Supplementary

Table S1 *RAS/RAF* and dominant gene pairs included in sensitivity analysis

Cancer type	*RAS/RAF* on Tissue	*RAS/RAF* SNV	cfDNA *RAS/RAF* MAF	cfDNA Greatest MAF Dominant Gene	Dominant Gene Variant	cfDNA Dominant Variant MAF
CRC	NRAS	p.G12D	0.172	TP53	p.R342*	0.165
CRC	NRAS	p.Q61L	0.5562	TP53	p.R175H	0.7532
CRC	NRAS	p.Q61R	0.2438	FBXW7	p.R689W	0.1369
CRC	KRAS	p.A146P	N/A	N/A	N/A	N/A
CRC	KRAS	p.A146T	0.0197	TP53	p.R273H	0.0131
CRC	KRAS	p.A146T	0.078	APC	p.T1445fs	0.06
CRC	KRAS	p.A146T	0.276	APC	p.T1465fs*28	0.1495
CRC	KRAS	p.A146V	0.179	TP53	p.R175H	0.3024
CRC	KRAS	p.G12A	0.025	TP53	p.H214R	0.025
CRC	KRAS	p.G12A	0.401	TP53	p.R273H	0.708
CRC	KRAS	p.G12C	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12C	0.0092	APC	p.C2071fs	0.0097
CRC	KRAS	p.G12C	N/A	SMAD4	p.D351H	0.003
CRC	KRAS	p.G12C	0.011	APC	p.E1306fs	0.017
CRC	KRAS	p.G12C	N/A	NOTCH1	p.R2156R	0.0018
CRC	KRAS	p.G12C	0.463	ATM	p.K2697*	0.502
CRC	KRAS	p.G12C	0.0157	TP53	p.R175H	0.0326
CRC	KRAS	p.G12C	0.218	TP53	p.R175H	0.142
CRC	KRAS	p.G12C	0.0287	TP53	p.S94fs	0.0315
CRC	KRAS	p.G12D	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12D	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12D	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12D	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12D	0.0021	SMAD4	p.C115S	0.0046
CRC	KRAS	p.G12D	0.3135	TP53	p.G245S	0.4596
CRC	KRAS	p.G12D	0.3298	TP53	p.G245S	0.5858
CRC	KRAS	p.G12D	0.009	TP53	p.G266E	0.026
CRC	KRAS	p.G12D	0.4378	EGFR	p.H773fs	0.3507
CRC	KRAS	p.G12D	0.0058	APC	p.I1287fs	0.0153
CRC	KRAS	p.G12D	0.4048	BRCA1	p.K339fs	0.2888
CRC	KRAS	p.G12D	0.2206	TP53	p.L206*	0.2974
CRC	KRAS	p.G12D	0.0079	EGFR	p.L989F	0.0078
CRC	KRAS	p.G12D	0.0029	NOTCH1	p.P2122L	0.0066
CRC	KRAS	p.G12D	N/A	CCND2	p.P281S	0.005
CRC	KRAS	p.G12D	0.2757	APC	p.Q1429*	0.4383
CRC	KRAS	p.G12D	0.1693	TP53	p.G331H	0.2131
CRC	KRAS	p.G12D	0.2512	TP53	p.G331H	0.3244
CRC	KRAS	p.G12D	0.3705	TP53	p.G331H	0.5334
CRC	KRAS	p.G12D	0.034	PTEN	p.R173C	0.027
CRC	KRAS	p.G12D	0.129	TP53	p.R196*	0.213
CRC	KRAS	p.G12D	0.0012	GNAS	p.R201H	0.0014
CRC	KRAS	p.G12D	0.1883	TP53	p.R213*	0.2754
CRC	KRAS	p.G12D	0.0509	TP53	p.R273H	0.0627
CRC	KRAS	p.G12D	0.158	TP53	p.R306*	0.1371
CRC	KRAS	p.G12D	0.1867	TP53	p.R306*	0.1739
CRC	KRAS	p.G12D	0.1923	TP53	p.R306*	0.253
CRC	KRAS	p.G12D	N/A	APC	p.R405*	0.001
CRC	KRAS	p.G12D	0.2784	FBXW7	p.R465C	0.3228
CRC	KRAS	p.G12D	0.001	APC	p.R499*	0.001
CRC	KRAS	p.G12D	0.0059	APC	p.S1495fs	0.0078
CRC	KRAS	p.G12D	0.0564	APC	p.Y1411fs	0.0699
CRC	KRAS	p.G12D	0.0461	TP53	p.V272M	0.0521
CRC	KRAS	p.G12D	0.0326	APC	p.Y1162fs	0.0747
CRC	KRAS	p.G12R	0.0073	TP53	p.T125T	0.0099
CRC	KRAS	p.G12S	0.0036	TP53	p.R282W	0.0039
CRC	KRAS	p.G12V	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12V	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12V	N/A	N/A	N/A	N/A
CRC	KRAS	p.G12V	0.429	TP53	p.C176R	0.483
CRC	KRAS	p.G12V	0.4947	TP53	p.F113V	0.4429
CRC	KRAS	p.G12V	0.0234	APC	p.G1106*	0.0154
CRC	KRAS	p.G12V	0.196	ATM	p.G2020D	0.1259
CRC	KRAS	p.G12V	0.0165	MET	p.G757E	0.0851
CRC	KRAS	p.G12V	0.0008	TP53	p.H179R	0.0101
CRC	KRAS	p.G12V	N/A	APC	p.K1462fs	0.0014
CRC	KRAS	p.G12V	0.0205	APC	p.K1462fs	0.0772
CRC	KRAS	p.G12V	0.0037	APC	p.L1489fs	0.0046
CRC	KRAS	p.G12V	0.023	TP53	p.L194F	0.031
CRC	KRAS	p.G12V	0.0131	TP53	p.R110L	0.0124
CRC	KRAS	p.G12V	0.0064	TP53	p.R175H	0.0055
CRC	KRAS	p.G12V	0.2587	TP53	p.R175H	0.4353
CRC	KRAS	p.G12V	N/A	TP53	p.R181H	0.0025
CRC	KRAS	p.G12V	N/A	NF1	p.R2698H	0.0017
CRC	KRAS	p.G12V	0.0815	TP53	p.R248Q	0.0974
CRC	KRAS	p.G12V	0.2938	TP53	p.R248W	0.109
CRC	KRAS	p.G12V	0.0053	TP53	p.R273C	0.0041
CRC	KRAS	p.G12V	N/A	TP53	p.R280I	0.0671
CRC	KRAS	p.G12V	0.016	APC	p.R499*	0.02
CRC	KRAS	p.G12V	0.0041	PDGFRA	p.R914W	0.0084
CRC	KRAS	p.G12V	0.1229	APC	p.T1556fs	0.0889
CRC	KRAS	p.G12V	0.097	APC	p.T1556fs	0.094
CRC	KRAS	p.G12V	0.3875	TP53	p.W53*	0.5589
CRC	KRAS	p.G12V	0.2643	TP53	p.Y220C	0.3238
CRC	KRAS	p.G12V	0.3705	TP53	p.Y220C	0.5845
CRC	KRAS	p.G13C	0.0318	ERBB2	p.T733I	0.046
CRC	KRAS	p.G13D	0.006	APC	Ap.R1114*	0.007
CRC	KRAS	p.G13D	0.0384	AR	p.A736T	0.0526
CRC	KRAS	p.G13D	0.218	TP53	p.C176F	0.292
CRC	KRAS	p.G13D	N/A	BRCA1	p.D1813G	0.0034
CRC	KRAS	p.G13D	0.004	APC	p.E1309fs	0.002
CRC	KRAS	p.G13D	0.115	APC	p.E1309fs	0.113
CRC	KRAS	p.G13D	N/A	MAPK1	p.F296V	0.0022
CRC	KRAS	p.G13D	0.356	APC	p.N813fs	0.0303
CRC	KRAS	p.G13D	N/A	APC	p.P1409fs	0.0018
CRC	KRAS	p.G13D	0.0599	TP53	p.R175H	0.0775
CRC	KRAS	p.G13D	0.1454	TP53	p.R196*	0.1584
CRC	KRAS	p.G13D	0.0379	APC	p.S1298fs	0.0456
CRC	KRAS	p.G13D	0.0027	TP53	p.Y220C	0.0161
CRC	KRAS	p.K117N	0.3962	TP53	p.N239D	0.3762
CRC	KRAS	p.K117N	0.256	TP53	p.R248W	0.132
CRC	KRAS	p.P34R	N/A	N/A	N/A	N/A
CRC	KRAS	p.Q61H	N/A	N/A	N/A	N/A
CRC	KRAS	p.Q61H	0.1604	TP53	p.R282W	0.1851
CRC	KRAS	p.Q61H	0.4848	TP53	p.V272M	0.6661
CRC	KRAS	p.Q61L	0.2704	BRCA1	p.A1823V	0.0013
CRC	KRAS	p.Q61L	0.581	TP53	p.V173L	0.768
CRC	BRAF	p.D594G	0.2466	TP53	p.E258*	0.3239
CRC	BRAF	p.D594G	0.3393	TP53	p.E258*	0.507
CRC	BRAF	p.D594G	0.1761	TP53	p.E286G	0.3526
CRC	BRAF	p.D594H	N/A	TP53	p.C124fs	0.0068
CRC	BRAF	p.D594N	0.0011	APC	p.T772fs	0.0008
CRC	BRAF	p.G466A	0.4065	APC	p.R259W	0.2854
CRC	BRAF	p.G466E	0.003	TP53	p.V272M	0.116
CRC	BRAF	p.G469E	0.1378	APC	p.E1464fs	0.1117
CRC	BRAF	p.G469E	0.0082	TP53	p.R342*	0.0077
CRC	BRAF	p.G469E	0.1496	TP53	p.R342*	0.1192
CRC	BRAF	p.K483E	0.577	TP53	p.C176G	0.542
CRC	BRAF	p.V600E	0.0028	N/A	N/A	N/A
CRC	BRAF	p.V600E	N/A	N/A	N/A	N/A
CRC	BRAF	p.V600E	N/A	N/A	N/A	N/A
CRC	BRAF	p.V600E	N/A	N/A	N/A	N/A
CRC	BRAF	p.V600E	0.0608	ARID1A	p.D1850fs	0.072
CRC	BRAF	p.V600E	0.0034	AKT1	p.E17K	0.0028
CRC	BRAF	p.V600E	0.0648	PIK3CA	p.E418K	0.0688
CRC	BRAF	p.V600E	0.0054	TP53	p.G266E	0.0019
CRC	BRAF	p.V600E	0.0013	NTRK3	p.G608S	0.0042
CRC	BRAF	p.V600E	0.0132	NTRK3	p.G608S	0.0349
CRC	BRAF	p.V600E	0.0387	NTRK3	p.G608S	0.0606
CRC	BRAF	p.V600E	0.0996	PIK3CA	p.N345K	0.1006
CRC	BRAF	p.V600E	0.3235	APC	p.N942fs	0.3228
CRC	BRAF	p.V600E	0.3904	APC	p.N942fs	0.4148
CRC	BRAF	p.V600E	0.423	APC	p.N942fs	0.421
CRC	BRAF	p.V600E	0.0008	ARID1A	p.R1528*	0.0032
CRC	BRAF	p.V600E	0.0973	ARID1A	p.R1528*	0.0924
CRC	BRAF	p.V600E	0.1688	ARID1A	p.R1528*	0.1488
CRC	BRAF	p.V600E	0.0016	TP53	p.R175H	0.002
CRC	BRAF	p.V600E	0.004	TP53	p.R175H	0.0039
CRC	BRAF	p.V600E	0.005	TP53	p.R175H	0.0048
CRC	BRAF	p.V600E	0.0206	TP53	p.R175H	0.0313
CRC	BRAF	p.V600E	0.0357	TP53	p.R175H	0.0375
CRC	BRAF	p.V600E	0.1222	TP53	p.R175H	0.1054
CRC	BRAF	p.V600E	0.2661	TP53	p.R175H	0.2318
CRC	BRAF	p.V600E	0.4084	TP53	p.R175H	0.4896
CRC	BRAF	p.V600E	0.2606	TP53	p.R248W	0.1705
CRC	BRAF	p.V600E	0.3617	TP53	p.R248W	0.2283
CRC	BRAF	p.V600E	0.4946	TP53	p.R248W	0.3502
CRC	BRAF	p.V600E	0.0816	TP53	p.R273C	0.0449
CRC	BRAF	p.V600E	0.1256	TP53	p.R273C	0.0664
CRC	BRAF	p.V600E	0.0616	TP53	p.R273C	0.0667
CRC	BRAF	p.V600E	0.1184	TP53	p.R273C	0.1331
CRC	BRAF	p.V600E	0.1132	TP53	p.R273C	0.1419
CRC	BRAF	p.V600E	0.263	TP53	p.R273C	0.1588
CRC	BRAF	p.V600E	0.4199	TP53	p.R273C	0.4077
CRC	BRAF	p.V600E	0.4799	TP53	p.R273C	0.4697
CRC	BRAF	p.V600E	0.4095	TP53	p.R342*	0.4817
CRC	BRAF	p.V600E	0.0305	SMAD4	p.R361C	0.0681
CRC	BRAF	p.V600E	0.0519	SMAD4	p.R361C	0.0885
CRC	BRAF	p.V600E	0.1136	SMAD4	p.R361C	0.2173
CRC	BRAF	p.V600E	0.0024	TP53	p.S90fs	0.0035
CRC	BRAF	p.V600E	0.049	APC	p.T1556fs	0.0496
CRC	BRAF	p.V600E	0.3524	APC	p.T1556fs	0.4254
CRC	BRAF	p.V600E	N/A	MYC	p.V160L	0.007
CRC	BRAF	p.V600E	0.0196	SMAD4	p.W524C	0.0078
CRC	BRAF	p.V600E	0.0033	TP53	p.Y163D	0.0014
CRC	BRAF	p.V600E	0.2866	TP53	p.Y205C	0.36
PDAC	KRAS	p.G12D	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12D	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12D	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12D	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12D	0.263	TP53	p.C277Y	0.0021
PDAC	KRAS	p.G12D	0.18	TP53	p.P153fs	0.11
PDAC	KRAS	p.G12D	0.441	TP53	p.P77fs	0.436
PDAC	KRAS	p.G12D	0.1	ARID1A	p.Q1519fs	0.119
PDAC	KRAS	p.G12D	0.012	TP53	p.R249S	0.008
PDAC	KRAS	p.G12D	N/A	TP53	p.R273C	0.004
PDAC	KRAS	p.G12D	0.006	TP53	p.R342*	0.002
PDAC	KRAS	p.G12D	0.129	TP53	p.R342*	0.023
PDAC	KRAS	p.G12D	0.176	TP53	p.T125P	0.159
PDAC	KRAS	p.G12D	0.0051	TP53	p.V173M	0.0081
PDAC	KRAS	p.G12D	0.221	TP53	p.W146*	0.356
PDAC	KRAS	p.G12D	0.0478	TP53	p.Y220C	0.0404
PDAC	KRAS	p.G12D	0.014	RB1	p.Y805fs	0.024
PDAC	KRAS	p.G12R	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12R	0.035	TP53	p.R248W	0.024
PDAC	KRAS	p.G12V	0.008	N/A	N/A	N/A
PDAC	KRAS	p.G12V	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12V	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12V	N/A	N/A	N/A	N/A
PDAC	KRAS	p.G12V	0.003	TP53	p.E286V	0.003
PDAC	KRAS	p.G12V	N/A	RB1	p.P25L	0.14
PDAC	KRAS	p.G12V	0.032	TP53	p.Q167fs	0.072
PDAC	KRAS	p.G12V	N/A	IDH1	p.R132S	0.001
PDAC	KRAS	p.G12V	0.127	TP53	p.R273H	0.07
PDAC	KRAS	p.G12V	0.319	TP53	p.S166*	0.15
PDAC	KRAS	p.Q61H	N/A	N/A	N/A	N/A
PDAC	KRAS	p.Q61R	N/A	N/A	N/A	N/A

SNV, single nucleotide variant; MAF, mutant allele frequency; cfDNA, cell-free DNA; CRC, colorectal cancer; PDAC, pancreatic ductal adenocarcinoma; N/A, no variant detected.

https://dx.doi.org/10.21037/jgo-23-114

Table S2 Sites of metastatic disease at time of cfDNA NGS

RAS/RAF SNV detected in cfDNA, yes/no	CRC (n=135)			PDAC (n=30)		
	Total	Yes	No	Total	Yes	No
Metastatic disease, n (%)						
Locally advanced/unknown	1 (0.7%)	1 (100%)	0	2 (6.7%)	1 (50%)	1 (50%)
1 site	28 (20.7%)	14 (50.0%)	14 (50.0%)	13 (43.3%)	8 (61.5%)	5 (38.5%)
Liver only	4 (3.0%)	2 (50.0%)	2 (50.0%)	8 (26.7%)	7 (87.5%)	1 (12.5%)
Lung only	11 (8.1%)	6 (54.5%)	5 (45.5%)	3 (10.0%)	0	3 (100%)
Peritoneum only	8 (5.9%)	2 (25.0%)	6 (75%)	1 (3.3%)	0	1 (100%)
Lymph node only	5 (3.7%)	4 (80%)	1 (20%)	0	0	0
Bone only	0	0	0	1 (3.3%)	1 (100%)	0
2 sites	70 (51.9%)	58 (82.9%)	12 (17.1%)	10 (33.3%)	3 (30%)	7 (70%)
3+ sites	36 (26.7%)	34 (94.4%)	2 (5.6%)	5 (20.0%)	4 (80%)	1 (20%)

cfDNA, cell-free DNA; NGS, next-generation sequencing; SNV, single nucleotide variant; CRC, colorectal cancer; PDAC, pancreatic ductal adenocarcinoma.

Table S3 Colorectal patients treated with anti-EGFR therapy

Received anti-EGFR mAb therapy, n (%)	30/135 (22.2%)
Received on clinical trial	4 (3.0%)
BRAF/MEK inhibitor + Anti-EGFR mAb	4 (3.0%)
BRAF V600E, received anti-EGFR mAb per past SOC	11 (8.1%)
BRAF non-V600E, received anti-EGFR per SOC	6 (4.4%)
Treated off-label/other	5 (3.7%)

EGFR, epidermal growth factor receptor; mAb, monoclonal antibody; SOC, standard of care.

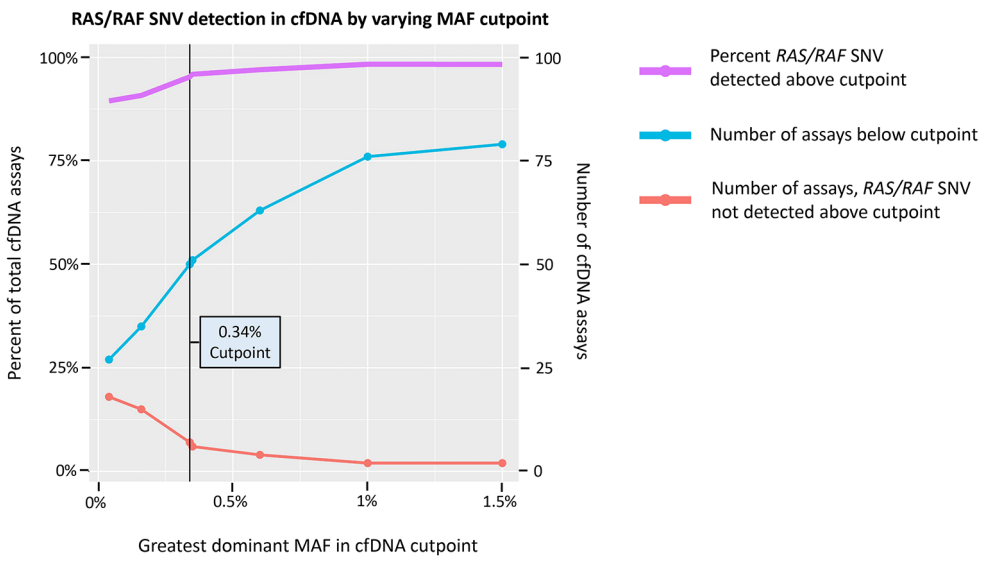

Figure S1 Relative partitioning by greatest dominant MAF, depicted as testing characteristics above varying MAF cutpoints. Percent *RAS/RAF* SNV detected among assays with MAF above cutpoint is shown by the purple line. Number of assays with MAF falling below cutpoint (teal line) or not detected despite MAF above cutpoint (red line) is shown, out of total 198 assays. Sensitivity based on greatest MAF above cutpoint falls gradually as cutpoint decreases between 1% and 0.34%, and falls more sharply below 0.34%. Number below detection threshold increases significantly with increasing cutpoint. The optimal cutpoint by maximally selected Wilcoxon rank statistic and recursive partitioning is indicated by a vertical line (P<0.0001). MAF, mutant allele frequency; SNV, single nucleotide variant; cfDNA, cell-free DNA.

https://dx.doi.org/10.21037/jgo-23-114