Received: 1 March 2023 | Revised: 4 August 2023 | Accepted: 7 August 2023

DOI: 10.1002/ijc.34733

RESEARCH ARTICLE

Cancer Genetics and Epigenetics

IJC INTERNATIONAL JOURNAL of CANCER

Atypical (non-V600E) *BRAF* mutations in metastatic colorectal cancer in population and real-world cohorts

Emerik Osterlund[1,2] | Ari Ristimäki[3,4] | Markus J. Mäkinen[5,6,7] | Soili Kytölä[8,9] | Juha Kononen[10,11] | Per Pfeiffer[12,13] | Leena-Maija Soveri[14,15] | Mauri Keinänen[16] | Halfdan Sorbye[17,18] | Luís Nunes[1] | Tapio Salminen[19,20] | Lasse Nieminen[21,22] | Aki Uutela[2,23,24] | Päivi Halonen[15,25] | Annika Ålgars[26,27] | Jari Sundström[28,29] | Raija Kallio[30,31] | Raija Ristamäki[26,27] | Annamarja Lamminmäki[32,33] | Hanna Stedt[32,33] | Eetu Heervä[26,27] | Teijo Kuopio[34,35] | Tobias Sjöblom[1] | Helena Isoniemi[2,23] | Bengt Glimelius[1] | Pia Osterlund[19,20,23,24,36,37]

Correspondence
Emerik Osterlund, Uppsala University, Department of Immunology, Genetics and Pathology, Rudbecklaboratoriet, 751 85 Uppsala, Sweden.
Email: emerik.osterlund@igp.uu.se, emerik.osterlund@gmail.com

Funding information
Finska Läkaresällskapet, Grant/Award Numbers: 2016, 2018, 2019, 2020, 2021, 2022; Relander's Foundation, Grant/Award Number: 2020-2022; Syöpäsäätiö, Grant/Award Numbers: 2019-2020, 2021, 2022-2023; Tampere University Hospital Funds, Grant/Award Numbers: Tukisäätiö 2019, 2020, OOO 2020; The Competitive State Research Financing of the Expert Responsibility Area of Tampere, Helsinki and Turku, Grant/Award Numbers: 2016, 2017, 2018, 2019, 2020, 2021, 2022; The Research Fund of Helsinki University Hospital, Grant/Award Numbers: 2019, 2020, 2021; Amgen, Grant/Award Number: 2012-2020; Eli

Abstract

BRAF-V600E mutation (mt) is a strong negative prognostic and predictive biomarker in metastatic colorectal cancer (mCRC). Non-V600Emt, designated atypical *BRAF*mt (a*BRAF*mt) are rare, and little is known about their frequency, co-mutations and prognostic and predictive role. These were compared between mutational groups of mCRC patients collected from three Nordic population-based or real-world cohorts. Pathology of a*BRAF*mt was studied. The study included 1449 mCRC patients with 51 (3%) a*BRAF*mt, 182 (13%) *BRAF*-V600Emt, 456 (31%) *RAS&BRAF* wild-type (wt) and 760 (52%) *RAS*mt tumours. a*BRAF*mt were seen in 2% of real-world and 4% of population-based cohorts. Twenty-six different a*BRAF*mt were detected, 11 (22%) class 2 (serrated adenocarcinoma in 2/9 tested), 32 (64%) class 3 (serrated in 15/25) and 4 (8%) unclassified. a*BRAF*mt patients were predominantly male, had more rectal primaries, less peritoneal metastases, deficient mismatch repair in one (2%), and better survival after metastasectomy (89% 5-year overall survival [OS]-rate) compared with *BRAF*-V600Emt. a*BRAF*mt and *BRAF*-V600Emt had poorer performance status

Abbreviations: a*BRAF*, atypical v-rapidly accelerated fibrosarcoma (RAF) murine sarcoma viral oncogene homologue B; *BRAF*, v-rapidly accelerated fibrosarcoma (RAF) murine sarcoma viral oncogene homologue B; BSC, best supportive care; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; dMMR, deficient mismatch repair; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinases; ESMO, European Society for Medical Oncology; *KRAS*, Kirsten rat sarcoma viral oncogene; LAT, local ablative therapy; MAPK, mitogen-activated protein kinase; mCRC, metastatic colorectal cancer; MEK, mitogen-activated protein kinase kinase; MMR, mismatch repair; *MLH1*, MutL protein homologue 1; mt, mutant, mutation; NGS, next generation sequencing; *NRAS*, neuroblastoma *RAS* viral oncogene homologue; OS, overall survival; PCR, polymerase chain reaction; PFS, progression free survival; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; pMMR, proficient mismatch repair; *RAS*, rat sarcoma virus; wt, wildtype.

This work has been presented in part at the European Society for Medical Oncology World Congress on Gastrointestinal Cancer, held online, July 1 to 4, 2020.

Bengt Glimelius and Pia Osterlund have shared last authorship.

For affiliations refer to page 500

Lilly and Company, Grant/Award Number: 2012-2017; Merck KGaA, Grant/Award Number: 2012-2020; Roche Oy, Grant/Award Number: 2012-2020; Sanofi, Grant/Award Number: 2012-2017; Servier, Grant/Award Number: 2016-2020

and received fewer treatment lines than *RAS*&*BRAF*wt and *RAS*mt. OS among a*BRAF*mt (median 14.4 months) was longer than for *BRAF*-V600Emt (11.2 months), but shorter than for *RAS*&*BRAF*wt (30.5 months) and *RAS*mt (23.4 months). Addition of bevacizumab trended for better OS for the a*BRAF*mt. Nine patients with a*BRAF*mt received cetuximab/panitumumab without response. a*BRAF*mt represents a distinct subgroup differing from other *RAS*/*BRAF* groups, with serrated adenocarcinoma in only half. OS for patients with a*BRAF*mt tumours was slightly better than for *BRAF*-V600Emt, but worse than for *RAS*mt and *RAS*&*BRAF*wt. a*BRAF*mt should not be a contraindication for metastasectomy.

KEYWORDS

a*BRAF*, *BRAF* mutation, colorectal cancer, metastatic, non-V600E

What's new?

In colorectal cancer, the *BRAF*-V600E mutation is a strong prognostic indicator, but little is known about other *BRAF* mutations. Here, the authors analysed the characteristics of atypical *BRAF* mutations in patients with metastatic colorectal cancer. These mutations were found in approximately 3% of cases, and these patients were predominantly male and had more rectal primary tumours and fewer peritoneal metastases. They also had better survival after surgical removal of metastases, compared with patients who had the *BRAF*-V600E mutation. Treatment with bevacizumab improved overall survival in patients with atypical *BRAF* mutations.

1 | INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer death worldwide.[1] Activation of the mitogen-activated protein kinase (MAPK) pathway plays a key role in the tumorigenesis of CRC, often caused by mutations in the oncogenes rat sarcoma virus (*RAS*) or rapidly accelerated fibrosarcoma murine sarcoma viral oncogene homologue B (*BRAF*).[2] *RAS* mutations (mainly Kirsten rat sarcoma viral oncogene [*KRAS*mt] and neuroblastoma *RAS* viral oncogene homologue [*NRAS*mt]) are seen in about 50% of CRC patients and cause resistance to epidermal growth factor receptor (EGFR)-inhibitors.[2,3]

BRAF-mutations (*BRAF*mt) are seen in about 15% of primary CRC and in 4% to 10% of metastatic (mCRC) trial patients, but higher frequencies (above 20%) have been noted in population-based series.[4-6] The *BRAF*-V600E mutation (*BRAF*-V600Emt) accounts for up to 90% of all *BRAF*mt.[2,5] In CRC, *BRAF*-V600Emt are associated with female sex, right-sided primary tumours and higher tumour grade, and in mCRC with poor prognosis.[7,8] About 20% of *BRAF*-V600Emt also have deficient mismatch repair (dMMR), which is almost exclusively due to CpG island methylator phenotype (CIMP) with hypermethylation of the MutL protein homologue 1 (*MLH1*)-gene.[7] *BRAF*-V600Emt predicts less effect from EGFR-inhibitors, however debated.[9]

Around 15% to 30% of CRCs arise from serrated precursor lesions, that is, sessile serrated lesions and traditional serrated adenomas. The formation is driven through genetic and epigenetic alterations.[10,11] Sessile serrated lesions mostly arise due to a *BRAF*-V600Emt, and CIMP-high leads to sporadic dMMR in 80%.[11,12] Traditional serrated adenomas, in contrast often arise through CIMP-low, *BRAF*-V600Emt or *KRAS*mt in combination with other events.[11-13]

With increased use of next-generation sequencing (NGS) in mCRC, other less common *BRAF*-mutations have been identified. The clinical significance of these atypical *BRAF*-mutations (a*BRAF*mt), also called non-V600E, is only partly known.[5,14-16] *BRAF*mt can be classified based on their signalling properties. Class 1 consists of *BRAF*-V600Emt, which signal as monomers, feedback inhibit *RAS* and are *RAS*-independent.[5,15,17] Class 2 mutations signal as constitutively active dimers, with medium to high level of kinase activity, and are also *RAS*-independent. Class 3 mutations are either kinase impaired or kinase dead and sensitive to extracellular signal-regulated kinases (ERK) mediated feedback, making their signalling activation *RAS*-dependent.

A study from 2015 described 10 a*BRAF*mt patients and showed that they had more rectal primary tumours, less peritoneal metastases and longer overall survival (OS) compared with *BRAF*-V600Emt.[18] In another study, 9643 patients with mCRC from three NGS databases were analysed, yielding 208 a*BRAF*mt (2.2% of all and 21.6% of all *BRAF*mt).[14] The a*BRAF*mt subgroup was younger, more often male, had lower tumour grade, less right-sided primary tumours and less peritoneal metastases compared with *BRAF*-V600Emt. They also had longer OS compared with *BRAF*-V600Emt and *BRAF*-wildtype (wt) (median 60.7, 11.4 and 43.0 months, respectively), however, only half of the a*BRAF*mt had follow-up data.[14] In a third study, a*BRAF*mt was seen in 1.7% and they had left-sided primaries and concomitant *RAS*-mutations more often than *BRAF*-V600Emt.[5] In this study no responses were seen in 11 patients receiving EGFR-inhibitors. In yet another study, 40 a*BRAF*mt received EGFR-inhibitors and responses were seen in 8% with class

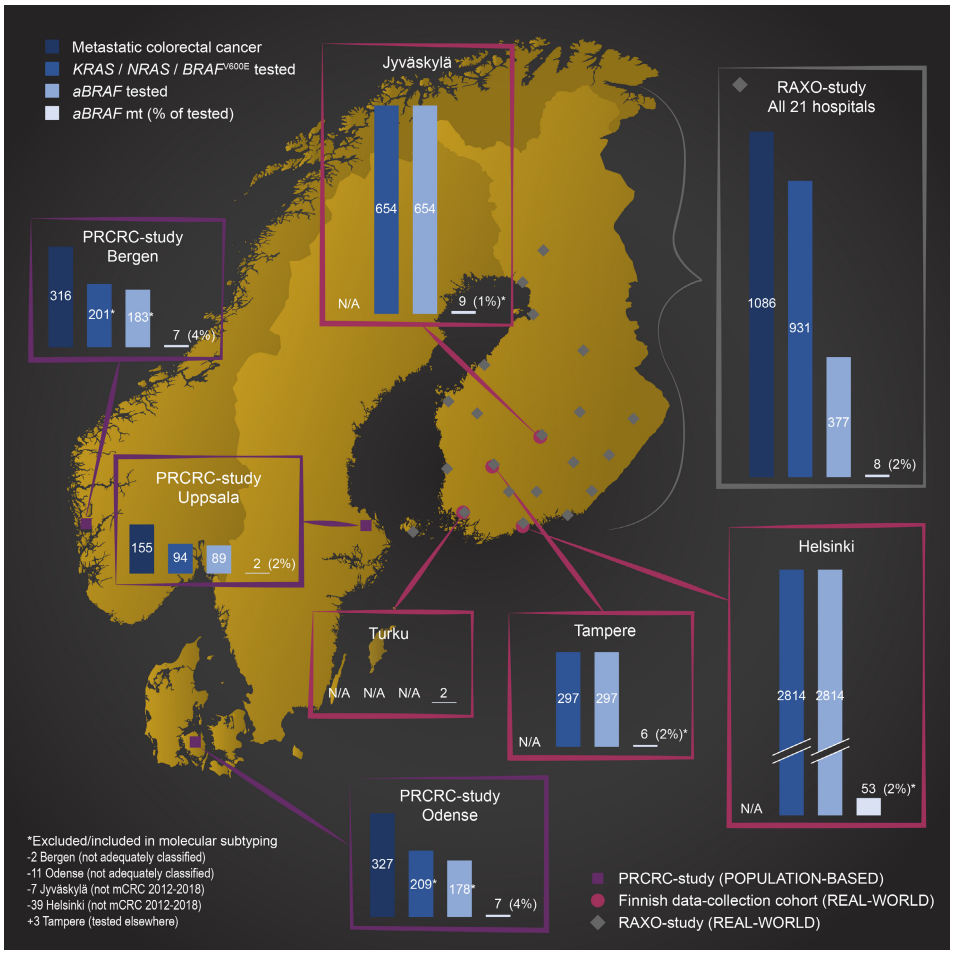

FIGURE 1 Patient flow and inclusion in the cohorts including a*BRAF* mutation rates per tested tumours (with methods detecting a*BRAF* mutations).

2 mutations (1/12) and in 50% with class 3 (14/28).[15] A study including 25 patients with a*BRAF*mt reported that class 3 mutations frequently were left-sided, and that class 2 mutations were more similar to class 1 mutations and showed poorer OS compared with class 3.[16]

Until now, a*BRAF*mt have mostly been studied in selected patient populations from trials or from collections of patients where analyses for *RAS*- and *BRAF*-mutations were performed, that is, usually prior to treatment initiation. Given the marked difference in the frequency of *BRAF*-V600Emt in mCRC between population-based and trial/hospital series[4-6] and the limited and non-conclusive knowledge about a*BRAF*mt, the aim was to study demographics, clinical characteristics, pathology and outcomes for a*BRAF*mt in less selected patients from populations-based and real-world cohorts.

2 | MATERIALS AND METHODS

2.1 | Description of cohorts

Patients from three Nordic cohorts were included: the prospective real-world Finnish RAXO-study, the population-based Scandinavian Prospective Registration of mCRC (PRCRC-study), and a real-world data collection cohort of all Finnish patients planned for treatment and thus molecularly tested at four hospitals (Helsinki, Jyväskylä, Tampere and Turku; Finnish data-collection cohort). Patient cohorts are presented in Figure 1.

The RAXO-study is a nationwide real-world study with 1086 mCRC patients referred between 2012 and 2018 for oncological treatments to the 21 hospitals treating cancer in Finland.[19]

The PRCRC-study is a population-based cohort from three Scandinavian university hospital regions, Uppsala, Sweden, Bergen, Norway and Odense, Denmark, with 798 patients included between 2003 and 2006.[20] All patients with mCRC in these three Scandinavian geographic areas, providing all cancer care in these university hospital regions, were included in the study, and molecular analyses were performed for both treatable and non-treatable patients with sufficient tumour material available.

The third cohort, the Finnish data-collection cohort consists of all molecularly tested (with a*BRAF* testing included) mCRC patients included 2012 to 2018 from four regions covering 62% of the Finnish population. The RAXO data-collection protocol and preliminary results from Tampere and Turku have been published,[19] and preliminary demographics and outcome of this cohort has been presented.[21] The Finnish data-collection cohort ($n = 6698$) partly overlaps the RAXO-study for mutation frequencies, as most hospitals recruiting patients to the RAXO-study were within these regions. Duplicate a*BRAF*mt in the Finnish data-collection cohort, where exact frequencies could not be calculated since validated data for non-a*BRAF*mt were not available, were removed and kept in the RAXO-study.

2.2 | Molecular testing

Testing for *RAS*- and *BRAF*-mutations in the RAXO-study and the Finnish data-collection cohort was done in clinical routine using accredited techniques. The indication for testing was planned systemic therapy for mCRC. In the RAXO-study reverse transcriptase polymerase chain reaction (PCR) or similar methods for *RAS* and *BRAF*-V660E testing was used during the first years and from around 2014 onward NGS, also identifying a*BRAF*mt, was used. The composition of the NGS panels varied but always contained analyses for hotspot mutations in *KRAS* and *NRAS* exons 2-4, and for *BRAF*-V600Emt, details described.[22] In the RAXO-study during NGS testing years, *BRAF* exons 11-15 were tested in 325 patients, exon 15 in 52. For the Finnish data-collection cohort, a*BRAF*mt was identified from the NGS databases at Helsinki (exons 11-15), Tampere (exon 15), and Turku University Hospitals (codons 464-469 and 600), and Central Finland Central Hospital (exons 7, 11, 12, 15 and 16). In the PRCRC-study cohort, the analyses were performed using a custom designed Ampliseq hotspot panel for most (*BRAF* exons 11 and 15),[23] with pyrosequencing for *KRAS* and *BRAF*-V600E in 44,[4] and Illumina sequencing (2661 bases) in one.[24]

2.3 | Mismatch repair testing

Mismatch repair (MMR)-status was tested either with immunohistochemistry for the four MMR proteins or PCR for the microsatellite genomic regions. Testing in the PRCRC-study has been described.[23,25] In the RAXO-study and the Finnish data-collection cohort testing for MMR-status was performed either in routine healthcare, or later using the same methods.

2.4 | Histopathology of a*BRAF*-mutant tumours

Haematoxylin and eosin-stained CRC specimens or whole slide images from 44 patients harbouring an a*BRAF*mt were reviewed by an expert GI pathologist (MJM), who was blind for clinical and mutational data (other than a*BRAF*mt). CRCs were subtyped according to WHO-2019 criteria,[26] for serrated adenocarcinoma more detailed criteria were used.[27] Three specimens were excluded due to insufficient amount of tumour tissue or due to a preoperative radiotherapy effect. In 29 cases, analysis was performed from resection material and in 12 cases from a biopsy.

2.5 | Statistical analyses

Demographics, treatments and outcomes between a*BRAF*mt and *BRAF*-V600Emt were compared using logistic regression. Chi-square or Fisher's exact tests were used for comparison of categorical variables between cohorts and Mann-Whitney *U*-test for comparison of continuous variables. Median follow-up time was estimated with the reverse Kaplan-Meier method. OS analyses were performed for the time interval from date of metastatic disease to date of death or censored if the patient was alive at last follow-up (cut-off August 15, 2008, in PRCRC, and October 7, 2020, in the RAXO-study and data-collection-cohort), using the Kaplan-Meier method. Progression free survival (PFS) was calculated from initiation of treatment to progression or death. Survival comparisons were done with log-rank tests (Tables S1 and S2) or with Cox regression for other comparisons. A multivariable cox regression was also constructed including clinically relevant variables without missing values (except for one patient with missing ECOG PS), where all variables were forced to the model. Proportional hazard assumption was assessed using Schoenfeld residuals with no clear violations shown and the hazard was interpreted as an average of the time-varying hazard ratio.[28] Two-tailed *P* values of <.05 and 95% confidence intervals (CI) not crossing 1 were considered statistically significant. The analyses were performed with SPSS statistical software (versions 25 and 27; IBM Corp, Armonk, NY).

3 | RESULTS

3.1 | Characteristics of patients in the cohorts

Characteristics for all 1911 patients in the RAXO and PRCRC cohorts and the background population for the Finnish data-collection cohort are shown in Table S1. Differences in demographics and treatments between population-based vs real-world, and between older and newer cohorts were seen due to different proportions of patients being non-treatable or treatable with different treatment modalities.

Characteristics for all accurately *RAS* and *BRAF*-V600E characterised patients (n = 1449) from the cohorts are presented in Table S2. *BRAF*-V600Emt were less common in the real-world RAXO-study than in the other cohorts, but no other statistically significant differences in mutation frequencies were noted. Patients in the RAXO-study were younger, more often male, had less right colon and more rectal primary tumours and synchronous presentation, had better Eastern Cooperative Oncology Group performance status (ECOG PS), and were treated with systemic therapy and had metastasectomies more often, compared with the older PRCRC-study.

Median OS was longer in the more recent RAXO-study (30.5 vs 11.9 months) compared with the older PRCRC-study, but there were no statistically significant OS differences when analysing treatment groups separately (cytotoxics only, cytotoxics combined with targeted agents and metastasectomy and/or local ablative therapy [LAT]) (Tables S1 and S2).

As there were no statistically significant differences among similarly treated patients in the cohorts they were combined for further analyses.

3.2 | a*BRAF*-mutation frequency in the different cohorts

The a*BRAF*mt frequency in the uniformly tested cohorts was 16/450 (4%) in the PRCRC-cohort, and 75/3765 (2%) in the Finnish data-

TABLE 1 Demographics for patients with different tumour mutations.

	Total		a*BRAF* mt		*BRAF*-V600E mt		RAS&*BRAF* wt		RAS mt		
	1449	100%	51	100%	182	100%	456	100%	760	100%	*P* value*
Age, median (range)	67	(21-97)	69	(51-89)	69	(33-86)	66	(22-93)	67	(21–97)	0.533
Agegroups											
≤70	886	61%	28	55%	100	55%	301	66%	457	60%	ref
>70	563	39%	23	45%	82	45%	155	34%	303	40%	0.996
Sex											
Female	621	43%	20	39%	113	62%	165	36%	323	43%	ref
Male	828	57%	31	61%	69	38%	291	64%	437	58%	0.004
Primary tumour location											
Right colon	461	32%	17	33%	128	71%	75	17%	241	32%	ref
Left colon	513	36%	11	22%	37	21%	210	46%	255	34%	0.061
Rectum	462	32%	23	45%	15	8%	167	37%	257	34%	0.004
Multiple or unknown	13	-	0	-	2	-	4	-	7	-	-
Tumour grade											
Low-grade	983	80%	30	70%	92	58%	318	82%	543	85%	ref
High-grade	243	20%	13	30%	67	42%	69	18%	94	15%	0.159
Missing	223	-	8	-	23	-	69	-	123	-	-
Primary surgery											
No	365	25%	13	25%	35	19%	114	25%	203	27%	ref
Yes	1084	75%	38	75%	147	81%	342	75%	557	73%	0.330
Adjuvant chemotherapy											
Yes	306	21%	10	20%	36	20%	110	24%	150	20%	0.978
Adjuvant radiotherapy[a]											
Yes	170	37%	14	61%	5	33%	58	35%	93	36%	0.102
Appearance of metastases											
Synchronous	900	62%	30	59%	114	63%	262	57%	494	65%	ref
Metachronous	549	38%	21	41%	68	37%	194	43%	266	35%	0.620
Number of metastatic sites											
1	717	49%	22	43%	88	48%	225	49%	382	50%	ref
2	475	33%	24	47%	56	31%	141	31%	254	33%	0.114
≥3	257	18%	5	10%	38	21%	90	20%	124	16%	0.228
Metastatic sites											
Liver	1019	70%	31	61%	99	54%	346	76%	543	71%	0.417
Lung	421	29%	18	35%	42	23%	106	23%	255	34%	0.080
Lymph nodes	399	28%	19	37%	73	40%	132	29%	175	23%	0.712
Peritoneal	275	19%	6	12%	58	32%	77	17%	134	18%	0.007
Bone	54	4%	4	8%	5	3%	20	4%	25	3%	0.110
Other	179	12%	5	10%	27	15%	60	13%	87	11%	0.360
Performance status											
0	446	31%	12	24%	49	27%	142	31%	243	32%	ref
1	672	46%	22	43%	71	39%	212	46%	367	48%	0.560
2-3	330	23%	17	33%	62	34%	102	22%	149	20%	0.789
Missing	1	-	-	-	-	-	-	-	1	-	-
Haemoglobin											
<11 g/L	236	17%	7	14%	32	18%	68	15%	129	17%	0.545

TABLE 1 (Continued)

	Total		a*BRAF* mt		*BRAF*-V600E mt		RAS&*BRAF* wt		RAS mt		
	1449	100%	51	100%	182	100%	456	100%	760	100%	*P* value*
Leukocytes											
>10 E9	297	21%	13	27%	49	28%	85	19%	150	20%	0.856
Platelets											
>400 E9	355	26%	13	27%	52	31%	94	21%	196	27%	0.639
Alkaline phosphatase											
>Upper limit of normal	540	39%	16	35%	68	40%	182	41%	274	38%	0.502
Carcinoembryonic antigen											
>5 ng/ml	869	72%	28	67%	92	69%	274	72%	475	72%	0.809
Mismatch repair status											
pMMR	737	93%	50	98%	93	73%	248	97%	346	96%	ref
dMMR	55	7%	1	2%	34	27%	7	3%	13	4%	0.005
Not tested	657	-	0	-	55	-	201	-	401	-	-
RAS-status											
Wild-type	664	46%	30	59%	178	98%	456	100%	0	0%	ref
Mutated	785	54%	21	41%	4	2%	0	0%	760	100%	<0.001

Note: **P* value between a*BRAF*mt and *BRAF*-^{V600E}mt.
Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.
[a]For rectal primaries.

collection cohort. In the RAXO-study, 8/377 (2%) had an a*BRAF*mt. No statistically significant differences in the frequency of a*BRAF*mt were seen between these cohorts ($P = .097$).

3.3 | Baseline demographics

The final study cohort consisted of 51 (3%) a*BRAF*mt, 182 (13%) *BRAF*-V600Emt, 456 (31%) *RAS*&*BRAF*wt (of which 22 were not tested for *NRAS* due to insufficient tumour tissue) and 760 (52%) *RAS*mt tumours among the 1449 adequately tested patients. Baseline demographics for all included patients are presented in Table 1 and after excluding the 27 patients with a*BRAF*mt from the Finnish data-collection cohort (as less complete information of other mutations and/or clinical information was available in these cohorts) in Table S3.

The a*BRAF*mt compared with *BRAF*-V600Emt were more often male (61% vs 38%, $P = .004$), had more rectal primary tumours (45% vs 8%, $P = .004$) and less peritoneal metastases (8% vs 24%, $P = .007$). When excluding patients from the Finnish data-collection cohort they also had more low-grade tumours (82% vs 58%, $P = .039$).

The a*BRAF*mt compared with *RAS*&*BRAF*wt had less left colon primary tumours (22% vs 46%, $P < .001$), and liver metastases (61% vs 76%, $P = .021$). The a*BRAF*mt compared with *RAS*mt more often had distant lymph node metastases (37% vs 23%, $P = .023$), ECOG PS 2-3 (33% vs 20%, $P = .032$) and less low-grade tumours (70% vs 85%, $P = .009$).

3.4 | a*BRAF*-mutation class

Among the 51 patients with a*BRAF*mt, D594G was the most common mutation (24%), followed by D594N (10%), G466E (6%), and G649R (6%), with rarer mutations presented in Table S4. Class 2 mutations were seen in 11 (22%) patients, class 3 in 32 (63%, of which 7 had conflicting information and are sometimes denoted unclassified), and four (8%) were unclassified. Five (10%) patients had two *BRAF*mt, four patients (8%) had both a class 2 or 3, and class 1 mutation (*BRAF*-V600Emt), and one had two different class 2 mutations (Table S4).

Demographics for class 2 and 3 a*BRAF*mt are shown in Table 2. The class 2 group (n = 11) compared with class 3 (n = 32) had non-significant trends of higher median age at mCRC diagnosis (74 vs 67 years), less ECOG PS 0 (18% vs 31%), fewer males (36% vs 63%) and rectal primary tumours (36% vs 56%). There were also trends for more liver metastases (73% vs 59%), but fewer lung metastases (9% vs 44%) for class 2 compared with class 3. Removing the 7 class 3 a*BRAF*mt that could also be unclassified did not alter the results (data not shown).

3.5 | Concomitant *RAS*-mutations and deficient mismatch repair

Concomitant *RAS*mt were common in a*BRAF*mt, and as expected rare in *BRAF*-V600Emt (41% vs 2%, $P < .001$, Table 1 and Table S3). A trend was seen for class 2 having concomitant *RAS*mt less often than class 3 (27% vs 44%, Table 2). a*BRAF*mt&*RAS*mt compared with

TABLE 2 Demographics for classes of *aBRAF* mutations.

	Total		Class 2		Class 3		Class 1 + 2/3		Unclassified		
	51	100%	11	100%	32	100%	4	100%	4	100%	*P* value*
Age											
Median (range)	69	(51–89)	74	(55-87)	67	(51–89)	68	(57-86)	73	(59-75)	.117
≤70	28	55%	4	36%	21	66%	2	50%	1	25%	ref
>70	23	45%	7	64%	11	34%	2	50%	3	75%	.098
Sex											
Female	20	39%	7	64%	12	38%	1	25%	0	0%	ref
Male	31	61%	4	36%	20	63%	3	75%	4	100%	.140
Primary tumour											
Right colon	17	33%	4	36%	8	25%	3	75%	2	50%	ref
Left colon	11	22%	3	27%	6	19%	1	25%	1	25%	1.000
Rectum	23	45%	4	36%	18	56%	0	0%	1	25%	.330
Tumour grade											
Low-grade	30	70%	7	88%	20	71%	1	25%	2	67%	ref
High-grade	13	30%	1	13%	8	29%	3	75%	1	33%	.370
Missing	8	-	3	-	4	-	0	-	1	-	-
Serrated adenoma											
No[a]	17	41%	6	67%	10	40%	0	0%	1	33%	ref
Yes	24	59%	3	33%	15	60%	4	100%	2	67%	.178
Missing	10	-	2	-	7	-	0	-	1	-	-
Primary surgery											
No	13	25%	4	36%	8	25%	0	0%	1	25%	ref
Yes	38	75%	7	64%	24	75%	4	100%	3	75%	.471
Adjuvant chemotherapy											
Yes	16	31%	3	27%	13	41%	0	0%	0	0%	.646
Adjuvant radiotherapy[b]											
Yes	14	61%	2	50%	12	67%	0	0%	0	0%	.535
Presentation of metastases											
Synchronous	30	59%	8	73%	18	56%	1	25%	3	75%	ref
Metachronous	21	41%	3	27%	14	44%	3	75%	1	25%	.340
Number of metastatic sites											
1	22	43%	6	55%	13	41%	2	50%	1	25%	ref
2	24	47%	5	45%	15	47%	2	50%	2	50%	.649
≥3	5	10%	0	0%	4	13%	0	0%	1	25%	NE
Metastatic sites											
Liver	31	61%	8	73%	19	59%	2	50%	2	50%	.430
Lung	18	35%	1	9%	14	44%	1	25%	2	50%	.064
Lymph nodes	19	37%	3	27%	13	41%	0	0%	3	75%	.433
Peritoneal	6	12%	2	18%	3	9%	1	25%	0	0%	.440
Bone	4	8%	0	0%	3	9%	0	0%	1	25%	.999
Other	5	10%	2	18%	2	6%	1	25%	0	0%	.260
Performance status											
0	12	24%	2	18%	10	31%	0	0%	0	0%	ref
1	22	43%	5	45%	12	38%	2	50%	3	75%	.435
2-3	17	33%	4	36%	10	31%	2	50%	1	25%	.477

TABLE 2 (Continued)

	Total		Class 2		Class 3		Class 1 + 2/3		Unclassified		
	51	100%	11	100%	32	100%	4	100%	4	100%	*P* value*
Haemoglobin											
<11 g/L	7	14%	3	27%	4	13%	0	0%	0	0%	.282
Leukocytes											
>10 E9	13	25%	3	27%	8	25%	2	50%	0	0%	.924
Platelets											
>400 E9	13	25%	3	27%	7	22%	2	50%	1	25%	.636
Alkaline phosphatase											
>Upper limit of normal	16	31%	5	45%	7	22%	3	75%	1	25%	.135
Carcinoembryonic antigen											
>5 ng/ml	28	55%	6	55%	19	59%	2	50%	1	25%	.947
Mismatch repair status											
pMMR	50	98%	11	100%	32	100%	4	100%	3	75%	ref
dMMR	1	2%	0	0%	0	0%	0	0%	1	25%	NE
RAS-status											
Wild-type	30	59%	8	73%	18	56%	2	50%	2	50%	ref
Mutated	21	41%	3	27%	14	44%	2	50%	2	50%	.340

Note: **P* value between class 2 and class 3.
Abbreviations: dMMR, deficient mismatch repair; NE, no estimate; pMMR, proficient mismatch repair.
[a]Includes adenocarcinoma not otherwise specified, mucinous and undifferentiated.
[b]For rectal primaries.

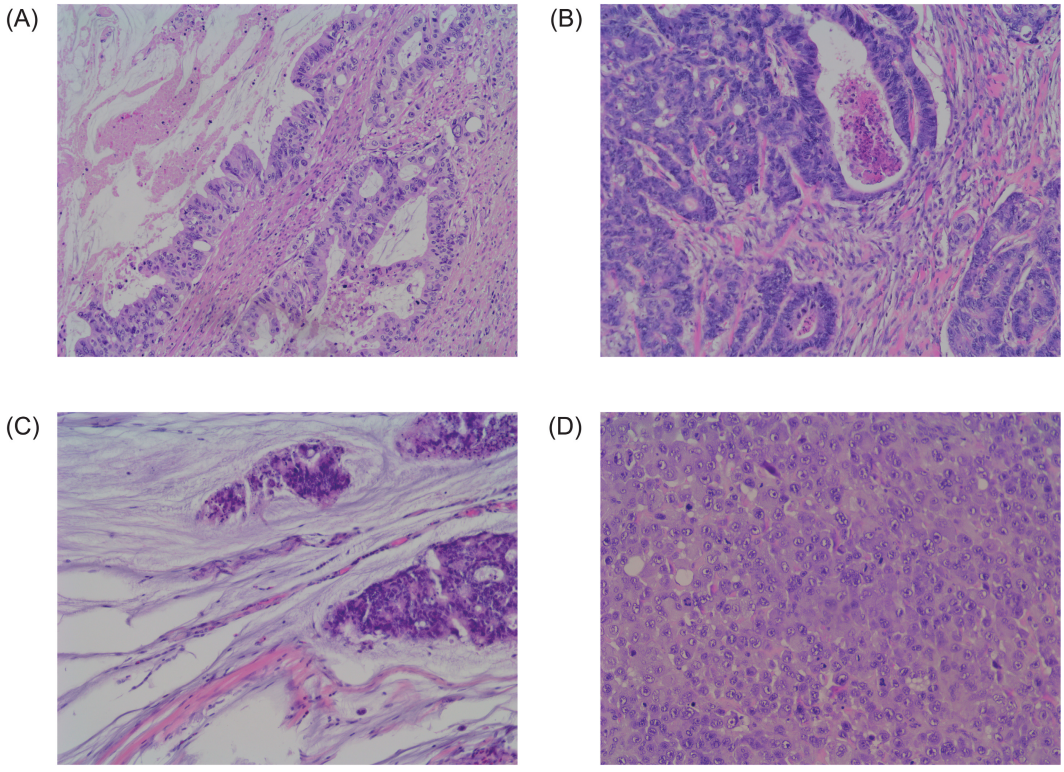

FIGURE 2 Typical histopathological features according to the WHO-2019 classification for the a*BRAF* mutated, with serrated adenocarcinoma (A), adenocarcinoma not otherwise specified (NOS, B), mucinous (C) and medullary (D) histology.

TABLE 3 Treatments divided by groups.

	Total		a*BRAF* mt		*BRAF*-V600E mt		RAS & *BRAF* wt		RAS mt		
	1449	100%	51	100%	182	100%	456	100%	760	100%	*P* value*
Type of treatment											
Systemic therapy only	866	60%	29	57%	126	69%	262	57%	449	59%	ref
Metastasectomy and/or LAT	385	27%	9	18%	15	8%	146	32%	215	28%	.041
Best supportive care	198	14%	13	25%	41	23%	48	11%	96	13%	.398
Total chemotherapy	1244	100%	37	100%	141	100%	406	100%	660	100%	NE
Number of lines											
1	492	40%	17	46%	59	42%	172	42%	244	37%	ref
2	337	27%	9	24%	54	38%	89	22%	185	28%	.227
≥3	415	33%	11	30%	28	20%	145	36%	231	35%	.491
First line chemotherapy											
Fluoropyrimidine	1228	99%	37	100%	137	97%	399	98%	655	99%	NE
Oxaliplatin	756	61%	24	65%	87	62%	232	57%	413	63%	.724
Irinotecan	319	26%	10	27%	40	28%	122	30%	147	22%	.872
Bevacizumab	606	49%	18	49%	67	48%	154	38%	367	56%	.902
EGFR-inhibitor	125	10%	0	0%	11	8%	101	25%	13	2%	NE
Best response first line											
PR/CR/NED	713	59%	18	51%	59	43%	274	69%	362	56%	.376
SD	377	31%	14	40%	52	38%	89	22%	222	34%	ref
PD	125	10%	3	9%	26	19%	36	9%	60	9%	
Not available	28	-	2	-	3	-	7	-	16	-	-
Chemotherapy all lines											
Fluoropyrimidine	1233	99%	37	100%	139	99%	400	99%	657	100%	NE
Oxaliplatin	985	79%	31	84%	111	79%	308	76%	535	81%	.497
Irinotecan	884	71%	26	70%	97	69%	294	72%	467	71%	.863
Bevacizumab	736	59%	23	62%	75	53%	213	52%	425	64%	.330
EGFR-inhibitor	305	25%	9	24%	25	18%	229	56%	42	6%	.366

Note: **P* value between a*BRAF* mt and *BRAF*-V600E mt.
Abbreviations: CR, complete response; NE, no estimate; NED, no evidence of disease; PD, progressive disease; PR, partial response; SD, stable disease.

a*BRAF*mt&*RAS*wt were less often male (37% vs 75%, $P = .011$), more often had ≥3 metastatic sites (21% vs 4%, $P = .044$) and more lymph node metastases (58% vs 29%, $P = .048$, Table S5).

A sporadic dMMR was seen in one (2%) of 51 a*BRAF*mt (unclassified a*BRAF*mt), being significantly lower compared with *BRAF*-V600Emt (27%, $P = .005$). For *RAS*&*BRAF*wt and *RAS*mt, the frequencies of dMMR were 3% and 4%, respectively.

3.6 | Pathology

Serrated adenocarcinoma was the most common subtype among a*BRAF*mt mCRC (n = 24; 59%), followed by adenocarcinoma not otherwise specified (NOS, n = 15; 37%), and single cases of mucinous and undifferentiated carcinomas (2% each, Figure 2A-D). There was a trend for more serrated adenocarcinomas among class 3, 15/25 (65%) compared with class 2, 3/9 (33%, $P = .250$, Table 2). Among patients without co-mutations in *RAS* or *BRAF*-V600E, 7/14 (50%) with class 3 and 2/6 (33%, $P = .642$) of class 2 were serrated adenocarcinomas. All four a*BRAF*mt&*BRAF*-V600Emt were serrated adenocarcinomas. The histopathological subtypes were compared for demographics and no statistically significant differences were noted (Table S6). Trends for more right-sided and less left-sided colon primary tumours, and more lung and fewer liver metastases were noted for serrated vs adenocarcinoma NOS groups.

3.7 | Treatments

Patients with a*BRAF*mt or *BRAF*-V600Emt received tumour controlling chemotherapy or had metastasectomies and/or LAT less often, and more seldom received three or more lines of chemotherapy than those with *RAS*&*BRAF*wt or *RAS*mt tumours (Tables 3 and S7). If treated with systemic therapy, nearly all patients received a

fluoropyrimidine, alone or usually in combination with oxaliplatin or irinotecan, with no major differences between molecular groups. Bevacizumab was used more often in patients with any mutation, whereas the opposite was true for EGFR-inhibitors. a*BRAF*mt had similar response rate (51%) to first-line chemotherapy as *BRAF*-V600Emt (43%, $P = .376$), and significantly worse than *RAS*&*BRAF*wt (69%, $P = .040$) and *RAS*mt (56%, $P < .001$).

3.8 | Overall survival and progression free survival

Median follow-up was 58.9 months (95% CI 55.7-62.1), with complete follow-up available for all patients. OS differed markedly according to mutation (Figures 3A and S1A) with the poorest survival for patients with *BRAF*-V600Emt (median 11.2 months), followed by those with a*BRAF*mt (median 14.4 months), *RAS*mt (median 23.4 months) and *RAS*&*BRAF*wt (median 30.5 months, Figures 3A and S1A), a*BRAF*mt differed significantly from all other groups. Similar differences between mutations could be seen when divided by treatment groups (metastasectomy and/or LAT, systemic therapy only, or BSC, Figures 3B-D and S1B-D). Similar survival trends were also noted when analysed separately for the PRCRC- and RAXO-studies (data not shown).

Median OS in a*BRAF*mt differed statistically significantly according to treatment and was 62.0 months for metastasectomy and/or LAT, 14.8 months for systemic therapy only and 2.7 months for BSC (Figure S2). The a*BRAF*mt had a 5-year OS rate of 89% for the metastasectomy and/or LAT group in line with *RAS*mt and *RAS*&*BRAF*wt, whereas the *BRAF*-V600Emt did worse (14% 5-year OS rate, Figures 3C and S1C). In the a*BRAF*mt group liver, lung, peritoneal, or distant lymph node resections were performed in nine patients, without recurrences in four patients. Median PFS in systemic therapy only patients did not differ according to mutation group (Figure S3A,B).

In an adjusted multivariable OS analysis (Table S8), a*BRAF*mt did significantly worse than *RAS*&*BRAF*wt (HR 0.60, 95% CI 0.43-0.84), almost significantly worse than *RAS*mt (HR 0.73, 95% CI 0.53-1.01), but not significantly better than *BRAF*-V600Emt (HR 1.24, 95% CI 0.88-1.76), when adjusting for age, sex, primary tumour location, ECOG PS, presentation of metastases, number of metastatic sites, treatment and newer (RAXO-study and Finnish data-collection cohort) vs older cohorts (PRCRC-study).

It was not possible to detect any differences in OS or PFS between the class 2 or class 3 a*BRAF*mt cases (Figures 3E and S4). There were too few cases in the other a*BRAF*mt subgroups to make any conclusions. Removing the seven class 3 a*BRAF*mt that also could be unclassified did not alter the results (data not shown). Neither could we detect any differences in OS or PFS within the a*BRAF*mt group whether *RAS* was concomitantly mutated or not (Figure S5A,B). *RAS*&*BRAF*-V600Ewt patients that also were a*BRAF*mt had significantly worse OS than those that did not. The same was also seen for *RAS*mt that had concomitant a*BRAF*mt (Figure 3F).

3.9 | EGFR-inhibitors in a*BRAF*-mutants

Nine patients with an a*BRAF*mt tumour received an EGFR-inhibitor (in combination with chemotherapy for eight patients), without tumour responses. Four patients had stable disease (all class 3) and five had immediate disease progression (Table S9). Response rate and PFS for a*BRAF*mt that received EGFR-inhibitors in second or later line was similar to that of *BRAF*-V600Emt and *RAS*mt (Table S10).

One class 3 a*BRAF*mt, that received FOLFOX+panitumumab with stable disease, later received encorafenib+cetuximab and had disease progression at 3 weeks. This patient then had a circulating tumour DNA analysis (FoundationOne Liquid CDx), revealing new *KRAS* (G12D, Q61H, G12A), *MAP2K1* (K57T, K57N), *EGFR* (V441G, G465E, V441D, I491R, G465R) and *PIK3CA* (M1043I) mutations, and a *GTF2IRD1-MET* fusion.

3.10 | Treatment with bevacizumab or regorafenib

Among patients receiving systemic therapy only, not including EGFR-inhibitors, the addition of bevacizumab in first line was associated with better OS compared with only cytotoxics in the *BRAF*-V600Emt, *RAS*&*BRAF*wt and *RAS*mt groups and showed a trend of better OS for a*BRAF*mt (Figure S6A-D), with the caveat of treatment selection and addition of bevacizumab in younger and fitter patients and less usage in the PRCRC-study. Significantly longer PFS could be seen for the *RAS*&*BRAF*wt and *RAS*mt groups (Figure S7A-D). Response in first-line systemic therapy only was seen in 62% (8/13 patients) of a*BRAF*mt receiving bevacizumab-containing treatment and disease control in 92%. Two a*BRAF*mt received regorafenib monotherapy. No responses were seen. One had stable disease for 8 months in third line and the other had immediate progression in fourth line.

4 | DISCUSSION

This study reports the results of patients with a*BRAF*mt detected in 4% in one population-based and in 2% in two real-world cohorts in the Nordic countries. A total of 51 a*BRAF*mt were found and the a*BRAF*mt frequency varied between 1% and 4% in the different subcohorts, which is in line with earlier studies.[5,14,18] We saw that in the truly population-based cohort with complete testing of all tumours with sufficient material, the a*BRAF*mt frequency was slightly higher (4%) compared with the real-world cohorts testing treatable patients only (1%-2%). In the three largest hospital-based series reported to date, the frequencies were 1.6%, 1.7% and 2.2%, thus around 2%.[5,14,18] Although our frequencies are uncertain due to a small number of cases and the number of patient series being limited, we suggest that a*BRAF*mt are more common in population-based materials, than in clinical trial/hospital-based patient materials.

OS was the longest for *RAS*&*BRAF*wt followed by *RAS*mt and a*BRAF*mt and shortest for *BRAF*-V600Emt (median 31 vs 23 vs 14 vs 11 months, respectively), and results for the a*BRAF*mt were

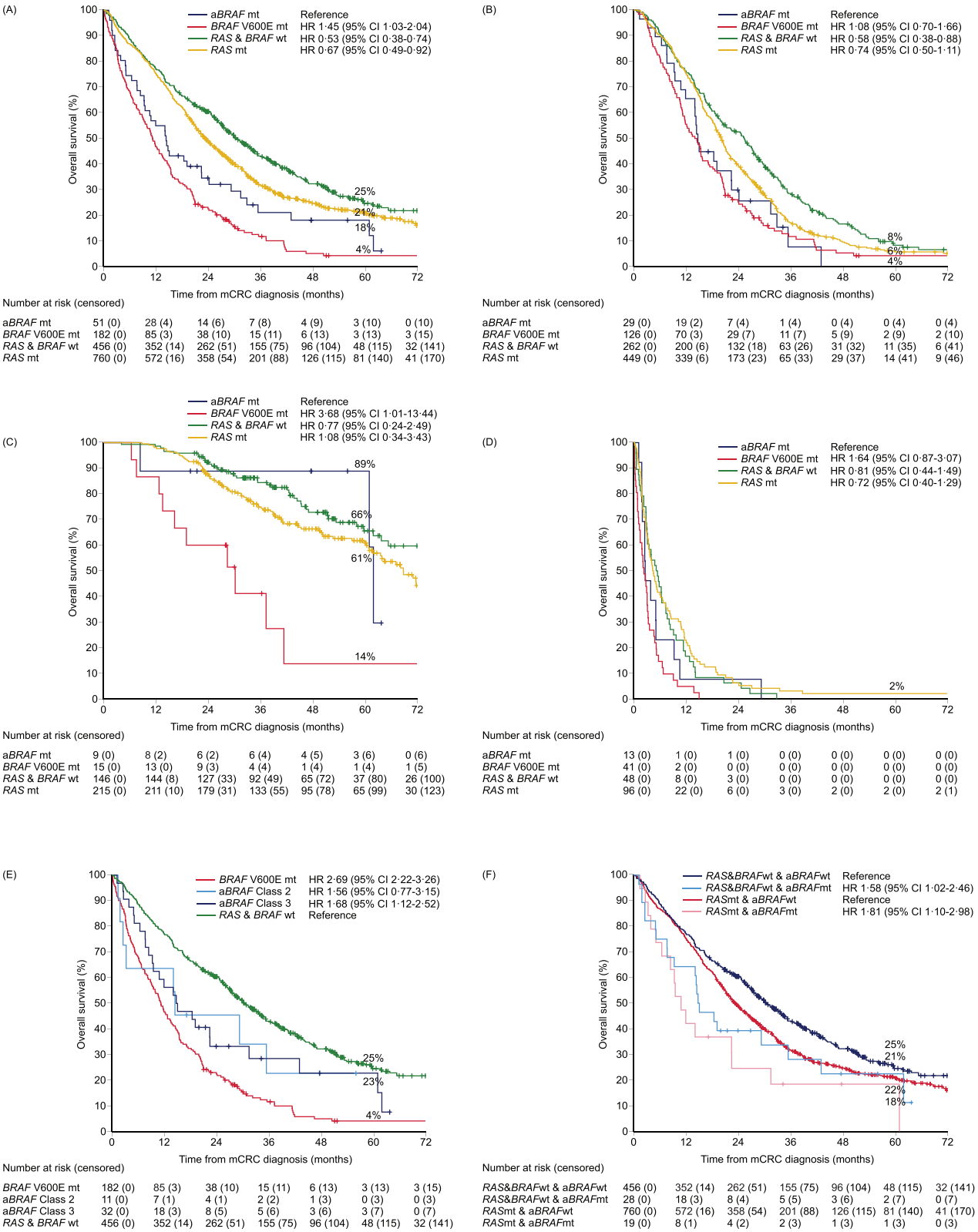

FIGURE 3 Overall survival by mutation group: all patients (A), systemic therapy only (B), metastasectomy and/or local ablative therapy (C), and best supportive care (D), for classes of *aBRAF* mutations (E) and for *RAS&BRAF* wildtype and *RAS* mutants with and without concomitant *aBRAF* mutations (F).

statistically significantly different from the other groups. *aBRAF*mt also have a better OS than *BRAF*-V600Emt in other studies.[5,14,18,29] But compared with the *BRAF*wt group (including *RAS*wt and *RAS*mt), one study reported better survival for *aBRAF*mt contrary to our results,[14] and similar or numerically worse survival in other studies,[5,16,18,29,30] more in line with our findings. We were also able

to show significantly worse OS among *RAS*&*BRAF*wt and *RAS*mt with concomitant a*BRAF*mt than for those without, an aspect not reported before. We could not detect any differences in OS or PFS between class 2 and class 3 a*BRAF*mt, whereas one study showed a trend for worse OS in class 2,[16] and others in line with us.[5,15] We could also not detect any differences between a*BRAF*mt with or without concomitant *RAS*mt, while a trend for a*BRAF*mt with concomitant *RAS*mt doing worse was seen in another study.[15]

Indirect support for this higher prevalence of a*BRAF*mt in populations comes from the survival differences seen here. We and others have previously reported that certain mutations/molecular traits with a poor prognosis, as, for example, *BRAF*-V600Emt and dMMR are about twice as common in population-based than in trial/hospital materials.[20,23] The clearly worse prognosis in patients with a*BRAF*mt tumours compared with *RAS*mt and *RAS*&*BRAF*wt tumours similarly indicate that a*BRAF*mt may be about twice as common in population-based than in other series, that is, the about 2% in trial/hospital series will be 4% in the background population as also seen in PRCRC. In the same patient materials as used here, we saw no differences in the proportion of tumours with *KRAS*-G12Cmt and also no worse prognosis for that subgroup relative to other *RAS*mt.[22]

In contrast to two earlier studies showing that patients with a*BRAF*mt tumours were younger than patients with *BRAF*-V600Emt tumours and about as old as patients with *RAS*mt and *RAS*&*BRAF*wt tumours,[5,14] we could not detect any age difference between a*BRAF*mt and *BRAF*-V600Emt (median 69 years), which was slightly higher than for those with *RAS*mt and *RAS*&*BRAF*wt tumours. a*BRAF*mt, *RAS*&*BRAF*wt and *RAS*mt were more often seen in males, whereas female sex was more common for *BRAF*-V600Emt, similar to one study,[14] but opposite to another study where female sex dominated among a*BRAF*mt.[18] Limited number of patients having a*BRAF*mt tumours may explain the disparate results between studies, although it appears as if the a*BRAF*mt group age and sex distributions are more similar to the *RAS*mt and *RAS*&*BRAF*wt groups than the *BRAF*-V600Emt group.

The similarity between the a*BRAF*mt, *RAS*&*BRAF*wt and *RAS*mt groups also relates to the location of the primary tumour, again contrasting to the *BRAF*-V600Emt group predominantly seen in the right colon.[5,14,16,18,29] Possibly because of this, peritoneal metastases were more common in the *BRAF*-V600Emt group than in the other groups, also noted in other studies.[14,16,18] There was also a trend towards lung metastases being more common for a*BRAF*mt than for *BRAF*-V600Emt and *RAS*&*BRAF*wt (35% vs 23% vs 23%, respectively). The same pattern could not be seen in other studies. In one study, lung metastases were more common for a*BRAF*mt than for *BRAF*-V600Emt but less common than for *RAS*&*BRAF*wt,[14] and in another study, the frequencies were roughly the same in all groups.[16] We could also see a trend for less poorly/undifferentiated tumours in a*BRAF*mt compared with *BRAF*-V600Emt, seen earlier.[14]

Concomitant *RAS*mt were considerably more common for a*BRAF*mt than for *BRAF*-V600Emt (41% vs 2%), which is in line with most other studies,[5,14,15,18,29,31] except one with only 1/25 a*BRAF*mt with concomitant *RAS*mt.[16] There was also a trend for concomitant *RAS*mt being more common for class 3 a*BRAF*mt than for class 2 a*BRAF*mt in our study, in line with.[15]

In our study, where all a*BRAF*mt were tested for MMR-status, dMMR was more common for *BRAF*-V600Emt compared with a*BRAF*mt, *RAS*&*BRAF*wt and *RAS*mt (27% vs 2% vs 3% vs 4%, respectively). In other studies, the dMMR frequency for a*BRAF*mt was 0% to 8%.[5,14-16] Compared with *BRAF*wt, it was slightly lower for a*BRAF*mt in one study,[14] and slightly higher in another study.[5]

In this series, serrated adenocarcinoma was the most common (59%) histological subtype among a*BRAF*mt tumours. While it is acknowledged that *BRAF*-V600Emt are nearly exclusively found in serrated adenocarcinomas,[32] the role of a*BRAF*mt in the serrated pathway has not been studied before. *BRAF*-V600Emt are mostly and *KRAS*mt rarely found in the sessile serrated pathway, often combined with CIMP-high and sporadic dMMR.[11,12] The *BRAF*-V600Emt in this study and a*BRAF*mt with concomitant *BRAF*-V600Emt have demographics and cancer features (female sex, older age, dMMR, right-sided primary tumours and peritoneal metastases) in line with the sessile serrated pathway cancers in literature.[12,33] The demographics of the a*BRAF*mt in the serrated group, especially those with concomitant *RAS*mt seen in half, align with the traditional serrated pathway demographics characterised by proficient mismatch repair (pMMR), *BRAF*-V600Emt in 1/3 and *KRAS*mt in 1/4.[11,32] Most *KRAS*mt are though found in the tubulovillous adenoma pathway harbouring *KRAS*mt in half and rarely *BRAF*-V600Emt, resembling the adenocarcinoma NOS group in our study with *RAS*mt in 27% and no *BRAF*-V600Emt.[11] The adenocarcinoma NOS in this study resemble this group also regarding demographics. Most class 2 (82%) and class 3 (70%) a*BRAF*mt patients had adenocarcinoma NOS in an Italian patient series, though without addressing serrated adenocarcinoma histology.[16] Serrated adenocarcinoma were seen in half of the class 3 a*BRAF* only mutants, and in a third of class 2 only mutants. In conclusion, our a*BRAF*mt serrated adenocarcinomas resemble more the traditional serrated adenocarcinomas with pMMR in all and concomitant *RAS*mt in nearly half of the cases. Based on our results a*BRAF*mt may represent a previously unrecognised driver mutation for serrated adenocarcinomas rising via the traditional serrated pathway, analogous to the sessile serrated pathway where most tumours have *BRAF*-V600Emt.[11]

Observed differences in the treatments provided for the various mutation groups may reflect the different natural courses of the disease. It is known that *BRAF*-V600Emt means fewer lines of chemotherapy and less frequent metastasectomies and, as a consequence more often BSC, than both *RAS*&*BRAF*wt and *RAS*mt mean.[4] Our finding of an intermediate pattern of treatments being closer to *BRAF*-V600Emt than the other groups fits with the survival noted.[18] It should be noted that the presence of an a*BRAF*mt was not known to any clinician, thus, not having any bearing on the choice of therapy. The outcomes in the a*BRAF*mt group, albeit based on only nine cases, being similar to the *RAS*mt and *RAS*&*BRAF*wt groups after metastasectomy/LAT, in line with one previous study[29] prompt that we should aim at maximising metastasectomy in a*BRAF*mt.[18]

So far, the number of patients having an a*BRAF*mt tumour being treated with an EGFR-inhibitor is limited and none within a prospective design, therefore, it is difficult to draw firm conclusions about

resistance to EGFR-inhibitors. Our nine cases without response add to the idea that an a*BRAF*mt means resistance, as previously reported in two studies,[5,30] but contrasts with two other studies reporting 50% to 67% response rate for class 3 and 0% to 8% response rate for class 2.[15,16] Collectively, it appears as if class 2 a*BRAF*mt rarely respond to EGFR-inhibitors, but that some responses can be seen in class 3 a*BRAF*mt. In this respect, discrepancies in whether the uncommon but still much more common *BRAF*-V600Emt is a negative predictive factor for EGFR-inhibition still exist.[34,35]

a*BRAF*mt showed a trend towards better OS when bevacizumab was added to first-line treatment, in line with *RAS*&*BRAF*wt, *RAS*mt or *BRAF*mt subgroups. This needs to be interpreted with caution as it probably has been added to younger and fitter patients. Benefit related to added bevacizumab in a*BRAF*mt has not been clearly reported before. Shimada et al[29] report similar PFS in *BRAF*-V600Emt, a*BRAF*mt and *BRAF*wt, supporting our findings, and Schirripa et al[16] report first-line responses in 33% and disease control rate in 89%, also supporting our findings. Our mixed results in two patients with a*BRAF*mt treated with regorafenib are in line with a case report.[36]

In the second-line BEACON-study in *BRAF*-V600Emt, a combination of *BRAF*- and EGFR-inhibitors with or without a mitogen-activated protein kinase kinase (MEK)-inhibitor for mCRC yielded significantly improved OS, PFS and response rate.[37] One class 3 a*BRAF*mt in our material that had received panitumumab, later also received encorafenib+cetuximab. This patient, however, had an immediate progression on the treatment, but showed multiple acquired resistance mutations on FoundationOne performed after treatment (most likely due to previous EGFR-inhibitor treatment). The use of MAPK-kinase pathway inhibitors has been discussed in recent reviews by Yaeger et al and Dankner et al, with caution for use of *BRAF*- and *RAS*-inhibitors presented.[38,39]

An ongoing phase II study investigates the combination of binimetinib, encorafenib and cetuximab among a*BRAF*mt.[40] There are also other drug candidates for a*BRAF*mt.[41] One is ulixertinib, an ERK 1 and 2 inhibitor, where a*BRAF*mt can be included.[42,43] BGB3245, an inhibitor of both mono- and dimeric RAF, is explored in a phase I trial.[44] A RAF inhibitor (PLX8349/FORE8349) targeting dimers that can disrupt both class 2 and class 3 *BRAF* heterodimers, is also studied.[45,46] It has been suggested that treatments for the group of patients with a*BRAF*mt should be targeted against a specific class of mutation,[34,38] a proper suggestion in our view.

This study consists of real-world and population-based materials, better reflecting the background population than all earlier work. We also have high-quality information on background demographics, treatment and outcome, with no patients lost to follow-up. Further, during the first time period when the PRCRC-study cohort was collected, knowledge of *RAS*mt or *BRAF*mt status was not present for any patient,[47] whereas during the second period (RAXO), many patients had their tumours investigated for *KRAS*-, *NRAS*- and *BRAF*-mutations but none had knowledge about whether an a*BRAF*mt was present. Thus, treatment selection for those with a*BRAF*mt could only be made based on *RAS*-/*BRAF*-mutation status besides clinical factors. All results and conclusions are robust in that they are seen also when cases derived from cohorts where not all patients with sufficient tumour material have been analysed are removed.

A weakness of this study is that the testing in the RAXO-study was done in clinical routine, however according to European Society for Medical Oncology-guidelines.[48,49] Thus, all patients were not tested for a*BRAF*mt, and we could only estimate the proportion among fully tested patients. Some a*BRAF*mt have therefore probably been missed. Scientifically it would also have been better if only one molecular technique was used, but this can also be seen as a strength since we wanted to explore the relevance of this specific mutation in the background population.

5 | CONCLUSION

a*BRAF*mt were seen in 2% to 4% in the different cohorts and are probably more common in population-based than in the real-world situations including only treatable patients. Patients with a*BRAF*mt tumours were clinically more alike patients with *RAS*&*BRAF*wt and *RAS*mt, than with *BRAF*-V600Emt tumours. As opposed to *BRAF*-V600Emt, a*BRAF*mt co-exists with *RAS*mt. Our a*BRAF*mt serrated adenocarcinomas resemble more the traditional serrated pathway adenocarcinomas with pMMR in all and concomitant *RAS*mt in nearly half. The a*BRAF*mt group had worse OS than both *RAS*&*BRAF*wt and *RAS*mt, but slightly better than the *BRAF*-V600Emt. It therefore seems as if the a*BRAF*mt is a distinct group, however, heterogenous. a*BRAF*mt are rare, but important to find as they appear relevant for prognosis, and treatment decision, especially regarding the use of EGFR-inhibitors. a*BRAF*mt should not be a contraindication for metastasectomy. However, larger, and prospective studies are needed for firm conclusions about therapy choice.

AUTHOR CONTRIBUTIONS

The work reported in the paper has been performed by the authors, unless clearly specified in the text. Emerik Osterlund, Ari Ristimäki, Markus J. Mäkinen, Tapio Salminen, Annika Ålgars, Raija Kallio, Raija Ristamäki, Helena Isoniemi, Bengt Glimelius and Pia Osterlund comprised the steering committee and participated in all phases of the study, including the design or conduct of the study, analyses, and interpretation of the data and preparation of the manuscript. All authors recruited patients or gathered data for the study. Emerik Osterlund and Pia Osterlund did the statistical analyses. All authors interpreted the data and were involved in the review and writing of the manuscript and the decision to submit for publication.

AFFILIATIONS

[1]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden
[2]Department of Transplantation and Liver Surgery, Helsinki University Hospital, Helsinki, Finland
[3]Department of Pathology, HUSLAB, HUS Diagnostic Center, Helsinki University Hospital, Helsinki, Finland

[4]Faculty of Medicine, Applied Tumor Genomics Research Program, Research Programs Unit, University of Helsinki, Helsinki, Finland
[5]Department of Pathology, Oulu University Hospital, Oulu, Finland
[6]Translational Medicine Research Unit, Department of Pathology, University of Oulu, Oulu, Finland
[7]Medical Research Center Oulu, Oulu, Finland
[8]Department of Genetics, HUSLAB, HUS Diagnostic Center, Helsinki University Hospital, Helsinki, Finland
[9]Department of Genetics, University of Helsinki, Helsinki, Finland
[10]Departemnt of Oncology, Central hospital of Central Finland, Jyväskylä, Finland
[11]Docrates hospital, Helsinki, Finland
[12]Department of Oncology, Odense University Hospital, Odense, Denmark
[13]Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[14]Home Care, Geriatric Clinic and Palliative Care, Joint Municipal Authority for Health Care and Social Services in Keski-Uusimaa, Hyvinkää, Finland
[15]Department of Oncology, Helsinki University Hospital, Helsinki, Finland
[16]Department of Genetics, Fimlab Laboratories, Tampere, Finland
[17]Department of Oncology, Haukeland University Hospital, Bergen, Norway
[18]Department of Clinical Science, University of Bergen, Bergen, Norway
[19]Department of Oncology, Tampere University Hospital, Tampere, Finland
[20]Department of Oncology, University of Tampere, Tampere, Finland
[21]Department of Pathology, Tampere University Hospital, Tampere, Finland
[22]Department of Pathology, University of Tampere, Tampere, Finland
[23]Department of Surgery, University of Helsinki, Helsinki, Finland
[24]Department of Transplant and HPB Surgery, Royal Infirmary of Edinburgh, Edinburgh, UK
[25]Department of Oncology, University of Helsinki, Helsinki, Finland
[26]Department of Oncology, Turku University Hospital, Turku, Finland
[27]Department of Oncology, University of Turku, Turku, Finland
[28]Department of Pathology, Turku University Hospital, Turku, Finland
[29]Institute of Biomedicine, University of Turku, Turku, Finland
[30]Department of Oncology, Oulu University Hospital, Oulu, Finland
[31]Department of Oncology, University of Oulu, Oulu, Finland
[32]Department of Oncology, Kuopio University Hospital, Kuopio, Finland
[33]Department of Medicine, University of Eastern Finland, Kuopio, Finland
[34]Department of Pathology, Central Finland Hospital Nova, Jyväskylä, Finland
[35]Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[36]Department of Gastrointestinal Oncology, Karolinska Universitetssjukhuset, Stockholm, Sweden
[37]Department of Oncology/Pathology, Karolinska Institutet, Stockholm, Sweden

ACKNOWLEDGEMENTS

We want to thank the RAXO-study, and PRCRC-study investigators, study nurses and patients. We also thank Celina Österlund for making the figures for the manuscript.

FUNDING INFORMATION

This RAXO-study was supported by Finska Läkaresällskapet (2016, 2018, 2019, 2020, 2021, 2022); Cancer Foundation Finland (2019-2020, 2021, 2022-2023); Relander's Foundation (2020-2022); the Competitive State Research Financing of the Expert Responsibility Area of Tampere, Helsinki and Turku (2016, 2017, 2018, 2019, 2020, 2021, 2022); Tampere University Hospital Funds (Tukisäätiö 2019, 2020; OOO 2020); and the Research Fund of Helsinki University Hospital (2019, 2020, 2021). The infrastructure with database and study nurses were partly supported by pharmaceutical companies (Amgen unrestricted grant 2012-2020, Eli Lilly and Company 2012-2017, Merck KGaA 2012-2020, Roche Oy 2012-2020, Sanofi 2012-2017 and Servier unrestricted grant 2016-2020). The funding sources had no role in the design and conduct of the study, collection, analysis and interpretation of the data or decision to submit the manuscript for publication.

CONFLICT OF INTEREST STATEMENT

Authors Emerik Osterlund, Ari Ristimäki, Markus J. Mäkinen, Soili Kytölä, Juha Kononen, Leena-Maija Soveri, Tapio Salminen, Lasse Nieminen, Aki Uutela, Päivi Halonen, Annika Ålgars, Jari Sundström, Raija Kallio, Raija Ristamäki, Annamarja Lamminmäki, Hanna Stedt, Eetu Heervä, Teijo Kuopio, Helena Isoniemi and Pia Osterlund all report institutional research funding from Eli Lilly, Merck KGaA, Nordic Drugs, Roche Oy, Sanofi and unrestricted grants from Amgen and Servier, during the conduct of the RAXO-study. The following authors report grants, personal fees, or non-financial support; Emerik Osterlund: Amgen; Per Pfeiffer: Taiho, Servier, Nordic Drugs, Shire, MSD, BMS, PledPharma, Egetis, Isofol, Lilly, Roche, Merck-Serono, Amgen and Celgene; Halfdan Sorbye: Pierre Fabre and Bayer; Päivi Halonen: Bayer, MSD, Servier and Eli Lilly; Annika Ålgars: Amgen, Merck, Roche, Servier and Bayer; Raija Ristamäki: Amgen, Astra-Zeneca, Eli Lilly, Eisai, Incyte, Merck, MSD and Servier; Hanna Stedt: Amgen, Bayer, Bristol-Myers Squibb, Daiichi Sankyo, Eisai, Merck, MSD, Pierre Fabre, Roche and Servier; Eetu Heervä: Astra-Zeneca, Eisai, Pfizer and Daiichi Sankyo; Teijo Kuopio: Amgen, Bayer, Roche, MSD and Pfizer; Pia Osterlund: Amgen, Astra-Zeneca, Bayer, Celgene, Daiichi Sankyo, Eli Lilly, Eisai, Erytech Pharma, Incyte, Fresenius, Jansen-Cilag, Merck, MSD, Nordic Drugs/Pharma, Nutricia, Pierre-Fabre, Roche, Sanofi, Servier and Sobi. The other authors do not declare a conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request, with approval by the steering committee.

ETHICS STATEMENT

The clinical trial identification codes were NCT01531595 and EudraCT 2011-003137-33 for the RAXO-study. The Finnish data-collection cohort was approved (THL/2305/5.05.00/2019). Ethical permissions were obtained from the ethical committees at Helsinki and Odense University Hospitals, Uppsala University and Committee for Medical Research Ethics Western Norway. The permissions included retrospective identification of all patients living in the catchment areas of the Scandinavian PRCRC and RAXO cohorts at the time of diagnosis of their primary tumour and to perform molecular analyses of their tumours. All studies were conducted in accordance with the declaration of Helsinki.

ORCID

Emerik Osterlund https://orcid.org/0000-0003-0973-6332
Luís Nunes https://orcid.org/0000-0002-3391-1607
Eetu Heervä https://orcid.org/0000-0002-6134-1170

REFERENCES

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71:209-249.
2. Tie J, Gibbs P, Lipton L, et al. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. *Int J Cancer*. 2011;128:2075-2084.
3. Zhao B, Wang L, Qiu H, et al. Mechanisms of resistance to anti-EGFR therapy in colorectal cancer. *Oncotarget*. 2017;8:3980-4000.
4. Sorbye H, Dragomir A, Sundstrom M, et al. High BRAF mutation frequency and marked survival differences in subgroups according to KRAS/BRAF mutation status and tumor tissue availability in a prospective population-based metastatic colorectal cancer cohort. *PLoS One*. 2015;10:e0131046.
5. Johnson B, Loree JM, Jacome AA, et al. Atypical, non-V600 BRAF mutations as a potential mechanism of resistance to EGFR inhibition in metastatic colorectal cancer. *JCO Precis Oncol*. 2019;3:1-10.
6. Levin-Sparenberg E, Bylsma LC, Lowe K, Sangare L, Fryzek JP, Alexander DD. A systematic literature review and meta-analysis describing the prevalence of KRAS, NRAS, and BRAF gene mutations in metastatic colorectal cancer. *Gastroenterol Res*. 2020;13:184-198.
7. Corcoran RB, Andre T, Atreya CE, et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. *Cancer Discov*. 2018;8:428-443.
8. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG Island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet*. 2006;38:787-793.
9. Therkildsen C, Bergmann TK, Henrichsen-Schnack T, Ladelund S, Nilbert M. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: a systematic review and meta-analysis. *Acta Oncol*. 2014;53:852-864.
10. de Palma FDE, D'Argenio V, Pol J, Kroemer G, Maiuri MC, Salvatore F. The molecular hallmarks of the serrated pathway in colorectal cancer. *Cancers (Basel)*. 2019;11:11.
11. Bond CE, Whitehall VLJ. How the BRAF V600E mutation defines a distinct subgroup of colorectal cancer: molecular and clinical implications. *Gastroenterol Res Pract*. 2018;2018:9250757.
12. Nguyen HT, Duong HQ. The molecular characteristics of colorectal cancer: implications for diagnosis and therapy. *Oncol Lett*. 2018;16:9-18.
13. Nazemalhosseini Mojarad E, Kuppen PJ, Aghdaei HA, Zali MR. The CpG Island methylator phenotype (CIMP) in colorectal cancer. *Gastroenterol Hepatol Bed Bench*. 2013;6:120-128.
14. Jones JC, Renfro LA, Al-Shamsi HO, et al. (non-V600) BRAF mutations define a clinically distinct molecular subtype of metastatic colorectal cancer. *J Clin Oncol*. 2017;35:2624-2630.
15. Yaeger R, Kotani D, Mondaca S, et al. Response to anti-EGFR therapy in patients with BRAF non-V600-mutant metastatic colorectal cancer. *Clin Cancer Res*. 2019;25:7089-7097.
16. Schirripa M, Biason P, Lonardi S, et al. Class 1, 2, and 3 BRAF-mutated metastatic colorectal cancer: a detailed clinical, pathologic, and molecular characterization. *Clin Cancer Res*. 2019;25:3954-3961.
17. Yao Z, Yaeger R, Rodrik-Outmezguine VS, et al. Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature*. 2017;548:234-238.
18. Cremolini C, di Bartolomeo M, Amatu A, et al. BRAF codons 594 and 596 mutations identify a new molecular subtype of metastatic colorectal cancer at favorable prognosis. *Ann Oncol*. 2015;26:2092-2097.
19. Osterlund P, Salminen T, Soveri LM, et al. Repeated centralized multidisciplinary team assessment of resectability, clinical behavior, and outcomes in 1086 Finnish metastatic colorectal cancer patients (RAXO): a nationwide prospective intervention study. *Lancet Reg Health Eur*. 2021;3:100049.
20. Sorbye H, Pfeiffer P, Cavalli-Bjorkman N, et al. Clinical trial enrollment, patient characteristics, and survival differences in prospectively registered metastatic colorectal cancer patients. *Cancer*. 2009;115:4679-4687.
21. Heervä E, Ristimäki A, Kytölä S, et al. PD-19 Finnish population-based metastatic colorectal cancer data collection study: comparison with the prospective RAXO study. *Ann Oncol*. 2023;34:S8-S9.
22. Osterlund E, Ristimäki A, Kytölä S, et al. KRAS-G12C mutation in one real-life and three population-based Nordic cohorts of metastatic colorectal cancer. *Front Oncol*. 2022;12:826073.
23. Nunes L, Aasebo K, Mathot L, et al. Molecular characterization of a large unselected cohort of metastatic colorectal cancers in relation to primary tumor location, rare metastatic sites and prognosis. *Acta Oncol*. 2020;59:417-426.
24. Mathot L, Kundu S, Ljungström V, et al. Somatic Ephrin receptor mutations are associated with metastasis in primary colorectal cancer. *Cancer Res*. 2017;77:1730-1740.
25. Birgisson H, Edlund K, Wallin U, et al. Microsatellite instability and mutations in BRAF and KRAS are significant predictors of disseminated disease in colon cancer. *BMC Cancer*. 2015;15:125.
26. Nagtegaal ID, Odze RD, Klimstra D, et al. The 2019 WHO classification of tumours of the digestive system. *Histopathology*. 2020;76:182-188.
27. Mäkinen MJ. Colorectal serrated adenocarcinoma. *Histopathology*. 2007;50:131-150.
28. Stensrud MJ, Hernán MA. Why test for proportional hazards? *JAMA*. 2020;323:1401-1402.
29. Shimada Y, Tajima Y, Nagahashi M, et al. Clinical significance of BRAF non-V600E mutations in colorectal cancer: a retrospective study of two institutions. *J Surg Res*. 2018;232:72-81.
30. Shinozaki E, Yoshino T, Yamazaki K, et al. Clinical significance of BRAF non-V600E mutations on the therapeutic effects of anti-EGFR monoclonal antibody treatment in patients with pretreated metastatic colorectal cancer: the biomarker research for anti-EGFR monoclonal antibodies by comprehensive cancer genomics (BREAC) study. *Br J Cancer*. 2017;117:1450-1458.
31. Franczak C, Kandathil SM, Gilson P, et al. Uncommon mutational profiles of metastatic colorectal cancer detected during routine genotyping using next generation sequencing. *Sci Rep*. 2019;9:7083.

32. Stefanius K, Ylitalo L, Tuomisto A, et al. Frequent mutations of KRAS in addition to BRAF in colorectal serrated adenocarcinoma. *Histopathology*. 2011;58:679-692.
33. Jia M, Gao X, Zhang Y, Hoffmeister M, Brenner H. Different definitions of CpG Island methylator phenotype and outcomes of colorectal cancer: a systematic review. *Clin Epigenetics*. 2016;8:25.
34. Johnson B, Kopetz S. Applying precision to the management of BRAF-mutant metastatic colorectal cancer. *Target Oncol*. 2020;15:567-577.
35. Yu IS, Kopetz S. The emergence of targetable pathways in colorectal cancer. *Clin Adv Hematol Oncol*. 2021;19:774-783.
36. Callebout E, Ribeiro SM, Laurent S, et al. Long term response on Regorafenib in non-V600E BRAF mutated colon cancer: a case report. *BMC Cancer*. 2019;19:567.
37. Kopetz S, Grothey A, van Cutsem E, et al. BEACON CRC: a randomized, 3-arm, phase 3 study of encorafenib and cetuximab with or without binimetinib vs. choice of either irinotecan or FOLFIRI plus cetuximab in BRAF V600E-mutant metastatic colorectal cancer. *Ann Oncol*. 2019;30:LBA-006.Iv137-51.
38. Yaeger R, Corcoran RB. Targeting alterations in the RAF-MEK pathway. *Cancer Discov*. 2019;9:329-341.
39. Dankner M, Wang Y, Fazelzad R, et al. Clinical activity of mitogen-activated protein kinase-targeted therapies in patients with non-V600 BRAF-mutant tumors. *JCO Precis Oncol*. 2022;6:e2200107.
40. Kotani D, Bando H, Taniguchi H, et al. BIG BANG study (EPOC1703): multicentre, proof-of-concept, phase II study evaluating the efficacy and safety of combination therapy with binimetinib, encorafenib and cetuximab in patients with BRAF non-V600E mutated metastatic colorectal cancer. *ESMO Open*. 2020;5:e000624.
41. Rodriquenz MG, Ciardiello D, Latiano TP, et al. Exploring biological heterogeneity and implications on novel treatment paradigm in BRAF-mutant metastatic colorectal cancer. *Crit Rev Oncol Hematol*. 2022;173:103657.
42. BioMed Valley Discoveries I. *Study of Ulixertinib for Patients with Advanced Malignancies Harboring MEK or Atypical BRAF Alterations*. Bethesda (MD): National Library of Medicine; 2023.
43. BioMed Valley Discoveries I. *Trial of Ulixertinib in Combination with Hydroxychloroquine in Patients with Advanced Gastrointestinal (GI) Malignancies*. Bethesda (MD): National Library of Medicine; 2024.
44. MapKure LLC. *Study of Safety, Pharmacokinetics, and Antitumor Activity of BGB-3245 in Participants with Advanced or Refractory Tumors*. Bethesda (MD): National Library of Medicine; 2023.
45. Yao Z, Gao Y, Su W, et al. RAF inhibitor PLX8394 selectively disrupts BRAF dimers and RAS-independent BRAF-mutant-driven signaling. *Nat Med*. 2019;25:284-291.
46. Fore B. *A Study of FORE8394 as a Single Agent in Patients with Advanced Unresectable Solid Tumors*. Bethesda (MD): National Library of Medicine; 2022.
47. Tveit KM, Guren T, Glimelius B, et al. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol*. 2012;30:1755-1762.
48. van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol*. 2016;27:1386-1422.
49. Schmoll HJ, Van Cutsem E, Stein A, et al. ESMO consensus guidelines for management of patients with colon and rectal cancer. A personalized approach to clinical decision making. *Ann Oncol*. 2012;23:2479-2516.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Osterlund E, Ristimäki A, Mäkinen MJ, et al. Atypical (non-V600E) *BRAF* mutations in metastatic colorectal cancer in population and real-world cohorts. *Int J Cancer*. 2024;154(3):488-503. doi:10.1002/ijc.34733