Research Article

RAS mutation prevalence among patients with metastatic colorectal cancer: a meta-analysis of real-world data

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Aim: A confirmed wild-type *RAS* tumor status is commonly required for prescribing anti-EGFR treatment for metastatic colorectal cancer. This noninterventional, observational research project estimated *RAS* mutation prevalence from real-world sources. **Materials & methods:** Aggregate *RAS* mutation data were collected from 12 sources in three regions. Each source was analyzed separately; pooled prevalence estimates were then derived from meta-analyses. **Results:** The pooled *RAS* mutation prevalence from 4431 tumor samples tested for *RAS* mutation status was estimated to be 43.6% (95% CI: 38.8–48.5%); ranging from 33.7% (95% CI: 28.4–39.3%) to 54.1% (95% CI: 51.7–56.5%) between sources. **Conclusion:** The *RAS* mutation prevalence estimates varied among sources. The reasons for this are not clear and highlight the need for further research.

Colorectal cancer (CRC) was the third most common malignancy among men and the second most common malignancy among women globally in 2012 [1]. Approximately, 60% of CRC cases occur in developed regions and incidence rates can vary up to tenfold around the world, with the lowest rates observed in South Central Asia and Africa (excluding Southern Africa) [2]. A quarter of patients with CRC already have metastatic disease at the time of their initial presentation and diagnosis, and almost 50% will subsequently develop metastases [3]. Once

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Table 1. Data sources included in the analysis.				
Data source	**Country**	**Type of data source**	**Study period**	**Number of samples†**
Pathology & clinical data				
Argentina	Argentina	Amgen-sponsored study	March 2014 to November 2014	565
Czech Republic I	Czech Republic	Pathology center	January 2014 to December 2014	100
Greece	Greece	Amgen-sponsored study	December 2013 to August 2014	514
Hungary I	Hungary	Pathology center	September 2013 to June 2014	150
Hungary II	Hungary	Pathology center	September 2013 to June 2014	150
Middle East Registry	Algeria, Egypt, Saudi Arabia, UAE, Lebanon, Kuwait, Bahrain	mCRC registry	2013 to 2014	188
Pathology-only data				
Czech Republic II	Czech Republic	Pathology center	January 2014 to December 2014	100
Czech Republic III	Czech Republic	Pathology center	January 2014 to December 2014	100
Middle East Pathology I	Algeria, Saudi Arabia, UAE, Lebanon, Kuwait, Bahrain	mCRC registry	2012 to 2015	447
Middle East Pathology II	Egypt	mCRC registry	2012 to 2015	303
Poland I	Poland	Pathology center	January 2014 to December 2014	1664
Poland II	Poland	Pathology center	January 2014 to December 2014	150

†Patients may have had more than one sample taken and tested for *RAS* mutation status, and therefore may be represented in the aggregate data more than once.
mCRC: Metastatic colorectal cancer.

metastatic disease has emerged, the 5-year survival rate is approximately 5–15%, with a median overall survival of 9–30 months [4].

In the past decade, new treatment options have become available to patients with metastatic CRC (mCRC) that include the use of irinotecan- and oxaliplatin-containing chemotherapies, the use of antiangiogenic drugs, such as bevacizumab, and the introduction of monoclonal antibodies (mAbs), such as cetuximab and panitumumab, which target the EGFR [5]. A number of studies have shown that *RAS* biomarker status (exons 2, 3 and 4 of the *KRAS* and *NRAS* oncogenes) is predictive of patients' treatment response to anti-EGFR mAbs specifically, and patients who have *RAS* wild-type tumors are significantly more likely to benefit from anti-EGFR treatment [6–10]. As a result, treatment guidelines for anti-EGFR therapies recommend that patients with mCRC should have confirmed wild-type *RAS* tumor status prior to the initiation of treatment [11]. There have been a limited number of studies that have attempted to use real-world data to estimate the prevalence of *RAS* mutations among patients with mCRC. The majority of data relating to *RAS* mutation prevalence in mCRC, which have been published previously, are reported from clinical trials.

The primary objective of this research project was to estimate the prevalence of *RAS* mutations in patients with mCRC using different real-world data sources. These estimates include an overall estimate and estimates calculated according to patients' demographic and clinical characteristics. Additional objectives included estimating the prevalence of non-*KRAS* exon 2 mutations (*KRAS* exons 3, 4 and *NRAS* exons 2, 3, 4) in patients with a confirmed wild-type *KRAS* exon 2 status and estimating the prevalence of *BRAF* mutations among patients with mCRC.

Materials & methods
Data sources
This noninterventional, observational research project used aggregate patient data, rather than individual patient data, from multiple real-world data sources to estimate *RAS* mutation prevalence. Data were collected from 12 data sources (three local mCRC registries, two industry-sponsored studies [Amgen] and seven pathology centers) across 12 countries (Algeria, Argentina, Bahrain, Czech Republic, Egypt, Greece, Hungary, Kuwait, Lebanon, Poland, Saudi Arabia and the United Arab Emirates) (Table 1). Recruitment periods for the observational studies and registries providing the aggregate data spanned a range of different time periods (from 2012 to 2015). The study period for the majority of the data sources was between January and December 2014.

The data were collected using a standardized online data collection form for all samples according to both their *RAS* mutation status and a number of demographic and clinical variables. In addition, the turnaround times for *RAS* testing of the samples were also requested. The online data collection form was distributed to each data source, with data entry organized locally. A pilot trial of the online form was conducted to ensure any issues with the

electronic data collection were addressed before full implementation of the form. Data checks were subsequently carried out after receiving data from each source to determine if there were any errors or missing data that required correction or clarification. Turnaround time was defined as the number of days between the laboratory receiving the request for a *RAS* test to be performed and the result being reported to the requesting oncologist. Only aggregate data were requested with no possible link to individual patient information.

Patient eligibility criteria

To be eligible for the study, patients were required to have a recorded diagnosis of mCRC and a tumor sample that had been tested for *RAS* mutation status. Some samples were initially tested for *KRAS* exon 2 status only, with testing for the remaining *RAS* exons (*KRAS* exons 3, 4 and *NRAS* exons 2, 3, 4) conducted at a later time point. In practice, samples were likely to have been collected at different time points (e.g., time of surgery or immediately prior to initiation of anti-EGFR therapy), including some which may have been collected prior to metastatic diagnosis (most likely stage III CRC).

Statistical analysis

The prevalence of *RAS* and *BRAF* mutations (defined generically herein as 'biomarker' [*bm*]) was estimated as follows:

$$\frac{100 \times \text{Number of samples with bm mutation}}{\text{Number of samples with bm mutation} + \text{Number of samples with bm wild type}}$$

The prevalence of *RAS* and *BRAF* mutations was estimated from all samples taken from patients with mCRC. The prevalence of non-*KRAS* exon 2 mutations (i.e., mutations of *KRAS* exons 3, 4 and *NRAS* exons 2, 3, 4) was also estimated for all samples from patients with mCRC and a confirmed wild-type *KRAS* exon 2 status. The prevalence estimates are reported with their corresponding 95% CIs. The Clopper–Pearson (exact) method was used for CI calculation. For each data source, prevalence was also estimated according to a number of covariate patient characteristics, including patient age, gender, the site the tumor sample was taken from, the clinical indication for sampling, presence or absence of metastases, tumor staging and line of treatment at the time of testing. Comparison between the prevalence estimates by different variables was carried out based on 95% CIs and p-values (using Pearson χ^2 testing).

The meta-analysis was performed using a random effects model to consolidate the results of the primary outcome analysis, and account for the heterogeneity of the data sources, particularly with respect to variables such as testing methods, time periods and patient characteristics. The Freeman–Tukey double arcsine transformation method was used to stabilize the variance. The Hodges–Lehman estimator – a robust and nonparametric estimator of a population's location parameter – was used for the overall point estimate of prevalence. The meta-analysis was carried out using the 'meta' command in Stata (StataCorp. 2013. Stata Statistical Software: Release 2013, StataCorp LP, TX, USA).

Results

Aggregate data from 4431 tumor samples from 12 data sources were included in the *RAS* mutation prevalence analysis. Aggregate data from 2561 tumor samples from six data sources were included in the *BRAF* analysis. Aggregate data from 2606 tumor samples from 11 data sources were included in the analysis of non-*KRAS* exon 2 (*KRAS* exons 3, 4 and *NRAS* exons 2, 3, 4) samples with *KRAS* exon 2 wild-type samples. The size of these data sources varied from 100 tumor samples (Czech Republic pathology centers) to 1664 tumor samples (Polish pathology center) (Table 1).

RAS mutation prevalence

The overall pooled *RAS* mutation prevalence across the 12 data sources included in the analysis was estimated to be 43.6% (95% CI: 38.8–48.5%). *RAS* mutation prevalence varied between data sources, ranging from 33.7% (95% CI: 28.4–39.3%) in the Middle Eastern pathology dataset and 34.6% (95% CI: 27.7–42.1%) in the Middle Eastern mCRC registry to 53.6% (95% CI: 43.2–63.8%) and 54.1% (95% CI: 51.7–56.5%) in two European pathology centers, in the Czech Republic and Poland, respectively (Figure 1).
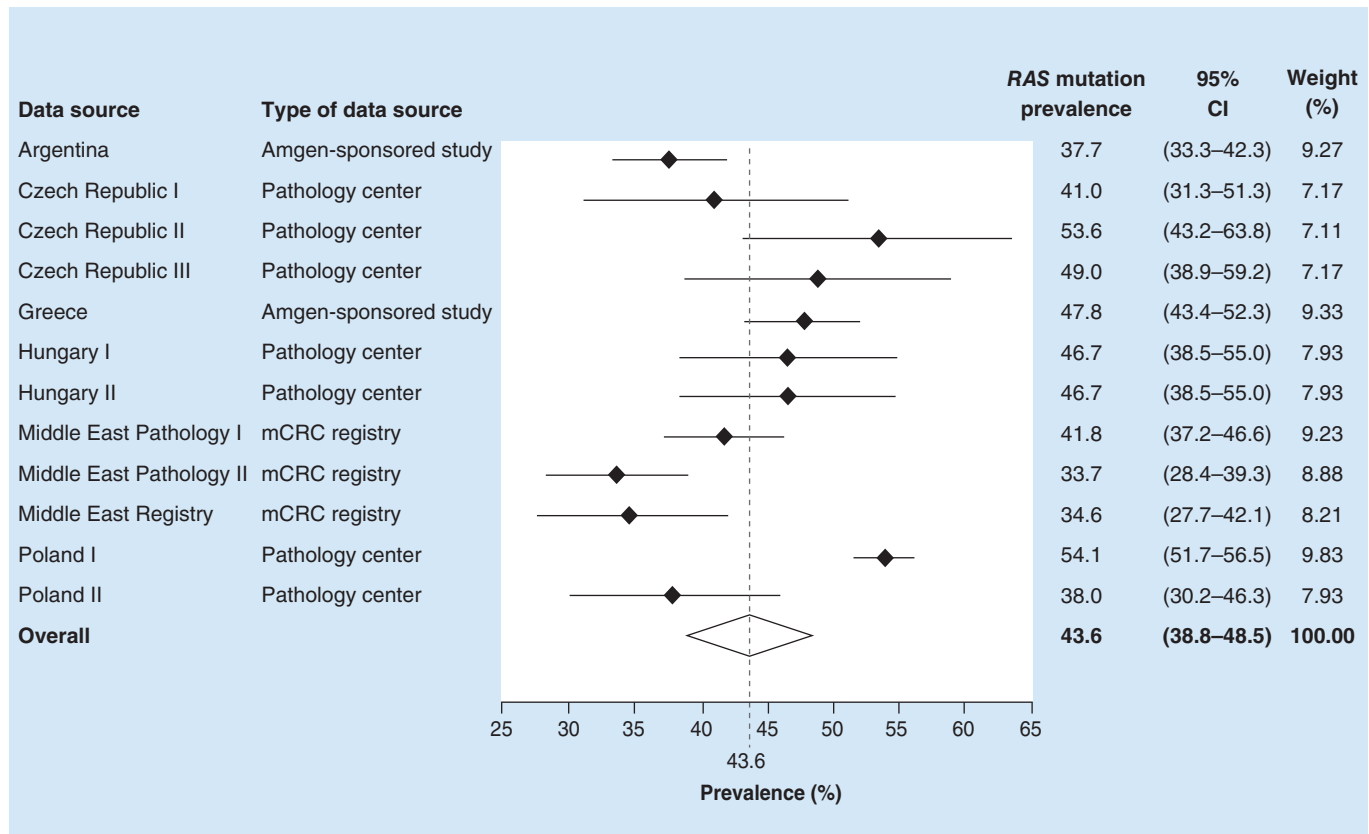
Figure 1.   Overall *RAS* mutation prevalence. Forest plot showing biomarker prevalence by data source.
mCRC: Metastatic colorectal cancer.

RAS prevalence by patient characteristics

Pooled *RAS* mutation prevalence estimates varied according to certain demographics and clinical variables. Although none of the differences were statistically significant, numerically greater *RAS* mutation prevalence estimates were observed
in: patients who were aged ≥70 years compared with younger patients; tumor samples isolated from metastatic rather than primary tumor sites; and tumor samples with a ratio of ≥20% neoplastic cells to normal cells in the tumor sample versus <20% (Table 2).

There were no differences in prevalence estimates for any of the other variables assessed including: patient gender; the tissue from which the sample DNA was isolated (left colon, right colon or rectum); whether or not there was evidence of liver metastases at the time the sample was taken; if there were synchronous or metachronous metastases; or tumor stage (Table 2).

BRAF mutation prevalence

The overall pooled *BRAF* mutation prevalence was estimated as 5.8% (95% CI: 2.7–9.9%) across the six data sources. The *BRAF* mutation prevalence estimates varied from 2.7% (95% CI: 0.7–6.7%) in one of the Poland pathology datasets to 14.3% (95% CI: 6.4–26.2%) in one of the Czech Republic pathology datasets (Figure 2).

Non-*KRAS* exon 2 mutation prevalence in *KRAS* exon 2 wild-type samples

The overall pooled non-*KRAS* exon 2 mutation prevalence, in *KRAS* exon 2 wild-type samples, was estimated as 14.2% (95% CI: 10.4–18.4%). The prevalence estimates varied from 8.6% (95% CI: 5.3–13.2%) in one of the Middle Eastern mCRC pathology datasets to 22.3% (95% CI: 14.7–31.6%) in a Hungarian pathology center (Figure 3).

Table 2. Pooled *RAS* prevalence estimates by patient characteristics.

Variable	Subgroups	Pooled *RAS* prevalence estimate (%)	95% CI	Number of data sources included in analysis
Overall *RAS* mutation prevalence		43.6	38.8–48.5	12
Age (years)	18–49	42.9	29.7–56.5	6
	50–69	40.9	33.9–48.2	12
	18–69	42.1	36.8–47.5	12
	≥70	49.6	44.8–54.3	10
Gender	Female	43.3	36.8–49.9	10
	Male	43.8	39.0–48.6	12
Tissues from which DNA was isolated	Left colon	42.7	37.8–47.6	9
	Right colon	45.5	35.5–55.7	9
	Rectum	44.2	38.1–50.3	11
Site tumor sample was taken from	Primary	43.3	36.9–49.9	9
	Metastatic	51.3	43.0–59.5	3
Liver metastases[†]	Liver metastasis	39.7	30.3–49.4	7
	No liver metastasis	43.2	36.5–50.1	6
Metastatic development	Synchronous	47.5	40.1–55.0	5
	Metachronous	42.0	32.4–51.9	6
Tumor stage[†]	Stage I/II	44.0	33.5–54.7	5
	Stage III	40.4	32.4–48.6	5
	Stage IV	43.7	35.8–51.8	6
Line of treatment[†]	1st line	49.3	40.7–57.8	2
	2nd line	35.8	31.2–40.6	2
	3rd line or later	39.2	33.4–45.1	2
Ratio of neoplastic cells as a percentage	<20%	43.0	33.3–52.9	2
	≥20%	47.0	42.6–51.4	9

[†]Subgroups were based on patient status at the time the tumor sample was taken.

Table 3. RAS mutation testing turnaround time by data source.

Data source	Number of samples	Turnaround time (working days), n (%)		
		≤5	6–10	>10
Czech Republic I	100	37 (37.0)	53 (53.0)	10 (10.0)
Czech Republic II	100	43 (43.0)	47 (47.0)	10 (10.0)
Czech Republic III	100	56 (56.0)	39 (39.0)	5 (5.0)
Greece	510	0 (0)	510 (100.0)	0 (0)
Hungary I	150	37 (24.7)	113 (75.3)	0 (0)
Hungary II	150	46 (30.7)	73 (48.7)	31 (20.7)
Middle East Pathology I	447	262 (58.6)	185 (41.4)	0 (0)
Middle East Pathology II	303	274 (90.4)	29 (9.6)	0 (0)
Middle East Registry	158	33 (20.9)	61 (38.6)	64 (40.5)
Poland I	1664	1368 (82.2)	274 (16.5)	22 (1.3)
Poland II	150	27 (18.0)	71 (47.3)	52 (34.7)
Overall	3832	2183 (57.0)	1455 (38.0)	194 (5.1)

RAS testing turnaround time

Eleven data sources reported *RAS* testing turnaround times for 3832 samples. Nearly all (94.9%) of the *RAS* mutation test results were reported to the requesting physician within 10 working days of the test being requested, with 57.0% being reported in 1–5 days, 38.0% in 6–10 days and only 5.1% in >10 days (Table 3).
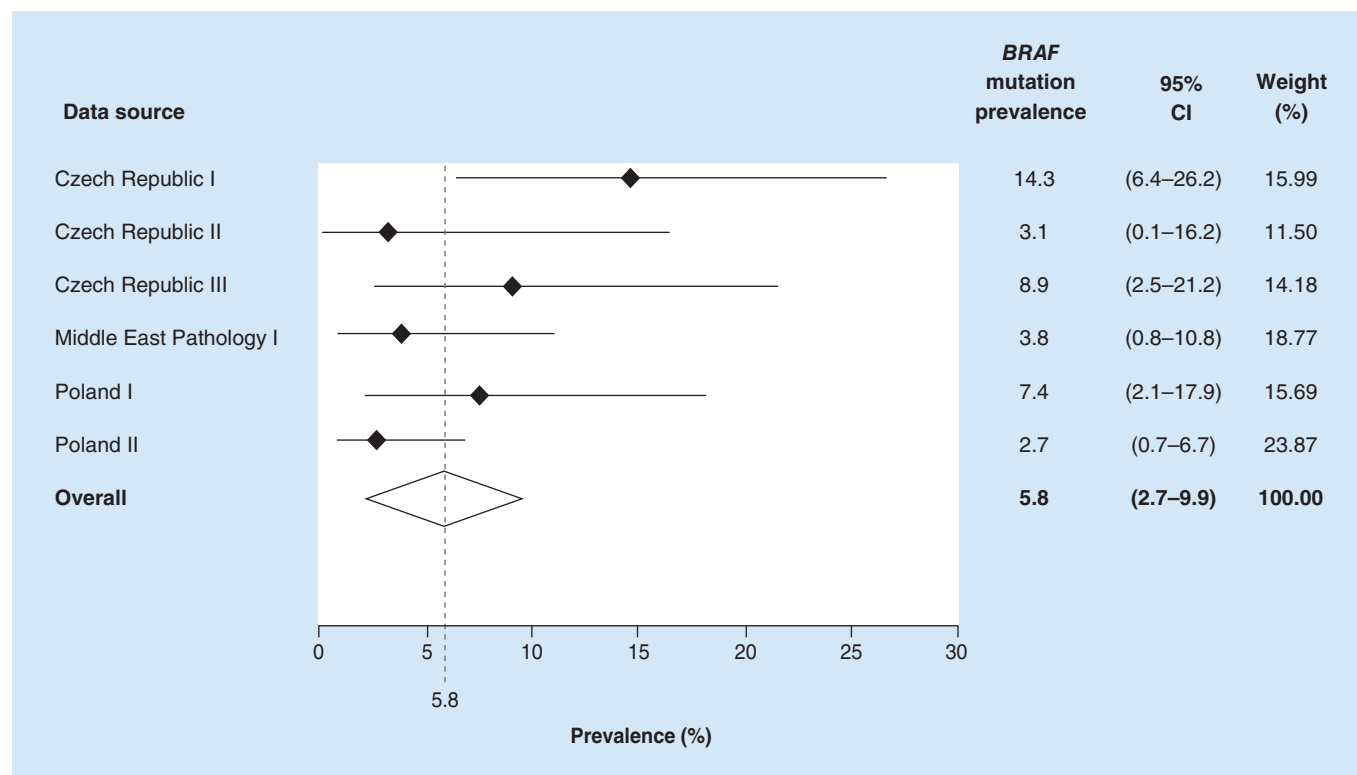
Figure 2.   Overall *BRAF* mutation prevalence.

Discussion

To our knowledge, this is the largest research project to date to carry out a pooled analysis of *RAS* mutation prevalence specifically using real-world data. The overall pooled *RAS* mutation prevalence across all data sources included in the study was estimated to be 43.6% (95% CI: 38.8–48.5%). However, the *RAS* mutation prevalence estimates for individual data sources included in this analysis varied considerably. Elucidating the reasons for this variability was beyond the scope of the current study but may relate to the ethnic diversity of the patient cohorts in the various countries/regions from which the data were derived. Numerous epidemiological data show major geographical variation with significantly higher risk of CRC in affluent societies and prospective cohort data have linked dietary habits and lifestyle factors to CRC, which may contribute to *RAS* mutation heterogeneity [12]. The largely lower rate of CRC seen in developing countries of Asia and Africa compared with western countries may also relate to the fact that studies on the prevalence and raw data are very blurred and not definite [13]. The current study highlights the need for further research regarding the reasons for geographical variation in *RAS* mutation prevalence.

The overall prevalence estimate from this study is somewhat different to estimates reported from past clinical trials. A retrospective analysis of pooled data from five randomized controlled studies (RCTs) of panitumumab estimated the overall unadjusted *RAS* mutation prevalence in patients with mCRC as 55.9% (95% CI: 53.9–57.9%); however, the prevalence estimate presented here is more consistent with the results of another recent real-world study, which found the overall *RAS* mutation prevalence reported by European pathology laboratories to be 48.5% (95% CI: 46.4–50.6%) [11,12]. This suggests that the actual prevalence in real-world clinical practice may be lower, meaning that more patients are potential candidates for anti-EGFR treatment than might have been previously thought. Potential reasons for this apparent imbalance may relate to the necessary application of restrictive RCT selection criteria to determine which patients are eligible to participate in controlled trials and which may result in study cohorts not representing the wider patient population observed in routine clinical practice. The wider availability of next-generation sequencing for patient selection or characterization in an RCT setting may also have driven the apparent bias toward an overestimate of the prevalence of *RAS* mutation among patients with mCRC. This approach may have a lower detection limit than standard laboratory tests for the detection of
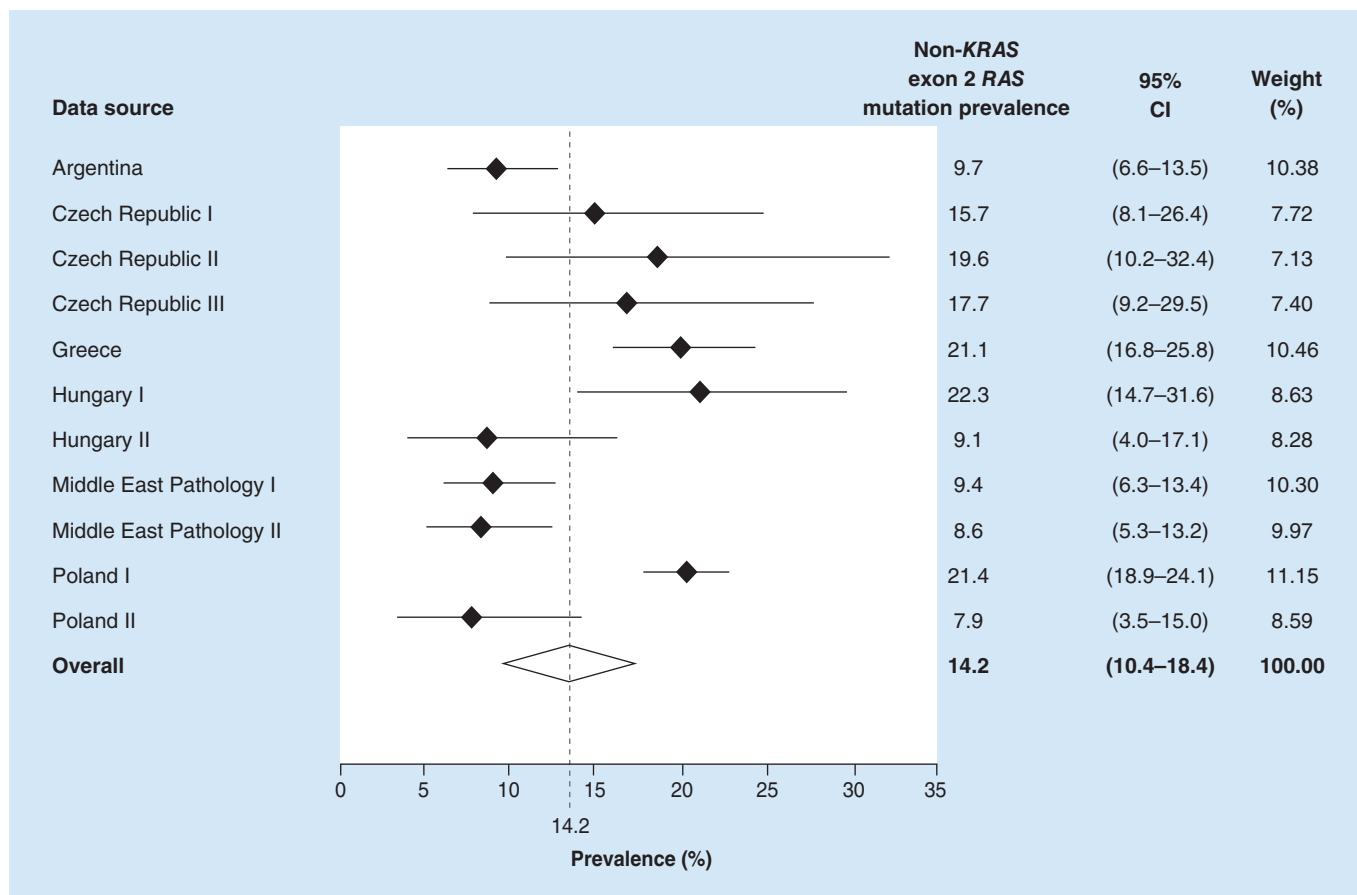
Data source		Non-*KRAS* exon 2 *RAS* mutation prevalence	95% CI	Weight (%)
Argentina		9.7	(6.6–13.5)	10.38
Czech Republic I		15.7	(8.1–26.4)	7.72
Czech Republic II		19.6	(10.2–32.4)	7.13
Czech Republic III		17.7	(9.2–29.5)	7.40
Greece		21.1	(16.8–25.8)	10.46
Hungary I		22.3	(14.7–31.6)	8.63
Hungary II		9.1	(4.0–17.1)	8.28
Middle East Pathology I		9.4	(6.3–13.4)	10.30
Middle East Pathology II		8.6	(5.3–13.2)	9.97
Poland I		21.4	(18.9–24.1)	11.15
Poland II		7.9	(3.5–15.0)	8.59
Overall		**14.2**	**(10.4–18.4)**	**100.00**

Prevalence (%)

Figure 3.   Overall non-*KRAS* exon 2 *RAS* mutation prevalence, in patients with a confirmed wild-type *KRAS* exon 2 status.

low copy number mutant alleles [13]. Alternatively, standard laboratory tests may be less sensitive than those used during RCTs.

The overall pooled *BRAF* mutation prevalence of 5.8% was similar to findings from previous RCTs, where *BRAF* mutation prevalence was estimated as 8.1% (95% CI: 6.7–9.6%) [11]. Similarly, the prevalence of other *RAS* mutations (*KRAS* exons 3, 4 and *NRAS* exons 2, 3, 4) in samples with a confirmed *KRAS* exon 2 wild-type status was numerically lower than the estimate reported by a recent meta-analysis of both panitumumab and cetuximab RCTs, which found that 19.9% (95% CI: 16.7–23.4%) of samples had a non-*KRAS* exon 2 mutation. However, the results are within the margin of error of each other [14].

The *RAS* testing turnaround time was no greater than 10 working days for nearly all of the samples (94.9%) included in the different data sources. This is marginally higher than reported in a survey of pathology centers in Europe, carried out at the end of 2014, which estimated that for 90.8% of the laboratories the turnaround time was less than 10 working days [12]. However, this estimate only includes the time between a laboratory receiving the sample and reporting back to the physician. Hence, it is likely that the time from a physician making the request to receiving the sample result is actually longer.

Data collection methodology among pathology laboratories has previously been shown to be highly variable, with a number of different DNA extraction and mutation testing methods being used [15]. This is important to note given the recognized differences in sensitivity among different genotype-ascertainment methodologies [16,17]. In addition, potential quality issues of sample preparation could explain some of the variability. One of the limitations of this research project is that it did not collect data relating to the quality of the DNA samples tested or ascertain if the pathology laboratories involved in the testing were participating in an external quality assurance scheme. This highlights the importance of external quality assurance schemes, particularly given the number of *RAS* testing methods that have not yet been validated and the lack of standardization among pathology laboratories [16,18,19].

Blood-based *RAS* mutation analysis is now available in many countries and may address some of these concerns if used in future clinical trials [20]. Although institutes may or may not be accredited by an independent body, it is a requirement of the European Medicines Authority that *RAS* mutational status should be determined by an experienced laboratory using validated test methods.

Additionally, the prevalence estimates reported here should be interpreted with caution as the data sources are highly heterogeneous. The heterogeneity of the data could be explained by differences in data collection methodology and the original purposes of the data collection. Differences between eligibility criteria, patient characteristics and study periods may have contributed to *RAS* prevalence differences. Some of the included samples may have been initially tested for *KRAS* exon 2 status with testing for the remaining *RAS* exons (*KRAS* exons 3, 4 and NRAS exons 2, 3, 4) conducted at a later time point. In practice, samples were likely to have been collected at different time points (e.g., time of surgery), including some which may have been collected prior to metastatic diagnosis (most likely stage III CRC).

Conclusion

This pooled analysis of *RAS* mutation prevalence estimates from real-world data sources found the overall *RAS* mutation prevalence among patients with mCRC to be somewhat lower than reported in clinical trials.

Future perspective

New knowledge of biomarkers involved in mCRC will impact the future screening, diagnosis and treatment of the disease. This may lead to new anticancer agents that are selective for certain subgroups of patients and to more precise use of the currently available agents. This will increase treatment options available to clinicians and may lead to tailored individualistic approaches, decreasing treatment cost and the adverse effects related to mAb therapy.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Summary points

- *RAS* mutation status is an important predictive biomarker of successful outcome for patients with metastatic colorectal cancer (mCRC) treated with anti-EGFR therapies such as cetuximab and panitumumab.
- Therefore, in many countries, a confirmed wild-type *RAS* (exons 2, 3, 4 of *KRAS* and *NRAS*) tumor status is a requirement for prescribing anti-EGFR treatment to patients with mCRC.
- Our currently limited understanding of *RAS* mutation prevalence in mCRC is largely derived from clinical trials; this research project aims to estimate the prevalence on *RAS* mutations based on real-world data sources.
- Aggregate patient data were collected from a range of geographically diverse real-world sources, by means of an online standardized form, and included in an overall meta-analysis.
- The overall pooled *RAS* mutation prevalence for 4431 tumor samples across 12 data sources was estimated to be 43.6%.
- *RAS* mutation prevalence varied between data sources, ranging from low estimates of 33.7% in a Middle Eastern pathology dataset and 34.6% in a Middle Eastern mCRC registry to the highest estimates of 53.6% and 54.1% in two European pathology centers, in the Czech Republic and Poland, respectively.
- The overall pooled *RAS* mutation prevalence was lower than previously reported in a pooled analysis of randomized controlled studies of anti-EGFR treatment; however, it was more consistent with the results of a similar, albeit smaller, real-world study.
- Therefore, the actual real-world prevalence may be lower than reported in randomized controlled studies, meaning that more patients are potential candidates for anti-EGFR treatment than might have been previously thought.

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