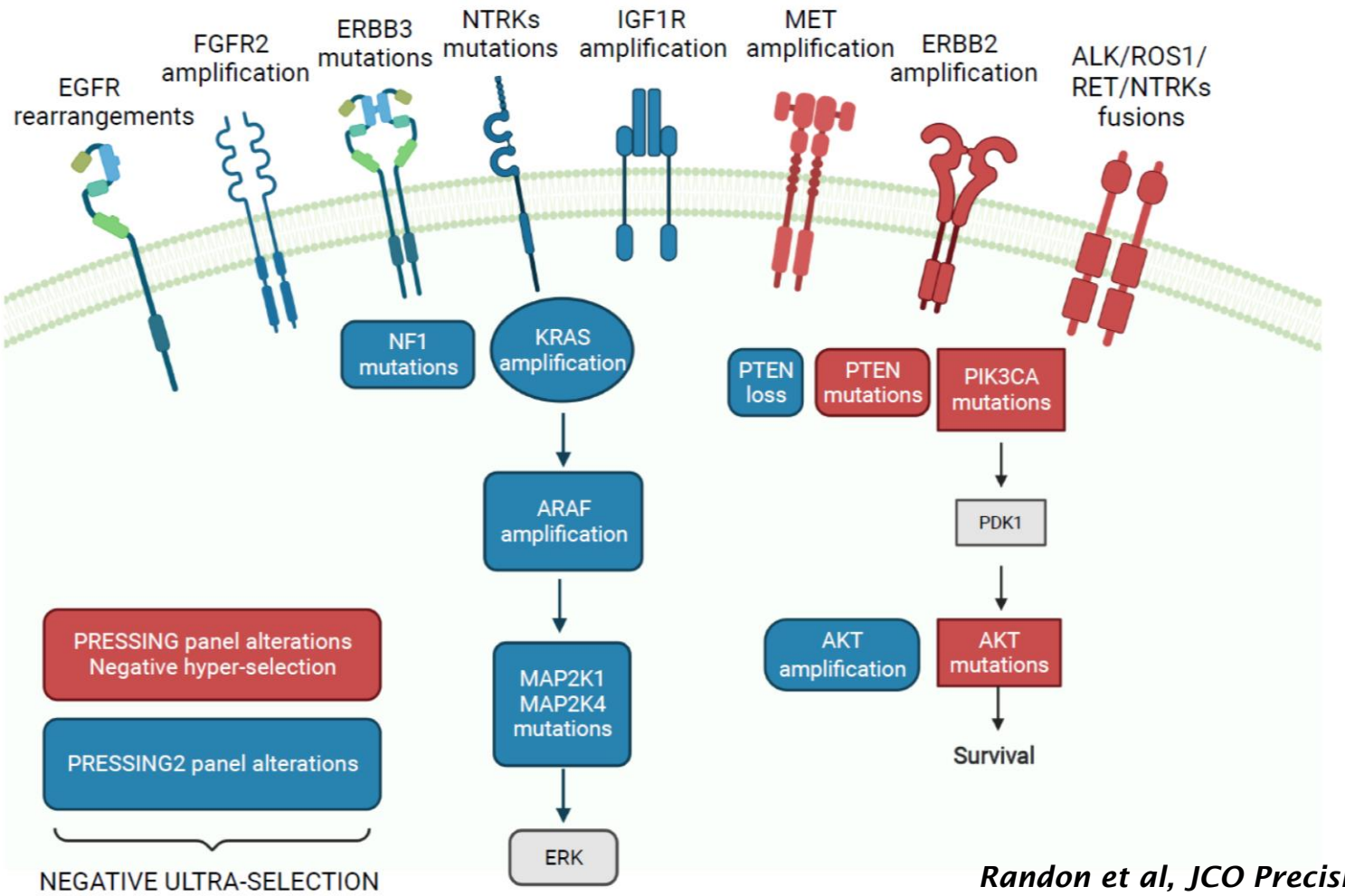
MSI-high and/or *RAS* & *BRAF* wt

Right colon

Marchiò et al, ESMO guideline 2019

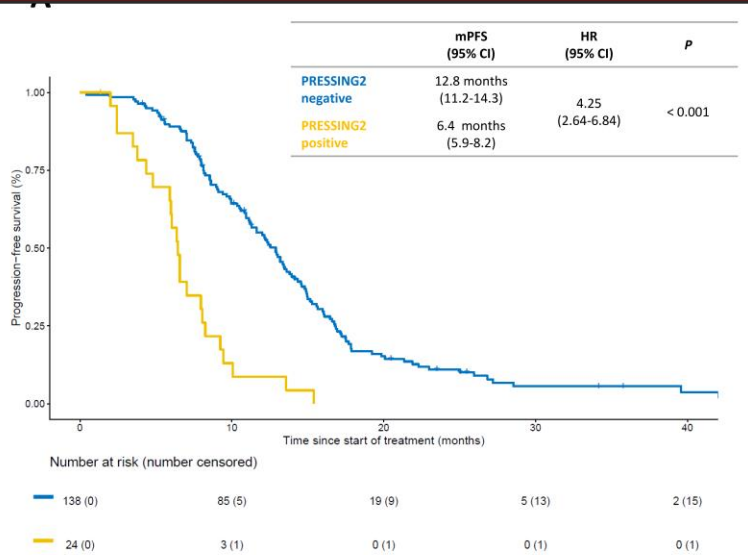
Negative ultra-selection for anti-EGFR-based therapy: PRESSING-2 study

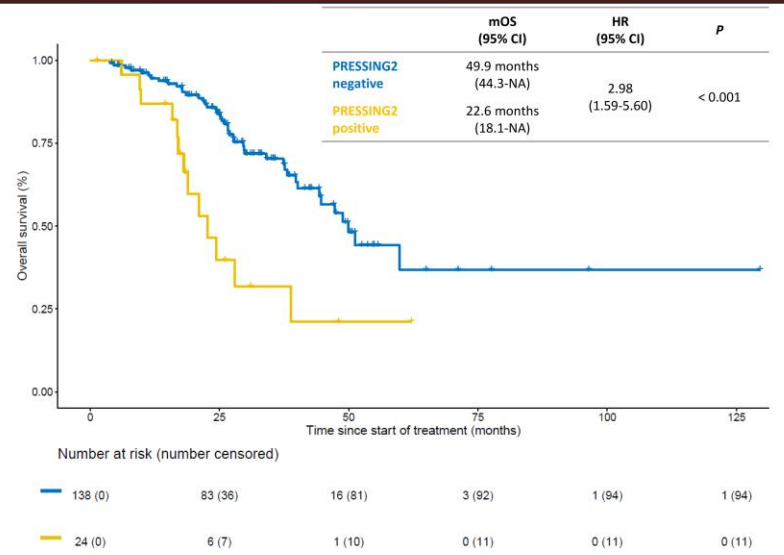
among 650 samples profiled by means of FoundationOne® CDx,
162 were *RAS*/*BRAF* wt, MSS, PRESSING panel neg. and treated with an anti-EGFR-based treatment
PRESSING-2 pos.=24 (15%) [numerically enriched in right-sided]

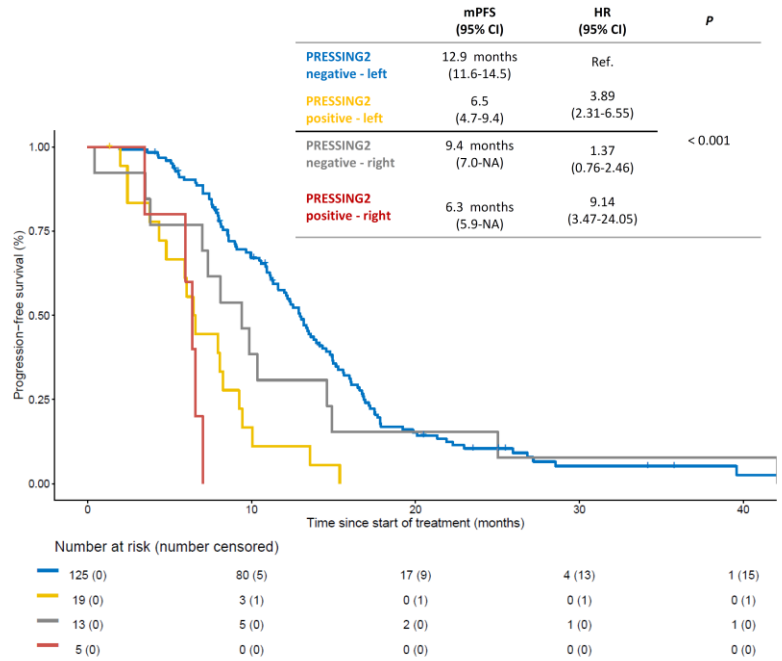


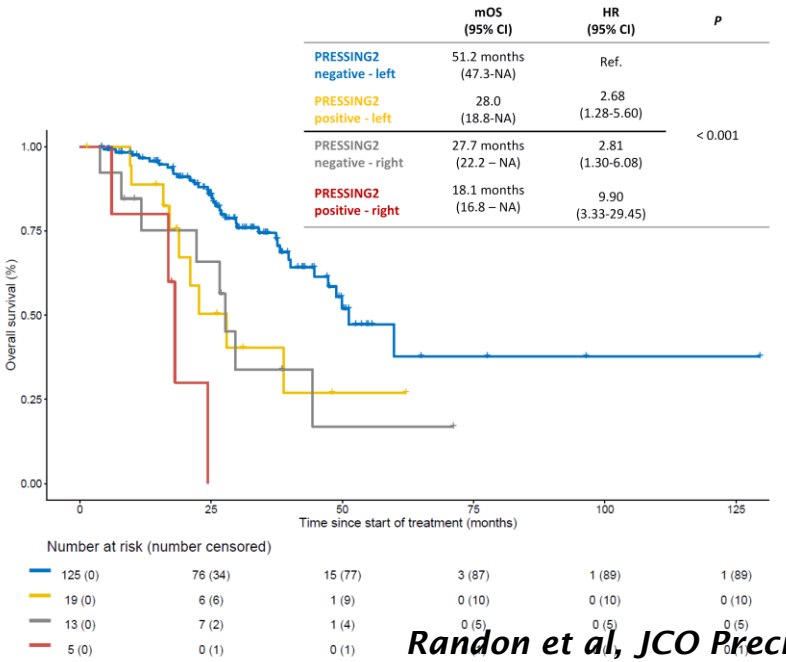
Randon et al, JCO Precision Oncol, under publication

Negative ultra-selection for anti-EGFR-based therapy: PRESSING-2 study





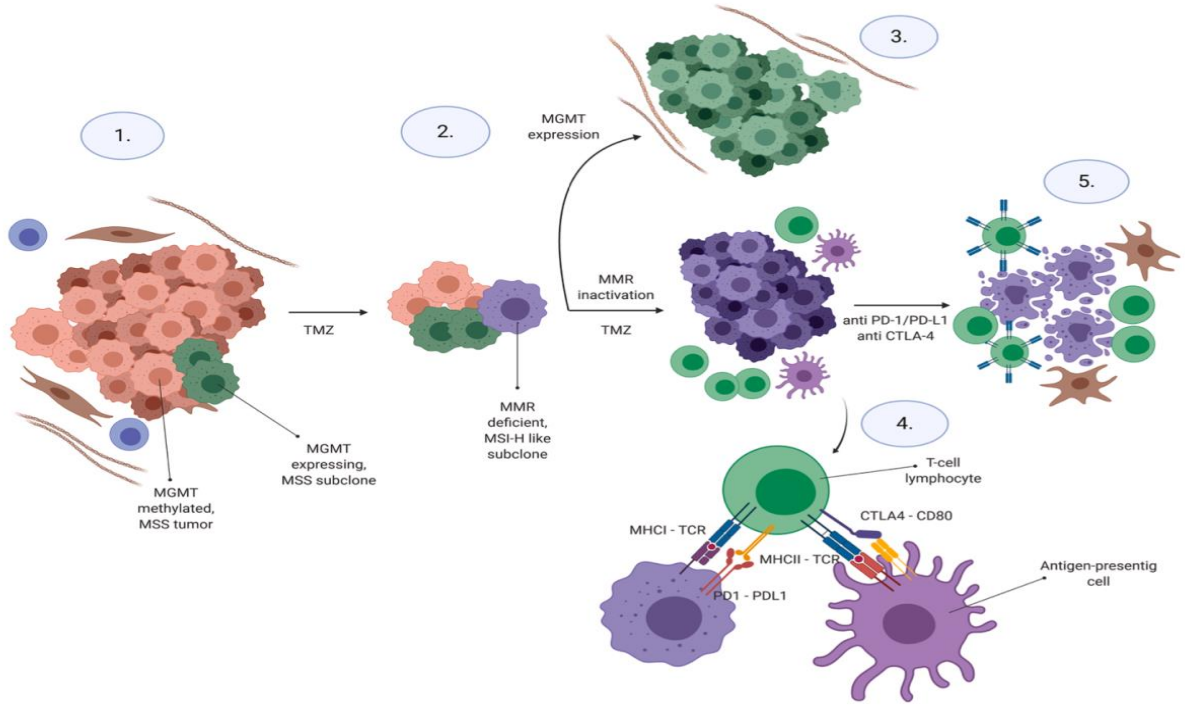




Randon et al, JCO Precision Oncol, under publication

Potential use of TEMOZOLOMIDE as a priming therapy for ICIs in MGMT silencing mCRC

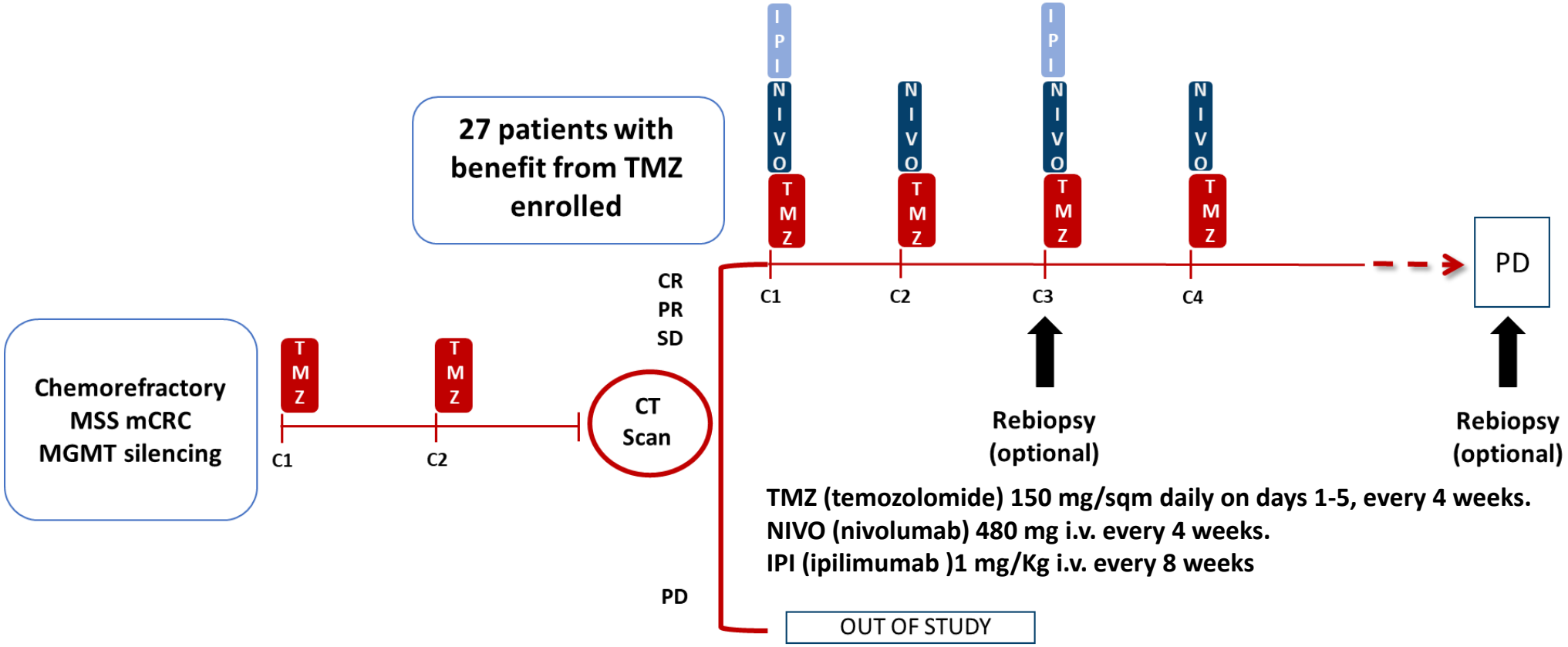
Secondary resistance to TMZ may induce a **hypermutated status** (TMZ mutational signature #8 characterized by T>C transitions), frequently coupled by **acquired mutations in MMR genes** in diverse tumor types, including GBM, CRC and NECs



The induction of hypermutation (TMB-high) by a TMZ priming phase provided the rationale for immune-sensitization of MSS mCRCs

Pietrantonio et al. Cancer Treat Rev 2020; Alexandrov et al, Nature 2013; Germano G et al, Nature 2017; Campbell et al, Cell 2017; Klempner et al, JCO PO 2020

MAYA trial

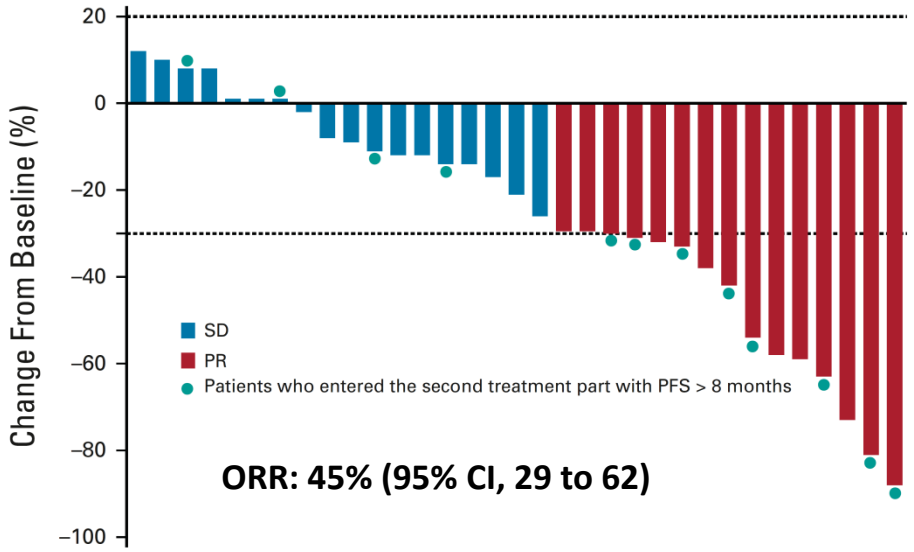


Primary endpoint: 8-month PFS rate in patients entered the Second Treatment Phase.

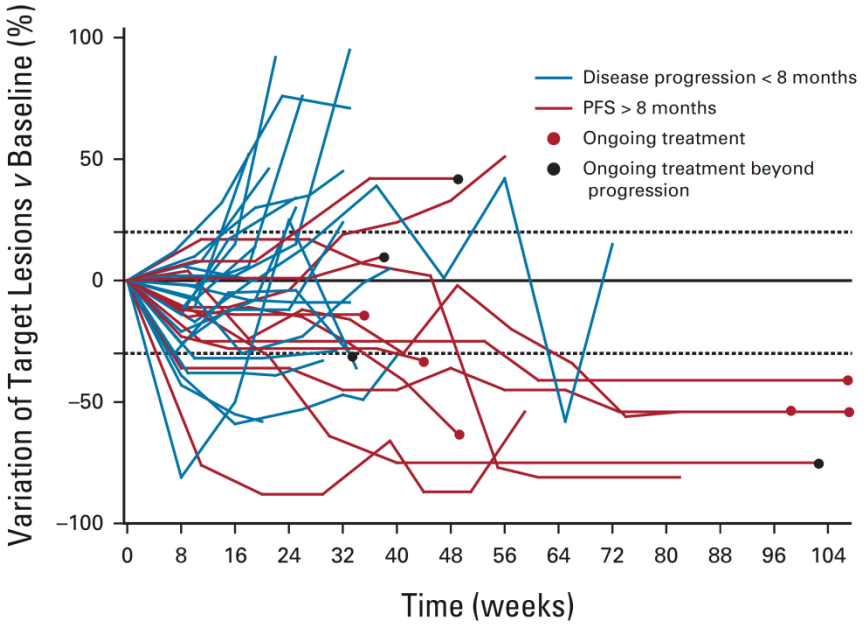
According to Fleming single-stage design, p0 (8-months PFS rate in the null hypothesis) = 5%, and p1 (8-months PFS rate in the alternative hypothesis) = 20%, a total of **27 patients** were required. Null hypothesis was rejected if at least **4 patients** were progression-free at the 8-month timepoint

Morano et al. JCO 2022

MAYA trial: results in whole treatment strategy

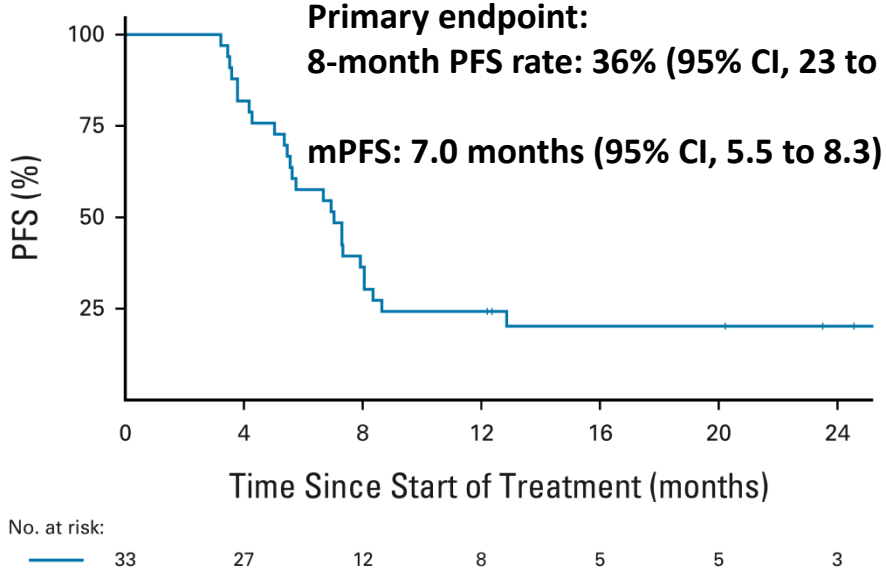


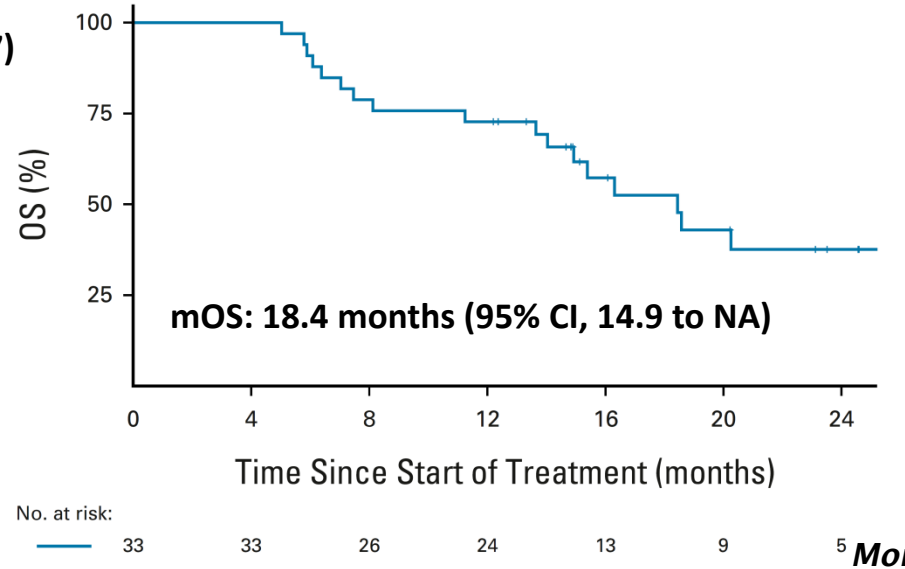
ORR: 45% (95% CI, 29 to 62)



Primary endpoint:
8-month PFS rate: 36% (95% CI, 23 to 57)

mPFS: 7.0 months (95% CI, 5.5 to 8.3)

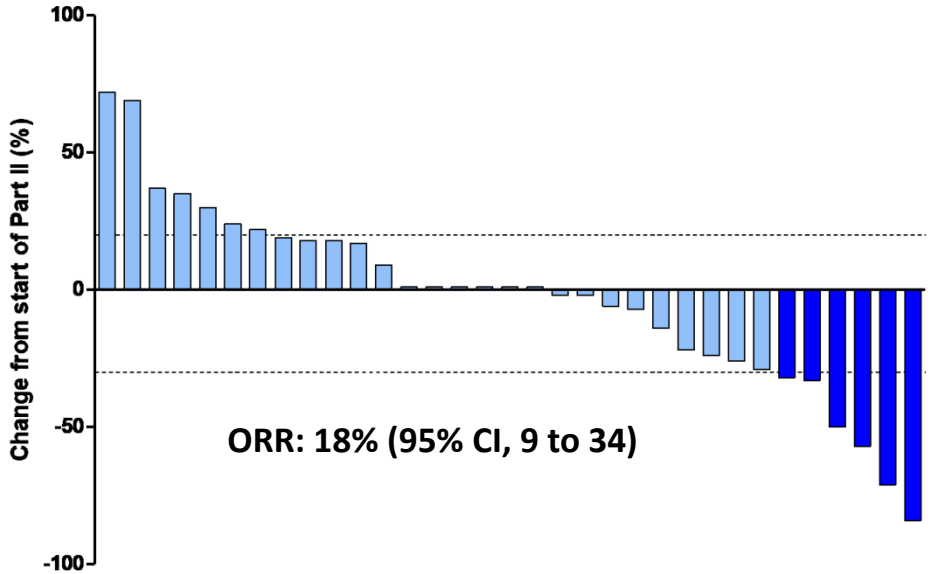




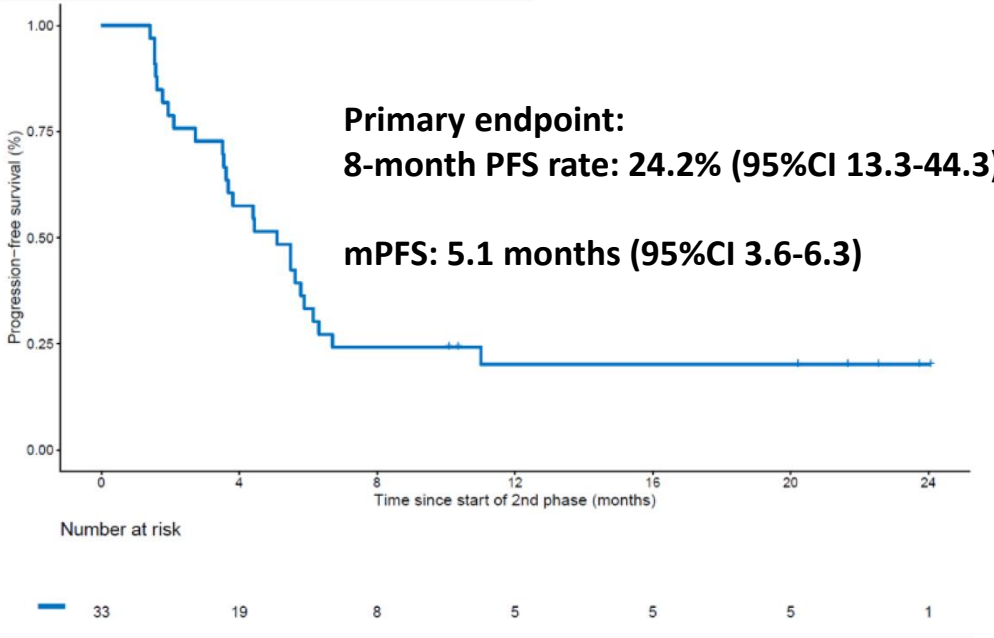
mOS: 18.4 months (95% CI, 14.9 to NA)

Morano et al. JCO 2022

MAYA trial: results in the second treatment part

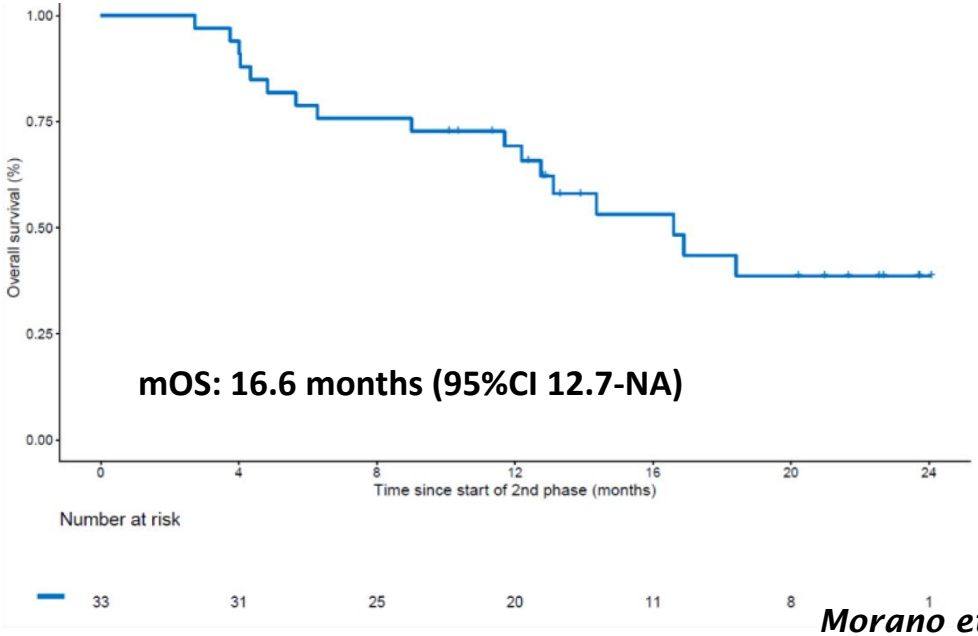


ORR: 18% (95% CI, 9 to 34)



Primary endpoint:
8-month PFS rate: 24.2% (95%CI 13.3-44.3)

mPFS: 5.1 months (95%CI 3.6-6.3)



mOS: 16.6 months (95%CI 12.7-NA)

Morano et al. JCO 2022

Biomarkers in colorectal cancer

Tissue-based

- Microsatellite instability
- *POLE* mutation
- TMB
- *RAS* (*KRAS-G12C*) mutation
- *BRAF* (*V600E*) mutation
- HER2 iperexpression/amplification
- Gene fusions
- *MGMT* deficiency
- CMS
- Other gene alterations

Plasma-based

- CEA and CA19.9
- ctDNA
- Circulationg Tumor Cells
- Exosome
- Circulating RNA
- Protein

CEA increase from nadir as a marker of PD after first-line induction therapy

among 733 patients with baseline CEA ≥10 ng/ml enrolled in TRIBE e TRIBE2 studies,
434 have at least one paired CEA and radiological assessment during maintenance or treatment break

Paired evaluable CEA and radiological assessment
N=1178

Progression disease by CEA	Change in CEA from nadir	AUC (95% CI)	Sensitivity, *n* (%, 95% CI)	Specificity *n* (%, 95% CI)	Negative predictive value, *n* (%, 95% CI)	Positive predictive value, *n* (%, 95% CI)	CT scan avoided, *n* (%)	Radiological PD and no CEA increase among cases with no CEA increase, *n* (%)	Radiological PD and no CEA increase among all radiological PDs, *n* (%)
Cut-off by optimal AUC	≥120%	0.81 (0.79–0.83)	187/254 (74%, 68–79)	723/924 (78%, 75–81)	723/790 (92%, 90–93)	187/388 (48%, 45–52)	790/1178 (67%)	67/790 (8%)	67/254 (26%)
Cut-off by maximum sensibility	Any increase	0.64 (0.61–0.67)	235/254 (93%, 89–95)	327/924 (35%, 32–39)	327/346 (95%, 92–96)	235/832 (28%, 27–29)	346/1178 (29%)	19/346 (5%)	19/254 (7%)

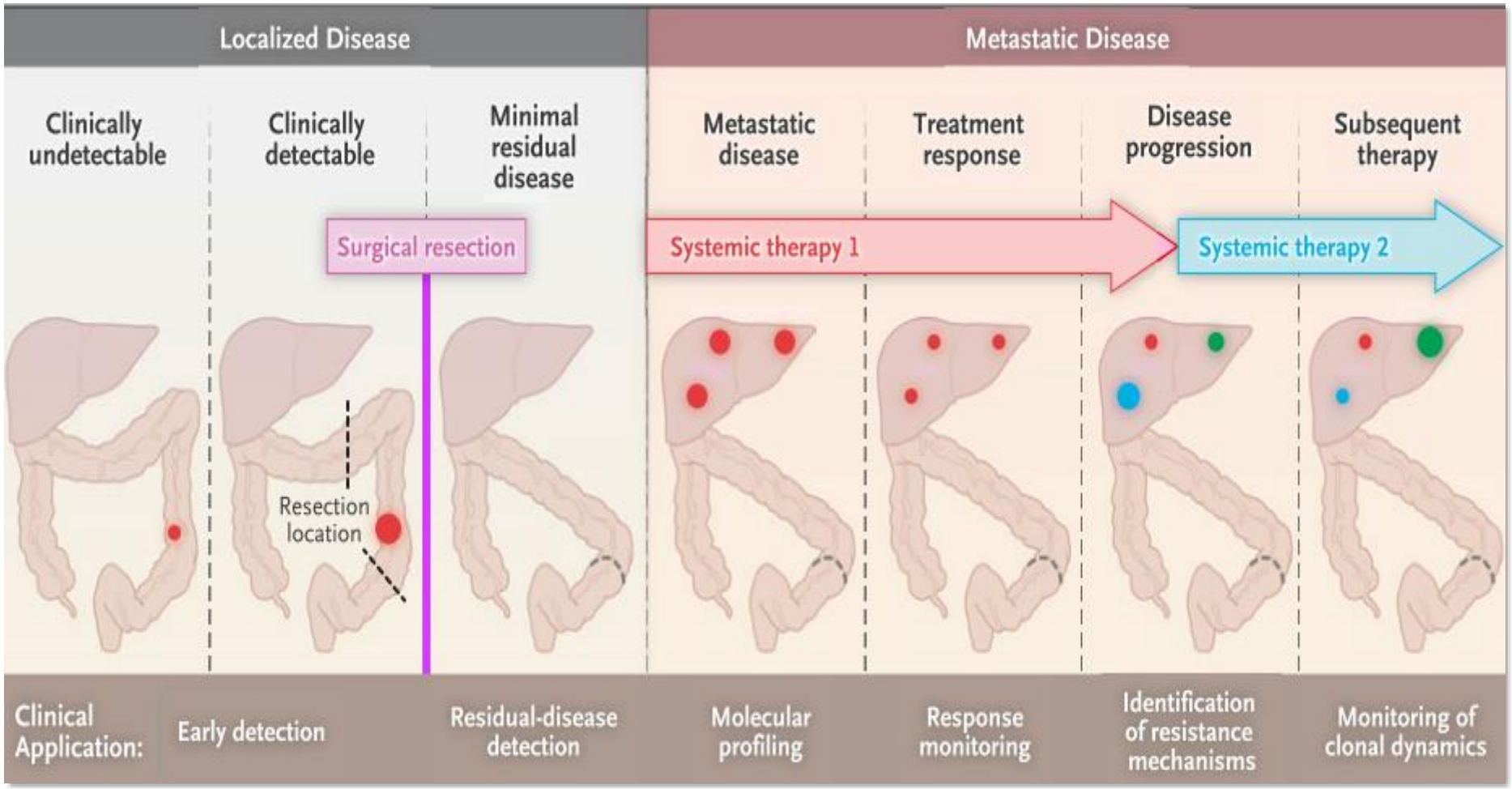
- Cut-off ≥120% from nadir could be used during follow-up in most patients after the end of induction chemotherapy thus sparing a relevant amount of radiological assessments.

- Any increase of CEA from nadir as cut-off, increasing the sensitivity while reducing specificity, should be especially evaluated when missing PD may cause immediate deterioration of patients'conditions due to a high risk of disease-related symptoms (i.e. liver failure due to multiple liver metastases, intestinal occlusion due to peritoneal carcinomatosis, uncontrolled pain due to pelvic relapse).

Moretto et al., Br J Cancer 2021

ctDNA – potential clinical applications in CRC

What questions could liquid biopsies answer?



Corcoran et al, N Eng J Med 2018

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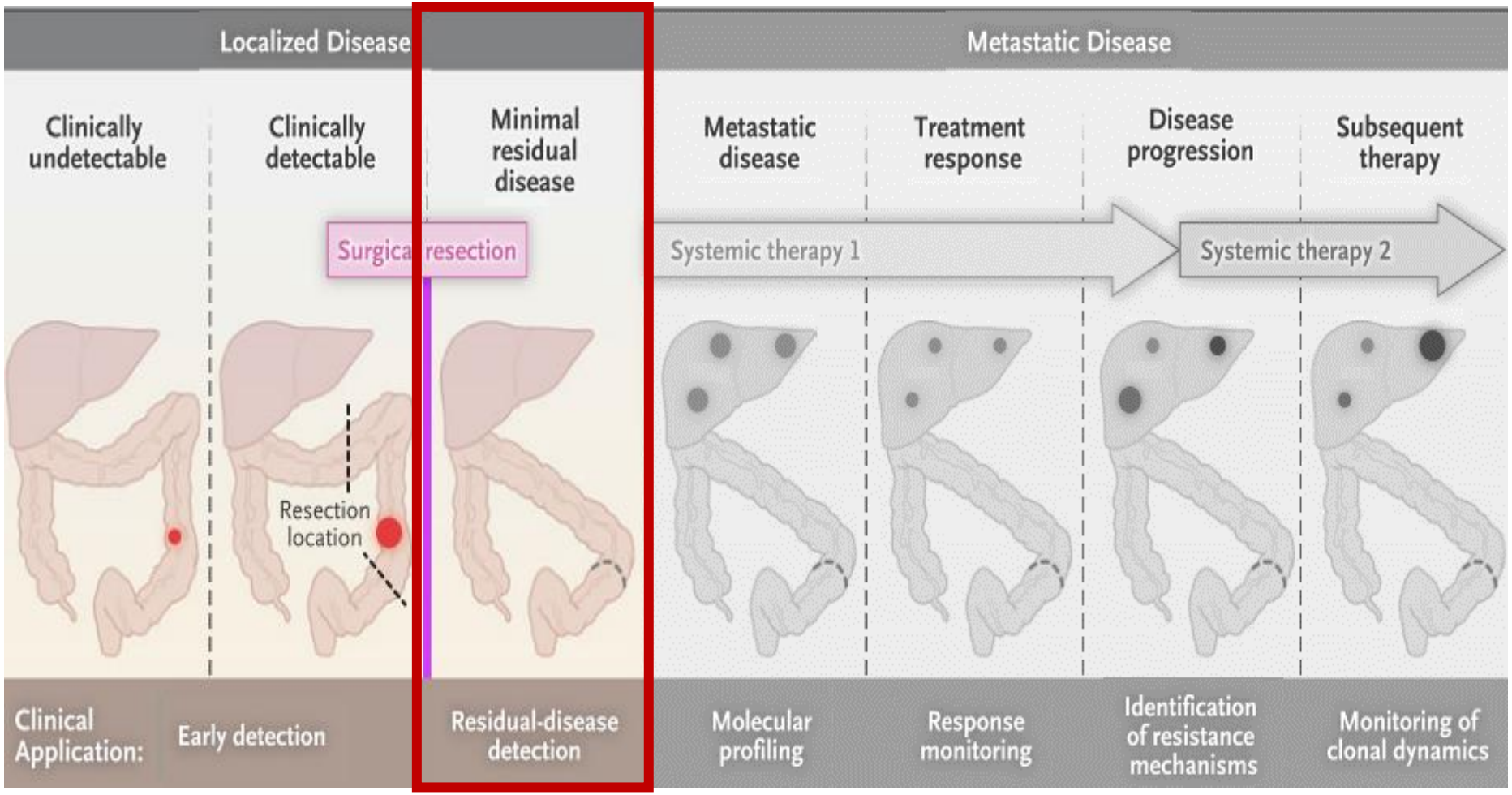
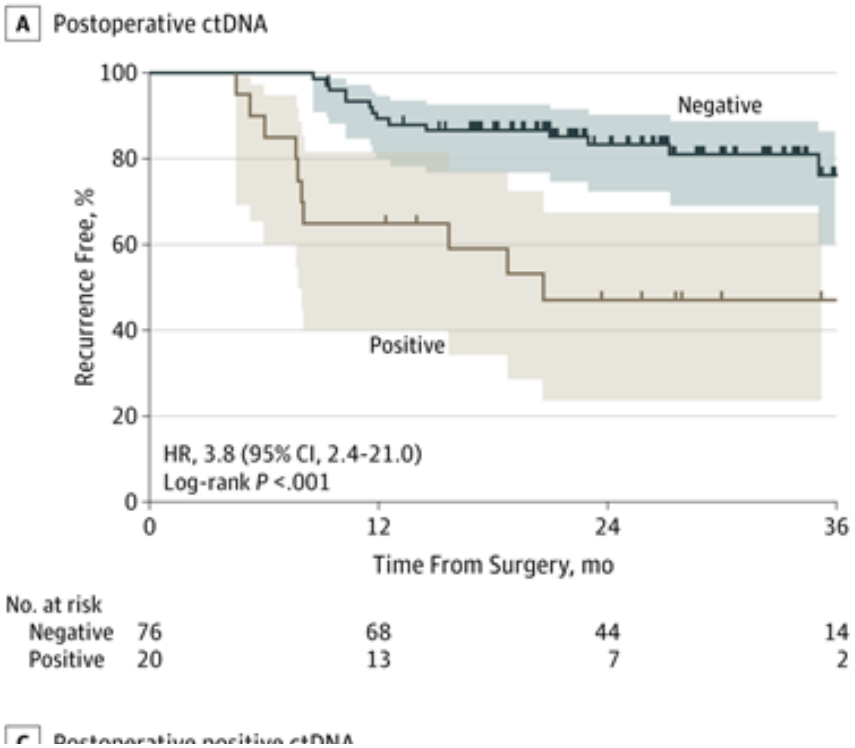


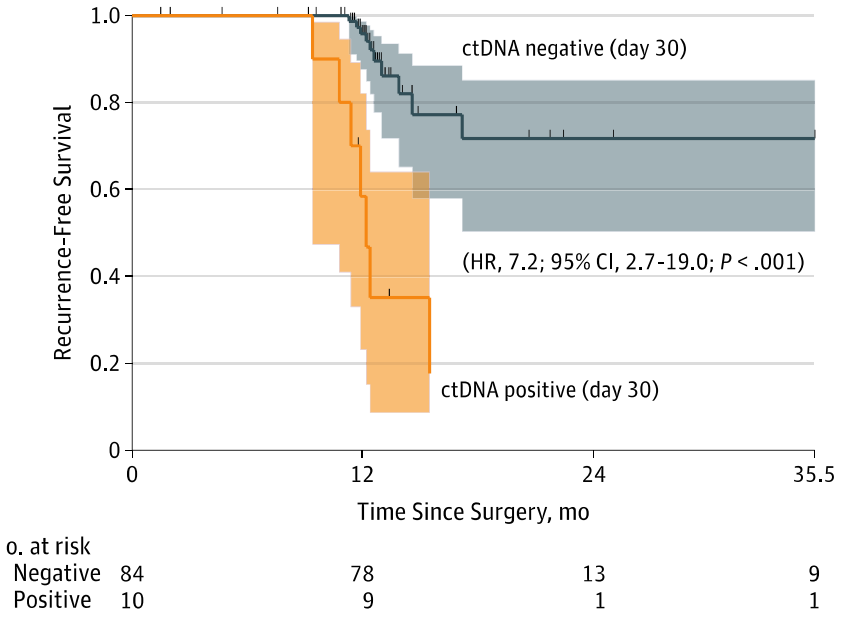
Corcoran et al, N Eng J Med 2018

ctDNA in early stages CRC

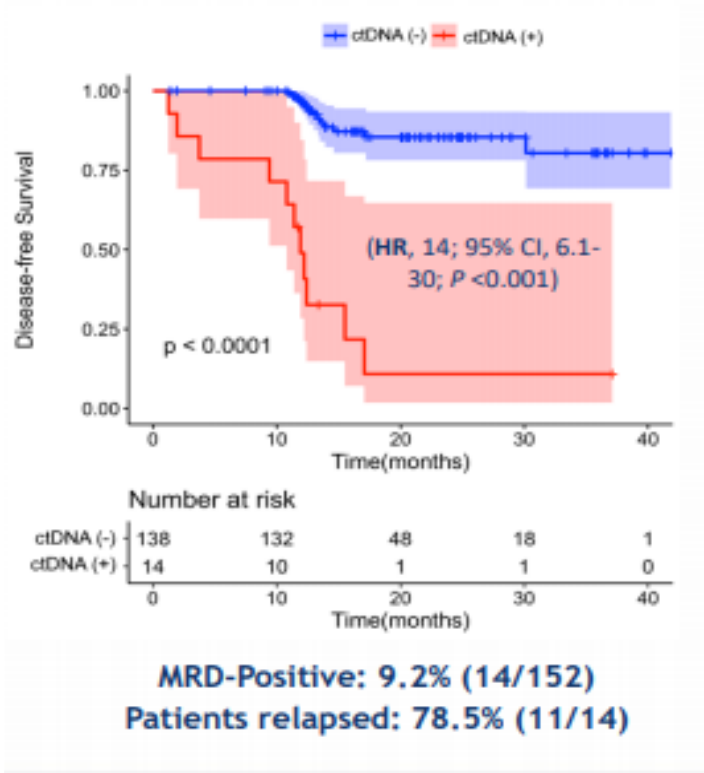
Recurrence Free Survival according to post-op ctDNA status



Tie et al, JAMA Oncol 2019



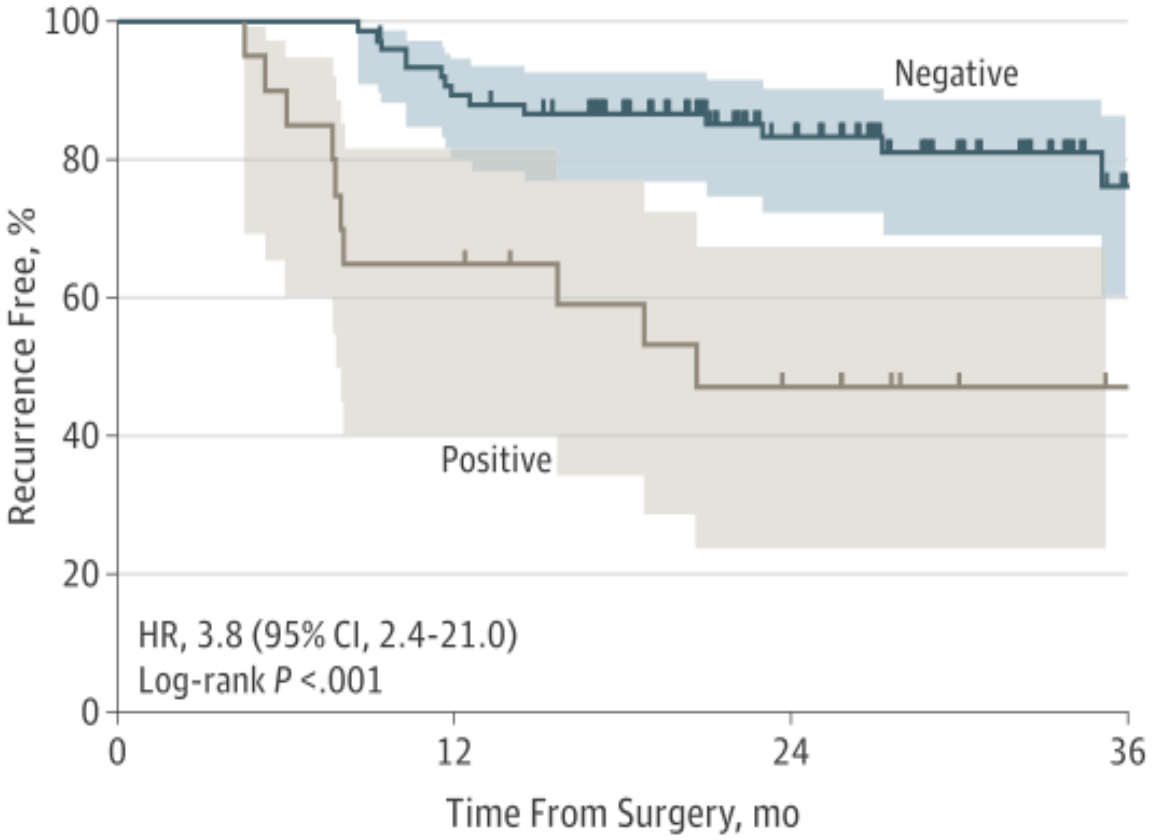
Reinert et al, JAMA Oncol '19



Tarazona N et al. ASCO 2020

Where are we going? Perspective #1

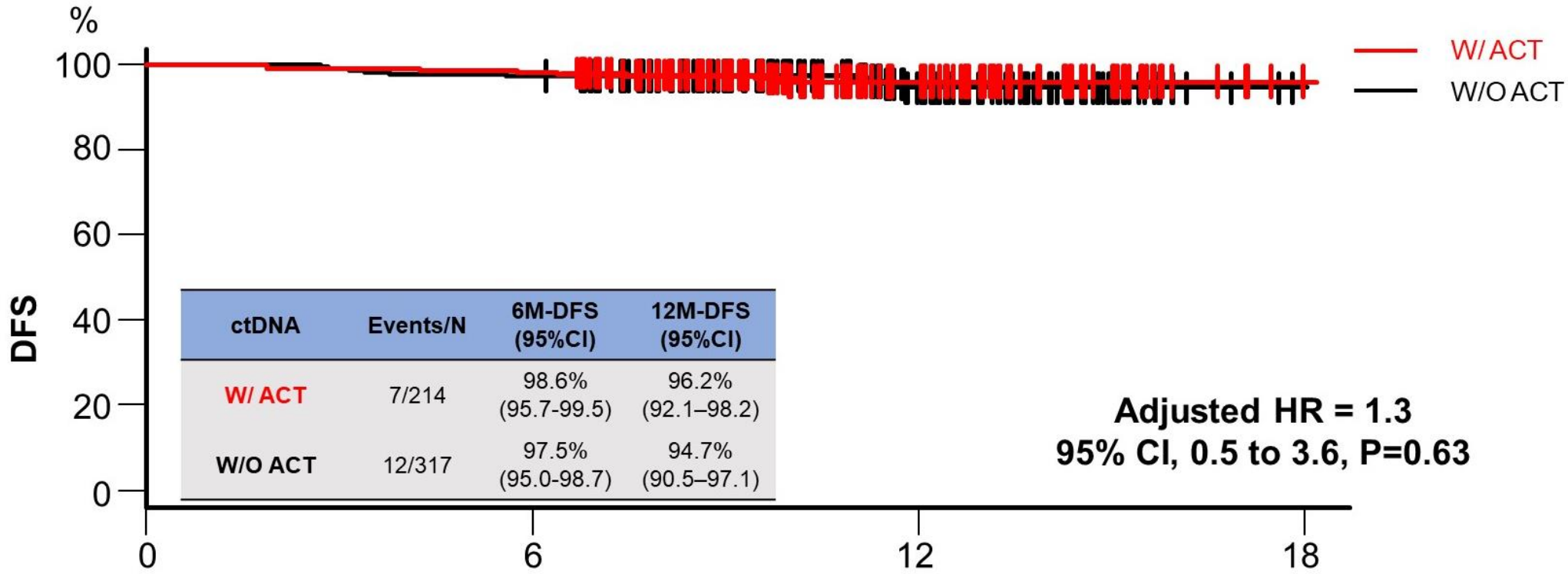
Stage III CRC (n=96) - Post-surgery ctDNA



To avoid/de-intensify chemotherapy in ctDNA-negative pts

Tie et al, JAMA Oncol 2019

CIRCULATE-Japan – GALAXY study – post-op ctDNA neg



ctDNA	Events/N	6M-DFS (95%CI)	12M-DFS (95%CI)
W/ ACT	7/214	98.6% (95.7-99.5)	96.2% (92.1–98.2)
W/O ACT	12/317	97.5% (95.0-98.7)	94.7% (90.5–97.1)

Adjusted HR = 1.3
95% CI, 0.5 to 3.6, P=0.63

Median follow-up time: 11.4 months
Data cutoff: Nov 19, 2021

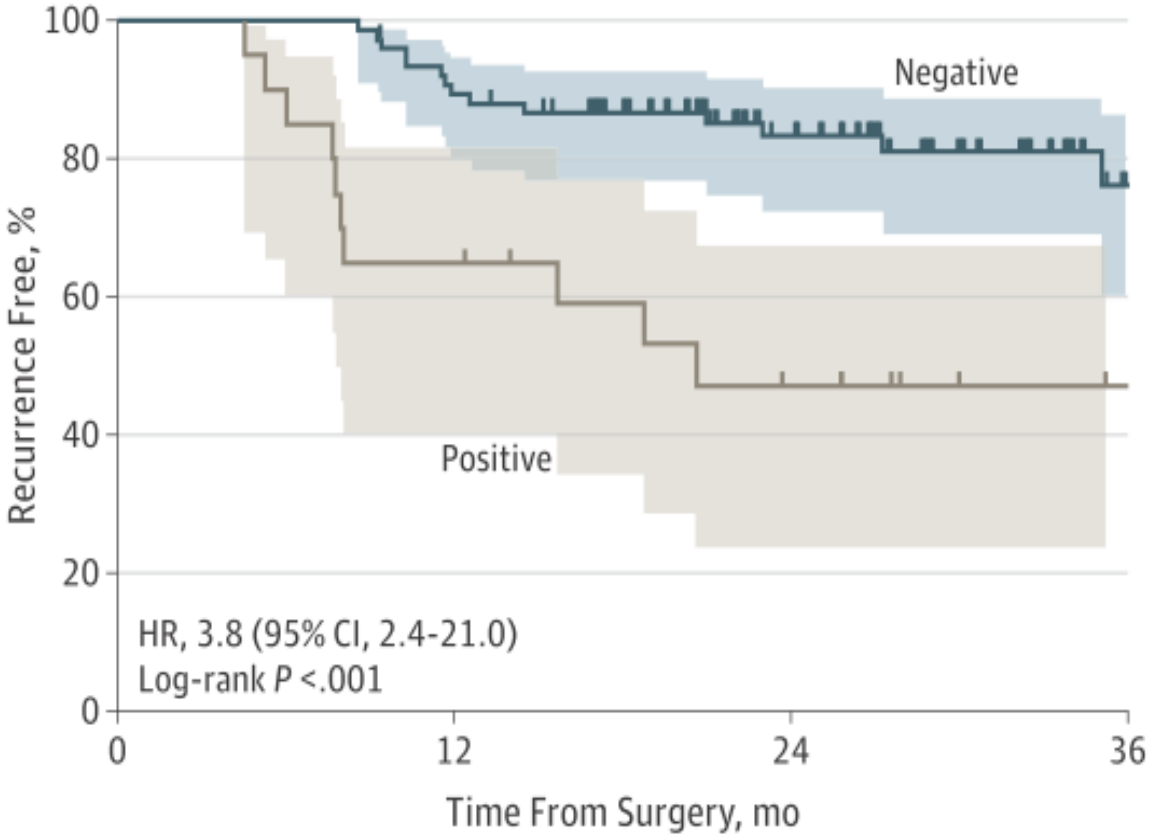
Number at risk

W/ ACT	214	211	79	0
W/O ACT	317	309	117	0

Kotaka et al, ASCO GI 2022

Where are we going? Perspective #2

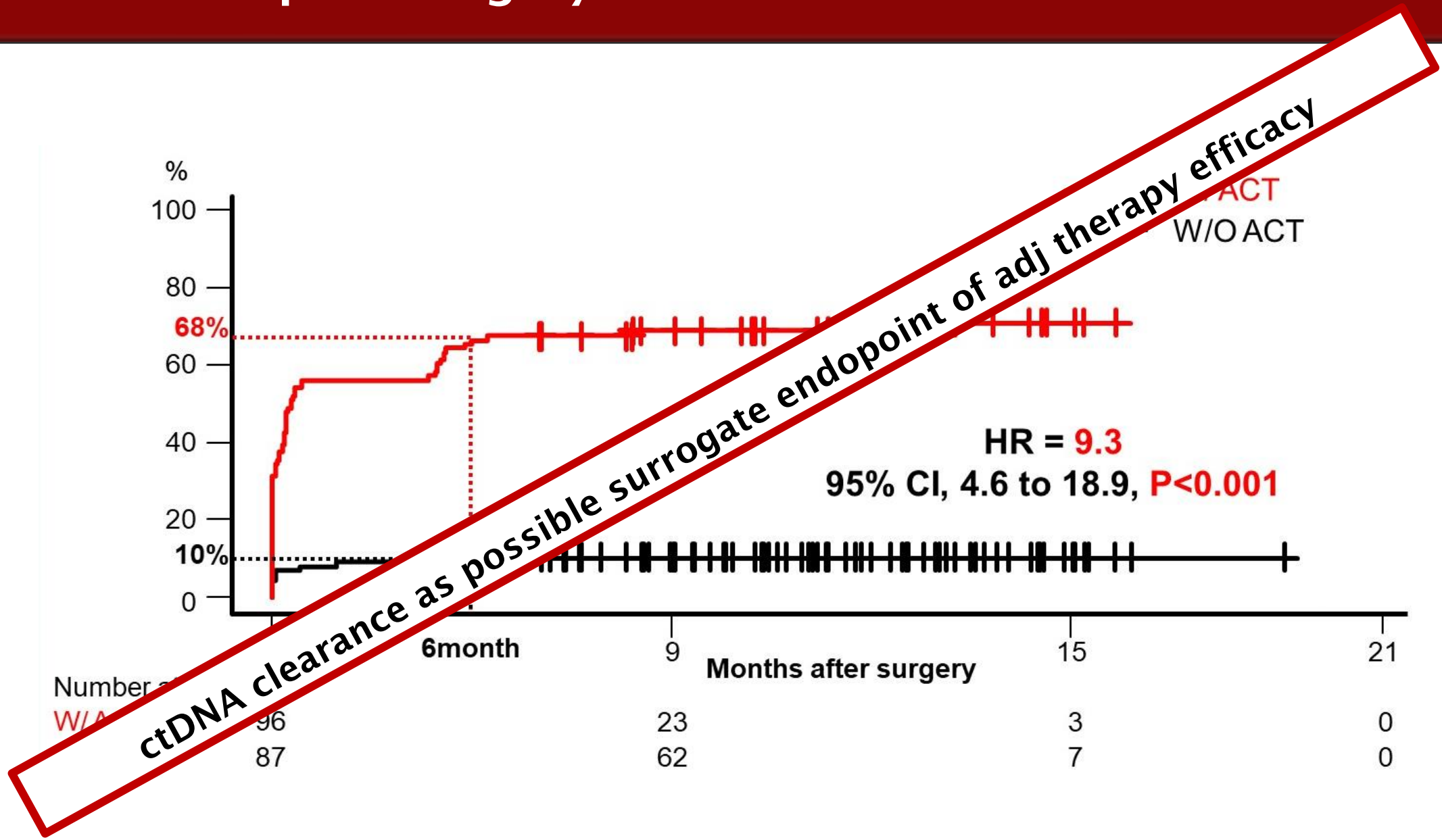
Stage III CRC (n=96) - Post-surgery ctDNA



To administer/intensify chemotherapy in ctDNA-positive pts

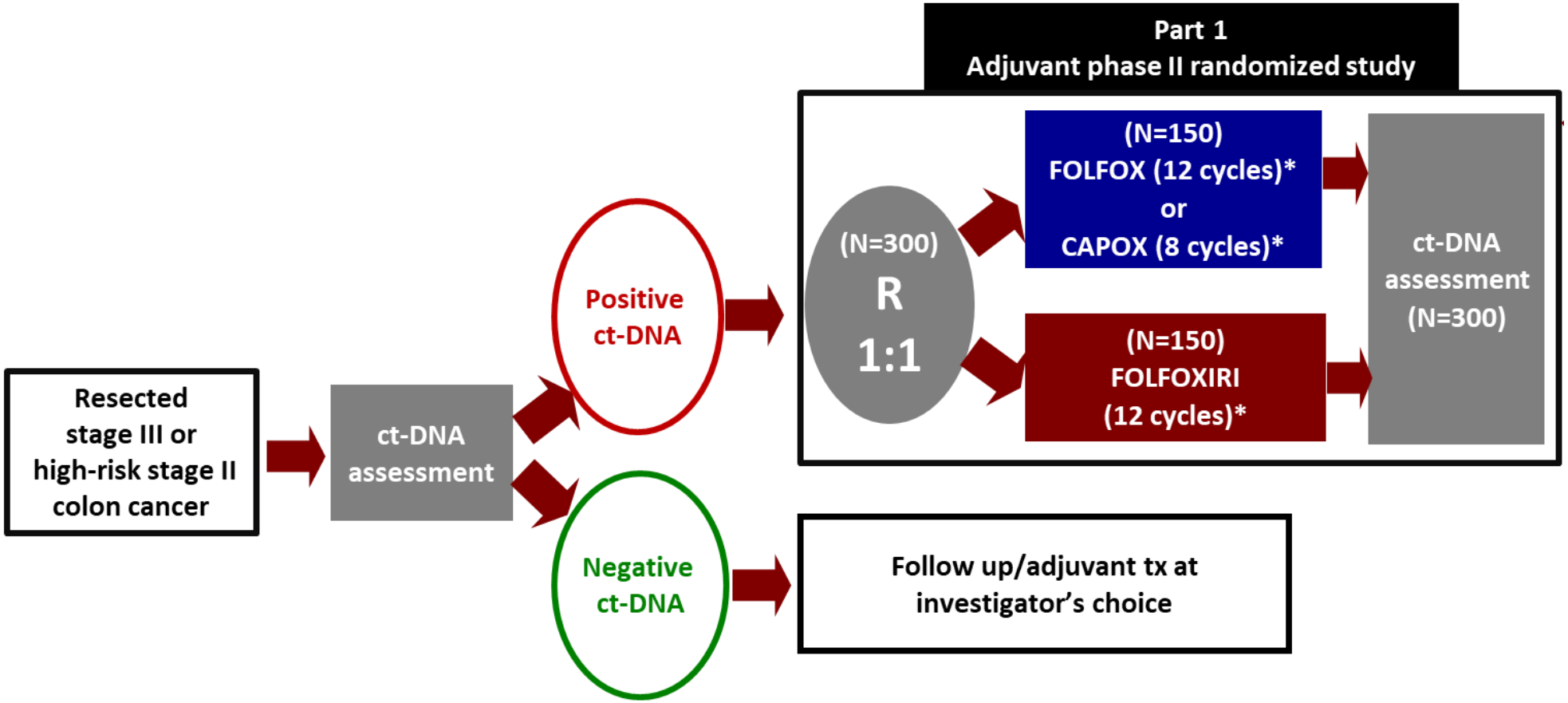
Tie et al, JAMA Oncol 2019

Clearance of post-surgery ctDNA with or without chemotherapy



Kotaka et al, ASCO GI 2022

ERASE-CRC – Part 1 – Adjuvant phase II study



Primary endpoint: ctDNA clearance rate

Additional info:

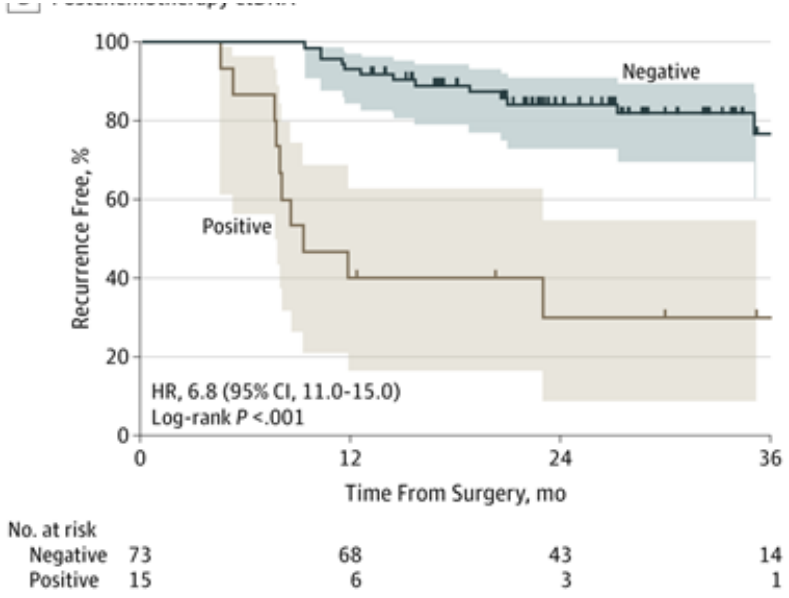
- **Prognostic impact of ctDNA clearance**
- **<u>"Surrogacy" of ctDNA clearance for DFS</u>**
- **Prognostic impact of post-op and post-adj ctDNA**

Part 1, adjuvant randomized study:

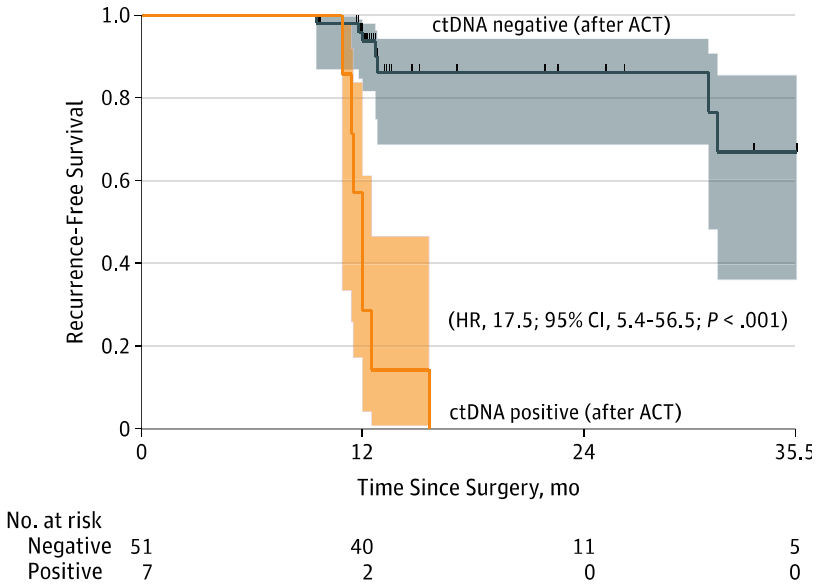
- ✓ **Stratification factors**:
  - High risk stage III vs low-risk stage III vs high-risk stage II
  - Center
- ✓ **Primary endpoint:** rate of ct-DNA clearance
- ✓ **Target accrual:** 300 patients

ctDNA in early stages CRC

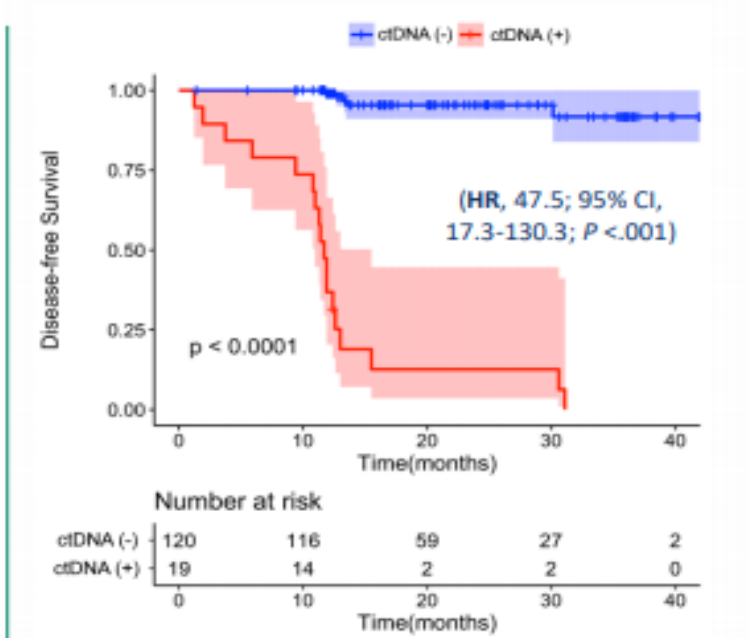
Recurrence Free Survival according to post-adjuvant ctDNA status



Tie et al, JAMA Oncol 2019



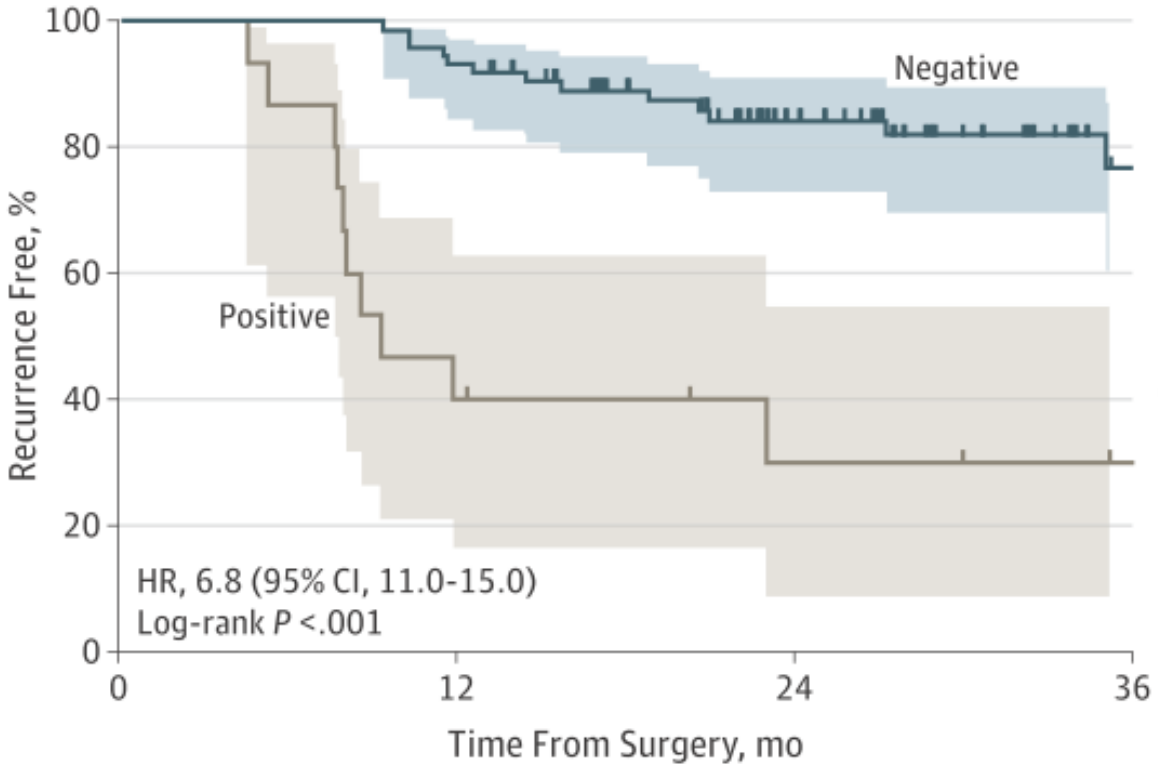
Reinert et al, JAMA Oncol '19



Tarazona N et al. ASCO 2020

Where are we going? Perspective #3

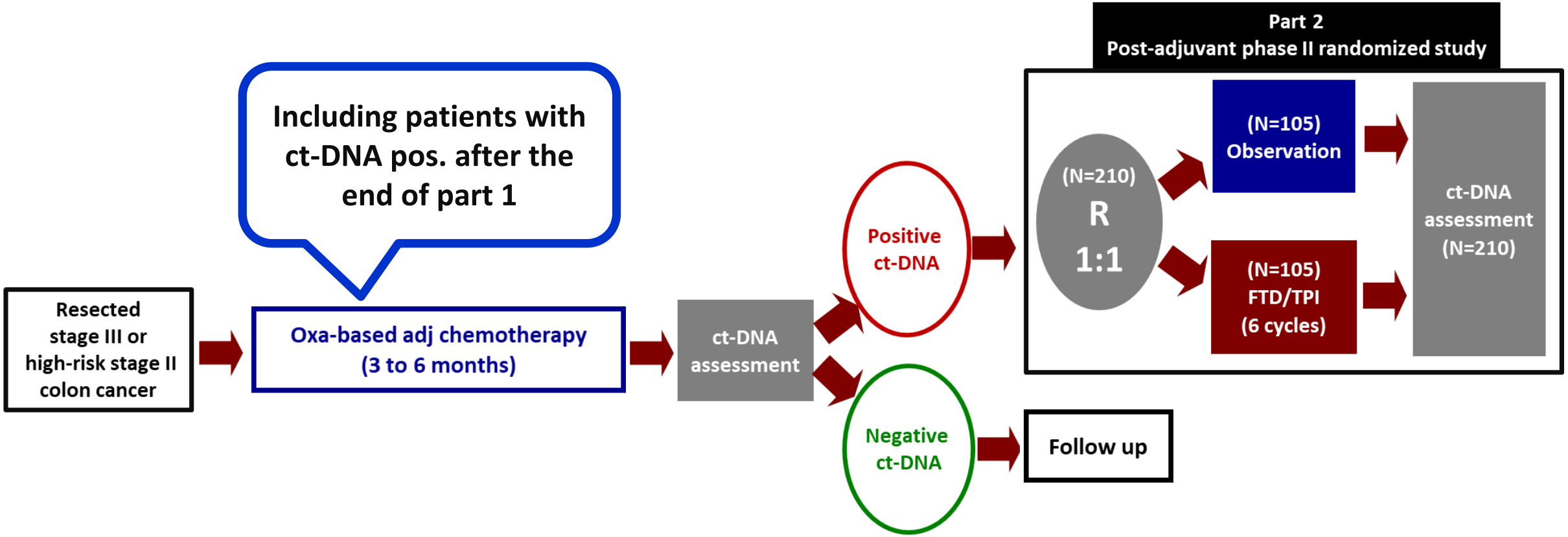
Stage III CRC (n=88) - Post-adjuvant tx ctDNA



To intensify follow-up/additional therapies in ctDNA-positive pts

Tie et al, JAMA Oncol 2019

ERASE-CRC – Part 2 – Post-Adjuvant phase II study



Primary endpoint: ctDNA clearance rate

Additional info:
Prognostic impact of ctDNA clearance
<u>"Surrogacy" of ctDNA clearance with a post-adjuvant tx for DFS</u>

Part 2, post-adjuvant randomized study:

- ✓ **Stratification factors**:
  - Previous adjuvant therapy (FOLFOX/CAPOX vs FOLFOXIRI)
  - High risk stage III vs low-risk stage III vs high-risk stage II
- ✓ **Primary endpoint:** rate of ct-DNA clearance
- ✓ **Target accrual:** 210 patients

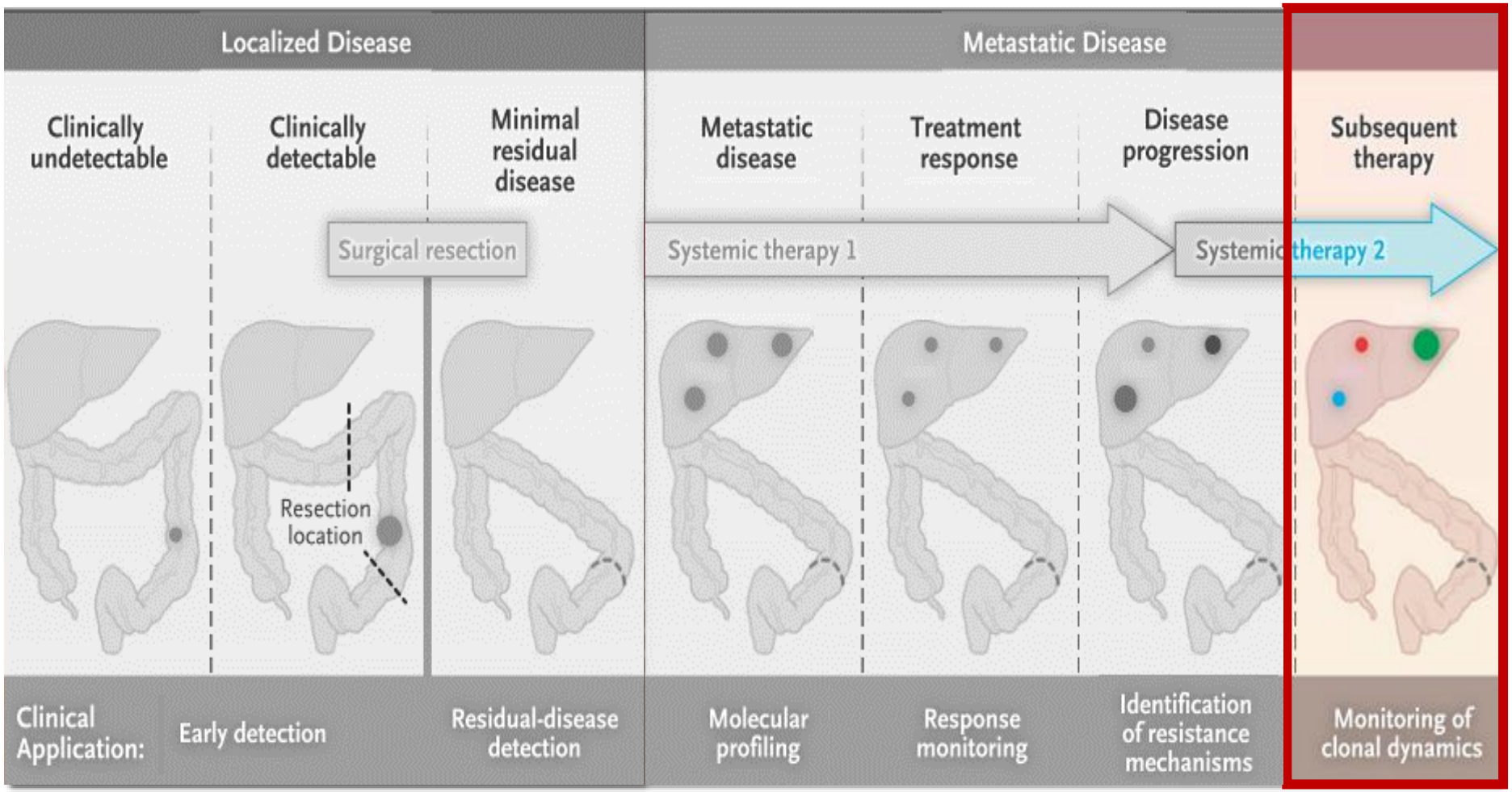
Ongoing studies assessing the role of MRD with ctDNA

Study	Phase	Assay	Population	N pts	Drugs	Primary endpoint	Country
COBRA	II/III	Lunar 1 Guardant Health	Colon STAGE II	1408	Exp: ctDNA pos.: FOLFOX/CAPOX; ctDNA neg.: obs Control: obs	ctDNA clearance (phase II) RFS (phase III)	
CIRCULATE	III	Gene panel (NGS)	Colon STAGE II	4812	ctDNA pos.: Exp: Cape or CAPOX (at investigator choice) Control: Obs ctDNA neg.: FU or off-study	DFS	
CIRCULATE	III	ddPCR	Colon STAGE II	554	ctDNA pos.: Exp: FOLFOX Control: Obs ctDNA neg.: FU or off-study	DFS in ctDNA pos.	
GALAXY, ALTAIR, VEGA	III	Signatera	CRC STAGE II-IV	VEGA 1240 ALTAIR 240	ctDNA neg (VEGA): Exp. Obs Control: CAPOX 3 months ctDNA pos, (ALTAIR) - CAPOX x3 months : Exp: FTD/TPI Control: obs	DFS	
BESPOKE	II Case-control	Signatera	CRC STAGE II-III	1000	Tx or obs recommended based on ctDNA status (control arm as per clinical practice without ctDNA analysis)	Tx decision based on ctDNA status	
DYNAMIC II	III	ddPCR	Colon STAGE II	450	Exp: ctDNA pos: adj 5FU/Cape +/- oxa ctDNA neg.: obs Comparator: at physician's discrection	N. Pts treated with Tx RFS	
DYNAMIC III	II/III	ddPCR	Colon STAGE III	1000	Exp: ctDNA informed Tx (escalation or descalation) Control: Tx blinded on ctDNA status	RFS	
PEGASUS	II	Lunar 1 Guardant Health	Colon STAGE III STAGE II HR	140	ctDNA pos. CAPOX (3 months)→ Cape (3 months) if ctDNA neg; FOLFIRI (6 months) if ctDNA pos ctDNA neg. Cape (6 months)→ FU if ctDNA neg; CAPOX (6 months) if ctDNA pos	ctDNA neg. rate	
TRACC	III non-inferiority	Signatera	Colon STAGE III STAGE II HR	1620	Exp: ctDNA informed Tx (escalation or descalation) Control: Tx blinded on ctDNA status	DFS	
NCT03803553	II	Lunar 1 Guardant Health	CRC STAGE III-IV	500	ctDNA post-adj pos.: Exp: FOLFIRI (Nivo is MSI-H; Enco+Bini+Cet if BRAFmut) Control: Obs ctDNA post-adj neg.: Obs	DFS ctDNA clearance	

Kasi et al, JCO Precision Oncol 2022

ctDNA – potential clinical applications in CRC

What questions could liquid biopsies answer?

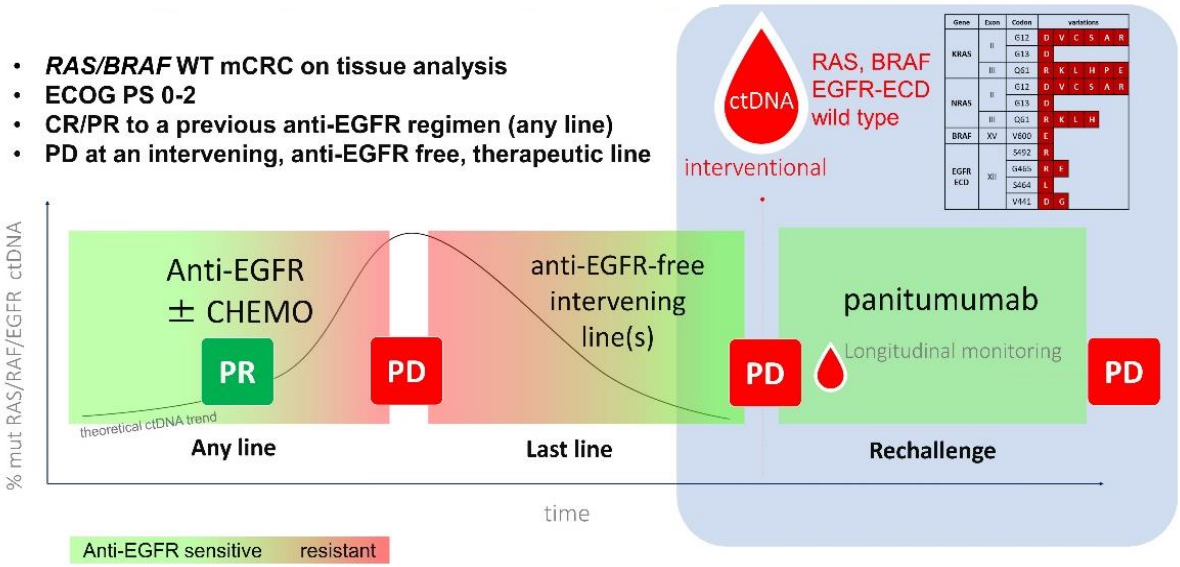


Corcoran et al, N Eng J Med 2018

Prospective evaluation of ctDNA for antiEGFR rechallenge: the CHRONOS trial

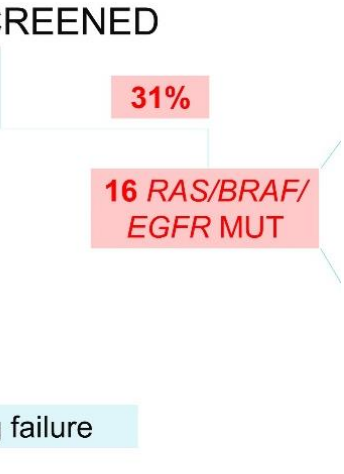
- ***RAS/BRAF*** **WT mCRC on tissue analysis**
- **ECOG PS 0-2**
- **CR/PR to a previous anti-EGFR regimen (any line)**
- **PD at an intervening, anti-EGFR free, therapeutic line**

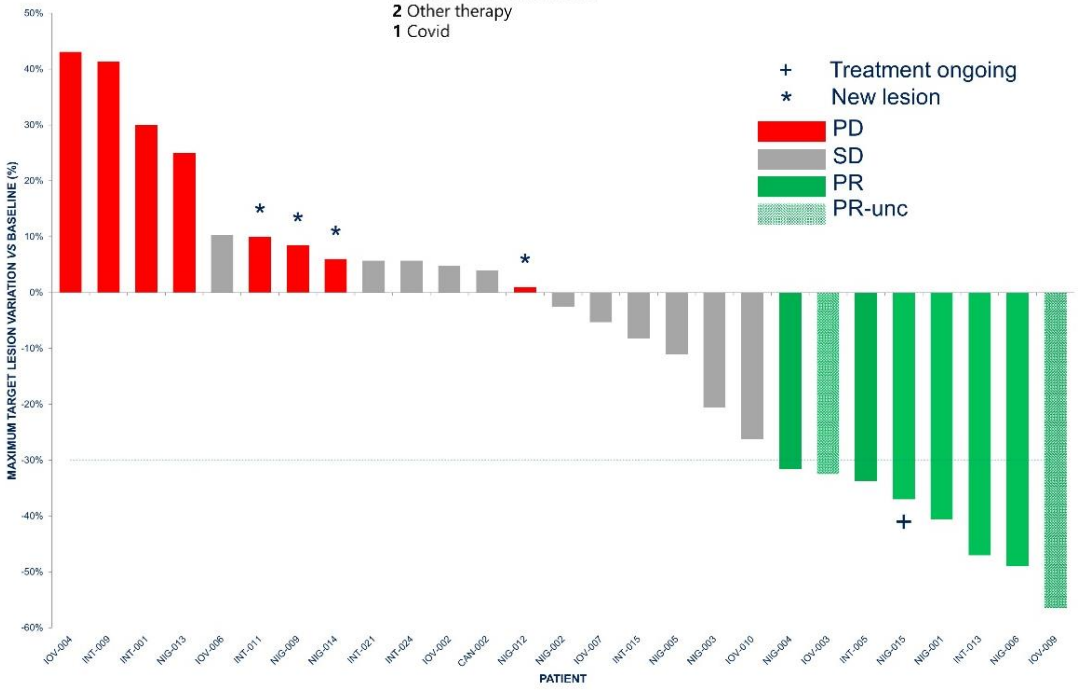
Phase II trial single-stage



Best Response RECIST 1.1 by centralized revision	N	%
Responses (PR+CR)	**8**	**30%**
Partial Response	8*	30%
Stable Disease ≥4 mos	9	33%
Stable Disease <4 mos	2	7%
Control of disease (PR+SD≥4 mos)	**17**	**63%**
Progressive Disease	8	30%
Total	27	100%

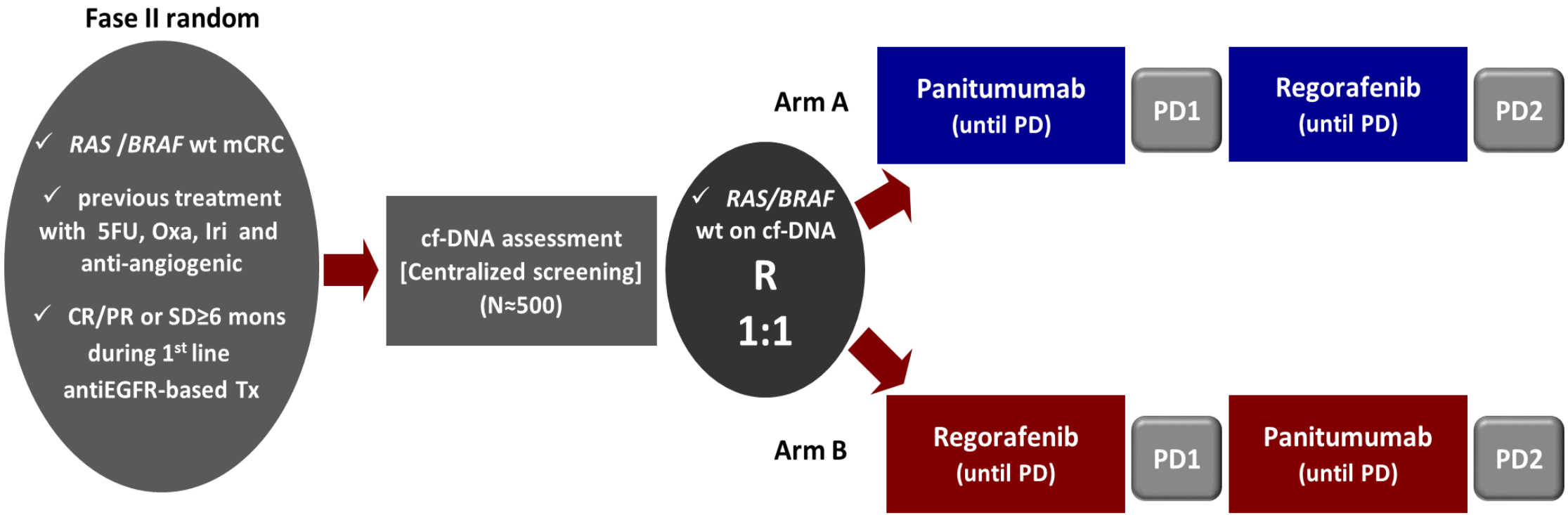
* Two PR were unconfirmed





Sartore-Bianchi et al, ASCO 2021

Ongoing study: the PARERE trial



Stratification factors:
- **ECOG PS: 0 vs 1**

Primary endpoint: OS

Target accrual: 214 pts

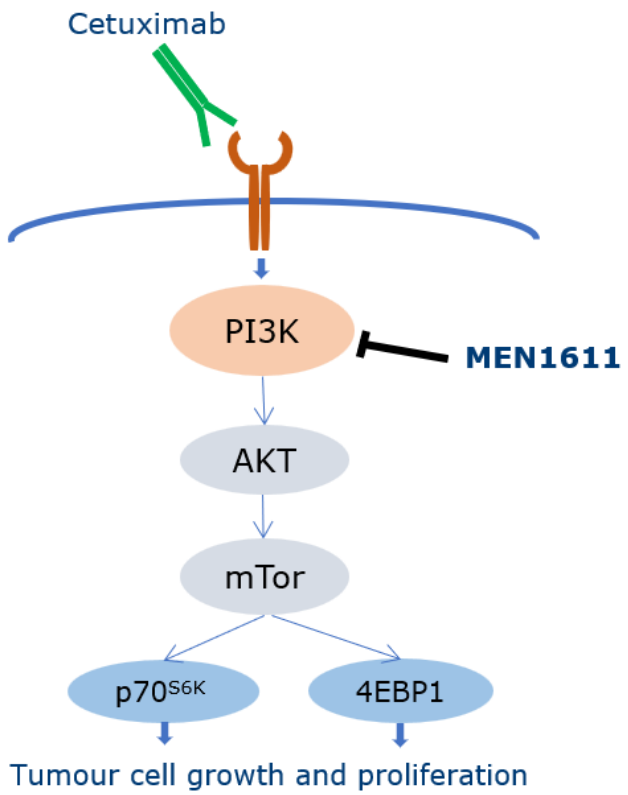
Moretto et al, Clin Colorectal Cancer 2021

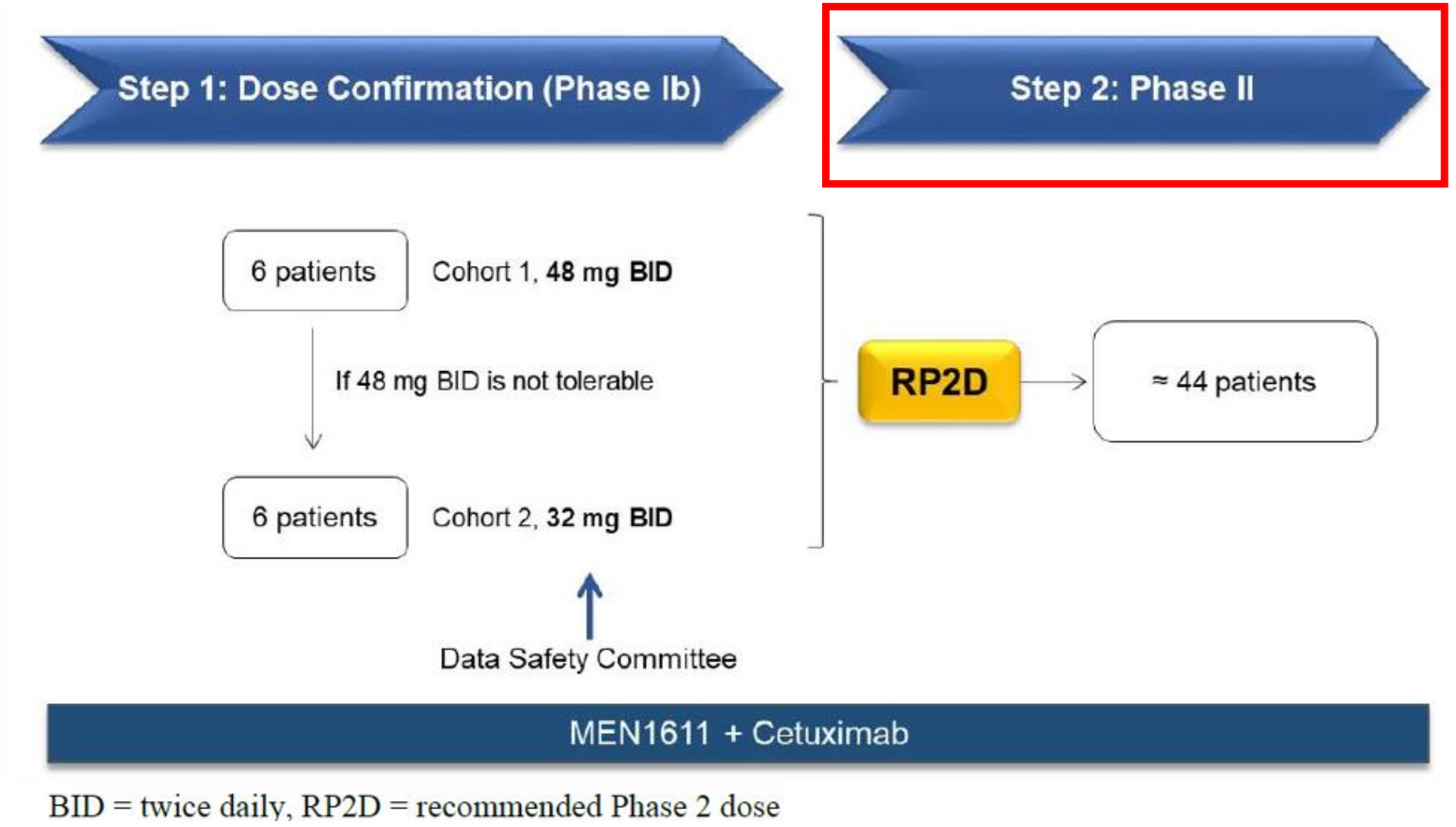
Ongoing study in *PI3K* mut and *RAS/BRAF* wt pts on ctDNA: the C-PRECISE-01trial

C·PRECISE·01

COLON-A PI3K INHIBITOR WITH TARGETED COMBINATIONS IN SOLID TUMORS TREATMENT

C-PRECISE-01 is an open-label, multicentre, phase Ib/II study of MEN1611, a PI3K Inhibitor, and cetuximab in patients with PIK3CA mutated, N-K-RAS and BRAF wild-type metastatic colorectal cancer (mCRC) failing irinotecan, oxaliplatin, 5-FU and anti-EGFR containing regimens.





BID = twice daily, RP2D = recommended Phase 2 dose

Primary endpoints:

- STEP 1: To determine MEN1611 RP2D in combination with cetuximab (DLT incidence ≤1 out of 6 patients treated)
- STEP 2: To assess antitumor activity
  - ORR according to RECIST 1.1

Secondary endpoints:

- Safety and tolerability of the combination
- Phamacokinetics profile of MEN1611
- * DCR, DoR, PFS and OS

NCT04495621

Take home messages

✓Several tissue biomerkers were routinarely assessed in clinical practice for their prognostic and predictive value (Microsatellite instability, *RAS* and *BRAF* mutations, NTRK fusions)

✓Other biomarkers may soon enter clinical practice for their predictive value of response to target therapy (HER-2 overexpression/amplification, KRAS-G12C, POLE mutation, MGMT silencing)

✓However, prevalence of therapeutically actionable alterations is low and fragmentation of mCRC in multiple molecular entities will imply a paradigm shift in the analysis of alterations from “single gene analysis” to “multigene panel analysis”.

✓ Liquid biopsy is a potential source of clinically relevant information that could drive clinicians’ decision making in different settings of CRC patients’ care.

✓However, the clinical reliability of a liquid biopsy-based therapeutic approach should be challenged in properly designed trials.