*Short Review*

# Validation of Blood Testing for *K-ras* Mutations in Colorectal and Pancreatic Cancer

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**Abstract**. *Background: K-ras mutation in a tumour is a powerful negative predictor for treatment success. Identifying tumour K-ras mutation is complex, and could be simplified by an appropriate blood test. Clinical studies were identified in which K-ras mutation status was assessed in both blood and tumour to ascertain whether blood K-ras mutation is predictive of tumour K-ras mutation. Between 29% and 100% of patients with a tumour K-ras mutation in 11 studies presented the same mutation in peripheral blood. Only 5/272 patients presented blood K-ras mutation in the absence of the same tumour mutation, possibly due to sampling errors. K-ras mutation in blood appears to indicate K-ras mutation in tumour, while the absence of blood K-ras mutation does not prove lack of mutation in the tumour. This suggests that a blood test for the detection of tumour K-ras may be possible, and could direct cancer treatment strategies.*

With the number of cases of cancer estimated to double globally between 2000 and 2020, and to nearly triple by 2030 (1), the need to develop targeted treatment strategies for the efficient use of costly cancer therapies has never been greater. Under such a strategy, the ineffective use of costly treatments would be reduced by directing drug regimens at those patients who are most likely to benefit, in particular, those with a similar genetic profile to other patients who have been successfully treated in this way.

The *ras* gene carries a mutation in up to 80-90% of cancer patients, usually at codon 12 or 13 of the *K-ras* gene (2, 3). The presence of a *K-ras* mutation has been shown to be a powerful negative predictor of treatment success with epidermal growth factor receptor (EGFR) inhibitors such as panitumumab and cetuximab in certain types of cancer, including prostate cancer and non-small cell lung cancer (NSCLC) (4-19). This opens up the possibility of increasing the efficiency of treatment allocation based on the assessment of a patient's *K-ras* mutation status.

*K-ras* mutation is currently usually determined by tissue biopsy, which is invasive, costly and potentially subjective. Furthermore, it can be difficult to obtain suitable tumour samples for mutational analysis from all cancer patients. These concerns create a strong case for the development of an alternative method to detect mutant *K-ras* from more readily accessible patient samples than the tumour itself.

A non-invasive technique for the determination of the *K-ras* mutation status would offer several advantages, including greater speed and reduced cost. It would also be technically easier for the health care professional to perform, would cause less distress to the patient, could be performed without a tumour specimen, and would allow repeated assessments to be made for the same patient before and after anticancer treatments. Repeated tests after surgery could provide an early warning for recurrence of disease.

Several studies have investigated *K-ras* levels in non-tumour tissues as well as in the tumour tissue. We reviewed all reported studies investigating *K-ras* mutation status in the plasma, serum or urine in addition to the tumour to determine whether a blood or urine test for the presence of *K-ras* mutation would be a viable alternative to a tissue biopsy. If a correlation between tissue and non-tissue *K-ras* mutation could be shown, the possibility of a simple blood test to identify candidates for anticancer treatment with EGFR inhibitors comes a step closer.

## Study Identification and Selection

Relevant published trials were identified through a literature search of the PubMed database, conducted during the period July–December 2009. We limited the review to studies

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published in the English language. In order to identify studies which assessed circulating levels of the mutated *K-ras* gene in cancer patients, the combinations of key-words shown in Table I were used. Relevant articles not identified by this strategy, but referenced in the bibliographies of articles which were located in this way, were also included.

Table I. *Summary of search terms used in PubMed search.*

| Combinations of PubMed search terms used |
|---|
| *K-ras* + plasma + cancer |
| *K-ras* + serum + cancer |
| *K-ras* + peripheral blood + cancer |
| *K-ras* + urine + cancer |

## Results

A total of 11 studies were identified which investigated *K-ras* mutation status in the tumour tissue as well as the plasma or the serum in colorectal cancer and pancreatic cancer (Table II). No relevant studies were identified which assessed *K-ras* mutation status within the urine and tumour.

*Colorectal cancer.* Mulcahy *et al*. reported a study of 14 patients with colorectal cancer, in which the presence of *K-ras* mutations in the plasma DNA was assessed using polymerase chain reaction (PCR) techniques (20). Eleven healthy subjects were also included as controls. *K-ras* mutations were found in the tumour tissue of 7 out of the 14 colorectal cancer patients. Of these patients, 6 (86%) also displayed the same mutation in the DNA extracted from plasma samples. No mutations were detected in the plasma of patients whose primary tumour was negative for *K-ras* or among the 11 healthy controls.

In 2009, Lefebure *et al*. published the results of a study in which serum *K-ras* mutation and *RASSF2A* methylation status were assessed using real-time PCR performed in the presence of a peptide nucleic acid specific of the wild-type sequence in 29 patients presenting an unresectable metastatic colorectal cancer treated by chemotherapy (21). Among 12 patients presenting a *K-ras* mutation in their primary tumour, 7 (58%) presented the same mutation in the serum. Six patients were found to have no *K-ras* mutation detectable in the primary tumour, and absence of *K-ras* mutation in the serum was confirmed in all of these patients.

In a study conducted by Trevisiol *et al*., *K-ras* mutations were examined in DNA samples extracted from the serum of 86 patients with colorectal cancer and were compared with the *K-ras* status of their primary tumours. *K-ras* mutations were found in tissue samples of 28 patients (33%), of whom, 10 (36%) were also found to have serum *K-ras* mutations (22). Among the 58 patients whose primary tumours were negative for *K-ras* mutations, one had a *K-ras* mutation in the DNA serum sample. The authors suggest that this apparently discrepant case may be explained either by the presence of multiple intratumoural clones or multiple foci of tissue with mutated genes or by a sampling error of the tumour specimen.

Ryan *et al*. conducted a prospective study of 94 patients who underwent putative curative resection for colorectal carcinoma (23). *K-ras* mutations were analysed in matched tumour and serum samples. *K-ras* mutation was found in 41/78 (53%) tumours, of which 31 (76%) had an identical mutation detectable in the serum. A further serum mutant *K-ras*-positive result was found in 1 out of 37 (3%) tumour *K-ras* mutation-negative cases. Again, the authors suggest sampling error of the paraffin-embedded tumour specimen may account for the failure to detect a mutation within the tumour in this discrepant case. The authors in this study also reported that repeated post-surgical testing for serum *K-ras* mutation indicated that patients in whom a serum *K-ras* mutation could be detected after surgery had a highly significantly greater chance of cancer recurrence than patients with no detectable serum *K-ras* mutation during follow-up (odds ratio, 71.6; 95% confidence interval, 7.7-663.9; $p<0.0001$).

In 2009, Yen *et al*. published results of a study in which *K-ras* mutation status in the peripheral blood of 76 metastatic colorectal cancer patients receiving chemotherapy was analysed using membrane-arrays, and that in tumours was analysed by DNA sequencing (24). Among 76 patients with metastatic colorectal cancer, *K-ras* mutations were identified in the tumour of 33, of whom 28 also presented positive *K-ras* mutations in the peripheral blood. Two patients with no detectable *K-ras* in the tumour presented a positive *K-ras* mutation in the peripheral blood. A highly statistically significant correlation between *K-ras* mutations in the tumour and the peripheral blood was observed ($p<0.0001$).

Ryan *et al*. reported the results of a trial in which *K-ras* mutations at codon 12 were detected using an enriched PCR-restriction fragment length polymorphism (RFLP) technique in 123 patients with colorectal cancer (Dukes' stages A-D) or dysplastic colorectal adenoma (25). Among 76 patients evaluated prior to tumour resection, 49% had *K-ras* mutation in the primary tumour, with a serum mutant *K-ras* was detected in "almost all" of these patients (86% correlation). Among patients evaluated post-surgery, 62% were *K-ras* mutation-positive in the primary tumour, and serum mutant *K-ras* was detected in 29% of these patients. The exact number of patients presenting with *K-ras* mutations in both tumour and serum is not reported, nor whether any patients had a *K-ras* mutation in the serum but not in the tumour.

In a summary conducted by Sorenson of published results of assays used to detect mutated *Kras-2* sequences in

Table II. *Summary of studies in which K-ras mutation was assessed in the tumour tissue and in the plasma or serum.*

| Study | Cancer type | No. of patients* | Medium (method) of *K-ras* mutation detection | |
|---|---|---|---|---|
| Mulcahy *et al*. (20) | Colorectal cancer | 14 | Plasma (MASA-PCR) | Tumour tissue (MASA-PCR) |
| Lefebure *et al*. (21) | Unresectable or refractive metastatic colorectal cancer | 29 | Serum (PCR) | Tumour tissue (PCR) |
| Trevisiol *et al*. (22) | Colorectal cancer | 86 | Serum (ME-PCR) | Tumour tissue (RFLP-PCR) |
| Ryan *et al*. (23) | Colorectal neoplasia | 94 | Serum (PCR) | Tumour tissue (PCR) |
| Yen *et al*. (24) | Metastatic colorectal cancer | 76 | Peripheral blood (membrane array-based multi marker assay) | Tumour tissue (mRNA isolation and first-strand DNA synthesis) |
| Ryan *et al*. (25) | Colorectal or dysplastic colorectal adenoma | 123 | Plasma/serum (enriched PFLP-PCR) | Tumour tissue (enriched RFLP-PCR) |
| Sorensen *et al*. (26) | Colorectal cancer | 131 | Plasma/serum (NR) | Tumour tissue (NR) |
| Lecomte *et al*. (27) | Resected primary colorectal cancer | 58 | Plasma (MASA-PCR) | Tumour tissue (MASA-PCR) |
| Olsen *et al*. (28) | Unresectable pancreatic adenocarcinoma with no evidence of metastatic disease | 12 | Plasma (two-stage PCR) | Tumour tissue (two-stage PCR) |
| Mulcahy *et al*. (20) | Pancreatic cancer | 21 | Plasma (PCR, gel electrophoresis) | Tumour tissue (PCR, gel electrophoresis) |
| Sorenson *et al*. (26) | Pancreatic cancer | 144 | Plasma/serum (NR) | Tumour tissue (NR) |

*Total number of patients in the study. The number valid for inclusion in this review may be lower in some cases. PCR: Polymerase chain reaction; MASA: mutant allele-specific amplification; ME-PCR: mutant-enriched PCR; RFLP-PCR: restriction fragment length polymorphism PCR; NR: not reported.

plasma/serum samples from patients with colorectal cancer, 131 suitable patients investigated by five different groups were identified (26). Forty-four of these patients had tumours that demonstrated a *K-ras* mutation, 55% of whom also presented positive plasma/serum assay for mutated *K-ras*. The presence of any positive plasma/serum *K-ras* mutation in the absence of a corresponding tumour mutation is not reported.

Lecomte *et al*. conducted a study of the prognostic value of free-circulating tumour-associated DNA in the plasma of colorectal cancer patients (27). In this study, patients with a tumour presenting *K-ras* mutations, as detected by the mutant allele-specific amplification method, were selected for plasma screening. Of the 58 tumours analysed, 22 (38%) were mutated at *K-ras*, and an identical alteration was detected in 10 (45%) out of the 22 corresponding plasma samples. It is not reported whether any serum samples positive for *K-ras* mutation were associated with an absence of *K-ras* mutation in the tumour.

*Pancreatic cancer.* In 2009, Olsen *et al*. published the results of a phase I trial in 12 patients with locally advanced pancreatic carcinoma treated with gefitinib, paclitaxel and three-dimensional conformal radiation. The authors reported the detection of plasma *K-ras* mutations using a two-stage RFLP-PCR assay on patients' plasma both before and after therapy (28). Mutations were confirmed by direct sequencing. *K-ras* mutations were detected in the pre-gefitinib plasma of 5/11 patients and in the matched tumour tissue of 3/4 patients.

In a study of 21 patients with pancreatic cancer reported by Mulcahy *et al*., the presence of *K-ras* mutations in the plasma DNA was assessed using PCR (20). Biopsy tissues

Table III. *Summary of K-ras mutation status in the tumour and plasma/serum of studies in this review.*

| Study | Methodology | *K-ras* mutation | | |
|---|---|---|---|---|
| | | Plasma/ serum | Tumour | |
| | | | Positive | Negative |
| Colorectal cancer | | | | |
| Mulcahy *et al*. (20) | MASA-PCR | Positive | 6 | 0 |
| | | Negative | 1 | 7 |
| Lefebure *et al*. (21) | PCR | Positive | 7 | 0 |
| | | Negative | 5 | 6 |
| Trevisiol *et al*. (22) | ME-PCR/ RFLP-PCR | Positive | 10 | 1 |
| | | Negative | 14 | 57 |
| Ryan *et al*. (23) | PCR | Positive | 31 | 1 |
| | | Negative | 10 | 36 |
| Yen *et al*. (24) | Multi marker assay/ mRNA isolation & 1st-strand DNA synthesis | Positive | 28 | 2 |
| | | Negative | 5 | 41 |
| Ryan *et al*. (25) | Enriched RFLP-PCR | Positive | NR* | NR* |
| | | Negative | NR* | NR* |
| Sorensen *et al*. (26) | NR | Positive | 24 | NR |
| | | Negative | 20 | NR |
| Lecomte *et al*. (27) | MASA-PCR | Positive | 10 | NR |
| | | Negative | 12 | NR |
| Pacreatic cancer | | | | |
| Olsen *et al*. (28) | Two-stage PCR | Positive | 3 | 1 |
| | | Negative | NR | NR |
| Mulcahy *et al*. (20) | PCR, gel electrophoresis | Positive | x** | 0 |
| | | Negative | 0 | y** |
| Sorenson *et al*. (26) | NR | Positive | 40 | NR |
| | | Negative | 39 | NR |

PCR: Polymerase chain reaction; MASA: mutant allele-specific amplification; RFLP-PCR: restriction fragment length polymorphism PCR; ME-PCR: mutant-enriched PCR; NR: not reported; *86% correlation reported; **x+y=10.

were available for 10 patients, and plasma and tumour DNA alterations were reported to correspond in every case.

In a summary of results of the detection of mutated *K-ras* sequences in the plasma/serum of 144 patients with pancreatic carcinoma published by Sorenson (26), the tumour tissue in 79 patients was found to contain mutated *K-ras* gene. Of these patients, 51% were also found to have a positive assay in the plasma or serum for *K-ras* mutation.

## Discussion

A series of 11 studies was identified in which *K-ras* mutation status was assessed in the tumour tissue, as well as the plasma or serum of cancer patients. No studies were identified in which *K-ras* mutation status in the urine was assessed. In each of the studies identified, patients already had a confirmed diagnosis of cancer (colorectal or pancreatic). Across all studies, the proportion of patients with a positive *K-ras* mutation in the tumour who also had a positive *K-ras* mutation in the serum or plasma ranged from 29% to 100%. While these figures indicate that the absence of a *K-ras* mutation in the plasma or serum does not prove absence of the mutation in the tumour, it is interesting to investigate whether a positive *K-ras* mutation in the plasma or serum can predict a tumour *K-ras* mutation. A summary of the findings relating to the presence of absence of *K-ras* mutations in the tumour and plasma/serum in the 11 papers discussed in this review is given in Table III. From this table we can see that a *K-ras* mutation was observed in the plasma or serum of just 5 patients where it was not found to exist in the tumour, out of a minimum total of 272 patients examined. Of necessity, this calculation excludes those studies in which the plasma/serum *K-ras* mutation status was not investigated in patients previously identified as having no tumour *K-ras* mutation.

The very small number of patients presenting with a plasma or serum *K-ras* mutation in the absence of a similar tumour mutation may potentially be explained by sampling errors, as acknowledged by the authors themselves (22, 23). Indeed, the very rationale for this review was to seek alternatives to the currently clinically impractical and unreliable methods for the detection of *K-ras* mutation in tumour tissue.

These findings allow us to tentatively draw the conclusion that the presence of a plasma or serum *K-ras* mutation strongly indicates a tumour *K-ras* mutation, while the absence of a plasma or serum *K-ras* mutation does not necessarily prove the absence of a tumour *K-ras* mutation. In other words, a positive blood test for *K-ras* mutation would strongly suggest *K-ras* mutation was present in the tumour, while a negative blood test would require further investigation. This knowledge opens the possibility of a blood test in which a positive result for *K-ras* mutation would preclude treatment with EGFR inhibitors, while a negative blood test would require further investigation. While not definitive in itself, the development of such a test could have important implications for the practical management of cancer cases according to their response to treatment.

One study investigated in this review also showed that postoperatively, *K-ras* mutation is a strong predictor of disease recurrence, stronger even than Dukes' stage of disease (23). Thus, repeated testing for *K-ras* mutation *via* a blood test in postoperative patients may prove an inexpensive and convenient method for the early detection of disease recurrence. Clearly, such a test as this is not possible through tumour tissue sampling.

The number of studies available for this review is clearly relatively small and interpretation of the findings should be made with caution. Moreover, due to the diverse nature of the studies, no statistical analysis is possible. Because of the potential value of these findings in the practical management of cancer patients, a larger dedicated, prospective trial should ideally be instigated to confirm the findings discussed in this review.

## Conclusion

From a review of studies in which the *K-ras* mutation status was assessed in both the tumour tissue and the plasma or serum, it is concluded that a positive *K-ras* mutation in the plasma or serum suggests a *K-ras* mutation in the tumour, while the absence of *K-ras* mutation in the plasma or serum does not necessarily prove a lack of similar mutation in the tumour tissue. This finding could open the way for a clinically useful blood test for the detection of tumour *K-ras* and the targeting of chemotherapeutical agents. Future trials are also needed to determine the optimal method of testing for *K-ras* mutations in the blood.

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