




## RESEARCH ARTICLE

## Cancer Genetics and Epigenetics

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

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## 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.

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Bengt Glimelius and Pia Osterlund have shared last authorship.

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

#### KEYWORDS

*aBRAF*, *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.<sup>1</sup> 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*).<sup>2</sup> *RAS* mutations (mainly Kirsten rat sarcoma viral oncogene [*KRASmt*] and neuroblastoma *RAS* viral oncogene homologue [*NRASmt*]) are seen in about 50% of CRC patients and cause resistance to epidermal growth factor receptor (EGFR)-inhibitors.<sup>2,3</sup>

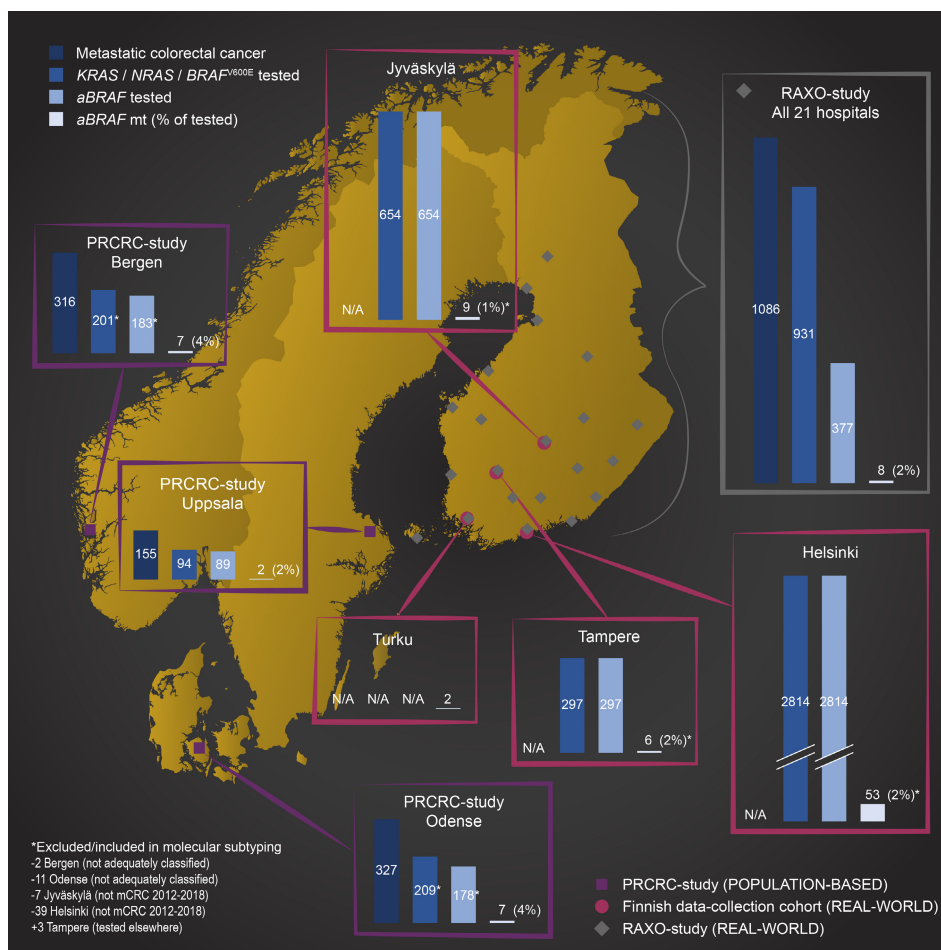
*BRAF*-mutations (*BRAFmt*) are seen in about 15% of primary CRC and in 4% to 10% of metastatic (mCRC) trial patients, but higher frequencies (above 20%) have been noted in population-based series.<sup>4-6</sup> The *BRAF-V600E* mutation (*BRAF-V600Emt*) accounts for up to 90% of all *BRAFmt*.<sup>2,5</sup> In CRC, *BRAF-V600Emt* are associated with female sex, right-sided primary tumours and higher tumour grade, and in mCRC with poor prognosis.<sup>7,8</sup> 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.<sup>7</sup> *BRAF-V600Emt* predicts less effect from EGFR-inhibitors, however debated.<sup>9</sup>

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.<sup>10,11</sup> Sessile serrated lesions mostly arise due to a *BRAF-V600Emt*, and CIMP-high leads to sporadic dMMR in 80%.<sup>11,12</sup> Traditional serrated

adenomas, in contrast often arise through CIMP-low, *BRAF-V600Emt* or *KRASmt* in combination with other events.<sup>11-13</sup>

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 (*aBRAFmt*), also called non-V600E, is only partly known.<sup>5,14-16</sup> *BRAFmt* 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.<sup>5,15,17</sup> 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 *aBRAFmt* patients and showed that they had more rectal primary tumours, less peritoneal metastases and longer overall survival (OS) compared with *BRAF-V600Emt*.<sup>18</sup> In another study, 9643 patients with mCRC from three NGS databases were analysed, yielding 208 *aBRAFmt* (2.2% of all and 21.6% of all *BRAFmt*).<sup>14</sup> The *aBRAFmt* 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 *aBRAFmt* had follow-up data.<sup>14</sup> In a third study, *aBRAFmt* was seen in 1.7% and they had left-sided primaries and concomitant *RAS*-mutations more often than *BRAF-V600Emt*.<sup>5</sup> In this study no responses were seen in 11 patients receiving EGFR-inhibitors. In yet another study, 40 *aBRAFmt* received EGFR-inhibitors and responses were seen in 8% with class



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

2 mutations (1/12) and in 50% with class 3 (14/28).<sup>15</sup> A study including 25 patients with aBRAFmt 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.<sup>16</sup>

Until now, aBRAFmt 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<sup>4-6</sup> and the limited and non-conclusive knowledge about aBRAFmt, the aim was to study demographics, clinical characteristics, pathology and outcomes for aBRAFmt 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.<sup>19</sup>

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.<sup>20</sup> 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 aBRAF 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,<sup>19</sup> and preliminary demographics and outcome of this cohort has been presented.<sup>21</sup> 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 aBRAFmt in the Finnish data-collection cohort, where exact frequencies could not be calculated since validated data for non-aBRAFmt 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 aBRAFmt, 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*-V600E, details described.<sup>22</sup> 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, aBRAFmt 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),<sup>23</sup> with pyrosequencing for *KRAS* and *BRAF*-V600E in 44,<sup>4</sup> and Illumina sequencing (2661 bases) in one.<sup>24</sup>

## 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.<sup>23,25</sup> 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 aBRAF-mutant tumours

Haematoxylin and eosin-stained CRC specimens or whole slide images from 44 patients harbouring an aBRAFmt were reviewed by an expert GI pathologist (MJM), who was blind for clinical and mutational data (other than aBRAFmt). CRCs were subtyped according to WHO-2019 criteria,<sup>26</sup> for serrated adenocarcinoma more detailed criteria were used.<sup>27</sup> 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 aBRAFmt and *BRAF*-V600E 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.<sup>28</sup> 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*-V600E 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 | aBRAF-mutation frequency in the different cohorts

The aBRAFmt 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 |         | aBRAF mt |         | BRAF-V600E mt |         | RAS&BRAF wt |         | RAS mt |         | P value* |
|------------------------------------|-------|---------|----------|---------|---------------|---------|-------------|---------|--------|---------|----------|
|                                    | 1449  | 100%    | 51       | 100%    | 182           | 100%    | 456         | 100%    | 760    | 100%    |          |
| 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 <sup>a</sup> |       |         |          |         |               |         |             |         |        |         |          |
| 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 |      | aBRAF mt |      | BRAF-V600E mt |      | RAS&BRAF wt |      | RAS mt |      | P value* |
|--------------------------|-------|------|----------|------|---------------|------|-------------|------|--------|------|----------|
|                          | 1449  | 100% | 51       | 100% | 182           | 100% | 456         | 100% | 760    | 100% |          |
| 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 aBRAFmt and BRAF-V600E mt.

Abbreviations: dMMR, deficient mismatch repair; pMMR, proficient mismatch repair.

<sup>a</sup>For rectal primaries.

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

### 3.3 | Baseline demographics

The final study cohort consisted of 51 (3%) aBRAFmt, 182 (13%) BRAF-V600E mt, 456 (31%) RAS&BRAFwt (of which 22 were not tested for NRAS due to insufficient tumour tissue) and 760 (52%) RASmt 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 aBRAFmt 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 aBRAFmt compared with BRAF-V600E mt 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 aBRAFmt compared with RAS&BRAFwt had less left colon primary tumours (22% vs 46%,  $P < .001$ ), and liver metastases (61% vs 76%,  $P = .021$ ). The aBRAFmt compared with RASmt 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 | aBRAF-mutation class

Among the 51 patients with aBRAFmt, 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 BRAFmt, four patients (8%) had both a class 2 or 3, and class 1 mutation (BRAF-V600E mt), and one had two different class 2 mutations (Table S4).

Demographics for class 2 and 3 aBRAFmt 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 aBRAFmt that could also be unclassified did not alter the results (data not shown).

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

Concomitant RASmt were common in aBRAFmt, and as expected rare in BRAF-V600E mt (41% vs 2%,  $P < .001$ , Table 1 and Table S3). A trend was seen for class 2 having concomitant RASmt less often than class 3 (27% vs 44%, Table 2). aBRAFmt&RASmt compared with

**TABLE 2** Demographics for classes of aBRAF mutations.

|  | Total |         | Class 2 |         | Class 3 |         | Class 1 + 2/3 |         | Unclassified |         | P value* |
|--|-------|---------|---------|---------|---------|---------|---------------|---------|--------------|---------|----------|
|  | 51    | 100%    | 11      | 100%    | 32      | 100%    | 4             | 100%    | 4            | 100%    |          |
| <b>Age</b>                               |       |         |         |         |         |         |               |         |              |         |          |
| 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     |
| <b>Sex</b>                               |       |         |         |         |         |         |               |         |              |         |          |
| Female                                   | 20    | 39%     | 7       | 64%     | 12      | 38%     | 1             | 25%     | 0            | 0%      | ref      |
| Male                                     | 31    | 61%     | 4       | 36%     | 20      | 63%     | 3             | 75%     | 4            | 100%    | .140     |
| <b>Primary tumour</b>                    |       |         |         |         |         |         |               |         |              |         |          |
| 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     |
| <b>Tumour grade</b>                      |       |         |         |         |         |         |               |         |              |         |          |
| 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            | -       | -        |
| <b>Serrated adenoma</b>                  |       |         |         |         |         |         |               |         |              |         |          |
| No <sup>a</sup>                          | 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            | -       | -        |
| <b>Primary surgery</b>                   |       |         |         |         |         |         |               |         |              |         |          |
| No                                       | 13    | 25%     | 4       | 36%     | 8       | 25%     | 0             | 0%      | 1            | 25%     | ref      |
| Yes                                      | 38    | 75%     | 7       | 64%     | 24      | 75%     | 4             | 100%    | 3            | 75%     | .471     |
| <b>Adjuvant chemotherapy</b>             |       |         |         |         |         |         |               |         |              |         |          |
| Yes                                      | 16    | 31%     | 3       | 27%     | 13      | 41%     | 0             | 0%      | 0            | 0%      | .646     |
| <b>Adjuvant radiotherapy<sup>b</sup></b> |       |         |         |         |         |         |               |         |              |         |          |
| Yes                                      | 14    | 61%     | 2       | 50%     | 12      | 67%     | 0             | 0%      | 0            | 0%      | .535     |
| <b>Presentation of metastases</b>        |       |         |         |         |         |         |               |         |              |         |          |
| Synchronous                              | 30    | 59%     | 8       | 73%     | 18      | 56%     | 1             | 25%     | 3            | 75%     | ref      |
| Metachronous                             | 21    | 41%     | 3       | 27%     | 14      | 44%     | 3             | 75%     | 1            | 25%     | .340     |
| <b>Number of metastatic sites</b>        |       |         |         |         |         |         |               |         |              |         |          |
| 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       |
| <b>Metastatic sites</b>                  |       |         |         |         |         |         |               |         |              |         |          |
| 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     |
| <b>Performance status</b>                |       |         |         |         |         |         |               |         |              |         |          |
| 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)

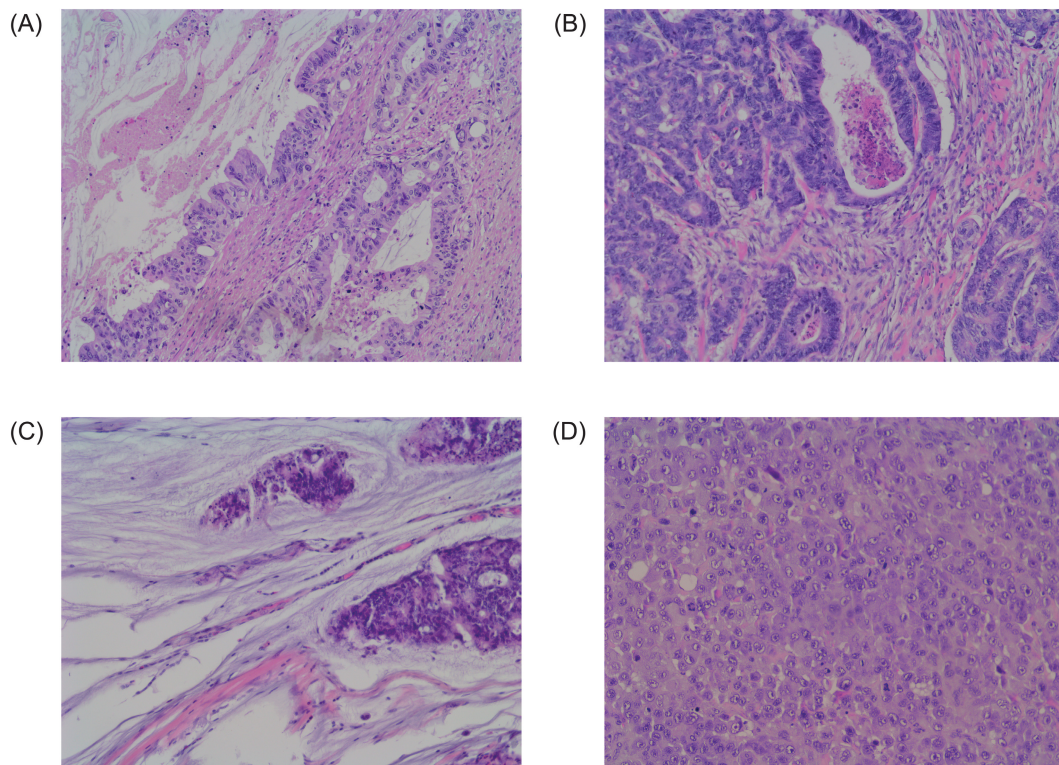
|                          | Total |      | Class 2 |      | Class 3 |      | Class 1 + 2/3 |      | Unclassified |      | P value* |
|--------------------------|-------|------|---------|------|---------|------|---------------|------|--------------|------|----------|
|                          | 51    | 100% | 11      | 100% | 32      | 100% | 4             | 100% | 4            | 100% |          |
| 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.

<sup>a</sup>Includes adenocarcinoma not otherwise specified, mucinous and undifferentiated.

<sup>b</sup>For rectal primaries.



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



**TABLE 3** Treatments divided by groups.

|                                 | Total       |             | aBRAF mt  |             | BRAF-V600E mt |             | RAS & BRAF wt |             | RAS mt     |             | P value*  |
|---------------------------------|-------------|-------------|-----------|-------------|---------------|-------------|---------------|-------------|------------|-------------|-----------|
|                                 | 1449        | 100%        | 51        | 100%        | 182           | 100%        | 456           | 100%        | 760        | 100%        |           |
| <b>Type of treatment</b>        |             |             |           |             |               |             |               |             |            |             |           |
| 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      |
| <b>Total chemotherapy</b>       | <b>1244</b> | <b>100%</b> | <b>37</b> | <b>100%</b> | <b>141</b>    | <b>100%</b> | <b>406</b>    | <b>100%</b> | <b>660</b> | <b>100%</b> | <b>NE</b> |
| <b>Number of lines</b>          |             |             |           |             |               |             |               |             |            |             |           |
| 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      |
| <b>First line chemotherapy</b>  |             |             |           |             |               |             |               |             |            |             |           |
| 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        |
| <b>Best response first line</b> |             |             |           |             |               |             |               |             |            |             |           |
| 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         | -           | -         |
| <b>Chemotherapy all lines</b>   |             |             |           |             |               |             |               |             |            |             |           |
| 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 aBRAF 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.

aBRAFmt&RASwt were less often male (37% vs 75%,  $P = .011$ ), more often had  $\geq 3$  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 aBRAFmt (unclassified aBRAFmt), being significantly lower compared with BRAF-V600E mt (27%,  $P = .005$ ). For RAS&BRAFWt and RASmt, the frequencies of dMMR were 3% and 4%, respectively.

### 3.6 | Pathology

Serrated adenocarcinoma was the most common subtype among aBRAFmt 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 aBRAFmt&BRAF-V600E mt 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 aBRAFmt or BRAF-V600E mt 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&BRAFWt or RASmt 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. aBRAFmt had similar response rate (51%) to first-line chemotherapy as BRAF-V600Emt (43%,  $P = .376$ ), and significantly worse than RAS&BRAFWt (69%,  $P = .040$ ) and RASmt (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 aBRAFmt (median 14.4 months), RASmt (median 23.4 months) and RAS&BRAFWt (median 30.5 months, Figures 3A and S1A), aBRAFmt 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 aBRAFmt 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 aBRAFmt had a 5-year OS rate of 89% for the metastasectomy and/or LAT group in line with RASmt and RAS&BRAFWt, whereas the BRAF-V600Emt did worse (14% 5-year OS rate, Figures 3C and S1C). In the aBRAFmt 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), aBRAFmt did significantly worse than RAS&BRAFWt (HR 0.60, 95% CI 0.43-0.84), almost significantly worse than RASmt (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 aBRAFmt cases (Figures 3E and S4). There were too few cases in the other aBRAFmt subgroups to make any conclusions. Removing the seven class 3 aBRAFmt 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 aBRAFmt group whether RAS was concomitantly mutated or not (Figure S5A,B). RAS&BRAFWt patients that also were aBRAFmt had significantly worse OS than those that did not. The same was also seen for RASmt that had concomitant aBRAFmt (Figure 3F).

### 3.9 | EGFR-inhibitors in aBRAF-mutants

Nine patients with an aBRAFmt 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 aBRAFmt that received EGFR-inhibitors in second or later line was similar to that of BRAF-V600Emt and RASmt (Table S10).

One class 3 aBRAFmt, 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.

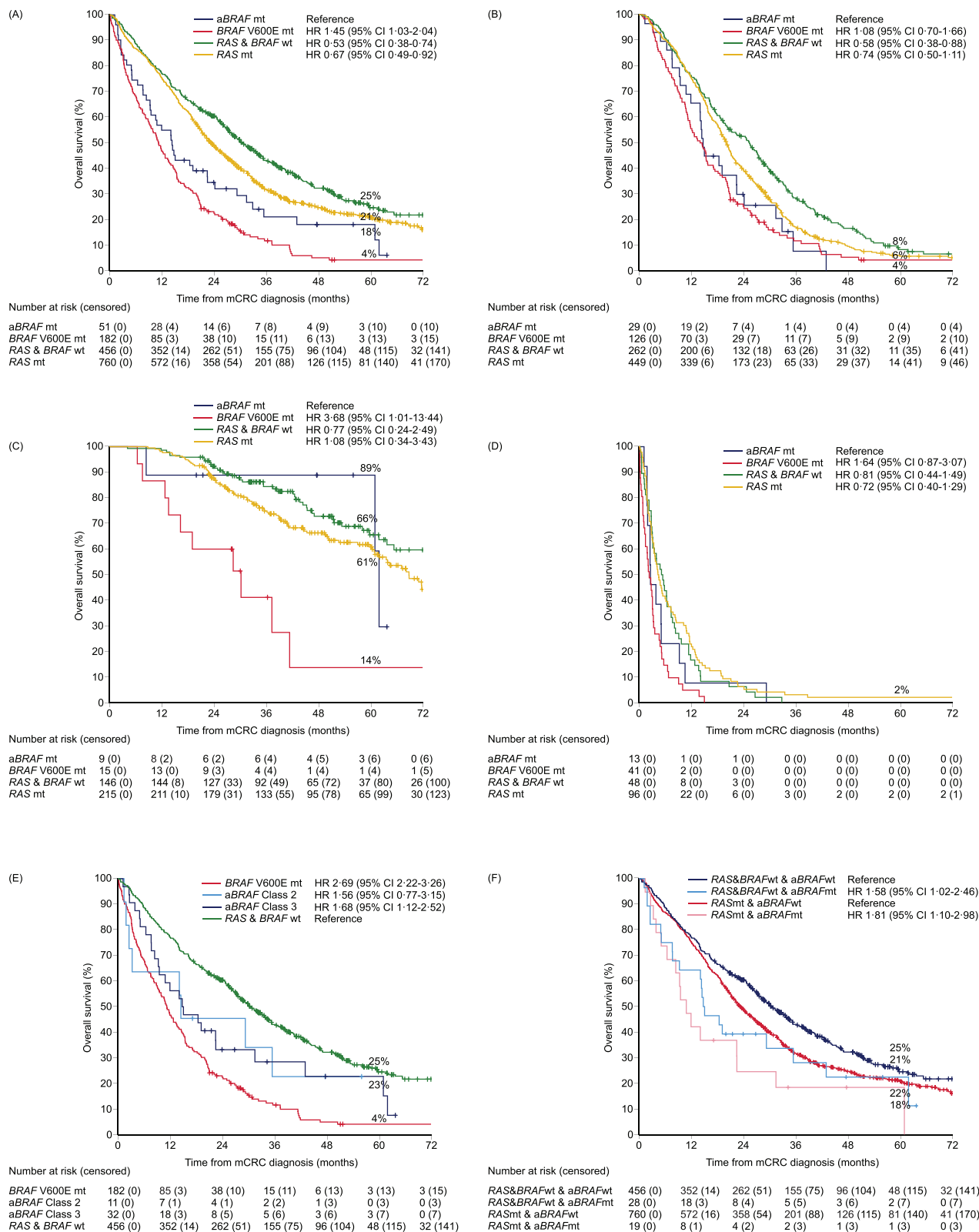
### 3.10 | Treatment with bevacizumab or regorafenib

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&BRAFWt and RASmt groups and showed a trend of better OS for aBRAFmt (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&BRAFWt and RASmt groups (Figure S7A-D). Response in first-line systemic therapy only was seen in 62% (8/13 patients) of aBRAFmt receiving bevacizumab-containing treatment and disease control in 92%. Two aBRAFmt 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 aBRAFmt detected in 4% in one population-based and in 2% in two real-world cohorts in the Nordic countries. A total of 51 aBRAFmt were found and the aBRAFmt frequency varied between 1% and 4% in the different subcohorts, which is in line with earlier studies.<sup>5,14,18</sup> We saw that in the truly population-based cohort with complete testing of all tumours with sufficient material, the aBRAFmt 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%.<sup>5,14,18</sup> Although our frequencies are uncertain due to a small number of cases and the number of patient series being limited, we suggest that aBRAFmt are more common in population-based materials, than in clinical trial/hospital-based patient materials.

OS was the longest for RAS&BRAFWt followed by RASmt and aBRAFmt and shortest for BRAF-V600Emt (median 31 vs 23 vs 14 vs 11 months, respectively), and results for the aBRAFmt 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*-V600E mt in other studies.<sup>5,14,18,29</sup> 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,<sup>14</sup> and similar or numerically worse survival in other studies,<sup>5,16,18,29,30</sup> more in line with our findings. We were also able

to show significantly worse OS among *RAS&BRAF*wt and *RAS*mt with concomitant *aBRAF*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 *aBRAF*mt, whereas one study showed a trend for worse OS in class 2,<sup>16</sup> and others in line with us.<sup>5,15</sup> We could also not detect any differences between *aBRAF*mt with or without concomitant *RAS*mt, while a trend for *aBRAF*mt with concomitant *RAS*mt doing worse was seen in another study.<sup>15</sup>

Indirect support for this higher prevalence of *aBRAF*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*-V600E and dMMR are about twice as common in population-based than in trial/hospital materials.<sup>20,23</sup> The clearly worse prognosis in patients with *aBRAF*mt tumours compared with *RAS*mt and *RAS&BRAF*wt tumours similarly indicate that *aBRAF*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*-G12C and also no worse prognosis for that subgroup relative to other *RAS*mt.<sup>22</sup>

In contrast to two earlier studies showing that patients with *aBRAF*mt tumours were younger than patients with *BRAF*-V600E tumours and about as old as patients with *RAS*mt and *RAS&BRAF*wt tumours,<sup>5,14</sup> we could not detect any age difference between *aBRAF*mt and *BRAF*-V600E (median 69 years), which was slightly higher than for those with *RAS*mt and *RAS&BRAF*wt tumours. *aBRAF*mt, *RAS&BRAF*wt and *RAS*mt were more often seen in males, whereas female sex was more common for *BRAF*-V600E, similar to one study,<sup>14</sup> but opposite to another study where female sex dominated among *aBRAF*mt.<sup>18</sup> Limited number of patients having *aBRAF*mt tumours may explain the disparate results between studies, although it appears as if the *aBRAF*mt group age and sex distributions are more similar to the *RAS*mt and *RAS&BRAF*wt groups than the *BRAF*-V600E group.

The similarity between the *aBRAF*mt, *RAS&BRAF*wt and *RAS*mt groups also relates to the location of the primary tumour, again contrasting to the *BRAF*-V600E group predominantly seen in the right colon.<sup>5,14,16,18,29</sup> Possibly because of this, peritoneal metastases were more common in the *BRAF*-V600E group than in the other groups, also noted in other studies.<sup>14,16,18</sup> There was also a trend towards lung metastases being more common for *aBRAF*mt than for *BRAF*-V600E 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 *aBRAF*mt than for *BRAF*-V600E but less common than for *RAS&BRAF*wt,<sup>14</sup> and in another study, the frequencies were roughly the same in all groups.<sup>16</sup> We could also see a trend for less poorly/undifferentiated tumours in *aBRAF*mt compared with *BRAF*-V600E, seen earlier.<sup>14</sup>

Concomitant *RAS*mt were considerably more common for *aBRAF*mt than for *BRAF*-V600E (41% vs 2%), which is in line with most other studies,<sup>5,14,15,18,29,31</sup> except one with only 1/25 *aBRAF*mt with concomitant *RAS*mt.<sup>16</sup> There was also a trend for concomitant

*RAS*mt being more common for class 3 *aBRAF*mt than for class 2 *aBRAF*mt in our study, in line with.<sup>15</sup>

In our study, where all *aBRAF*mt were tested for MMR-status, dMMR was more common for *BRAF*-V600E compared with *aBRAF*mt, *RAS&BRAF*wt and *RAS*mt (27% vs 2% vs 3% vs 4%, respectively). In other studies, the dMMR frequency for *aBRAF*mt was 0% to 8%.<sup>5,14-16</sup> Compared with *BRAF*wt, it was slightly lower for *aBRAF*mt in one study,<sup>14</sup> and slightly higher in another study.<sup>5</sup>

In this series, serrated adenocarcinoma was the most common (59%) histological subtype among *aBRAF*mt tumours. While it is acknowledged that *BRAF*-V600E are nearly exclusively found in serrated adenocarcinomas,<sup>32</sup> the role of *aBRAF*mt in the serrated pathway has not been studied before. *BRAF*-V600E are mostly and *KRAS*mt rarely found in the sessile serrated pathway, often combined with CIMP-high and sporadic dMMR.<sup>11,12</sup> The *BRAF*-V600E in this study and *aBRAF*mt with concomitant *BRAF*-V600E 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.<sup>12,33</sup> The demographics of the *aBRAF*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*-V600E in 1/3 and *KRAS*mt in 1/4.<sup>11,32</sup> Most *KRAS*mt are though found in the tubulovillous adenoma pathway harbouring *KRAS*mt in half and rarely *BRAF*-V600E, resembling the adenocarcinoma NOS group in our study with *RAS*mt in 27% and no *BRAF*-V600E.<sup>11</sup> The adenocarcinoma NOS in this study resemble this group also regarding demographics. Most class 2 (82%) and class 3 (70%) *aBRAF*mt patients had adenocarcinoma NOS in an Italian patient series, though without addressing serrated adenocarcinoma histology.<sup>16</sup> Serrated adenocarcinoma were seen in half of the class 3 *aBRAF* only mutants, and in a third of class 2 only mutants. In conclusion, our *aBRAF*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 *aBRAF*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*-V600E.<sup>11</sup>

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*-V600E 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.<sup>4</sup> Our finding of an intermediate pattern of treatments being closer to *BRAF*-V600E than the other groups fits with the survival noted.<sup>18</sup> It should be noted that the presence of an *aBRAF*mt was not known to any clinician, thus, not having any bearing on the choice of therapy. The outcomes in the *aBRAF*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<sup>29</sup> prompt that we should aim at maximising metastasectomy in *aBRAF*mt.<sup>18</sup>

So far, the number of patients having an *aBRAF*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 aBRAFmt means resistance, as previously reported in two studies,<sup>5,30</sup> but contrasts with two other studies reporting 50% to 67% response rate for class 3 and 0% to 8% response rate for class 2.<sup>15,16</sup> Collectively, it appears as if class 2 aBRAFmt rarely respond to EGFR-inhibitors, but that some responses can be seen in class 3 aBRAFmt. 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.<sup>34,35</sup>

aBRAFmt showed a trend towards better OS when bevacizumab was added to first-line treatment, in line with RAS&BRAFWt, RASmt or BRAFmt 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 aBRAFmt has not been clearly reported before. Shimada et al<sup>29</sup> report similar PFS in BRAF-V600Emt, aBRAFmt and BRAFWt, supporting our findings, and Schirripa et al<sup>16</sup> report first-line responses in 33% and disease control rate in 89%, also supporting our findings. Our mixed results in two patients with aBRAFmt treated with regorafenib are in line with a case report.<sup>36</sup>

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.<sup>37</sup> One class 3 aBRAFmt 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.<sup>38,39</sup>

An ongoing phase II study investigates the combination of binimetinib, encorafenib and cetuximab among aBRAFmt.<sup>40</sup> There are also other drug candidates for aBRAFmt.<sup>41</sup> One is ulixertinib, an ERK 1 and 2 inhibitor, where aBRAFmt can be included.<sup>42,43</sup> BGB3245, an inhibitor of both mono- and dimeric RAF, is explored in a phase I trial.<sup>44</sup> A RAF inhibitor (PLX8349/FORE8349) targeting dimers that can disrupt both class 2 and class 3 BRAF heterodimers, is also studied.<sup>45,46</sup> It has been suggested that treatments for the group of patients with aBRAFmt should be targeted against a specific class of mutation,<sup>34,38</sup> 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 RASmt or BRAFmt status was not present for any patient,<sup>47</sup> 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 aBRAFmt was present. Thus, treatment selection for those with aBRAFmt could only be made based on RAS-/BRAFMutation 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.<sup>48,49</sup> Thus, all patients were not tested for aBRAFmt, and we could only estimate the proportion among fully tested patients. Some aBRAFmt 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

aBRAFmt 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 aBRAFmt tumours were clinically more alike patients with RAS&BRAFWt and RASmt, than with BRAF-V600Emt tumours. As opposed to BRAF-V600Emt, aBRAFmt co-exists with RASmt. Our aBRAFmt serrated adenocarcinomas resemble more the traditional serrated pathway adenocarcinomas with pMMR in all and concomitant RASmt in nearly half. The aBRAFmt group had worse OS than both RAS&BRAFWt and RASmt, but slightly better than the BRAF-V600Emt. It therefore seems as if the aBRAFmt is a distinct group, however, heterogeneous. aBRAFmt are rare, but important to find as they appear relevant for prognosis, and treatment decision, especially regarding the use of EGFR-inhibitors. aBRAFmt 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.

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## CONFLICT OF INTEREST STATEMENT

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## 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.

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#### SUPPORTING INFORMATION

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

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