RAS Testing Practices and *RAS* Mutation Prevalence Amongst mCRC Patients: Results from a Europe-Wide Survey

PD-015

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BACKGROUND

- Panitumumab and cetuximab are monoclonal antibodies (mAbs) that inhibit the epidermal growth factor receptor (EGFR), and are indicated for patients with metastatic colorectal cancer (mCRC).[1–3]
- In Europe, the therapeutic indication for anti-EGFR mAbs is restricted to patients with confirmed *RAS* wild-type mCRC only (exons 2, 3 and 4 of the *KRAS* and *NRAS* oncogenes).[4,5]

OBJECTIVES

- To describe the *RAS* testing practices currently used in Europe.
- To estimate the turnaround time for *RAS* testing results and identify factors associated with variations in turnaround time.
- To estimate the *RAS* mutation prevalence in patients with mCRC according to predefined clinical and demographic characteristics.

METHODS

Study participants

- Pathology laboratories invited to take part in this online survey were all current or recent participants in the European Society of Pathology's CRC biomarker testing external quality assurance scheme.

Survey design

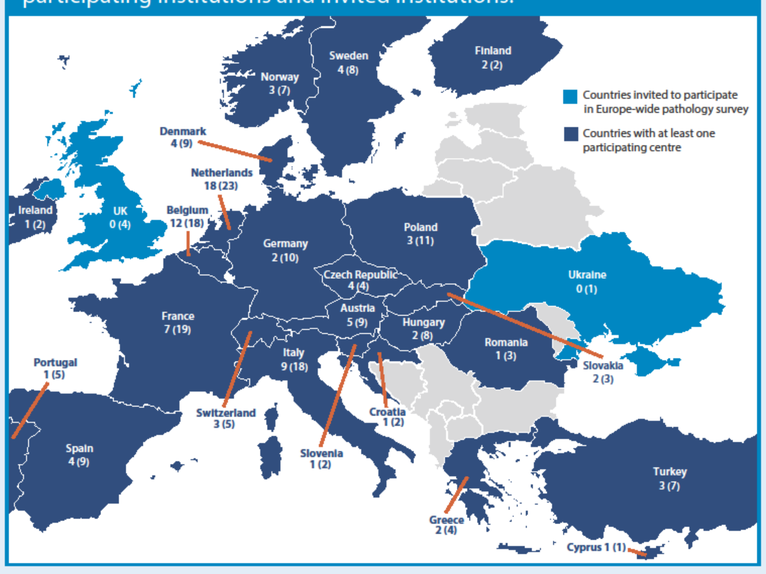
- The online study survey was divided into two parts.
- The first part, a questionnaire, covered the laboratory characteristics and the *RAS* testing methods being used.
  - Data were collected on number of patients tested for *RAS* status per year, testing location and indication, cut-off percentage of neoplastic cells for testing, *KRAS* and *NRAS* codons tested and DNA extraction method used.
- The second part requested aggregated data from the 20–30 most recent patients with mCRC assessed for *RAS* mutation status.
  - Aggregated data were collected on *RAS* mutation prevalence by codon, tumour site and type of tissue sampled, and also on the turnaround time for *RAS* testing results.

RESULTS

Study participants

- In total, 194 pathology laboratories across 26 European countries were invited to participate in the survey.
- Of the laboratories contacted, 96 (49.5%) in 24 countries satisfactorily completed the questionnaire between October and December 2014 (Figure 1).

FIGURE 1. Survey responses by country, showing number of participating institutions and invited institutions.



Laboratory characteristics

- The majority of pathology laboratories (71.9%) estimated testing >80 patients for *RAS* mutation status each year.
- Most laboratories perform testing within their own institute (93.8%) and on request by the treating oncologist (89.5%) (Table 1).
  - Routine testing of all patients with CRC for *RAS* mutations was performed in 5.3% of laboratories.
- In total, 89.6% of laboratories reported using a minimum cut-off percentage of neoplastic cells for *RAS* testing.
- Five DNA extraction methods were mainly used by the laboratories (Table 1).
  - The QIAamp DNA FFPE kit (Qiagen) was the most commonly used method (41.7%).

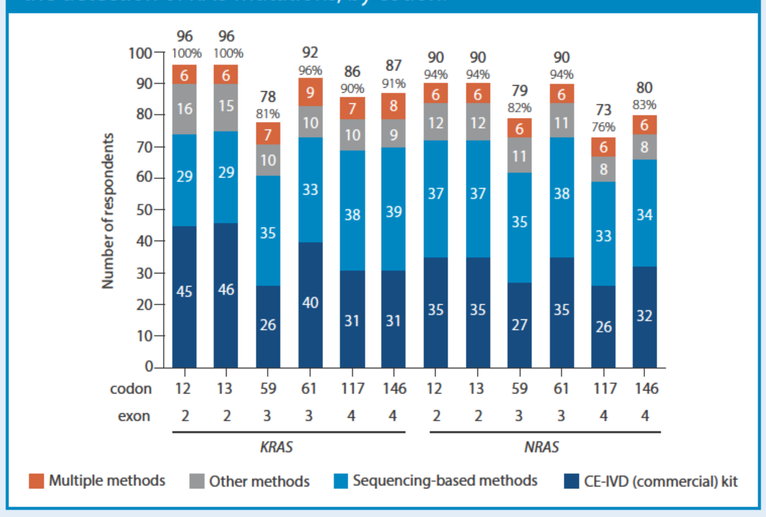
TABLE 1. Characteristics of participating laboratories.

Variable, n	Criterion	n	%
Patients with mCRC tested for *RAS* each year, n=96	≤80	27	28.1
	>80	69	71.9
DNA extraction method used, n=96	QIAamp DNA FFPE kit (Qiagen)	40	41.7
	Cobas DNA Sample Preparation kit (Roche)	12	12.5
	QIAamp DNA mini kit (Qiagen)	7	7.3
	Raw proteinase K lysate	6	6.3
	Maxwell 16 (Promega)	14	14.6
	MagnaPure (Roche)	1	1.0
	Other	16	16.7
***RAS* mutations tested, n=96**	All codons tested	70	72.9
	Not all codons tested	26	27.1
Place *RAS* testing was performed, n=96	Own institute	90	93.8
	Own institute and external	5	5.2
	External	1	1.0
Indication for *RAS* testing, n=95	On request from oncologist	85	89.5
	All patients with CRC tested	5	5.3
	Other	5	5.3
Minimum percentage of neoplastic cells, n=96	No cut-off defined	10	10.4
	<10%	18	18.8
	≥10%	68	70.8

RAS testing practices

- The majority of laboratories (72.9%) report implementation of *RAS* testing for all hot-spot mutations.
  - In total, 100% of participants reportedly test for *RAS* mutations in *KRAS* exon 2, codons 12 and 13 (Figure 2).
  - Only 3.1% of participants reported only testing *KRAS* exon 2, codons 12 and 13.
- Subsequent DNA testing for *RAS* hot-spot mutations is performed with either commercially available (CE-IVD) kits or sequencing-based methods (Figure 2).
  - Overall, no clear preference in DNA testing method was observed between these two categories.

FIGURE 2. Frequency of CE-IVD kits and sequencing-based methods for the detection of *RAS* mutations, by codon.

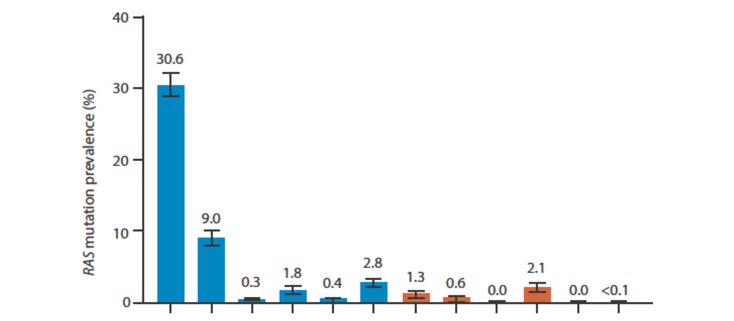


- Dideoxy sequencing of the PCR product was the most frequently used method (30%), followed by the Therascreen *KRAS*/*NRAS* pyro kit (15%).
  - A next-generation sequencing-based method is reportedly used by 10% of the participating laboratories.

RAS mutation prevalence

- For patients who had all *KRAS* and *NRAS* codons tested (n=2245), the overall *RAS* mutation prevalence was 48.5% (95% CI 46.4–50.6%).
- The mutation prevalence varied by codon; the highest rates were reported for *KRAS* exon 2, codons 12 and 13 (Figure 3).

FIGURE 3. *RAS* mutation prevalence, by codon, for patients who underwent complete testing of all *KRAS* and *NRAS* codons (n=2245).



- *RAS* mutation prevalence varied significantly by primary tumour location (p=0.043), indication for *RAS* testing (p=0.036) and approximate number of patients tested yearly (p<0.001) (Table 2).
  - *RAS* mutation prevalence did not vary significantly by type of tissue sampled, DNA extraction method, the place *RAS* testing was performed, or the minimum cut-off percentage of neoplastic cells used for testing.

TABLE 2. Patient and laboratory variables that significantly affect *RAS* mutation prevalence.

Variable, n	Criterion, n	*RAS* mutation prevalence, %	95% CI	p-value
Total, n=3244		46.0	(44.3–47.7)	
***RAS* mutations tested, n=3244**	All codons tested, n=2245	48.5	(46.4–50.6)	<0.001
	Not all codons tested, n=999	40.3	(37.3–43.5)	
Location of tumour[a,b], n=1393	Right colon, n=511	54.6	(50.2–59.0)	0.043
	Left colon, n=429	46.4	(41.6–51.2)	
	Rectum, n=453	51.0	(46.3–55.7)	
Patients with mCRC tested for *RAS* each year[a], n=2093	≤80, n=382	44.8	(40.5–49.0)	<0.001
	>80, n=1711	49.7	(47.3–52.0)	
Indication for *RAS* testing[a], n=2215	On request from oncologist, n=1983	48.6	(46.4–50.8)	0.036
	All patients with CRC tested, n=84	60.7	(49.5–71.2)	
	Other, n=148	43.2	(35.1–51.6)	

[a]Only includes laboratories testing all codons. Only includes wild-type and mutated results, patients with unknown/unavailable *RAS* mutation status have been excluded. [b]Excludes patients with unknown/unavailable primary tumour location.

Patients reported in aggregated data sample may have had *RAS*-family mutations affecting more than one oncogene.

Turnaround time

- For 90.8% of participants, turnaround time between the laboratory receiving the request and reporting the result to the oncologist was ≤10 (working) days.
  - Only 9.2% of laboratories reported turnaround times of >10 days.
- Turnaround time was significantly longer if participants estimated testing >80 mCRC tumour specimens per year (p<0.001), if all *KRAS* and *NRAS* codons were tested (p<0.001), if testing methods were different for different codons (p<0.001) and if testing was not performed exclusively at the participant's 'own institute' (p<0.001).

CONCLUSIONS

- **Full testing, for all *RAS* hot-spot mutations in *KRAS* and *NRAS* exons 2, 3 and 4, has been implemented in the majority of laboratories.**
- **Although most centres test for *RAS* mutation status on request from an oncologist, 5% are routinely testing all patients with CRC.**
- **The *RAS* mutation prevalence calculated in this study (48.5%) was similar to recent clinical trials, which reported mutation rates of 50–55%.[2,6]**
  - **In addition, the prevalence in this study was consistent with findings from sequenced CRC tumours in the TCGA database (49%),[7] and also with a smaller study of pathology centres in the Netherlands (47.6%).[8]**
- **Turnaround time was ≤10 working days for the majority of the samples tested.**
  - **Almost half of the laboratories report turnaround times of ≤5 days, which is desirable in clinical practice.**
- **Although the implementation of *RAS* testing has been broadly successful, testing practices are highly variable amongst pathology laboratories.**

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ACKNOWLEDGEMENTS

This study was funded by Amgen Ltd. Editorial assistance and support were provided by Adelphi Communications Ltd, Bollington, UK, supported by Amgen Ltd.

This poster was presented at the ESMO 17th World Congress on Gastrointestinal Cancer, Barcelona, Spain, July 1–4, 2015