

KRAS mutation analysis: a comparison between primary tumours and matched liver metastases in 305 colorectal cancer patients

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BACKGROUND: KRAS mutation is a negative predictive factor for treatment with anti-epidermal growth factor receptor antibody in metastatic colorectal cancer (CRC). KRAS mutation analysis is usually performed on primary tumour tissue because metastatic tissue is often not available. However, controversial data are available on the concordance of test results between primary tumours and corresponding metastases. We assessed the concordance of KRAS mutation status in a study of 305 primary colorectal tumours and their corresponding liver metastases.

METHODS: Patients with histologically confirmed CRC who underwent surgical resection of the primary tumour and biopsy or surgical resection of the corresponding liver metastasis were included. KRAS mutation analysis was performed for codons 12 and 13.

RESULTS: KRAS mutation was detected in 108 out of 305 primary tumours (35.4%). In 11 cases (3.6%), we found a discordance between primary tumour and metastasis: 5 primary tumours had a KRAS mutation with a wild-type metastasis, 1 primary tumour was wild type with a KRAS mutation in the metastasis, and in 5 cases the primary tumour and the metastasis had a different KRAS mutation.

CONCLUSION: We observed a high concordance of KRAS mutation status of 96.4% (95% CI 93.6–98.2%) between primary colorectal tumours and their corresponding liver metastases. In only six patients (2.0%; 95% CI 0.7–4.2%), the discordance was clinically relevant. In this largest and most homogenous study to date, we conclude that both primary tumours and liver metastases can be used for KRAS mutation analysis.

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Recent advances in specific signalling pathways of cancer cells have introduced targeted therapy into treatment regimes for patients with metastatic colorectal cancer (CRC) (Tol and Punt, 2010). Cetuximab and panitumumab are monoclonal antibodies that bind to the extracellular domain of the epidermal growth factor receptor (EGFR). They inhibit ligand-induced stimulation of several intracellular signalling pathways, such as RAS/RAF/MAPK and phosphoinositide-3 pathway, which results in decreased stimulation of cell cycle progression, proliferation, angiogenesis, and stimulation of apoptosis (Scaltriti and Baselga, 2006). The KRAS oncogene is currently the most relevant molecular biomarker that predicts the response to EGFR-targeted therapy in CRC. An oncogenic mutation in KRAS leads to constitutive activation of the RAS/RAF signalling pathway independent from EGFR activation by binding of the ligand (Benvenuti *et al*, 2007). KRAS mutations occur in approximately 38% of colorectal tumours and involve codon 12 and 13 in >96% of cases (Oliveira *et al*, 2004). Metastatic CRC patients with tumours harbouring a

KRAS mutation are resistant to treatment with anti-EGFR antibodies, showing lower response rates, decreased progression-free survival, and overall survival compared with patients with KRAS wild-type tumours (Karapetis *et al*, 2008; Tol *et al*, 2009; Van Cutsem *et al*, 2009). Therefore, the European Medicines Agency and the Food and Drug Administration have restricted the use of anti-EGFR antibodies in metastatic CRC to patients with KRAS wild-type tumours.

Cetuximab and panitumumab have shown efficacy both as monotherapy (Amado *et al*, 2008; Karapetis *et al*, 2008) and in combination with chemotherapy (Tol *et al*, 2009; Van Cutsem *et al*, 2009) in patients with KRAS wild-type metastatic CRC. Nevertheless, even among patients with KRAS wild-type tumours, the majority of patients do not respond to anti-EGFR therapy. Efficacy of anti-EGFR therapy was suggested to be further restricted to patients with BRAF wild-type tumours (Di Nicolantonio *et al*, 2008). An additional explanation for the suboptimal response rates to anti-EGFR antibodies in patients with KRAS wild-type tumours is discordance of KRAS mutation status between primary colorectal tumours and corresponding metastases. In the early dissemination model, tumour cells depart the primary lesion before the acquisition of a fully malignant phenotype to undergo new mutations and metastatic growth at a distant site (Klein, 2009). According to this model, a discordance in mutation status

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between primary tumours and metastases may occur, and as a consequence the mutation status of the primary tumour might not be adequate to predict the response of metastases to anti-EGFR treatment.

Current data on the concordance in *KRAS* mutation status between primary colorectal tumours and metastases are conflicting. Five studies showed a 100% concordance of *KRAS* mutation status in primary CRC and corresponding metastases (Losi *et al*, 1992; Suchy *et al*, 1992; Zauber *et al*, 2003; Weber *et al*, 2007; Etienne-Grimaldi *et al*, 2008). In contrast to these data, others have reported a discordance of *KRAS* mutation status in primary tumours and metastatic sites, with an overall discordance observed in 4–32% of the patients (Oudejans *et al*, 1991; Al-Mulla *et al*, 1998; Albanese *et al*, 2004; Oliveira *et al*, 2007; Artale *et al*, 2008; Santini *et al*, 2008; Cejas *et al*, 2009; Garm Spindler *et al*, 2009; Loupakis *et al*, 2009; Molinari *et al*, 2009; Perrone *et al*, 2009; Baldus *et al*, 2010; Italiano *et al*, 2010). These controversial results are probably due to the fact that these studies were underpowered with a small number of patients, and included a wide variety of metastatic sites. Therefore, it is still uncertain whether the evaluation of *KRAS* mutation status in the most commonly available primary tumour correctly reflects the *KRAS* mutation status of corresponding metastasis. This is highly relevant given the large number of CRC patients as well as the potential toxicity and costs of anti-EGFR therapy.

We assessed the concordance in *KRAS* mutation status in primary tumours and their corresponding liver metastases in an adequately powered study of 305 CRC patients.

PATIENTS AND METHODS

Patient selection

Patients with histologically confirmed CRC who underwent surgical resection of the primary tumour and biopsy or surgical resection of the corresponding liver metastasis were included in this analysis. Results were obtained from archived material of three large pathology laboratories and from material collected from the CAIRO2 study, a large multicentre trial of the Dutch Colorectal Cancer Group (Tol *et al*, 2009).

In patients with a discordance of *KRAS* mutation status between the primary tumour and metastasis, additional blocks of the primary tumour were obtained to exclude heterogeneity within the tumour. Lymph node metastases present at the time of diagnosis were also acquired in these patients.

Tumour DNA preparation

Formalin-fixed paraffin-embedded tissue blocks were cut at 4 μ m thickness and stained with haematoxylin and eosin (HE). The presence of tumour tissue was marked by a pathologist. Subsequently the blocks were cut at 20–40 μ m thickness and micro dissected for DNA extraction. Tumour tissue was dissolved in 200 μ l lysis buffer (QIAamp DNA Micro Kit, Qiagen, Venlo, The Netherlands) and incubated with proteinase K overnight at 56 °C for two nights. DNA was extracted according to the manufacturer's protocol (QIAamp DNA Micro Kit, Qiagen), and DNA concentration was determined at 260 nm using the Nanodrop 26 ND-1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, NC, USA).

KRAS mutation analysis

For *KRAS* mutation analysis, exon 2 (codon 12 and 13) was amplified using a 50 μ l reaction mixture containing 0.2 μ m forward (5'-TGTAACACGACGGCCAGTAGGCCTGCTGAAAATGACTG-3') and reverse (5'-CAGGAAACAGCTATGACCTGGATCATATTCG TCCACAAAA-3') primers (Invitrogen, Breda, The Netherlands); dATP, dCTP, dGTP and dTTP (GE Healthcare, Zeist, The Netherlands) at 0.2 mM each; 50 mM KCl; 10 mM Tris-HCl (pH

8.3); 2.5 mM MgCl₂; 1 U AmpliTaq Gold polymerase (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and 50 ng of template DNA. The PCR conditions were as follows: 94 °C for 10 min; 92 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min (40 cycles); and 72 °C for 10 min.

All PCR products were purified with the MultiScreen HTS, 96 well Filtration System (Millipore, Carrigtwohill, Ireland). Subsequently, the purified products were sequenced using fluorescently labelled terminators (BigDye Terminators (v 1.1); Applied Biosystems, Foster City, CA, USA) with both M13-forward and M13-reverse sequencing primers. The sequencing products were analysed on an ABI 3730 DNA Analyser (Applied Biosystems) and the data analysis was performed using Sequencing Analysis Software Sequencing Analysis Software v5.3.1 with KBTM Base-caller. Sequence results were scored by visual inspection of the chromatograms (Applied Biosystems).

Statistical analysis

We considered a discordance level of 5% or more to be clinically relevant, that is, leading to substantial change in routine clinical practice. To exclude such level of discordance under the assumption that the true discordance was 2.5% or less, we set the sample size at 304 paired samples. With this sample size, the precision in the estimated percentage of discordance was 2.5% (i.e., s.e. 1.25, half-width of the 95% confidence interval equal to 2.5%).

The comparison of patient and primary tumour characteristics between patients with *KRAS* wild-type and *KRAS* mutant primary tumours was done using Wilcoxon's rank sum test or χ^2 for numerical or categorical variables, respectively. Differences in *KRAS* mutation status between the primary tumour and corresponding metastasis were analysed by calculating the percentage of concordance, and (clinically relevant) discordance, together with the corresponding Clopper–Pearson 95% confidence intervals. Differences were considered to be statistically significant when the *P*-value was below 0.05. All statistical tests were two-sided.

RESULTS

Patient characteristics

We analysed *KRAS* codon 12 and 13 mutations in 320 matched primary colorectal tumours and liver metastases. The tumour cell percentages in all primary tumours and metastases were above 30%. We failed to obtain a *KRAS* mutation status in 15 patients; therefore our further analyses were performed in 305 paired samples. Patient characteristics are shown in Table 1.

KRAS mutation and histopathological parameters

A total of 108 patients (35.4%) had a *KRAS* mutation in the primary tumour; of which 37 patients had a Gly12Asp mutation, 28 patients a Gly12Val mutation, 14 patients a Gly13Asp mutation, 10 patients a Gly12Cys mutation, 7 patients a Gly12Ser mutation, 7 patients a Gly12Ala mutation, 3 patients a Gly12Arg mutation, 1 patient a Gly12Asp and Gly12Ala mutation and 1 patient a Gly12Phe mutation (Table 2). Histopathological characteristics of the primary tumour were comparable between patients with and without a *KRAS* mutation (Table 1).

Concordance of *KRAS* status in primary tumours and corresponding liver metastases

In 294 patients (96.4%; 95% CI 93.6–98.2%), the same *KRAS* mutation status was obtained from the primary tumour and the corresponding liver metastasis. In 11 patients (3.6%; 95% CI 1.8–6.4%), of which 7 had synchronous metastases at diagnosis and 4 developed metachronous metastases, we found a discordance

Table 1 Distribution of tumour characteristics according to KRAS status of the primary tumour

	Overall, n = 305	KRAS mutation, n = 108	KRAS wild type, n = 197	P-value
Age				0.20
Median (IQR)	64 (57–70)	65 (58–71)	64 (57–70)	
Gender				0.37
Male	191 (62.6%)	64 (59.3%)	127 (64.5%)	
Female	114 (37.4%)	44 (40.7%)	70 (35.5%)	
Metastases presentation				0.45
Synchronous	169 (55.4%)	63 (58.3%)	106 (53.8%)	
Metachronous	136 (44.6%)	45 (41.7%)	91 (46.2%)	
Tumour location				0.63
Colon	167 (54.8%)	59 (54.6%)	108 (54.8%)	
Rectum	54 (17.7%)	16 (14.8%)	38 (19.3%)	
Rectosigmoid	80 (26.2%)	32 (29.6%)	48 (24.4%)	
Unknown	4 (1.3%)	1 (0.9%)	3 (1.5%)	
Histopathological subtype				0.12
Adenocarcinoma	271 (88.9%)	90 (83.3%)	181 (91.9%)	
Adenocarcinoma with muc. component	21 (6.9%)	10 (9.3%)	11 (5.6%)	
Mucinous adenocarcinoma	8 (2.6%)	5 (4.6%)	3 (1.5%)	
Unknown	5 (1.6%)	3 (2.8%)	2 (1.0%)	
Differentiation grade				0.21
Good	33 (10.8%)	13 (12.0%)	20 (10.2%)	
Moderate	196 (64.3%)	65 (60.2%)	131 (66.5%)	
Poor	52 (17.0%)	17 (15.7%)	35 (17.8%)	
Unknown	24 (7.9%)	13 (12.0%)	11 (5.6%)	
T stage				0.62
T1	4 (1.3%)	2 (1.9%)	2 (1.0%)	
T2	20 (6.6%)	9 (8.3%)	11 (5.6%)	
T3	231 (75.7%)	81 (75.0%)	150 (76.1%)	
T4	36 (11.8%)	11 (10.2%)	25 (12.7%)	
Unknown	14 (4.6%)	5 (4.6%)	9 (4.6%)	
N stage				0.10
N0	114 (37.4%)	46 (42.6%)	68 (34.5%)	
N1	87 (28.5%)	31 (28.7%)	56 (28.4%)	
N2	86 (28.2%)	26 (24.1%)	60 (30.5%)	
Unknown	18 (5.9%)	5 (4.6%)	13 (6.6%)	
Number of lymph nodes examined				0.28
Median (IQR)	10 (6–15)	10 (6–13)	10 (6–16)	
Number of lymph node metastases				0.15
Median (IQR)	1 (0–4)	1 (0–3)	1 (0–4)	

Abbreviation: IQR = interquartile range.

Table 2 Distribution of KRAS mutation types

Codon 12/13	Patients with KRAS mutation (n, %)
Gly12Asp	37 (34%)
Gly12Val	28 (26%)
Gly13Asp	14 (13%)
Gly12Cys	10 (9%)
Gly12Ser	7 (6%)
Gly12Ala	7 (6%)
Gly12Arg	3 (3%)
Gly12Phe	1 (1%)
Gly12Asp + Gly12Ala	1 (1%)

between primary tumours and metastases. Five patients had a KRAS mutation in the primary tumour and not in the liver metastasis. Only one patient had a wild-type status of the primary tumour, while the metastasis showed a KRAS mutation. In five patients, the primary tumours had different KRAS mutations compared with the metastases. One of these patients had two primary tumours. Both primary tumours had the same KRAS mutation (Gly13Asp), while the liver metastasis had a different KRAS mutation (Gly12Ser). In another patient, the primary tumour had a double mutation (Gly12Asp/Gly12Val) and the

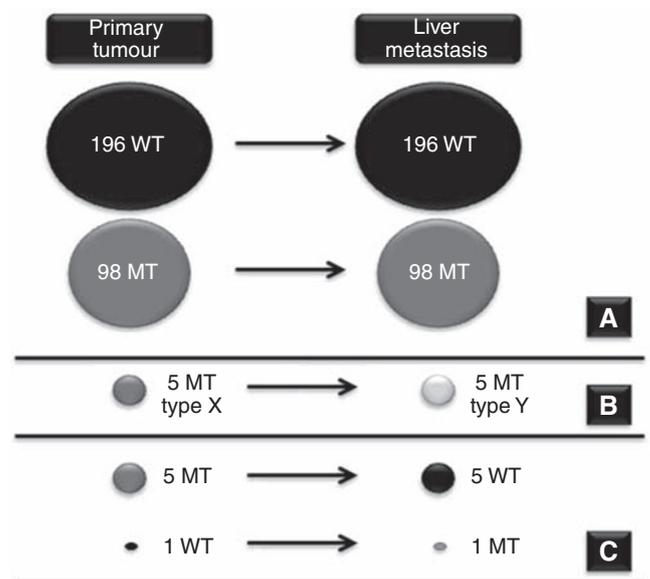
**Figure 1** Overall concordance of the KRAS mutation status between primary tumour and liver metastasis (A), discordance without clinical impact (B), and discordance with clinical impact (C). Abbreviations: WT, wild type; MT, mutation.

Table 3 Patients with a discordant KRAS status between primary tumour and liver metastasis. Multiple blocks of primary tumour tissue and lymph node metastases were tested when available

	KRAS status primary tumour	KRAS status 2nd tumour	KRAS status lymph node metastasis	KRAS status liver metastasis
1	Gly12Ala	—	LN 1: Gly12Ala LN 2: Gly12Ala LN 3: Gly12Ala	WT
2	Gly12Asp Gly12Asp WT	—	—	WT
3	Gly12Cys	—	—	WT
4	Gly12Asp Gly12Asp Gly12Asp Gly12Asp	—	LN 1: Gly12Asp LN 2: Gly12Asp LN 3: Gly12Asp LN 4: Gly12Asp LN 5: WT	WT
5	Gly12Ser	—	—	WT
6	WT	—	—	Gly12Cys
7	Gly12Asp	—	LN 1: WT LN 2: WT LN 3: WT	Gly12Ala
8	Gly13Asp	Gly13Asp	LN 1: Gly13Asp	Gly12Ser
9	Gly12Ser	—	—	Gly12Ala
10	Gly12Cys Gly12Asp	—	LN 1: Gly12Asp LN 2: Gly12Asp LN 3: Gly12Asp LN 4: Gly12Asp LN 5: Gly12Asp LN 6: WT	Gly12Asp
11	Gly12Asp/Gly12Val	—	LN 1: Gly12Val LN 2: Gly12Val LN 3: Gly12Val LN 4: Gly12Asp LN 5: Gly12Asp LN 6: Gly12Asp LN 7: Gly12Asp	Gly12Asp

Abbreviation: WT = wild type.

metastasis had a Gly12Asp mutation (Figure 1, Table 3). Taken together, the observed discordance was clinically relevant in only six patients (2.0%; 95% CI 0.7–4.2%).

Subsequent analyses in patients with a discordance of KRAS status

Several tests were performed to exclude bias of the test results. First, the HE coupes of all patients with a discordant KRAS mutation status between the primary tumour and liver metastasis were revised. The primary tumours and liver metastases had a mean tumour cell percentage of 65 and 60%, respectively. Subsequent independent reanalysis of the KRAS mutation status resulted in the same discordances.

Second, several mutation analyses were performed on different areas of the tumour and from different tumour blocks in order to establish possible tumour heterogeneity. Two patients showed heterogeneity of KRAS status within the primary tumour. One of these patients demonstrated two areas with a Gly12Asp mutation and one area with wild-type status, of which the latter resembled the liver metastasis. The other patient showed two different KRAS mutations within the same tumour, of which one is concordant with the liver metastasis (Table 3).

Third, 6 of the 11 patients with discordant results did have lymph nodes metastases at the time of diagnosis. KRAS mutation testing of all lymph nodes separately revealed overall concordant KRAS status between lymph node metastases and the primary tumour in three patients. The KRAS status of the lymph nodes in the other three patients showed heterogeneity, of which at least one lymph node metastases showed a different KRAS status compared

with the primary tumour. However, this explains the discordance between the primary tumour and liver metastasis only in one patient (Table 3).

DISCUSSION

This is the first adequately powered study in CRC that compares KRAS mutation status between primary tumours and their corresponding liver metastases. We showed that tissue from the primary tumour can reliably be used for KRAS mutation testing in order to select patients for anti-EGFR therapy.

We observed a concordant KRAS mutation status in 96.4% of 305 paired samples of colorectal tumours and liver metastases. However, the difference in KRAS status was not clinically relevant in 5 of the 11 patients with discordant results, because both primary tumour and metastasis had a different KRAS mutation. Given the high statistical power of our analysis, we were able to obtain a highly accurate estimate of the level of discordance that enabled us to conclude that the level of discordance was 2.0%. The high rate of concordance is in agreement with the notion that KRAS mutations are considered as early driving events in CRC progression, and associated with the growth of small adenoma to clinically significant size (Vogelstein *et al*, 1988). Therefore, KRAS mutation status is expected to be equal in both primary tumours and metastases (Klein, 2009).

The previously reported lower concordance levels between primary tumours and metastases are most likely due to bias caused by false-negative results in underpowered studies. We calculated that 304 paired cases were needed to reliably exclude a rate of discordance of >5%, while previous studies included only 10 to

Table 4 Overview of studies providing data on *KRAS* status of primary tumour and related metastasis

Author study	Year	No. of pts	Analysed metastatic site	Method	KRAS mutation in PT (%)	KRAS mutation in PT, WT in M	KRAS WT in PT, mutation in M	Total percentage of discordance
Albanese	2004	30	Liver	SSCP analysis	14 (47%)	5/14 (36%)	4/16 (25%)	9/30 (30%)
Al-Mulla	1998	26	Liver	ASO/direct seq	10 (38%)	2/10 (20%)	3/16 (19%)	5/26 (19%)
		31	Lymph node	ASO/direct seq	10 (32%)	1/10 (10%)	5/21 (24%)	6/31 (19%)
Artale	2008	48	Diverse, 81% liver	Direct seq	11 (23%)	1/11 (9%)	2/37 (5%)	3/48 (6%)
Baldus	2010	20	Visceral metastasis	Direct seq	9 (45%)	1/9 (11%)	1/11 (9%)	2/20 (10%)
		55	Lymph node	Direct seq	29 (53%)	15/29 (52%)	2/26 (8%)	17/55 (31%)
Cejas	2010	93	Liver	Direct seq	30 (32%)	1/30 (3%)	4/63 (6%)	5/93 (5%)
		17	Lung	Direct seq	10 (59%)	1/10 (10%)	1/7 (14%)	2/17 (12%)
Etienne-Grimaldi	2008	48	Liver biopsy	PCR-RFLP	16 (33%)	0 (0%)	0 (0%)	0 (0%)
Italiano	2009	59	Not specified	Seq	23 (39%)	1/23 (4%)	2/36 (6%)	3/59 (5%)
Losi	1992	19	Local recurrence	Multiplex-ASPCR	12 (63%)	0 (0%)	0 (0%)	0 (0%)
		16	Metastasis, 38% liver	Multiplex-ASPCR	13 (81%)	0 (0%)	0 (0%)	0 (0%)
Loupakis	2009	43	Liver	Seq	Not mentioned	0 (0%)	2/*	2/43 (5%)
Molinari	2009	37	Diverse, 74% liver	Seq	16 (43%)	2/16 (13%)	1/21 (5%)	3/37 (8%)
		15	Lymph node	Seq	8 (53%)	0 (0%)	0 (0%)	0 (0%)
Oliveira	2006	28	Lymph node	Not mentioned	18 (64%)	2/18 (11%)	7/10 (70%)	9/28 (32%)
Oudejans	1991	31	Liver and lung	Hybridization	14 (45%)	1/14 (7%)	1/17 (6%)	2/31 (6%)
Perrone	2008	10	Diverse, mainly liver	Direct seq	2 (20%)	1/2 (50%)	1/8 (13%)	2/10 (20%)
Santini	2008	99	Diverse, 80% liver	Seq	38 (38%)	3/38 (8%)	1/61 (2%)	4/99 (4%)
Garm Spindler	2009	31	Not specified	qPCR	11 (35%)	2/11 (18%)	0/20 (0%)	2/31 (6%)
Suchy	1992	58	Autopsy material, not specified	Dot-blot hybridization	15 (26%)	0 (0%)	0 (0%)	0 (0%)
Weber	2006	36	Liver	Seq	14 (39%)	0 (0%)	0 (0%)	0 (0%)
Zauber	2003	42	Diverse, 93% lymph node, 5% liver	SSCP analysis + seq	22 (52%)	0 (0%)	0 (0%)	0 (0%)
Overall		892	All sites	All methods	345/849 (41%)	39/345 (11%)	35/504 (7%)	76/892 (9%)
		276	Liver	All methods	84/233 (36%)	8/84 (10%)	11/149 (7%)	21/276 (8%)
		129	Lymph nodes	All methods	65/129 (50%)	18/65 (28%)	14/64 (22%)	32/129 (25%)

Abbreviations: ASO = allele-specific oligonucleotide; ASPCR = allele-specific polymerase chain reaction; M = metastasis; pts = patients; PT = primary tumour; qPCR = quantitative PCR; RFLP = restriction fragment length polymorphism; SSCP = single strand conformational polymorphism; seq = sequencing. *Total number of cases not specified.

110 patients (Table 4). Moreover, in these studies metastases of different sites were compared with the primary tumour. As the molecular patterns may differ between metastatic sites (Klein, 2009), more reliable results are obtained when *KRAS* mutation status is tested more rigorously for each metastatic site. The liver is the predominant site of metastases in the majority of metastatic CRC patients; therefore the results of our large series of 305 liver metastases provide a solid reference for clinical decision making as to anti-EGFR therapy. Another issue is the fact that *KRAS* testing is technically not as straightforward as is often assumed. Several quality assurance systems are now in place, and the first 'round robin' test indicates that at least 30% of the experienced pathology laboratories fail to pass the threshold level of the quality assurance programs (Bellon *et al*, 2011). Other important facts about *KRAS* testing are the correct evaluation of the amount of tumour tissue in the sample and the sensitivity of testing methods. In a previous study, we demonstrated in >500 samples that both sequencing and real-time PCR are reliable methods (Tol *et al*, 2010).

A discordant *KRAS* status between the primary tumour and metastasis was observed in a small number of patients (3.6%). In these cases, tumour cells may have departed the primary lesions before the acquisition of a fully malignant phenotype to undergo somatic mutations or deletions at a distant site (Klein, 2009). Another explanation for the discordant results may be heterogeneity of *KRAS* status within the primary tumour, although this was the case in only a small number of patients. Finally, a discordance may in theory be explained by metastases from a non-detected second primary.

Previously published data showed that a considerable fraction (25%, Table 4) of colorectal lymph node metastases does not resemble the primary tumour in terms of *KRAS* mutation status. In 5 of the 25 lymph node metastases that we tested the *KRAS* status was not concordant with the primary tumour, which is consistent

with the literature (Table 4). Therefore, lymph node metastases do not seem suitable for determination of the *KRAS* mutation status of colorectal carcinomas. Discordance in *KRAS* mutation status might be due to clonal selection during the process of metastasis, however, heterogeneity in lymph node metastases could explain this discordance in only one patient.

Eight different *KRAS* mutation types were observed in our study, of which Gly12Asp showed the highest frequency. Five patients (1.6%) harboured different *KRAS* mutation types in the primary tumour compared with the metastases. This confirms the findings of Cejas *et al* (2009) and Albanese *et al* (2004), who reported a small number of patients (4 and 7%, respectively) with different mutation types between primary tumours and metastases. A different *KRAS* mutation type between primary lung adenocarcinomas and corresponding lymph node metastases was also observed in only 1% of the patients (Schmid *et al*, 2009). Currently, all patients with a *KRAS* mutation are excluded from treatment with anti-EGFR antibodies, independently of the mutation type. However, a recent paper indicated that codon 13 mutated tumours may be sensitive to cetuximab treatment (De Roock *et al*, 2010). As we observed a low frequency in *KRAS* mutation type discrepancies between primaries and metastases, this is not of clinical importance in selecting patients for anti-EGFR therapy.

In conclusion, we demonstrated a high level of concordance of 96.4% between primary tumours and liver metastases, which for clinical purposes to select CRC patients for anti-EGFR therapy was even higher with 98%. The implication of these results for general oncology practice is that both tissue of primary tumour or liver metastasis may be used for *KRAS* mutation testing. The results of our study are only valid for liver metastases and cannot be extrapolated to other metastatic locations. Furthermore, we demonstrated that discordance of test results between primary

tumour and metastases cannot account for the failure rate of anti-EGFR therapy in patients with KRAS wild-type tumours. Therefore, novel predictive markers in addition to KRAS and BRAF mutation status are warranted.

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