Supplemental Online Content

Martinelli E, Martini G, Famiglietti V, et al. Cetuximab rechallenge plus avelumab in pretreated patients with *RAS* wild-type metastatic colorectal cancer: the phase 2 single-arm clinical CAVE trial. *JAMA Oncol*. Published online August 12, 2021. doi:10.1001/jamaoncol.2021.2915

This supplemental material has been provided by the authors to give readers additional information about their work.

Supplementary Figures and Tables (online only)

Supplementary Figures and Tables:

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Supplementary Table 1: Baseline characteristics

	Patients (n=77)
Sex	
Male	42 (55%)
Female	35 (45%)
Median Age	63 (54-69)
ECOG PS	
0	52 (68%)
1	25 (32 %)
Number of previous lines of treatment	
2	52 (67%)
≥3	25 (33%)
Site of primary tumor	
Right*	5 (6%)
Left †	47 (61%)
Rectum	25 (32%)
Synchronous Metastases	
yes	56 (73%)
no	21 (27%)
Number of metastatic sites	
≤2	45 (58%)
≥3	32 (42%)
Liver limited disease	16 (21%)
Mucinous histology	4 (5%)
Resected primary tumor	
Yes	48 (62%)
No	29 (38%)
First line anti-EGFR drug Cetuximab Panitumumab	 38 (49%) 39 (51%)
Microsatellite status	
MSI-H	3 (4%)
MSS	71(92%)
Unknown	3 (4%)
RAS/BRAF (ctDNA)** WT MUT	67/77 (87%) 48/67 (72%) 19/67 (28%)
EGFR S492R (ctDNA)**	
WT	67/67 (100%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group. PS= performance status, EGFR= epidermal growth factor receptor, MSS=microsatellite stable, MSI microsatellite instable-high, WT= wild type, MUT= mutated, ctDNA= circulating tumor DNA

*Located in caecum, ascending colon, liver flexure, and transverse colon, †located in splenic flexure, descending colon, and sigmoid colon

** retrospective analysis of samples collected at baseline

Supplementary Table 2: Baseline characteristics of 67 patients with ctDNA *RAS/BRAF/EGFRS492R* Wild Type and mutant tumors

	Wild type patients(n=48)	Mutant patients (n=19)
Sex		
Male	25 (52%)	12 (63%)
Female	23 (48%)	7 (37%)
Median Age	60 (52-68)	65 (60-75)
ECOG PS		
0	34 (71%)	10 (53%)
1	14 (29 %)	9 (47%)
Number of previous lines of treatment		
2	33 (69%)	18 (95%)
≥3	15 (31%)	1 (5%)
Site of primary tumor		
Right*	1 (2%)	3 (16%)
Left †	33 (69%)	9 (47%)
Rectum	14 (29%)	7 (37%)
Synchronous Metastases		
yes	30 (62%)	17 (89%)
no	18 (38%)	2 (11%)
Number of metastatic sites		
≤2	32 (67%)	10 (53%)
≥3	16 (33%)	9 (47%)
Liver limited disease	8 (17%)	6 (32%)
Mucinous histology	1 (2%)	3 (16%)
Resected primary tumor		
Yes	32 (67%)	9 (47%)
No	16 (33%)	10 (53%)
First line anti-EGFR drug Cetuximab Panitumumab	 23 (48%) 25 (52%)	 8 (42%) 11 (58%)
Microsatellite status		
MSI-H	2 (4%)	0 (0%)
MSS	44 (92%)	19 (100%)
Unknown	2 (4%)	0 (0%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group. ctDNA= circulating tumor DNA, PS= performance status, EGFR= epidermal growth factor receptor, MSS=microsatellite stable, MSI microsatellite instable-high, WT= wild type, MUT= mutated

*Located in caecum, ascending colon, liver flexure, and transverse colon, †located in splenic flexure, descending colon, and sigmoid colon

Supplementary table 3: analysis of patients with baseline mutated ctDNA

	CtDNA analysis of mutated patients at baseline									
ID patient	**KRAS G12C**	**KRAS G12D**	**KRAS G12A**	**KRAS G12S**	**KRAS G12V**	**KRAS G13D**	**KRAS Q61H**	**KRAS A146P**	**BRAF V600E**	**EGFR S492R**
11	■									
14							■			
16		■								
17		■								
20								■		
24			■							
26									■	
28					■					
30						■				
38			■							
45							■			
47				■						
55	■									
60							■			
62		■								
69						■				
76					■					
81							■		■	
82		■								

Supplementary table 4: CtDNA analysis at progression of patients with baseline wild type ctDNA

	CtDNA analysis at progression of patient with baseline wild type ctDNA									
ID patient	**KRAS G12C**	**KRAS G12D**	**KRAS G12A**	**KRAS G12S**	**KRAS G12V**	**KRAS G13D**	**KRAS Q61H**	**KRAS A146P**	**BRAF V600E**	**EGFR S492R**
1										
2									■	
3										
4										
5										
7		■								
9										
10										
12										
13										
18							■			■
19										
21										
29										
32										
33										
36		■								
37										
39										■
43										
44										
48										
52										
57							■			
59										
61		■								■
73					■					
77										
79										
80										

Supplementary Table 5: Treatment-emerged adverse events

	Grades 1-2	**Grade 3**
Skin disorders:		
Rash	46 (60%)	11 (14%)
Dry skin	13 (17%)	0
Nail disorders	11 (14%)	0
Pruritus	8 (10%)	0
Conjunctivitis	7 (10%)	0
Blepharitis	2 (3%)	0
Gastrointestinal disorders:		
Diarrhoea	19 (25%)	3 (4%)
Abdominal pain	6 (7%)	0
Nausea	5 (6%)	0
Vomiting	2 (3%)	0
AST/ALT increase	6 (7%)	1 (1%)
Blood bilirubin increase	3 (4%)	0
Lipase and/or amylase increase	2 (3%)	2 (3%)
Immune related disorders:		
Hypothyroidism	2 (3%)	0

Data are in n (%). The table lists any grade (1-2 and 3) adverse events that occurred. No grade 4 or 5 adverse events have been recorded so far in study population. AST= aspartate aminotransferase, ALT= alanine aminotransferase

Supplementary table 6: Dose delays and reductions

	Patients= 77
Dose delays:	35 (45%)
Dose delays related to study drugs:	28 (36%)
7 days delay	16 (21%)
14 days delay	9 (12%)
>14 days delay	3* (4%)
Cetuximab delay	23 (30%)
Avelumab delay	5 (6%)
Dose reductions of cetuximab:	7 (10%)
1 dose reduction	6 (8%)
2 dose reductions	1 (2%)

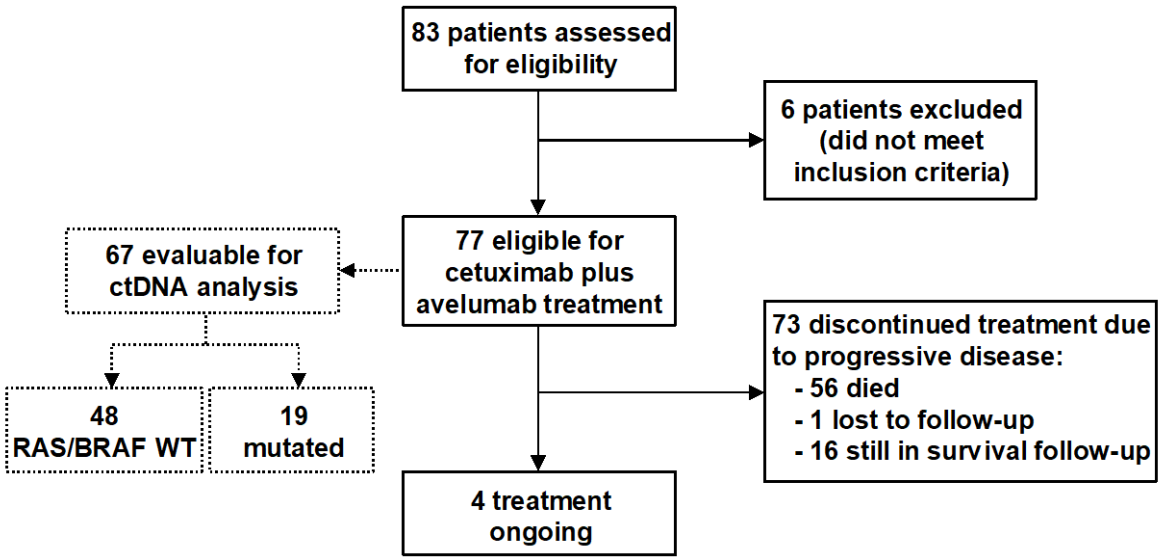
Data are n (%), * >14 days delays were Cetuximab related, drug was resumed after 21, 21 and 28 days

Supplementary table 7: Post CAVE mCRC treatments

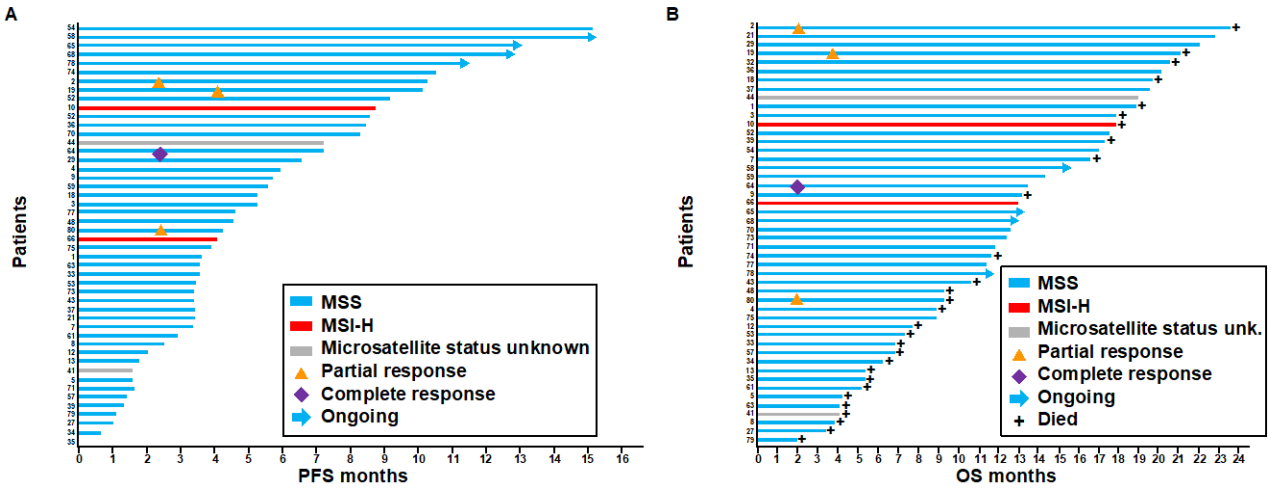
Patients who received therapy after CAVE mCRC trial	**49/73 (67%)**
Medical treatment* 1 treatment line >1 treatment line	45/49 (92%) 35/49 (71%) 10/49 (20%)
Non-medical treatment: Surgery	4/49 (8%) 2/49 (4%)
Radiotherapy	2/49 (4%)
CtDNA Wild Type patients that received therapy after CAVE mCRC trial	32/44 (73%)

Data are n (%) or). mCRC= metastatic colorectal cancer, ctDNA= circulating tumor DNA
* as medical treatment, patients received trifluridine/tipiracil (n=21), regorafenib (n=17), pembrolizumab (n=1), FOLFIRI, XELOX (n=1), FOLFOX (n=3), FOLFOX plus bevacizumab (n=2), FOLFIRI plus aflibercept (n=1), trifluridine/tipiracil plus bevacizumab (n=7), mitomycin C plus capecitabine (n=1), panitumumab (n=1), cabozantinib (n=4) (clinical trial).

Supplementary Figure 1: Trial profile



Supplementary Figure 2: Swimmer plot for Overall Survival and Progression Free Survival in patients with plasma ctDNA mutational status



Supplementary text

Patients and Methods

Patients monitoring and response assessment

Briefly, at least 6 mL of whole blood was collected, plasma was separated through two different centrifugation steps (the first at 1500×g and the second at 2000×g, both at room temperature for 10 min) and stored at -80^{0}C until analysis. ctDNA analyses of plasma were carried out using the automated Idylla^{TM} qPCR-based platform by Biocartis. Both ctKRAS and ctNRAS/BRAF/EGFR cartridges were used. The mutations detected by the cartridges are: ctKRAS: exon 2 (G12 > C/R/S/A/D/V, G13 > D), exon 3 (A59 > E/G/T, Q61 > K/L/R/H), exon 4 (K117 > N, A146 > P/T/V); ctNRAS/BRAF/EGFR: NRAS exon 2 (G12 > C/S/A/D/V, G13 > D/V/R), exon 3 (A59 > T, Q61 > K/L/R/H), exon 4 (K117 > N, A146 > T/V), BRAF V600 > E/D/K/R, EGFR S492 > R. The results of the analyses were visualized using the on-line tool Idylla^{TM} Explore. According to datasheet, for the *KRAS* gene the analytical sensitivity is ≤ 1% for mutations in exon 2 and 3 and ≤5% in exon 4. Regarding the *NRAS* gene, the analytical sensitivity is ≤5% for mutations in exons 2, 3 and 4: while for the *BRAF* gene codon 600 mutations, analytical sensitivity is ≤1% (idyllaexplore.biocartis.com).[15]

Statistical analysis

If the patient did not die, the survival time was censored on the last date the patient was known to be alive. Patients with clinical progression were considered to have progressed at the time of clinical deterioration. Patients lost to follow-up without any radiologic progression were censored at the date of the last follow-up visit for which information was available.