

RAS-amplified colorectal cancers: Microsatellite stability status, RAS/BRAF mutations, and prediction of anti-EGFR resistance

Alexa B. Schrock¹, Jaideep Singh Sandhu², Chongkai Wang², Ching Ouyang³, Vincent A. Miller¹, Siraj M. Ali¹, Marwan Fakhri²

¹Foundation Medicine, Inc., Cambridge, MA; ²City of Hope Comprehensive Cancer Center, Duarte, CA; ³Department of Computational and Quantitative Medicine, Beckman Research Institute of the City of Hope, Duarte, CA



Background

- RAS mutations are associated with worse prognosis than RAS wild type tumors in metastatic colorectal cancer (mCRC)
- RAS mutations predict resistance to anti-EGFR therapy in mCRC
- RAS amplification (RAS^{amp}) results in activation of the EGFR pathway and is associated with anti-EGFR resistance in preclinical models
- There are no reports on the impact of the presence or degree of amplification, as identified by next generation sequencing (NGS), on anti-EGFR therapy response.

Methods

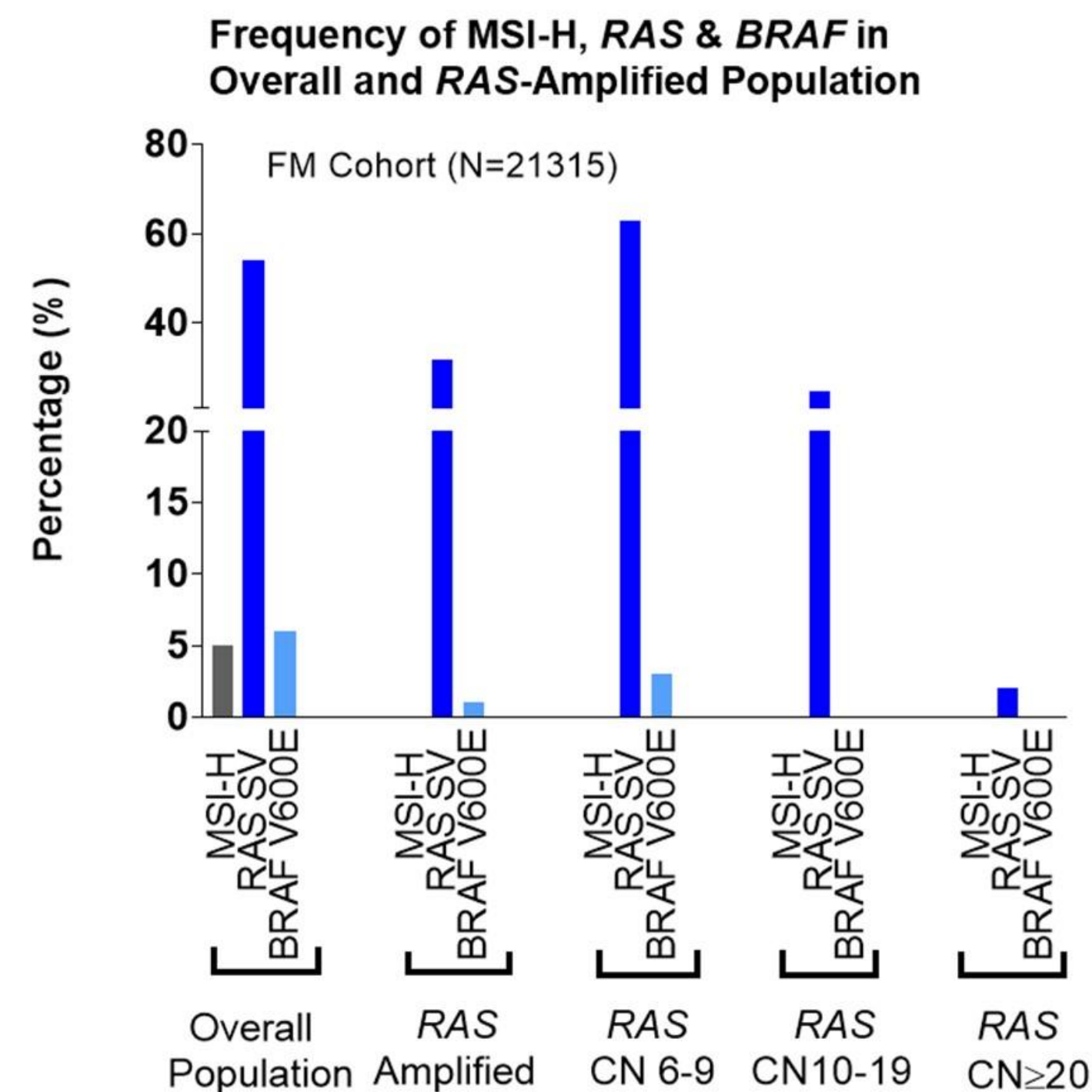
- Foundation Medicine (FM):** We investigated the FM database for RAS^a in CRC and characterized this population's demographics and tumor genomic profile
- Gene amplification was defined as gene copy number (CN) of ≥ 6 copies
- City of Hope (COH):** We reviewed a single center molecular database (2015-2018, cases analyzed by FM) of mCRC and described the prevalence of RAS amplified cases, associated patient characteristics, and their response to anti-EGFR therapy

Results

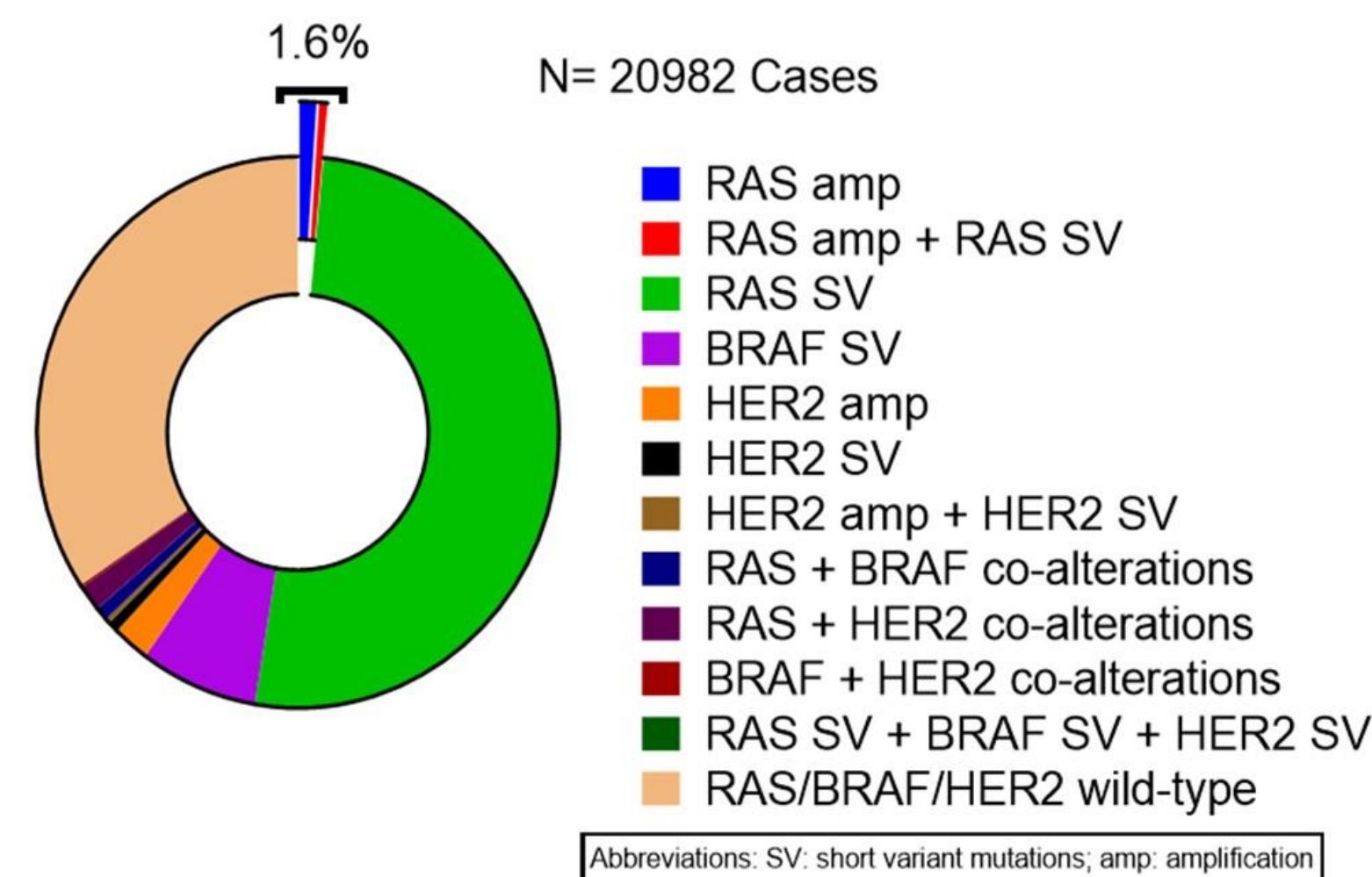
FM Cohort: Population Characteristics & Co-Existent Genomic Alterations in RAS Amplified Cases

FM Cohort	RAS ^a Cases* n(%)	Age (Years) Median	Gender (M:F)	RAS SV n(%)	BRAF V600E SV n(%)	MSI-H Status %	≥ 2 Gene Amps n(%)	≥ 5 Gene Amps n(%)
RAS ^a	365 (1.7)	56	210:155	117 (32)	4 (1)	0	254 (70)	107 (29)
RAS CN 6-9	139 (38)	56	75:64	88 (63)	4 (3)	0	96 (69)	47 (34)
RAS CN 10-19	103 (28)	54	51:52	26 (25)	0	0	63 (61)	24 (23)
RAS CN ≥ 20	123 (33)	57	84:39	3 (2)	0	0	95 (77)	36 (29)

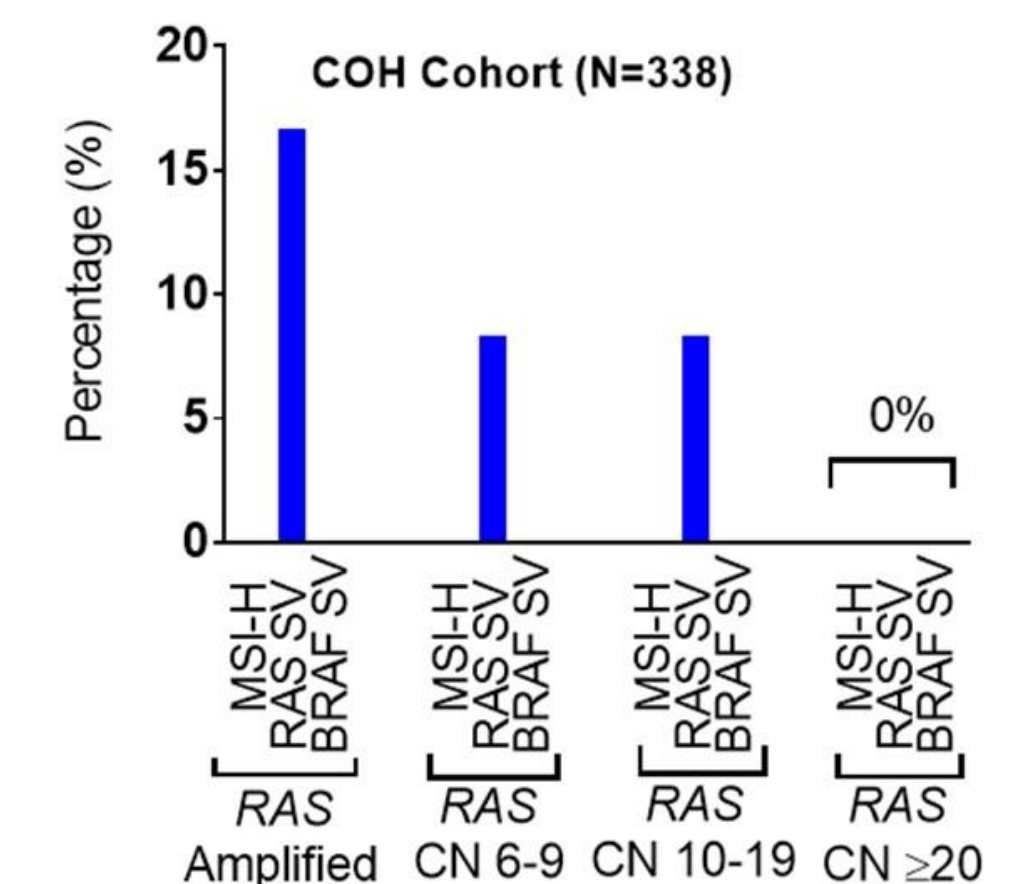
Abbreviations: M: male; F: female; mCRC: metastatic colorectal cancer; n: number; CN: copy number; SV: short variants (point mutation or indel); Amps: amplifications
* Total CRC cases=21315, median age 58, gender (M:F) 11666:9649



FM Cohort: Frequency of Genomic Alterations in mCRC



Frequency of MSI-H, RAS & BRAF Short Variant Mutations in RAS-Amplified Population



COH: Frequency of RAS Amplification

COH Cohort	Total CRC Cases	RAS Amplified Cases	RAS Amplified Cases with CN 6-9	RAS Amplified Cases with CN 10-19	RAS Amplified Cases with CN ≥ 20
N (%)	338	12 (3.6%)	2 (0.6%)	2 (0.6%)	8 (2.4%)

RAS-Amplifications: COH Experience with Anti-EGFR Therapy

Case #	Age	Sex	Sidedness	KRAS CN	RAS SV Status	BRAF SV Status	Prior OX or IRI Resistance*	Anti-EGFR Regimen/Line	Anti-EGFR Initiated After Sample Collection	Best Response	PFS/Months
1	53	Female	Left	13	WT	WT	NA	FOLFIRI & Pmab (1 st Line)	Yes	SD	4
2	53	Male	Right	20	WT	WT	OX & IRI resistant	FOLFIRI & Pmab (3 rd Line)	Yes	PD	2
3	50	Female	Left	25	WT	WT	OX & IRI Resistant	IRI & Cmab (2 nd Line)	Yes	SD	7
4	31	Male	Left	29	WT	WT	IRI Resistant	CAP & Cmab (3 rd Line)	Yes	PD	2.5
5	56	Male	Left	30	WT	WT	OX & IRI Resistant	FOLFIRI & Pmab (3 rd Line)	Yes	PD	2.5
6	53	Female	Left	42	WT	WT	OX Resistant	FOLFOX & Pmab (2 nd Line)	No	PD	3.5
7	36	Female	Left	54	WT	WT	OX & IRI Resistant	IRI & Pmab (4 th Line)	Yes	PD	0.5
8	62	Female	Left	7	KRAS G12S	WT	OX Exposed	No Anti-EGFR Regime	NA	NA	NA
9	61	Male	Left	8	KRAS G12V	WT	OX & IRI Resistant	No Anti-EGFR Regime	NA	NA	NA
10	38	Female	Left	8	WT	WT	OX & IRI resistant	No Anti-EGFR Regime	NA	NA	NA
11	50	Female	Left	13	KRAS G12S	WT	OX & IRI resistant	No Anti-EGFR Regime	NA	NA	NA
12	71	Male	Left	39	WT	WT	OX Exposed	No Anti-EGFR Regime	NA	NA	NA
13	46	Female	Left	79	WT	WT	OX & IRI Exposed	No Anti-EGFR Regime	NA	NA	NA

Abbreviations: OX: oxaliplatin; IRI: irinotecan; Cmab: cetuximab; Pmab: panitumumab; FOLFIRI: fluorouracil, leucovorin, and irinotecan; FOLFOX: fluorouracil, leucovorin, and oxaliplatin; CAP: capecitabine; CN: copy number; PD: progressive disease; SD: stable disease; SV: short variant (point mutation or indel); WT: wild-type; NA: not applicable; COH: City of Hope. * All patients were exposed to prior fluoropyrimidines

Conclusions

- NGS identifies RAS^a (≥ 6 CN) and RAS^a ≥ 20 CN in 1.5% and 0.6% of CRC, respectively
- RAS^a tumors occurred predominantly in the left colon in a single institute cohort
- RAS^a ≥ 20 CN tumors are MSS, generally lack RAS/BRAF short variant mutations, and predict for resistance to anti-EGFR therapy
- Our data suggests that tumors with elevated RAS CN may not drive a significant benefit from anti-EGFR therapy
- The data support the potential relevance of 20-CN cut-point for the exclusion of pts from anti-EGFR, or at least in guiding the sequencing of anti-EGFR therapy
- Additional validation through the retrospective analysis of completed phase II and III anti-EGFR clinical trials could provide support for our findings

References

- Phipps AI, Buchanan DD, Makar KW, et al. KRAS-mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers. *Br J Cancer*. 2013;108(8):1757-64.
- Sforza V, Martinelli E, Ciardiello F, et al. Mechanisms of resistance to anti-epidermal growth factor receptor inhibitors in metastatic colorectal cancer. *World J Gastroenterol*. 2016;22(28):6345-61.
- Valtorta E, Misale S, Sartore-Bianchi A, Nagtegaal ID, Paraf F, Lauricella C, et al. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. *Int J Cancer* 2013;133: 1259-65.
- Mekenkamp LJ, Tol J, Dijkstra JR, de Krijger I, Vink-Borger ME, van Vliet S, et al. Beyond KRAS mutation status: influence of KRAS copy number status and microRNAs on clinical outcome to cetuximab in metastatic colorectal cancer patients. *BMC Cancer* 2012;12:292.

Correspondence: Marwan Fakhri, MD Professor, Medical Oncology and Therapeutics Research City of Hope National Medical Center 1500 E Duarte St, Duarte, CA 91010 Email: mfakhri@coh.org