

A Systematic Literature Review and Meta-Analysis Describing the Prevalence of *KRAS*, *NRAS*, and *BRAF* Gene Mutations in Metastatic Colorectal Cancer

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Abstract

Background: Tumors of the metastatic colorectal cancer (mCRC) patients that are wildtype (WT) for *KRAS* or *NRAS* mutations respond more favorably to anti-epidermal growth factor receptor (EGFR) treatments. Treatment guidelines now recommend that all mCRC patients have WT *KRAS* and *NRAS* tumor status confirmed prior to initiating anti-EGFR therapy. Evidence also suggests that *BRAF* mutations may predict lack of response to anti-EGFR therapy. As such, there is now a need for comprehensive data on the prevalence of *KRAS*, *NRAS*, and *BRAF* mutations among patients with mCRC.

Methods: A systematic literature review was conducted among studies that described the prevalence of *KRAS*, *NRAS*, and *BRAF* gene mutations in mCRC patients. Observational cohort studies and standard of care arm of randomized clinical trials were included. Random effects meta-analysis models were used to create summary prevalence estimates for each of the mutation types. Subgroup analyses were also conducted to identify potential sources of heterogeneity. Exploratory analyses of overall and progression-free survival by mutation status were also conducted.

Results: This systematic review and meta-analysis included 275 studies comprising 77,104 mCRC patients. The summary prevalence estimate was 35.9% for *KRAS* mutations, 7.1% for *BRAF* mutations, and 4.1% for *NRAS* mutations. Female patients had significantly more *KRAS* and *BRAF* mutations than males, and significant variation by study location was observed for both *KRAS* and *BRAF* mutation

prevalence. Overall survival was significantly decreased for patients with *KRAS*, *BRAF*, and *NRAS* mutations compared to those with WT tumors. Progression-free survival was also significantly decreased among patients with *KRAS* and *BRAF* mutations.

Conclusions: *KRAS*, *NRAS*, and *BRAF* mutation statuses in patients with mCRC are important predictors of treatment success and may also have prognostic value. In this paper we present the first systematic and comprehensive literature review and meta-analysis of the prevalence of *KRAS*, *BRAF*, and *NRAS* mutations and demonstrate the prognostic impact of mutation status on survival.

Keywords: Metastatic colorectal cancer; *KRAS*; *BRAF*; *NRAS*; Meta-analysis

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide in men and the second most in women [1]. Approximately 20-25% of new CRC cases are metastatic (mCRC) at diagnosis and up to 50% of all patients eventually develop metastatic disease [2-5]. During the last decade, improvements in the treatment of patients with mCRC have increased median survival time from 12 to 21 months [6]. This improvement is partly due to the development of therapies that target the epidermal growth factor receptor (EGFR), primarily panitumumab and cetuximab [7]. Numerous studies now show that mutations in codon 12 or 13 of exon 2 of the *KRAS* gene render tumors essentially insensitive to treatment with EGFR inhibitors [8-17], while anti-EGFR therapies have demonstrated a clear clinical benefit in patients with *RAS* wildtype (WT) mCRC [8, 18, 19].

KRAS, a GTPase signaling protein, is the human homolog of the Kirsten rat sarcoma-2 virus oncogene and regulates cell proliferation, differentiation, and survival [20]. Approximately 30-50% of CRC tumors have mutations in exon 2, which result in constitutive signaling by decreasing GTPase activity [21-23]. More recent evidence shows that *NRAS* mutations and *KRAS* mutations outside of exon 2 also predict treatment failure with either cetuximab or panitumumab, which led the

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National Comprehensive Cancer Network and other major oncology societies to recommend genotyping of tumor tissue in all patients with mCRC for *RAS* (*KRAS* exon 2 and non-exon 2; *NRAS*) and *BRAF* at diagnosis of stage IV disease and prior to initiation of anti-EGFR treatment [8, 24-26]. In addition, the Food and Drug Administration (FDA) labels for cetuximab and panitumumab specifically state that these agents are not recommended for the treatment of mCRC characterized by *KRAS* exon 2 mutations. Although it is known that *KRAS/NRAS* mutations predict a lack of response to anti-EGFR treatments, many tumors that are WT with respect to both *KRAS* and *NRAS* still do not respond to these therapies causing researchers to look downstream of *KRAS/NRAS* for additional predictive biomarkers [8].

Mutations in the *BRAF* gene, specifically Val600Glu, are believed to render the protein product constitutively active thereby bypassing EGFR inhibition by anti-EGFR agents; however, the role of *BRAF* mutations as a predictive marker of anti-EGFR treatment failure is still uncertain [8, 27-29]. Other *BRAF* mutations, such as Asp594Gly, have not been well-characterized, and their significance in CRC is not well understood [30, 31]. Nevertheless, the presence of *BRAF* mutations is a strong prognostic factor as mCRC patients with a *BRAF* mutation have significantly shorter overall survival (OS) than those with WT tumors [32-40].

Results regarding the prognostic value of *KRAS* and *NRAS* mutations are mixed [8]. A recent meta-analysis identified that mutations in *KRAS* exons 3 and 4 and *BRAF* were associated with significantly worse odds of survival with anti-EGFR treatment compared to WT tumors (odds ratio (OR): 0.26 and 0.29, respectively) [41]. As such, there is now a need for more information on the prevalence of *KRAS*, *NRAS*, and *BRAF* mutations and their impact on survival among patients with mCRC.

The objectives of this systematic review and meta-analysis were to: 1) provide a comprehensive assessment of *KRAS*, *NRAS*, and *BRAF* mutation prevalence among mCRC patients as reported in the scientific literature; 2) identify potential sources of heterogeneity in mutation prevalence by conducting pre-specified subgroup analyses; and 3) conduct an exploratory analysis to describe OS and progression-free survival (PFS) for mCRC patients by *KRAS*, *NRAS*, and *BRAF* mutation status.

Materials and Methods

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [42]. Methods and article inclusion criteria were specified in advance and documented in a protocol. This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Eligibility criteria

We included peer-reviewed articles published between Janu-

ary 1, 2006 and May 10, 2016 (the most recent 10-year period at time of search) that described the prevalence of *KRAS*, *NRAS*, or *BRAF* gene mutations in mCRC patients in observational studies (either treated or untreated patients) and in the standard of care arm of clinical trials. Though not truly population-based, data on prevalence of the specific genetic mutations from the standard of care arm were considered for the review in order to understand and characterize the prevalence of these mutations in patients who may choose to participate in a clinical trial. Only clinical trials in which the patients were not selected based on mutation status were included.

Eligible analytical study designs were randomized trials, prospective or retrospective observational cohort studies, cross-sectional, and case-control studies. We excluded opinion pieces, editorials, articles without original data (except systematic reviews), case reports and/or case series with less than 20 cases, and experimental animal studies. We excluded studies that did not specifically report prevalence statistics on gene mutations for mCRC patients, studies conducted in pediatric populations, and studies published in languages other than English.

Literature searches and study selection

Electronic literature searches were conducted in the PubMed, Embase, Web of Science, Cochrane Library, and ClinicalTrials.gov databases to identify relevant articles. The bibliography sections from previously published systematic reviews were hand searched to identify additional individual articles of interest not captured by the electronic search engines. A PRISMA flow diagram for study inclusion is shown here (Supplementary Material 1, www.gastrores.org).

A variety of search string parameters were used in an effort to identify all eligible articles, including the following: colon, colorectal, neoplasms, cancer, secondary, metastatic, EGFR, proto-oncogene proteins p21 (RAS), proto-oncogene, proteins, p21 (ras), *KRAS*, *NRAS*, *BRAF*, epidemiology, prevalence, and frequency.

The titles and abstracts of all publications identified via the literature search databases were independently screened by two reviewers based on the inclusion/exclusion criteria outlined above. A standardized abstraction form, which included fields for data elements relevant to the key topics of interest was created in order to maximize reliability. All data elements were entered and then reviewed for quality control by a second researcher. Any disagreements were resolved by a senior epidemiologist. If more than one article from the same study population was published (based on author names, sample sizes, intervention comparisons or outcomes), data from the publication(s) with the longest follow-up or most relevant population and/or outcomes were extracted. Multiple articles from the same study population were included if unique outcomes from the same population (i.e., prevalence of different mutations) were included.

The following qualitative information and quantitative data were abstracted from each of the studies: 1) characteristics of the study (including study design, study dates, name of cohort, NCT number, location (country), study population

description, individual study inclusion/exclusion criteria, sample size, number of subjects with mutation results, reason why mutation results were unavailable for some subjects, and the number of subjects with inconclusive results; 2) characteristics of study participants (including sex, age, race/ethnicity, comorbidities, ECOG-PS, location of primary tumor, stage at first diagnosis, number of metastases, and treatment status); 3) characteristics of study outcomes (including length of follow-up, type of mutation, specific mutation location, mutation assessment method, exon/codon numbers, mutation prevalence, whether mutation data were from primary or metastatic tumor, whether mutation data were obtained from tissue or liquid biopsy using plasma, median overall and PFS, and survival hazard ratios (HRs)).

Statistical analysis

Prevalence data were extracted from each study for each mutation and for each of the subgroups of interest where available. Random effects models were used to generate summary prevalence estimates of *KRAS*, *BRAF*, and *NRAS* gene mutations among mCRC patients and within subgroups of interest (specific mutation location, sex, race, median age, study time period, length of follow-up time, treatment status, study location, study design, source of tumor tissue, mutation assessment method, and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) score) to identify potential sources of heterogeneity. Of note, the analysis for heterogeneity by mutation assessment method evaluated whether the prevalence differed by different assessment method across studies. No direct comparison of testing methods was performed and the results of these analyses should not be interpreted as sensitivity or specificity analyses of mutation assessment method testing. For median age, and length of follow-up time, the median value was calculated across studies and subgroup analyses were conducted for studies above and below the median value. Studies were weighted according to the inverse of their variance using the method proposed by DerSimonian and Laird [43]. Heterogeneity between studies and subgroups in each analysis was evaluated using the Cochran's Q and I² statistical tests. Meta-regression analyses were conducted to explore the incremental effect of median age and length of follow-up time on prevalence. All prevalence analyses were performed using Comprehensive Meta-Analysis Software (version 3.0; Biostat, Englewood, New Jersey, USA).

Exploratory survival analysis

In addition to prevalence of *KRAS*, *NRAS*, and *BRAF* gene mutations in mCRC patient population, we also conducted an exploratory analysis of the median OS and PFS in the mutant versus WT populations. Median OS, median PFS, HRs for survival, and corresponding 95% confidence intervals (CIs) were abstracted from the literature if available. This analysis was exploratory in that a specific search was not performed for survival in these populations nor was it necessary for inclusion in

the study. Random effects models were used to meta-analyze the HRs of OS and PFS for mutant versus WT populations using Comprehensive Meta-Analysis Software. Random effects models of the log-transformed rates were used for the meta-analyses of median survival, which were performed using the "metan" command in Stata 15.0 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Assessment of study quality and reporting bias

Individual study quality was assessed using STROBE guidelines for reporting in observational studies [44]. The STROBE statement is a checklist of 22 items considered as essential for good reporting of observational studies. In our assessment of study quality, individual articles were given 1 point for each of the 22 checklist items addressed in the article.

Publication bias was assessed visually with funnel plots and statistically using Egger's regression method. For analyses with statistically significant publication bias, the Duval and Tweedie trim and fill method was used to calculate the adjusted estimate after adding in hypothetical "missing" studies due to lack of publication [45].

Results

Study characteristics

The PRISMA flow diagram for study selection is shown in here (Supplementary Material 1, www.gastrores.org). From a total of 786 de-duplicated articles, 275 studies comprising 77,104 mCRC patients were included in the meta-analyses. Individual study characteristics are described in here (Supplementary Material 2, www.gastrores.org). The study designs consisted of randomized and non-randomized clinical trials (n = 64), cross-sectional studies (n = 11), and prospective (n = 66) and retrospective (n = 134) observational studies. Study populations were drawn from all continents apart from Antarctica and Africa. Most studies were conducted in Europe (n = 132), followed by Asia (n = 63), and the USA (n = 41). The studies included in our meta-analysis ranged in STROBE score from 5 to 22 with a mean of 15.2 and median of 16. All studies that received scores of less than 10, except Smith 2014 [46], were studies in which only the abstract was available. Therapies and combination regimens varied substantially across studies, with many studies not reporting specific treatments used by included patients. Thus, treatment status was classified into three groups: partial population treated, complete population treated, and unknown/not treated. Only two studies specified that patients were not treated [47, 48] and thus this category was collapsed with "unknown."

Synthesis of results

Mutations were categorized inconsistently in the included

articles. Some were described only by exon number, only by codon number, or a combination of exon and codon numbers, while other papers provided specific mutation locations. Additionally, the majority of papers placed codons 12 and 13 within exon 2 for *KRAS*, *BRAF*, and *NRAS*, while some papers placed these codons within exon 1 or did not specify an exon number. When papers provided only an exon number, it was not always clear whether the entire exon was evaluated for the presence of mutations or if only the presence of certain hot spot mutations was evaluated. To make the categories as consistent as possible, we only analyzed the prevalence of mutations exactly how they were described in the paper and did not collapse categories. For example, in the table of *KRAS* mutation prevalence (Table 1), the exon 2 category only includes the papers that specifically described the location of *KRAS* mutations as occurring within exon 2 and is the most global estimate of exon 2 provided within a paper. There are two categories each for codons 12 and 13. The first category for each includes only prevalence data where the codon was specified as specifically existing within exon 2; and the second category for each of codons 12 and 13 includes the papers that described this codon as existing within exon 1 or 2 or did not specify a particular exon. To identify potential patterns of heterogeneity with regard to mutation prevalence, we performed subgroup analyses on relevant study characteristics (i.e., sex, study location, study design, source, mutation assessment, and study quality). Subgroup analyses were only performed on the overall *KRAS*, *BRAF*, or *NRAS* mutation prevalence estimate for each study rather than by specific mutation location.

Prevalence of *KRAS* mutations

The overall summary prevalence estimate (P_{sum}) of *KRAS* mutations was 35.9% (95% CI: 34.6% - 37.3%) based on 288 individual prevalence estimates. *KRAS* mutations most frequently occurred in exon 2 ($P_{\text{sum}} = 39.1\%$, 95% CI: 37.1% - 41.3%) and exon 1 ($P_{\text{sum}} = 35.5\%$, 95% CI: 30.3% - 41.1%), with considerably lower prevalence estimates in exon 4 ($P_{\text{sum}} = 5.0\%$, 95% CI: 4.1% - 06.2%) and exon 3 ($P_{\text{sum}} = 2.9\%$, 95% CI: 1.9% - 4.4%). Within exon 2, mutations in codon 12 were more common than in codon 13 (Table 1). Of the specific codon 12 mutations that were examined, Gly12ASP and Gly12Val were the most prevalent. Of the specific codon 13 mutations, Gly13Asp was the most prevalent. Codon 61 and codon 146 mutations were relatively infrequent. *KRAS* mutation prevalence was slightly higher in females compared with males ($P = 0.011$), and in clinical trials compared with observational studies ($P = 0.004$). *KRAS* mutation prevalence varied significantly by study location ($P = 0.025$). *KRAS* mutations were more prevalent when strip assays or gel electrophoresis methods were used as the assessment method and least prevalent when assessed by next-generation sequencing or high-resolution melting. As studies used multiple assessment methods to identify mutations, a P value could not be calculated for the mutation assessment method subgroup as the groups were not mutually exclusive. The prevalence did not differ significantly by STROBE score, median age of

study population, race, treatment status, study time period, or length of follow-up.

Prevalence of *BRAF* mutations

The overall P_{sum} of *BRAF* mutations was 7.1% (95% CI: 6.5% - 07.8%). Val600Glu mutations were statistically significantly more common than Asp594Gly mutations ($P = 0.002$). *BRAF* mutations were more common in females than in males ($P = 0.018$) and varied by study location ($P = 0.002$). *BRAF* mutation prevalence did not differ significantly by median age, race, study design, treatment status, source of tumor tissue (primary, metastases, both), STROBE score, study time period, or median length of follow-up (Table 2).

Prevalence of *NRAS* mutations

The overall prevalence of *NRAS* mutations was 4.1% (95% CI: 3.5% - 4.8%). Mutation prevalence did not differ significantly by exon number ($P = 0.368$). Mutations were more prevalent in codons 12 and 61 compared with codon 13. *NRAS* mutation prevalence by sex was only assessed in one study which determined a similar prevalence in both males and females. *NRAS* mutation prevalence also did not differ significantly by median age, study location, study design, treatment status, source of tumor tissue (primary, metastases, both), STROBE score, study time period, or median length of follow-up ≥ 25 months (Table 3).

OS and PFS by mutation status

In our exploratory analysis of survival based on the studies included in our assessment, a significantly increased risk of mortality was observed among patients with *KRAS*, *BRAF*, and *NRAS* mutations compared to patients with the respective WT tumors (Table 4). The highest risk was observed among patients with *BRAF* mutations compared to *BRAF* WT patients (HR = 2.83, 95% CI: 2.23 - 3.58). The risk of disease progression was also significantly higher among patients with *BRAF* mutations compared to WT (HR = 2.90; 95% CI: 1.84 - 4.56) as well as among patients with *KRAS* mutations versus WT (HR = 1.62, 95% CI: 1.33 - 1.97). No significant differences in survival by study population treatment status (complete population treated, partial population treated, unknown/not treated) was observed for OS in the *KRAS* or *BRAF* populations. An insufficient number of studies were available to evaluate OS by study population treatment status in the *NRAS* population or for PFS for any mutation (data not shown).

The median length of survival associated WT status was significantly longer than that associated with mutant status for both *KRAS* mutations (WT: 17.00 vs. mutant: 13.41 months, $P = 0.022$) and *BRAF* mutations (WT: 17.19 vs. mutant: 8.71 months, $P < 0.001$). Similar trends were observed for PFS for *KRAS* mutations (WT: 5.98 vs. mutant: 4.48 months, $P = 0.04$) and *BRAF* mutations (WT: 8.26 vs. mutant: 5.05

Table 1. Overall Prevalence of *KRAS* Mutations in mCRC and Subgroups

	N studies ^f	Summary prevalence (95% CI)	P-Het; I ²	P value for heterogeneity within subgroups
Overall	288	35.9% (34.6% - 37.3%)	< 0.001; 92.6%	
Exon 1 ^a	14	35.5% (30.3% - 41.1%)	< 0.001; 75.4%	
Exon 2 ^a	76	39.1% (37.1% - 41.3%)	< 0.001; 85.4%	
Codon 12 ^b	24	29.1% (26.3% - 32.1%)	< 0.001; 86.2%	
Codon 12 ^c	50	29.0% (26.9% - 31.2%)	< 0.001; 83.9%	
Gly12Ala	59	3.3% (2.7% - 3.9%)	< 0.001; 77.8%	< 0.001
Gly12Arg	36	0.9% (0.6% - 1.4%)	< 0.001; 82.9%	
Gly12Asp	85	14.6% (13.3% - 16.1%)	< 0.001; 90.8%	
Gly12Cys	61	3.5% (3.0% - 4.1%)	< 0.001; 76.1%	
Gly12Phe	5	0.2% (0.1% - 0.6%)	0.035; 61.3%	
Gly12Ser	62	2.8% (2.2% - 3.6%)	< 0.001; 88.8%	
Gly12Val	87	10.2% (9.1% - 11.4%)	< 0.001; 89.6%	
Other codon 12	15	0.9% (0.3% - 2.9%)	< 0.001; 96.9%	
Codon 13 ^b	19	9.0% (7.9% - 10.2%)	< 0.001; 62.7%	
Codon 13 ^c	39	8.6% (7.7% - 9.5%)	< 0.001; 55.2%	
Gly13Arg	8	0.2% (0.1% - 0.7%)	0.001; 72.0%	< 0.001
Gly13Asp	82	8.6% (7.6% - 9.6%)	< 0.001; 85.0%	
Gly13Cys	15	0.7% (0.4% - 1.2%)	< 0.001; 82.7%	
Gly13Ser	4	0.3% (0.0% - 4.8%)	< 0.001; 90.9%	
Gly13Val	8	0.4% (0.1% - 1.8%)	< 0.001; 80.2%	
Exon 3 ^a	9	2.9% (1.9% - 4.4%)	0.009; 60.9%	
Codon 61	14	2.7% (2.1% - 3.5%)	0.001; 62.0%	
Gln61Arg	4	0.4% (0.2% - 0.6%)	0.810; 0.0%	0.016
Gln61His	11	1.6% (0.7% - 3.5%)	< 0.001; 92.3%	
Gln61Leu	5	0.7% (0.2% - 2.2%)	0.004; 73.9%	
Other codon 61	3	0.2% (0.0% - 1.8%)	< 0.001; 89.3%	
Exon 4 ^a	8	5.0% (4.1% - 6.2%)	0.228; 25.2%	
Codon 146	9	2.5% (1.9% - 3.3%)	0.134; 35.5%	
P value between exons ^d				< 0.001
Sex				
Male	55	37.3% (35.1% - 39.7%)	< 0.001; 76.9%	0.011
Female	57	42.2% (39.3% - 45.2%)	< 0.001; 81.7%	
Median age of study population				
< 62	77	37.0% (34.3% - 39.8%)	< 0.001; 92.6%	0.544
≥ 62	102	36.0% (33.8% - 38.1%)	< 0.001; 90.6%	
Meta-regression on median age	179	0.004 (-0.012 - 0.020)	N/A	0.612
Race				
< 88% White/Caucasian	21	37.8% (32.4% - 43.4%)	< 0.001; 94.1%	0.401
≥ 88% White/Caucasian	17	34.1% (28.1% - 40.7%)	< 0.001; 91.1%	
Study location				
Asia	64	31.8% (28.3% - 35.5%)	< 0.001; 93.2%	0.025
Australia	7	27.3% (19.1% - 37.5%)	< 0.001; 97.9%	

Table 1. Overall Prevalence of *KRAS* Mutations in mCRC and Subgroups - (continued)

	N studies ^f	Summary prevalence (95% CI)	P-Het; I ²	P value for heterogeneity within subgroups
Europe	146	37.3% (35.2% - 39.3%)	< 0.001; 92.0%	
Multi-country	21	39.4% (36.1% - 42.9%)	< 0.001; 88.2%	
North America	47	36.4% (33.4% - 39.6%)	< 0.001; 88.9%	
South America	3	37.2% (28.7% - 49.9%)	< 0.001; 70.8%	
Study design				
Observational	226	35.0% (33.5% - 36.6%)	< 0.001; 92.9%	0.004
Clinical trial	62	39.5% (36.9% - 42.1%)	< 0.001; 89.0%	
Treatment status				
Partial population treated	10	27.5% (18.0% - 39.6%)	< 0.001; 95.0%	0.115
Complete population treated	158	37.2% (35.6% - 38.9%)	< 0.001; 89.6%	
Unknown/not treated	120	35.1% (32.9% - 37.4%)	< 0.001; 94.2%	
Source				
Primary tumor	60	18.9% (15.6% - 22.7%)	< 0.001; 94.6%	0.012
Metastasis	37	21.2% (16.4% - 27.0%)	< 0.001; 94.3%	
Both primary tumors and metastases	42	26.5% (23.0% - 30.4%)	< 0.001; 96.4%	
Mutation assessment method ^e				
Gel electrophoresis methods	13	41.1% (35.7% - 46.6%)	0.001; 65.5%	
High-resolution melting	18	28.8% (23.7% - 34.6%)	< 0.001; 97.3%	
Mass spectrometry	17	36.0% (32.2% - 40.0%)	< 0.001; 92.2%	
Multiplex mutation assays	20	36.4% (33.1% - 39.9%)	< 0.001; 87.2%	
Mutant allele specific PCR	77	37.4% (35.1% - 39.8%)	< 0.001; 92.2%	
Next-generation sequencing	11	29.7% (20.1% - 41.7%)	< 0.001; 97.1%	
Pyrosequencing	38	37.2% (33.9% - 40.6%)	< 0.001; 88.9%	
Sanger/direct sequencing (PCR)	144	35.5% (34.0% - 37.0%)	< 0.001; 86.3%	
Strip assay	7	42.1% (38.9% - 45.3%)	0.045; 53.4%	
Other	16	44.0% (37.4% - 50.7%)	< 0.001; 96.3%	
Not reported	14	40.3% (33.1% - 47.9%)	< 0.001; 96.1%	
Study quality score				
≤ 16	172	35.0% (33.1% - 37.0%)	< 0.001; 94.5%	0.073
> 16	116	37.5% (35.7% - 39.3%)	< 0.001; 85.4%	
Study time period				
Pre-2007	22	35.5% (32.3% - 38.9%)	< 0.001; 63.9%	0.105
Includes 2007	115	36.0% (33.7% - 38.4%)	< 0.001; 90.0%	
Post-2007	61	39.3% (36.7% - 42.0%)	< 0.001; 91.5%	
Median length of follow-up time				
< 25 months	29	34.6% (31.1% - 38.3%)	< 0.001; 80.9%	0.608
≥ 25 months	34	33.0% (28.1% - 38.3%)	< 0.001; 91.7%	
Meta-regression on follow-up time	63	0.002 (-0.004 - 0.008)	N/A	0.583

^aGlobal exon estimate (most global estimate for a given exon contained within a manuscript); ^bCodon specified in papers as existing within exon 2; ^cCodon specified in papers as existing within exon 1, exon 2, or unspecified; ^dP values assess differences between global exon estimates only; ^eCategories not mutually exclusive as some studies used multiple forms of mutation assessment methods, P value not calculated; ^fIndividual studies may contribute multiple prevalence estimates derived from independent study arms; so N refers to the number of independent prevalence estimates included in summary measure and may exceed the actual number of studies. mCRC: metastatic colorectal cancer; CI: confidence interval.

Table 2. Overall Prevalence of *BRAF* Mutations in mCRC and Subgroups

	N studies ^c	Summary prevalence (95% CI)	P-Het; I ²	P value for heterogeneity within subgroups
Overall	142	7.1% (6.5% - 7.8%)	< 0.001; 66.3%	
Exon 15 ^a	44	7.0% (6.1% - 8.1%)	0.001; 45.8%	
Val600Glu (V600E)	75	6.8% (5.8% - 7.9%)	< 0.001; 76.9%	0.002
Asp594Gly	4	0.6% (0.1% - 2.8%)	0.064; 58.8%	
Sex				
Male	12	7.9% (6.5% - 9.7%)	0.076; 39.7%	0.018
Female	12	11.0% (9.2% - 13.1%)	0.260; 18.7%	
Median age of study population				
< 62	35	7.8% (6.6% - 9.1%)	< 0.001; 55.5%	0.970
≥ 62	57	7.8% (6.7% - 9.1%)	< 0.001; 68.6%	
Meta-regression on median age	92	0.012 (-0.014 - 0.036)	N/A	0.390
Race				
< 88% White/Caucasian	7	7.1% (5.5% - 9.2%)	0.915; 0.0%	0.855
≥ 88% White/Caucasian	5	7.6% (4.1% - 13.7%)	0.007; 71.7%	
Study location				
Asia	29	6.0% (5.1% - 7.0%)	0.175; 19.6%	0.002
Australia	7	11.1% (8.6% - 14.2%)	0.062; 49.9%	
Europe	79	7.2% (6.3% - 8.1%)	< 0.001; 68.2%	
Multi-country	8	8.3% (5.4% - 12.5%)	< 0.001; 83.3%	
North America	19	6.6% (5.1% - 8.4%)	0.001; 56.5%	
Study design				
Observational	114	7.2% (6.5% - 7.9%)	< 0.001; 65.4%	0.897
Clinical trial	28	7.1% (5.7% - 8.7%)	< 0.001; 70.5%	
Treatment status				
Partial population treated	8	5.9% (3.9% - 8.9%)	0.168; 32.6%	0.659
Complete population treated	76	7.2% (6.3% - 8.3%)	< 0.001; 72.1%	
Unknown/not treated	58	7.1% (6.3% - 8.0%)	< 0.001; 57.3%	
Source				
Primary tumor	34	7.5% (6.3% - 8.7%)	0.152; 20.1%	0.832
Metastasis	16	6.4% (3.8% - 10.6%)	< 0.001; 68.3%	
Both primary tumors and metastases	20	7.1% (5.8% - 8.5%)	0.039; 39.0%	
Mutation assessment method ^b				
Gel electrophoresis methods	6	4.5% (2.5% - 7.9%)	0.754; 0.0%	
High-resolution melting	13	8.0% (5.9% - 10.9%)	< 0.001; 79.0%	
Mass spectrometry	13	5.4% (4.4% - 6.6%)	0.044; 44.2%	
Multiplex mutation assays	10	7.5% (5.8% - 9.6%)	0.170; 29.9%	
Mutant allele specific PCR	35	8.1% (7.0% - 9.2%)	< 0.001; 57.0%	
Next-generation sequencing	6	7.4% (5.7% - 9.5%)	0.974; 0.0%	
Not reported	7	7.1% (4.6% - 10.9%)	0.010; 64.2%	
Other	6	3.9% (2.3% - 6.4%)	0.004; 71.2%	
Pyrosequencing	19	9.1% (7.2% - 11.4%)	< 0.001; 74.3%	
Sanger/direct sequencing (PCR)	69	6.8% (6.1% - 7.6%)	0.004; 33.6%	

Table 2. Overall Prevalence of *BRAF* Mutations in mCRC and Subgroups - (continued)

	N studies ^c	Summary prevalence (95% CI)	P-Het; I ²	P value for heterogeneity within subgroups
Study quality score				
≤ 16	70	7.2% (6.4% - 8.0%)	< 0.001; 58.5%	0.803
> 16	72	7.0% (6.1% - 8.1%)	< 0.001; 70.7%	
Study time period				
Pre-2007	10	6.3% (4.2% - 9.5%)	0.038; 49.4%	0.520
Includes 2007	59	7.4% (6.2% - 8.7%)	< 0.001; 73.1%	
Post-2007	28	6.3% (4.2% - 9.5%)	0.098; 26.7%	
Median length of follow-up time				
< 25 months	12	9.7% (6.1% - 15.0%)	< 0.001; 71.9%	0.271
≥ 25 months	24	7.4% (6.1% - 8.8%)	0.002; 51.6%	
Meta-regression on follow-up time	36	-0.005 (-0.015; 0.005)	N/A	0.314

^aGlobal exon estimate (most global estimate for a given exon contained within a manuscript); ^bCategories not mutually exclusive as some studies used multiple forms of mutation assessment methods, P value not calculated; ^cIndividual studies may contribute multiple prevalence estimates derived from independent study arms, so N refers to the number of independent prevalence estimates included in summary measure and may exceed the actual number of studies. mCRC: metastatic colorectal cancer; CI: confidence interval.

Table 3. Overall Prevalence of *NRAS* Mutations in mCRC and Subgroups

	N Studies ^c	Summary prevalence (95% CI)	P-Het; I ²	P value for heterogeneity within subgroups
Overall	49	4.1% (3.5% - 4.8%)	< 0.001; 56.0%	
Exon 2 ^a	6	2.3% (1.4% - 3.7%)	0.127; 41.8%	
Codon 12 ^b	7	2.2% (1.6% - 3.0%)	0.209; 28.7%	< 0.001 ^f
Codon 13 ^b	4	0.7% (0.5% - 1.0%)	0.927; 0.0%	
Exon 3 ^a	5	2.4% (1.1% - 5.0%)	0.003; 75.5%	
Codon 61 ^b	10	3.6% (2.6% - 5.0%)	0.065; 44.1%	
Exon 4 ^a	3	0.6% (0.1% - 3.9%)	0.991; 0.0%	
P value between exons ^c				0.368
Sex				
Male	*	*	*	*
Female	*	*	*	
Median age of study population				
< 62	12	3.7% (3.0% - 4.7%)	0.863; 0.0%	0.479
≥ 62	12	4.3% (3.2% - 5.6%)	0.036; 47.1%	
Meta-regression on median age	24	0.006 (-0.048 - 0.060)	N/A	0.822
Race				
< 88% White/Caucasian	*	*	*	*
≥ 88% White/Caucasian	3	5.8% (3.4% - 9.8%)	0.954; 0.0%	
Study location				
Asia	13	3.0% (2.0% - 4.5%)	0.009; 54.5%	0.108
Australia	*	*	*	
Europe	22	4.6% (3.7% - 5.8%)	< 0.001; 64.6%	
Multi-country	3	5.8% (3.4% - 9.8%)	0.954; 0.0%	
North America	10	3.6% (2.9% - 4.5%)	0.980; 0.0%	
Study design				

Table 3. Overall Prevalence of *NRAS* Mutations in mCRC and Subgroups - (continued)

	N Studies ^e	Summary prevalence (95% CI)	P-Het; I ²	P value for heterogeneity within subgroups
Observational	39	3.9% (3.3% - 4.7%)	< 0.001; 61.9%	0.105
Clinical trial	10	5.3% (3.9% - 7.1%)	0.544; 0.0%	
Treatment status				
Partial population treated	5	3.5% (2.1% - 6.0%)	0.940; 0.0%	0.840
Complete population treated	15	4.2% (3.3% - 5.3%)	0.336; 10.5%	
Unknown/not treated	29	4.1% (3.3% - 5.0%)	< 0.001; 69.5%	
Source				
Primary tumor	8	3.4% (2.4% - 4.8%)	0.026; 55.9%	0.318
Metastasis	6	4.9% (3.4% - 7.0%)	0.936; 0.0%	
Both primary tumors and metastases	9	3.5% (2.5% - 4.9%)	0.019; 56.3%	
Mutation assessment method ^d				
High-resolution melting	6	3.0% (1.7% - 5.1%)	< 0.001; 84.6%	
Mass spectrometry	11	4.0% (3.2% - 5.0%)	0.023; 51.8%	
Multiplex mutation assays	4	4.2% (3.3% - 5.4%)	0.233; 29.8%	
Mutant allele specific PCR	7	4.3% (3.4% - 5.3%)	0.253; 23.1%	
Next-generation sequencing	8	4.9% (3.9% - 6.2%)	0.309; 15.4%	
Other	3	4.5% (2.6% - 7.6%)	0.064; 63.6%	
Pyrosequencing	9	5.1% (3.6% - 7.3%)	< 0.001; 72.4%	
Sanger/direct sequencing (PCR)	20	4.5% (4.0% - 5.0%)	0.483; 0.0%	
Strip assay	*	*	*	
Study quality score				
≤ 16	29	3.8% (3.0% - 4.8%)	< 0.001; 69.0%	0.151
> 16	20	4.7% (4.0% - 5.5%)	0.535; 0.0%	
Study time period				
Pre-2007	*	*	*	
Includes 2007	9	3.7% (2.9% - 4.7%)	0.447; 0.0%	0.247
Post-2007	26	4.5% (3.6% - 5.6%)	< 0.001; 63.2%	
Median length of follow-up time				
< 25 months	*	*	*	*
≥ 25 months	7	4.1% (3.0% - 5.5%)	0.998; 0.0%	
Meta-regression on follow-up time		0.000 (-0.012 - 0.012)	N/A	0.957

^aGlobal exon estimate (most global estimate for a given exon contained within a manuscript); ^bCodon specified in papers as existing within exon 1, exon 2, exon 3 or unspecified; ^cP values assess differences between global exon estimates only; ^dCategories not mutually exclusive as some studies used multiple forms of mutation assessment methods, P value not calculated; ^eIndividual studies may contribute multiple prevalence estimates derived from independent study arms, so N refers to the number of independent prevalence estimates included in summary measure and may exceed the actual number of studies; ^fP value assesses differences between codon estimates. *N < 3, insufficient studies to run a meta-analysis. mCRC: metastatic colorectal cancer; CI: confidence interval.

months, P = 0.005).

Risk of bias across studies

To evaluate the presence of publication bias among the studies, we generated funnel plots for the logit of the event rate by the standard error separately for each of the mutations types.

Upon visual inspection of the association of the logit of the prevalence of *KRAS* mutations by precision for each of the studies, studies with higher precision (typically representing larger study populations) appeared to have a mutation prevalence closer to the mean although the plot was asymmetrical (Egger's regression test: P value = 0.014, Fig. 1). The Duval and Tweedie method added 64 "missing" (hypothetically unpublished) studies to the left of the mean, resulting in an ad-

Table 4. Hazard Ratios and Median Overall and Progression-Free Survival by Mutation Status

	Overall survival		Progression-free survival			
	N studies	HR (95% CI)	P-Het; I ²	N studies	HR (95% CI)	P-Het; I ²
Survival analysis (mutant vs. wild-type)						
<i>KRAS</i>	47	1.49 (1.37 - 1.64)	< 0.001; 50.2%	25	1.62 (1.33 - 1.97)	< 0.001; 83.1%
<i>BRAF</i>	32	2.83 (2.23 - 3.58)	< 0.001; 84.1%	12	2.90 (1.84 - 4.56)	< 0.001; 91.3%
<i>NRAS</i>	4	1.88 (1.35 - 2.62)	0.795; 0.0%	*	*	*
Median survival		Median months (95% CI)			Median months (95% CI)	
<i>KRAS</i> mutant	41	13.41 (11.35 - 15.83)	< 0.001; 96.8%	42	4.48 (3.58 - 5.62)	< 0.001; 99.2%
<i>KRAS</i> wild-type	42	17.00 (14.52 - 19.89)	< 0.001; 96.8%	49	5.98 (4.74 - 7.54)	< 0.001; 99.3%
P value for difference	0.022			0.04		
<i>BRAF</i> mutant	12	8.71 (6.54 - 11.59)	< 0.001; 90.1%	11	5.05 (3.67 - 6.96)	< 0.001; 88.6%
<i>BRAF</i> wild-type	13	17.19 (13.00 - 22.74)	< 0.001; 96.6%	12	8.26 (6.78 - 10.07)	< 0.001; 93.5%
P value for difference	< 0.001			0.005		

*N < 3 (studies), insufficient for meta-analysis. mCRC: metastatic colorectal cancer; HR: hazard ratio; CI: confidence interval.

justed prevalence of 31.7% (95% CI: 30.3% - 33.2%), which is slightly less than the unadjusted prevalence of 35.9% (95% CI: 34.6% - 37.3%). This implies that the smaller, less precise studies may have been driving the prevalence up while smaller studies with lower prevalence estimates were not published. However, these are merely theoretical estimates of unknown potential sources of data. Visual inspection of the funnel plot of *BRAF* mutation prevalence by the precision for each of the studies was also asymmetrical around the summary effect measure (Egger’s regression test: P value = 0.001, Fig. 2). The Duval and Tweedie method added 45 “missing” studies to the right of the mean resulting in an adjusted prevalence estimate of 8.6% (95% CI: 7.8% - 9.5%), which is slightly higher than the unadjusted prevalence of 7.1% (95% CI: 6.5% - 7.8%). This may be due to the smaller studies with higher prevalence estimates being more likely to be published than smaller studies with lower prevalence estimates. Publication bias associated with the *NRAS* prevalence is unlikely due to the symmetry of the funnel plot (Egger’s regression test: P value = 0.112, Fig. 3).

Discussion

To the authors’ knowledge, this is first global systemic literature review and meta-analysis of *KRAS*, *BRAF*, and *NRAS* mutation prevalence in mCRC patients. This extensive and systematic review included in 275 studies comprising 77,104 mCRC patients. Our study provided a pooled overall prevalence estimate of 35.9% for *KRAS* mutations, 7.1% for *BRAF* mutations, and 4.1% for *NRAS* mutations. Additionally, our exploratory survival analysis showed increased risk of death associated with *KRAS*, *BRAF*, and *NRAS* mutations when compared with the respective WT tumors. Tumors with *KRAS* and *BRAF* mutations were found to have significantly increased risk of disease progression compared to WT tumors.

The subgroup analyses performed in this study identified several potential sources of heterogeneity in mutation prevalence. Female patients had significantly more *KRAS* and *BRAF* mutations than males, and significant variation by study location was observed for both *KRAS* and *BRAF* mutation prevalence. Additionally, the prevalence of *KRAS* mutations was higher among patients enrolled in the standard of care arm of clinical trials compared with those in observational studies. The evaluation of potential variation in mutation prevalence by sex, geography, and study design is recommended for future studies. No significant sources of heterogeneity were identified in *NRAS* prevalence meta-analyses.

This systematic literature review and meta-analysis has many strengths. It includes systematic study selection resulting in a large number of studies and is highly representative of the global prevalence of these mutations. The large number of individual studies included in our analysis provided the opportunity to conduct a number of subgroup analyses to identify potential sources of heterogeneity. We also assessed the risk of bias in individual studies using the STROBE guidelines for reporting observational studies and were then able to use these scores in a subgroup analysis to determine that our prevalence

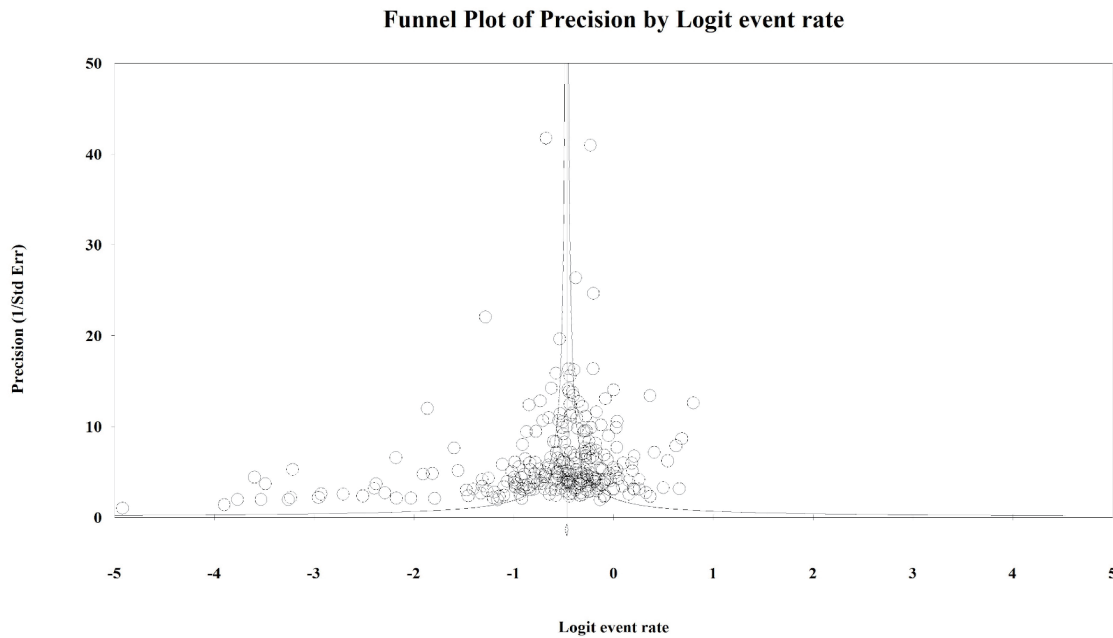


Figure 1. Publication bias: *KRAS*. Egger’s regression test: two-tailed P value = 0.014. Duval and Tweedie method added 64 “missing” studies to the left of the mean, resulting in an adjusted prevalence estimate of 31.7% (30.3% - 33.2%).

estimates were not significantly impacted by individual study bias.

We were limited in analyzing the data to how they were presented in the individual papers, despite any inconsistencies in nomenclature or mutation assessment methods. This could have resulted in lower overall mutation prevalence estimates as not all studies may have examined the presence of every

possible mutation within a given location. There also is some evidence that publication bias may have affected our summary prevalence estimates for *KRAS* and *BRAF* mutations.

Our analysis of pooled survival by *KRAS*, *NRAS*, and *BRAF* mutation status can only be considered exploratory in that an exhaustive search of studies that included survival data by mutation status was not conducted. If the studies we iden-

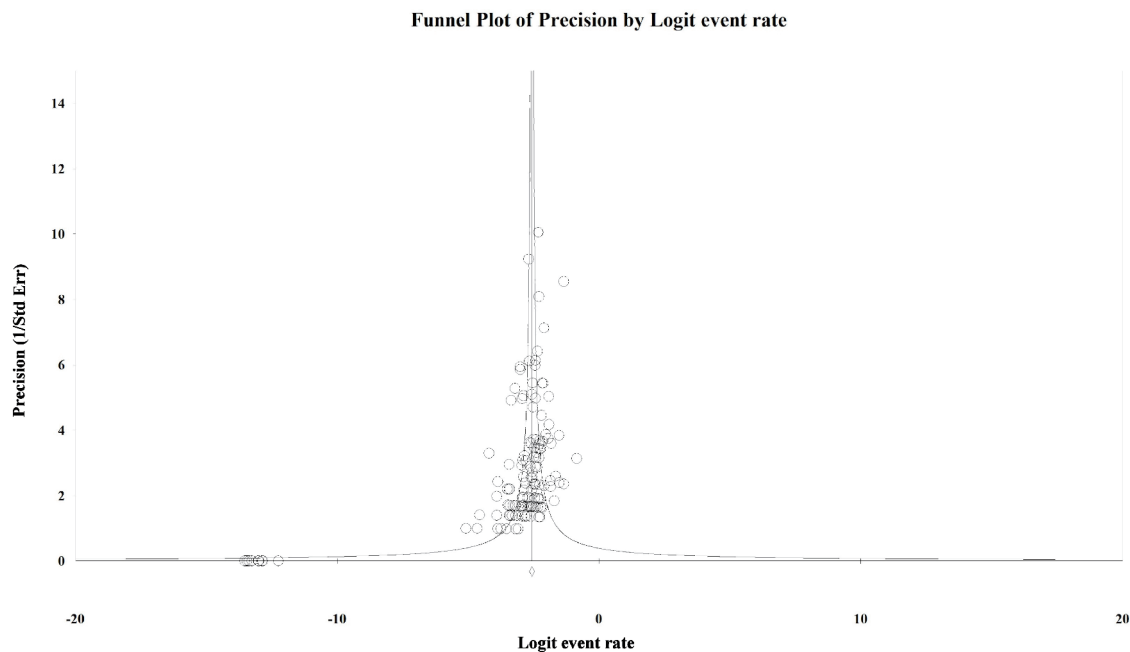


Figure 2. Publication bias: *BRAF*. Egger’s regression test: two-tailed P value = 0.001. Duval and Tweedie method added 45 “missing” studies to the right of the mean, resulting in an adjusted prevalence estimate of 8.6% (7.8% - 9.5%).

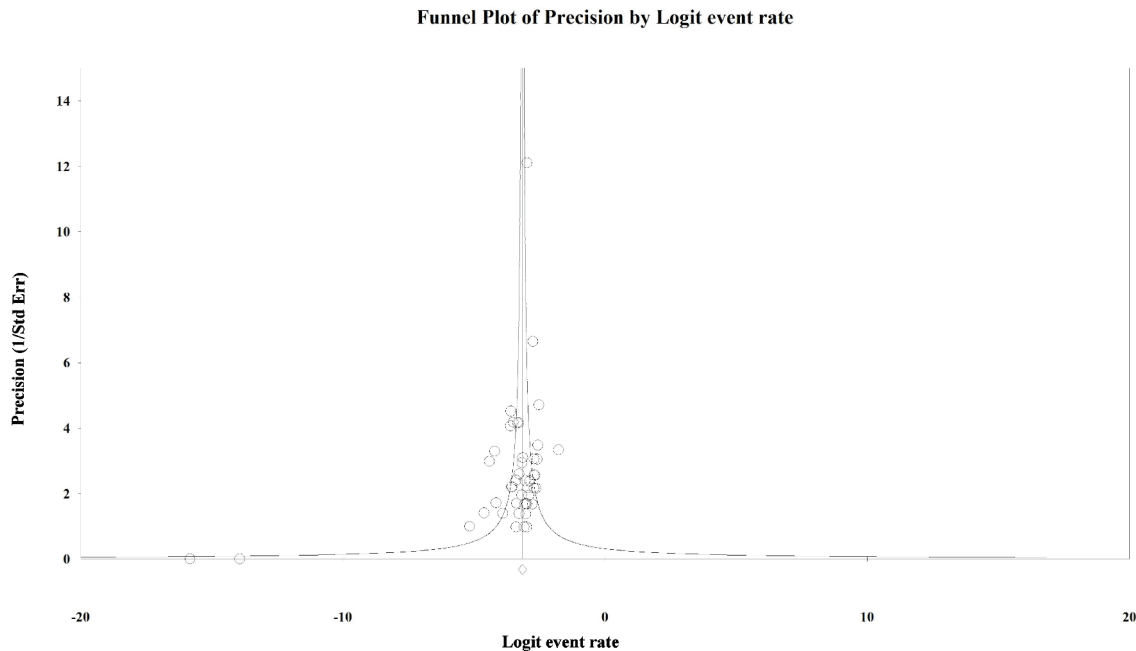


Figure 3. Publication bias: *NRAS*. Egger's regression test: two-tailed P value = 0.112.

tified as part of our comprehensive review happened to also include survival data, they were included in our analysis. The meta-analyses of HRs identified a significant risk of mortality among patients with *KRAS*, *BRAF*, and *NRAS* mutations compared to patients with the respective WT tumors. Patients with *BRAF* and *KRAS* mutations were also found to be at an increased risk of disease progression compared to WT tumors. Although subgroup analyses by population treatment status did not show a significant impact on survival, these analyses were limited by the variety of therapeutic regimens and combination therapies, as well as the lack of detail provided on treatment status in many studies, particularly as certain treatments such as anti-EGFR therapies are only effective in WT patients. However, the results of these exploratory analyses may inform future hypotheses on the prognostic impact of these mutations.

In this comprehensive literature review and meta-analysis, we provide the first global, comprehensive, summary prevalence estimates of *KRAS*, *BRAF*, and *NRAS* mutations in mCRC patients. These results provide important insight into the mCRC population for clinicians and researchers, particularly in light of the reduced treatment options for *KRAS* and *NRAS* mutant patients. Current clinical guidelines state that mutational testing for *BRAF* Val600Glu mutations “should be performed in colorectal cancer tissue in patients with colorectal carcinoma for prognostic stratification” [49]. The results of our exploratory survival analyses corroborate the significance of *BRAF* impact on survival, strengthening the recommendation that *BRAF* mutation testing should be performed to better inform patient prognosis. Our survival analyses also suggest that *KRAS* and *NRAS* mutations may also predict a poorer outcome when compared with their respective WT presentations. This meta-analysis provides representative estimates of mutation prevalence within different subgroups, which is important for making decisions about the implementation of current

treatment strategies and for developing new treatments that will be effective for the various patient subgroups.

Supplementary Material

Suppl 1. PRISMA flow diagram.

Suppl 2. Descriptive Characteristics of Included Studies.

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Conflict of Interest

The article processing charges were funded by Amgen, Inc. Individual conflict of interest disclosures are given below: ELS is employed by Epidstat Institute, work contracted through Amgen, Inc. LCB is employed by Epidstat Institute, work contracted through Amgen, Inc. KL is an employee of Amgen,

Inc. and owns stock in Amgen, Inc. LS is employed by Simul-Stat, work contracted through Amgen, Inc. JPF is employed by Epidstat Institute, work contracted through Amgen, Inc. DDA is employed by Epidstat Institute, work contracted through Amgen, Inc.

Informed Consent

This article is based on previously conducted studies and does not contain any studies with human participants or animals performed by any of the authors.

Author Contributions

Elizabeth Levin-Sparenberg: methodology, data curation, investigation, formal analysis, visualization, writing original draft, review and editing. Lauren C. Bylsma: methodology, data curation, investigation, formal analysis, visualization, writing original draft, review and editing. Kimberly Lowe: conceptualization, supervision, visualization, writing review and editing. Laura Sangare: visualization, writing review and editing. Jon P. Fryzek: methodology, visualization, writing-original draft, writing review and editing. Dominik D. Alexander: conceptualization, supervision, methodology, supervision, investigation, writing review and editing.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to Ms. Lauren C. Bylsma at lbylsma@epidstrategies.com.

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