KRAS mutation testing of tumours in adults with metastatic colorectal cancer: a systematic review and cost-effectiveness analysis

Marie Westwood, Thea van Asselt, Bram Ramaekers, Penny Whiting, Manuela Joore, Nigel Armstrong, Caro Noake, Janine Ross, Johan Severens and Jos Kleijnen

NHS

National Institute for Health Research

KRAS mutation testing of tumours in adults with metastatic colorectal cancer: a systematic review and cost-effectiveness analysis

Marie Westwood,[1]* Thea van Asselt,[2]
Bram Ramaekers,[2] Penny Whiting,[1] Manuela Joore,[2]
Nigel Armstrong,[1] Caro Noake,[1] Janine Ross,[1]
Johan Severens[3] and Jos Kleijnen[4]

[1]Kleijnen Systematic Reviews Ltd, York, UK
[2]Department of Clinical Epidemiology and Medical Technology Assessment,
  Maastricht University Medical Centre, Maastricht, the Netherlands
[3]Institute of Health Policy and Management, Erasmus University, Rotterdam,
  the Netherlands
[4]School for Public Health and Primary Care (CAPHRI), Maastricht University,
  Maastricht, the Netherlands

*Corresponding author

Declared competing interests of authors: none

Health Technology Assessment

Criteria for inclusion in the *Health Technology Assessment* journal
Reports are published in *Health Technology Assessment* (HTA) if (1) they have resulted from work for the HTA programme, and (2) they are of a sufficiently high scientific quality as assessed by the reviewers and editors.

Reviews in *Health Technology Assessment* are termed 'systematic' when the account of the search appraisal and synthesis methods (to minimise biases and random errors) would, in theory, permit the replication of the review by others.

HTA programme
The HTA programme, part of the National Institute for Health Research (NIHR), was set up in 1993. It produces high-quality research information on the effectiveness, costs and broader impact of health technologies for those who use, manage and provide care in the NHS. 'Health technologies' are broadly defined as all interventions used to promote health, prevent and treat disease, and improve rehabilitation and long-term care.

The journal is indexed in NHS Evidence via its abstracts included in MEDLINE and its Technology Assessment Reports inform National Institute for Health and Care Excellence (NICE) guidance. HTA research is also an important source of evidence for National Screening Committee (NSC) policy decisions.

For more information about the HTA programme please visit the website: www.hta.ac.uk/

This report

Abstract

KRAS mutation testing of tumours in adults with metastatic colorectal cancer: a systematic review and cost-effectiveness analysis

Marie Westwood,[1]* Thea van Asselt,[2] Bram Ramaekers,[2]
Penny Whiting,[1] Manuela Joore,[2] Nigel Armstrong,[1] Caro Noake,[1]
Janine Ross,[1] Johan Severens[3] and Jos Kleijnen[4]

[1]Kleijnen Systematic Reviews Ltd, York, UK
[2]Department of Clinical Epidemiology and Medical Technology Assessment, Maastricht University Medical Centre, Maastricht, the Netherlands
[3]Institute of Health Policy and Management, Erasmus University, Rotterdam, the Netherlands
[4]School for Public Health and Primary Care (CAPHRI), Maastricht University, Maastricht, the Netherlands

*Corresponding author

Background: Bowel cancer is the third most common cancer in the UK. Most bowel cancers are initially treated with surgery, but around 17% spread to the liver. When this happens, sometimes the liver tumour can be treated surgically, or chemotherapy may be used to shrink the tumour to make surgery possible. Kirsten rat sarcoma viral oncogene (*KRAS*) mutations make some tumours less responsive to treatment with biological therapies such as cetuximab. There are a variety of tests available to detect these mutations. These vary in the specific mutations that they detect, the amount of mutation they detect, the amount of tumour cells needed, the time to give a result, the error rate and cost.

Objectives: To compare the performance and cost-effectiveness of *KRAS* mutation tests in differentiating adults with metastatic colorectal cancer whose metastases are confined to the liver and are unresectable and who may benefit from first-line treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone.

Data sources: Thirteen databases, including MEDLINE and EMBASE, research registers and conference proceedings were searched to January 2013. Additional data were obtained from an online survey of laboratories participating in the UK National External Quality Assurance Scheme pilot for *KRAS* mutation testing.

Methods: A systematic review of the evidence was carried out using standard methods. Randomised controlled trials were assessed for quality using the Cochrane risk of bias tool. Diagnostic accuracy studies were assessed using the QUADAS-2 tool. There were insufficient data for meta-analysis. For accuracy studies we calculated sensitivity and specificity together with 95% confidence intervals (CIs). Survival data were summarised as hazard ratios and tumour response data were summarised as relative risks, with 95% CIs. The health economic analysis considered the long-term costs and quality-adjusted life-years associated with different tests followed by treatment with standard chemotherapy or cetuximab plus standard chemotherapy. The analysis took a 'no comparator' approach, which implies that the cost-effectiveness of each strategy will be presented only compared with the next most cost-effective strategy. The de novo model consisted of a decision tree and Markov model.

Results: The online survey indicated no differences between tests in batch size, turnaround time, number of failed samples or cost. The literature searches identified 7903 references, of which seven publications of five studies were included in the review. Two studies provided data on the accuracy of *KRAS* mutation testing for predicting response to treatment in patients treated with cetuximab plus standard chemotherapy. Four RCTs provided data on the clinical effectiveness of cetuximab plus standard chemotherapy compared with that of standard chemotherapy in patients with *KRAS* wild-type tumours. There were no clear differences in the treatment effects reported by different studies, regardless of which *KRAS* mutation test was used to select patients. In the 'linked evidence' analysis the Therascreen® *KRAS* RGQ PCR Kit (QIAGEN) was more expensive but also more effective than pyrosequencing or direct sequencing, with an incremental cost-effectiveness ratio of £17,019 per quality-adjusted life-year gained. In the 'assumption of equal prognostic value' analysis the total costs associated with the various testing strategies were similar.

Limitations: The results assume that the differences in outcomes between the trials were solely the result of the different mutation tests used to distinguish between patients; this assumption ignores other factors that might explain this variation.

Conclusions: There was no strong evidence that any one *KRAS* mutation test was more effective or cost-effective than any other test.

Study registration: PROSPERO CRD42013003663.

Funding: The National Institute for Health Research Health Technology Assessment programme.

Contents

List of tables

List of figures

Glossary

Cost-effectiveness analysis An economic analysis that converts effects into health terms and describes the costs for additional health gain.

Decision modelling A theoretical construct that allows the comparison of the relationship between costs and outcomes of alternative health-care interventions.

False negative Incorrect negative test result – number of diseased persons with a negative test result.

False positive Incorrect positive test result – number of non-diseased persons with a positive test result.

Incremental cost-effectiveness ratio The difference in the mean costs of two interventions in the population of interest divided by the difference in the mean outcomes in the population of interest.

Index test The test thats performance is being evaluated.

Markov model An analytic method particularly suited to modelling repeated events or the progression of a chronic disease over time.

Meta-analysis Statistical techniques used to combine the results of two or more studies and obtain a combined estimate of effect.

Meta-regression Statistical technique used to explore the relationship between study characteristics and study results.

Metastasis The spread of a disease from one organ or part to another, non-adjacent organ or part.

Opportunity costs The cost of forgone outcomes that could have been achieved through alternative investments.

Publication bias Bias arising from the preferential publication of studies with statistically significant results.

Quality-adjusted life-year A measure of health gain, used in economic evaluations, in which survival duration is weighted or adjusted by the patient's quality of life during the survival period.

Quality of life An individual's emotional, social and physical well-being and his or her ability to perform the ordinary tasks of living.

R0 No residual tumour.

R1 Microscopic residual tumour.

R2 Macroscopic residual tumour.

Receiver operating characteristic curve A graph which illustrates the trade-offs between sensitivity and specificity that result from varying the diagnostic threshold.

Reference standard The best currently available diagnostic test against which the index test is compared.

Sensitivity Proportion of people with the target disorder who have a positive test result.

Specificity Proportion of people without the target disorder who have a negative test result.

True negative Correct negative test result – number of non-diseased persons with a negative test result.

True positive Correct positive test result – number of diseased persons with a positive test result.

List of abbreviations

ARMS	amplification-refractory mutation system		HTA	Health Technology Assessment
ASCO	American Society of Clinical Oncology		ICER	incremental cost-effectiveness ratio
BRAF	v-raf murine sarcoma viral oncogene homolog B		IQR	interquartile range
			ITT	intention to treat
BSC	best supportive care		*KRAS*	Kirsten rat sarcoma viral oncogene
CEAC	cost-effectiveness acceptability curve		LY	life-year
CI	confidence interval		MALDI-TOF	matrix-assisted laser desorption ionisation time-of-flight
CR	complete response		mCRC	metastatic colorectal cancer
CRC	colorectal cancer		MDT	multidisciplinary team
CRD	Centre for Reviews and Dissemination		MRC	Medical Research Council
			MRI	magnetic resonance imaging
CT	computed tomography		NCIC CO.17	National Cancer Institute of Canada Clinical Trials Group CO.17
DNA	deoxyribonucleic acid			
ECOG	Eastern Cooperative Oncology Group		NEQAS	National External Quality Assurance Scheme
EGFR	epidermal growth factor receptor		NICE	National Institute for Health and Care Excellence
ESMO	European Society for Medical Oncology			
			OR	odds ratio
FDA	Food and Drug Administration		ORR	objective response rate
FFPE	formalin fixed paraffin embedded		OS	overall survival
FN	false negative		PCO	Provisional Clinical Opinion
FOLFIRI	irinotecan in combination with infusional fluorouracil plus folinic acid		PCR	polymerase chain reaction
			PD	progressive disease
			PFS	progression-free survival
FOLFOX	oxaliplatin in combination with infusional fluorouracil plus folinic acid		PR	partial response
			PRESS-EBC	Peer Review of Electronic Search Strategies Evidence-Based Checklist
FP	false positive		PSA	probabilistic sensitivity analysis
GERCOR	Groupe Coopérateur Multidisciplinaire en Oncologie		QALY	quality-adjusted life-year
			RCT	randomised controlled trial
HR	hazard ratio		RECIST	Response Evaluation Criteria in Solid Tumours
HRM	high-resolution melt (analysis)			

SD	stable disease	WHO	World Health Organization
TN	true negative	XELOX	oxaliplatin and capecitabine
TP	true positive		

Note

This monograph is based on the Technology Assessment Report produced for the National Institute for Health and Care Excellence (NICE). The full report contained a considerable number of data that were deemed commercial-in-confidence. The full report was used by the Appraisal Committee at NICE in their deliberations. The full report with each piece of commercial-in-confidence data removed and replaced by the statement 'commercial-in-confidence information (or data) removed' is available on the NICE website: www.nice.org.uk.

The present monograph presents as full a version of the report as is possible while retaining readability, but some sections, sentences, tables and figures have been removed. Readers should bear in mind that the discussion, conclusions and implications for practice and research are based on all the data considered in the original full NICE report.

Plain English summary

Bowel cancer is the third most common cancer in the UK, accounting for 13% of new cancer cases and around 10% of all cancer deaths. Around three-quarters of bowel cancers are initially treated with surgery, but around one in six will go on to spread to the liver. When this happens the cancer in the liver can sometimes be treated by further surgery, or, when surgery is not initially possible, chemotherapy may be used with the aim of shrinking the tumour to make surgery possible.

Tumours with mutations in a growth factor [Kirsten rat sarcoma viral oncogene (*KRAS*)] are less responsive to treatment with biological therapies, such as cetuximab. Before deciding on treatment options, patients are tested to see if their tumour has a mutation in the *KRAS* gene. There are a variety of tests available and different tests vary in the specific mutations that they attempt to detect, the amount of mutation they are able to detect, the amount of tumour cells needed for the test to work, the time that it takes to give a result, the error rate of the test and the cost of the test.

This project aimed to evaluate *KRAS* mutation tests to determine which should be recommended for use in the NHS in England and Wales. A survey of UK laboratories undertaking *KRAS* mutation testing, a systematic review of the literature and economic modelling found that there was no strong evidence that any one *KRAS* mutation test had greater accuracy, or was more cost-effective, than any other test.

Scientific summary

Background

Bowel cancer is the third most common cancer in the UK, accounting for 13% of new cancer cases and around 10% of all cancer deaths. The likelihood of surviving 1 year after diagnosis is around 73% and of surviving 5 years is around 55%. Most bowel cancers are initially treated with surgery, but around one in six will spread to the liver. When this happens the cancer in the liver can sometimes be treated by further surgery or, when surgery is not initially possible, chemotherapy may be used with the aim of shrinking the tumour to make surgery possible. Kirsten rat sarcoma viral oncogene (*KRAS*) mutations make some tumours less responsive to treatment with biological therapies, such as cetuximab. There are a variety of tests available to detect these mutations. These vary in the specific mutations that they detect, the amount of mutation they detect, the amount of tumour cells needed, the time to give a result, the error rate and cost.

Objectives

To compare the performance and cost-effectiveness of *KRAS* mutation tests (commercial or in-house) in differentiating adults with metastatic colorectal cancer (mCRC) whose metastases are confined to the liver and are unresectable and who may benefit from first-line treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone.

Methods

Assessment of clinical effectiveness

Thirteen databases, including MEDLINE and EMBASE, research registers and conference proceedings were searched from to January 2013. A web-based survey of UK laboratories gathered data on the technical performance of *KRAS* mutation tests. The systematic review included studies of tumour *KRAS* mutation testing in adults with colorectal cancer (CRC) and unresectable, liver-limited metastases. Eligible study designs were randomised controlled trials (RCTs)/controlled clinical trials comparing cetuximab plus standard chemotherapy with standard chemotherapy in participants with known tumour *KRAS* mutation status, and studies providing data on the accuracy of *KRAS* mutation testing to predict tumour response to cetuximab plus standard chemotherapy. Search results were screened for relevance independently by two reviewers. Full-text inclusion assessment, data extraction and quality assessment were conducted by one reviewer and checked by a second. RCTs were assessed for quality using the Cochrane risk of bias tool. Diagnostic accuracy studies were assessed using the QUADAS-2 tool. There were insufficient data for meta-analysis. For accuracy studies we calculated sensitivity and specificity together with 95% confidence intervals (CIs). Survival data were summarised as hazard ratios and tumour response data as relative risks with 95% CIs.

Assessment of cost-effectiveness

We considered the long-term costs and quality-adjusted life-years (QALY) associated with different tests followed by treatment with either standard chemotherapy or cetuximab plus standard chemotherapy. The analysis took a 'no comparator' approach, which implies that the cost-effectiveness of each strategy will be presented only compared with that of the next most cost-effective strategy. The de novo model consisted of a decision tree and a Markov model. The decision tree was used to model the test result (wild type, mutant or unknown) and the treatment decision. Patients with a *KRAS* wild-type test result received cetuximab plus standard chemotherapy; patients with a *KRAS* mutant or unknown

test result received standard chemotherapy. The long-term consequences in terms of costs and QALYs were estimated using a Markov model with a cycle time of 1 week and a lifetime time horizon (23 years). Health states in the Markov model were:

1. progression-free first line – never operated
2. progressive disease second line – never operated
3. progressive disease second line – unsuccessful resection
4. survival after curative resection
5. progression-free first line – unsuccessful resection
6. progressive disease third line – never operated
7. progressive disease third line – unsuccessful resection
8. dead.

We presented two analyses: 'linked evidence', including only tests for which data on test accuracy were available, and 'assumption of equal prognostic value', including all tests for which information on technical performance was available. In the linked evidence analysis, test accuracy and resection rates were test specific. Probabilities for (progression-free) survival were assumed to depend on the health state that a patient is in (e.g. survival after successful resection) and did not differ between the tests (test independent). In the assumption of equal prognostic value analysis, tests were assumed to differ solely by technical performance (i.e. proportion of failed tests), retrieved from the online survey of NHS laboratories in England and Wales. All other parameters were assumed to be equal.

Results

Five studies (seven publications) were included in the review.

What are the technical performance characteristics of the different KRAS *mutation tests?*

No studies assessed the technical performance of *KRAS* mutation tests. Fifteen UK-based laboratories completed the online questionnaire (response rate 50%). Pyrosequencing, using in-house methods, was the most commonly used test (nine laboratories) followed by the cobas® *KRAS* Mutation Test (Roche Molecular Systems, Branchburg, NJ, USA) (three laboratories). Sanger sequencing was used by two laboratories, one laboratory used the Therascreen® *KRAS* Pyro Kit (QIAGEN, Hilden, Germany) and one used high-resolution melt analysis (HRM) and direct sequencing. More than half of the responding laboratories reported that *KRAS* mutation testing was carried out on request (e.g. from a pathologist or oncologist); only one laboratory reported routine testing of all CRC samples. There were no clear differences between tests in terms of batch size, turnaround time, number of failed samples or test cost. With the exception of those using Sanger sequencing, all laboratories reported a limit of detection for percentage mutation of $\leq 10\%$.

What is the accuracy of KRAS *mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?*

Two studies provided data on the accuracy of *KRAS* mutation testing for predicting response to treatment in patients treated with cetuximab plus standard chemotherapy. The sensitivity and specificity estimates for the Therascreen kit for predicting objective response were 74.6% (95% CI 62.1% to 84.5%) and 35.5% (95% CI 19.2% to 54.6%) respectively. Estimates for pyrosequencing and matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry for predicting potentially curative resection following treatment were 52.0% (95% CI 31.3 to 72.2%) and 45.6% (95% CI 37.0 to 54.3%) respectively.

How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?

Four RCTs provided data on the clinical effectiveness of cetuximab plus standard chemotherapy compared with that of standard chemotherapy. Two trials used the LightMix® k-ras Gly12 assay (TIB MOLBIOL, Berlin, Germany), one used pyrosequencing together with MALDI-TOF mass spectrometry and one used pyrosequencing alone.

All studies reported improvements in objective response rate for patients with *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy compared with those treated with standard chemotherapy. There were no clear differences in the treatment effects reported by different studies, regardless of which *KRAS* mutation test was used to select patients.

What is the cost-effectiveness of the use of different KRAS *mutation tests to decide between standard chemotherapy or cetuximab plus standard chemotherapy?*

Linked evidence analysis

The linked evidence analysis included two tests, that is, only those tests for which evidence on test accuracy for prediction of either resection rate or objective response was available. We have data from the COIN and CELIM trials only; the COIN trial used pyrosequencing to test for *KRAS* mutations and the CELIM trial used an earlier version of the Therascreen *KRAS* RGQ PCR Kit. We assumed that the differences between the outcomes of these trials were exclusively caused by the different tests used. In addition, we assumed that all patients with *KRAS* wild-type tumours respond perfectly to cetuximab – or will all have a liver resection after cetuximab – and all patients with *KRAS* mutant tumours do not, and also that test accuracy based on objective response can be compared with accuracy based on resection rates.

Pyrosequencing results in the lowest total cost. The Therascreen *KRAS* RGQ PCR Kit is the more expensive but also more effective strategy, with an incremental cost-effectiveness ratio (ICER) of £17,019 per QALY gained. The cost-effectiveness acceptability curve (CEAC) indicates that, for lower values of the threshold, pyrosequencing is preferred and that at thresholds of ≥ £17,000 the Therascreen *KRAS* RGQ PCR Kit is the most cost-effective option. The results of the sensitivity analyses do not differ substantially from the base-case results in the sense that the Therascreen *KRAS* RGQ PCR Kit is consistently more expensive and more effective than pyrosequencing, with ICERs ranging from £14,860 to £20,528 per QALY gained.

Assumption of equal prognostic value analysis

The analysis based on the assumption of equal prognostic value included all tests for which information on technical performance was available from the online survey of NHS laboratories in England and Wales. This included the tests for which accuracy data, based on either objective response or resection rates, were not available. Therefore, this analysis assessed whether the tests were likely to be cost-effective given an assumption of equal prognostic value based on testing with pyrosequencing (as this was the only test for which full data were available on resection rates following treatment with chemotherapy, with and without cetuximab, for patients with initially inoperable liver metastases and both *KRAS* mutant and *KRAS* wild-type tumours) and test-specific information on technical failures within the laboratory only. In the base case and in the first sensitivity analysis, the total technical failure rate (pre-laboratory plus within-laboratory technical failures) is assumed to be equal for all tests. As a result, the strategies in these analyses differ only with respect to costs. In the base case the average QALYs for all comparators are 1.483. The total costs associated with the various testing strategies are very similar. The same applies to the first sensitivity analysis: costs are similar across strategies and average QALYs are equal by assumption at 1.278 (95% CI 1.115 to 1.446).

The second sensitivity analysis assumed that all of the technical failures that occurred were test specific. All other input parameters, such as test costs and test accuracy, were still considered equal. For this sensitivity analysis the cobas *KRAS* Mutation Test is the least costly and least effective strategy. The HRM analysis and Sanger sequencing have equal costs and effects and their ICER compared with the cobas *KRAS* Mutation Test is £69,815 per QALY gained. Pyrosequencing and the Therascreen *KRAS* RGQ PCR Kit are ruled out by extended dominance. From the CEAC it is apparent that the cobas *KRAS* Mutation Test is the preferred strategy for all threshold values of < £60,000.

Conclusions

Implications for service provision

There was no strong evidence that any one method of *KRAS* mutation testing had greater accuracy than any other for predicting tumour response or potentially curative resection, following treatment with cetuximab plus standard chemotherapy, in patients with mCRC whose metastases were limited to the liver and were unresectable before chemotherapy. The clinical effectiveness of cetuximab plus standard chemotherapy, in patients whose tumours are *KRAS* wild type, did not appear to vary according to which method was used to determine tumour *KRAS* mutation status.

The results of the linked evidence analysis indicated that the Therascreen *KRAS* RGQ PCR Kit was more costly and more effective than pyrosequencing with an ICER of £17,019 per QALY gained; sensitivity analyses did not show substantial differences compared with the base case. The results of the second sensitivity analysis for the equal prognostic value analysis (including all tests for which information on technical performance was available from the online survey of NHS laboratories in England and Wales) indicated that the cobas *KRAS* Mutation Test is the least expensive and least effective strategy. It should be noted that substantial assumptions were necessary to arrive at the economic results, in particular the assumption that the differences in resection rates observed between the different studies are solely due to the different tests used. This ignores all other factors that can explain variations in outcomes between the studies. Therefore, these outcomes of the assessment of cost-effectiveness should be interpreted with extreme caution.

Suggested research priorities

Retesting of stored samples from previous studies for which patient outcomes are already known could be used to provide information on the relative effectiveness of cetuximab plus standard chemotherapy and standard chemotherapy alone in patients with *KRAS* wild-type and *KRAS* mutant tumours, with mutation status determined using testing methods for which adequate data are currently unavailable. Should quantitative testing become part of routine practice, longitudinal follow-up studies relating the level of mutation and/or the presence of rarer mutations to patient outcomes would become possible. Studies of this type could help to assess which features of *KRAS* mutation tests are likely to be important in determining their clinical effectiveness. As the uncertainties associated with clinical effectiveness forced the major assumptions in the economic evaluation, this type of research would also facilitate economic analyses of *KRAS* mutation testing.

Study registration

This study is registered as PROSPERO CRD42013003663.

Funding

Chapter 1 Objective

The overall objective of this project was to summarise the evidence on the clinical effectiveness and cost-effectiveness of Kirsten rat sarcoma viral oncogene (KRAS) mutation tests (commercial or in-house) for the differentiation of adults with metastatic colorectal cancer (mCRC) whose metastases are confined to the liver and are unresectable and who may benefit from first-line treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone, as recommended in the National Institute for Health and Care Excellence (NICE) technology appraisal TA176.[1] To address clinical effectiveness, data on the clinical validity of the different KRAS mutation tests (sensitivity/specificity for detection of mutations known to be linked to insensitivity to cetuximab) are required. Because methods of testing KRAS mutation status differ in terms of both the mutations targeted and the limit of detection (the lowest proportion of tumour cells with a mutation that can be detected), the definitions of KRAS mutant and KRAS wild type vary according to which test is used. All testing methods are essentially reference standard methods for classifying mutation status, as defined by the specific test characteristics, and it is therefore not useful to select any particular test as the reference standard. In addition, the relationship between insensitivity to cetuximab and the presence of specific mutations or combinations of mutations, as well as the relationship between insensitivity to cetuximab and the level of mutation present, are uncertain. Therefore, the following research questions were formulated to address the review objectives:

1. What is the technical performance of the different KRAS mutation tests {e.g. proportion of tumour cells needed, limit of detection [minimum percentage mutation detectable against a background of wild-type deoxyribonucleic acid (DNA)], failures, costs, turnaround time}?
2. What is the accuracy (clinical validity) of KRAS mutation testing, using any test, for predicting response to treatment with cetuximab in combination with standard chemotherapy?
3. How do clinical outcomes from treatment with cetuximab in combination with standard chemotherapy and, when reported, from treatment with standard chemotherapy alone vary according to which test is used to select patients for treatment?
4. What is the cost-effectiveness of the use of the different KRAS mutation tests to decide between standard chemotherapy or cetuximab in combination with standard chemotherapy?

First-line chemotherapy of unresectable colorectal liver metastases seeks to achieve a tumour response such that the tumour is judged to be resectable. For this reason, resection rate is considered the ideal reference standard for question 2 and the optimal outcome measure for question 3.

Chapter 2　Background and definition of the decision problem

Population

The indication for this assessment is the detection of mutations in the *KRAS* oncogene in adults with mCRC, in whom metastases are confined to the liver and are unresectable. The presence or absence of *KRAS* mutations can affect the choice of first-line chemotherapy in these patients and mutation testing is used to direct the treatment pathway.[1]

The 2010 cancer registration data from the Office for National Statistics[2] showed that colorectal cancer (CRC) was the third most common cancer in both men and women, accounting for approximately 13% of all new cancer cases. The 2010 age-standardised incidence rate for CRC in England was 56.5 per 100,000 in men and 36.1 per 100,000 in women and this has remained constant for both sexes over the last 10 years.[2] In 2009 there were approximately 36,000 new cases of CRC recorded in England and Wales,[3] and in 2010 there were 14,691 recorded deaths from CRC in England and Wales, accounting for around 10% of all cancer deaths.[4] Age-standardised 5-year survival rates for CRC in England (2005–9) were 54.2% for men and 55.6% for women.[5] Approximately two-thirds of CRC cases (64% in 2009) are cancers of the colon and one-third (36%) are rectal (including the anus). Most (60%) rectal cancer cases occur in men and colon cancer cases are evenly distributed between the sexes.[3] CRC incidence is strongly related to age, with incidence rates increasing from age 50 years and peaking in the over 80s; in the UK (2007–9), 72% of new cases were diagnosed in people aged > 65 years.[3] There is some evidence in UK men of an association between incidence of CRC and deprivation; 2000–4 data show incidence rates approximately 11% higher for men living in more deprived areas than for men living in the least deprived areas.[6] National Bowel Cancer Audit data for 2011 included 28,260 new cases in England and Wales of which 21,306 (75.4%) were surgically treated; 3425 (16.1%) of these had confirmed liver metastases.[7] Reported estimates of the prevalence of *KRAS* mutations in codons 12 and 13 in the tumours of patients with mCRC range from 35% to 42%[8–10] and are similar (approximately 36%) when samples taken from metastases are considered separately.[8,9] The three most common mutations, G12D, G12V and G13D, account for approximately 75% of all *KRAS* mutations.[8] Because not all patients whose tumours are wild type for *KRAS* codons 12 and 13 respond to treatment with epidermal growth factor receptor (EGFR)-inhibiting monoclonal antibodies, the potential effects of mutations in codons 61 and 146 of *KRAS* have also been investigated. A US study,[11] which found *KRAS* codon 12 or 13 mutations in 900/2121 (42.4%) CRC patients, conducted further analysis of the 513 wild-type samples and found 19 additional mutations in *KRAS* codon 61 and 17 in *KRAS* codon 146; these additional mutations represent < 2% of the total study population.

Intervention technologies

There are a variety of tests available for *KRAS* mutation testing (*Table 1*) in NHS reference laboratories currently providing testing [laboratories participating in the UK National External Quality Assurance Scheme (NEQAS)]. The tests used can be broadly divided into two subgroups: mutation screening and targeted mutation detection. Mutation screening tests screen samples for all *KRAS* mutations (known and novel) whereas targeted tests analyse samples for specific known mutations. Successful mutation analysis is dependent on adequate sample quality and a sufficient quantity of tumour tissue in the sample. The sample requirements vary between test methods, with some (e.g. Sanger sequencing) requiring up to 25% tumour cells. The limit of detection (the percentage of mutation detectable in a tumour sample against a background of wild-type DNA) may also vary between different test methods, with some studies reporting

TABLE 1 Overview of *KRAS* mutation tests

Sequencing method	Targeted (mutations targeted)/screening test	Limits of detection (% mutation)	Number of laboratories using the method	
			NEQAS report[a]	Laboratory contact[b]
Commercial tests				
Therascreen® *KRAS* RGQ PCR Kit (QIAGEN, Hilden, Germany)	Targeted (7 mutations: six codon 12 and one codon 13)	0.77–6.43%	3	1
Therascreen® *KRAS* Pyro Kit (QIAGEN)	Targeted (12 mutations: six codon 12, one codon 13 and five codon 61)	1.0–3.5%		2
cobas® *KRAS* Mutation Test (Roche Molecular Systems, Branchburg, NJ, USA)	Targeted (19 mutations: six codon 12, six codon 13 and seven codon 61)	1.6–6.3% depending on mutation	4	4
KRAS LightMix® kit (TIB MOLBIOL, Berlin, Germany)	Targeted (9 mutations: seven codon 12 and two codon 13)	Unclear	0	0
KRAS StripAssay® (ViennaLab, Vienna, Austria)	Targeted (13 mutations: eight codon 12, two codon 13 and three codon 61)	Unclear	0	0
In-house tests				
Sanger sequencing	All mutations within specific codons of the *KRAS* gene	Unclear	6	1
Pyrosequencing	All mutations within specific codons of the *KRAS* gene	5–10%[b]	15	8
Real-time PCR	Targeted (details unclear)	Unclear	2	0
High-resolution melt analysis	All mutations within specific codons of the *KRAS* gene	~5%[b]	2	2
Next-generation sequencing	All mutations within specific codons of the *KRAS* gene	~5%[b]	0	0
MALDI-TOF mass spectrometry	All mutations within selected codons in the *KRAS* oncogene	~10%	1	0

MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; PCR, polymerase chain reaction.
a   NEQAS pilot scheme 2012–13, run 2.[12] Thirty UK-based laboratories participated in the scheme; some laboratories used more than one method.
b   NICE contact with laboratories October/November 2012. Fifteen laboratories provided information on methodologies used. Laboratories using pryosequencing frequently stated that the cobas *KRAS* Mutation Test was used as an alternative for samples with low tumour content.

mutation detection at as little as 1% against a background of wild-type DNA (see *Table 1*). This is an important issue as it is unclear whether detecting diminishingly small proportions of mutation is clinically useful – should patients with very low proportions of mutation be treated as mutant or wild type? There is some evidence that the results of *KRAS* mutation testing in plasma samples correlate well with those obtained from tumour tissue.[13,14] However, tissue samples remain the gold standard. Clinical opinion, provided by specialist advisors during scoping, suggested that plasma testing is currently a 'research only' application that should not be included in this assessment.

A Provisional Clinical Opinion (PCO) from the American Society of Clinical Oncology (ASCO), published in 2009,[15] recommended universal *KRAS* mutation testing in patients with mCRC in whom treatment with EGFR inhibitors is being considered. The recommendation also stated that testing should be carried out in an accredited laboratory and that patients whose tumours have *KRAS* mutations in codons 12 or 13

should not be treated with EGFR inhibitors. At the time that this guidance was published there were no US Food and Drug Administration (FDA)-approved tests for *KRAS* mutations. The ASCO PCO specified that samples should be selected by a pathologist to include predominantly tumour cells without significant necrosis or inflammation; be freshly extracted or stored in an appropriate preservation solution or rapidly frozen; be neutral-buffered formalin fixed and paraffin embedded and the area of interest selected by the pathologist.[15] Acceptable assay types were listed as real-time polymerase chain reaction (PCR) using probes specific for the most common mutations in codons 12 and 13; direct sequencing of exon 1 in the *KRAS* gene; and the Therascreen commercial kit (at that time manufactured by DxS, Manchester, UK).[15] Subsequently, the QIAGEN Therascreen *KRAS* Rotar-Gene Q (RGQ) PCR Kit has been approved by the FDA when used with the QIAGEN QIAamp® DSP DNA FFPE (formalin fixed paraffin embedded) Tissue Kit and the QIAGEN Rotor-Gene® Q MDx (software version 2.1.0) and *KRAS* Assay Package.[16]

Targeted mutation detection tests

All targeted tests are commercial kits and these look for different numbers of mutations within specific codons of the *KRAS* gene and have differing limits of detection. They may therefore differ in their ability to accurately differentiate patients who are likely to benefit from treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone.

The Therascreen *KRAS* RGQ PCR Kit is a CE-marked real-time PCR assay for the qualitative detection of seven mutations in codons 12 and 13 of the *KRAS* gene. It has been approved by the US FDA for the application covered by this assessment, that is, the selection of patients with mCRC for treatment with cetuximab. The Therascreen *KRAS* RGQ PCR Kit uses two technologies for the detection of mutations: ARMS (amplification-refractory mutation system) for mutation-specific DNA amplification and Scorpions for detection of amplified regions. Scorpions are bifunctional molecules containing a PCR primer covalently linked to a fluorescently labelled probe. A real-time PCR instrument [Rotor-Gene Q 5-Plex HRM (high-resolution melt) for consistency with CE marking] is used to perform the amplification and to measure fluorescence.[17] There is an earlier version of the Therascreen *KRAS* PCR Kit that also uses ARMS and Scorpions for the detection of *KRAS* mutations and is designed to detect the same *KRAS* mutations as the current, reformulated and revalidated version. Evidence for both versions will be included in this assessment.

The Therascreen *KRAS* Pyro Kit is a CE-marked test for the quantitative measurement of 12 mutations in codons 12, 13 and 61 of the *KRAS* gene. The kit is based on pyrosequencing technology and consists of two assays, one for detecting mutations in codons 12 and 13 and a second for detecting mutations in codon 61. The two regions are amplified separately by PCR and then amplified DNA is immobilised on streptavidin sepharose high-performance beads. Single-stranded DNA is prepared and sequencing primers added. The samples are then analysed using the PyroMark® Q24 System (QIAGEN). The *KRAS* Plug-in Report is recommended by the manufacturer for the analysis of the results; however, the analysis tool within the pyrosequencer can also be used.[18]

The cobas *KRAS* Mutation Test from Roche Molecular Systems is a CE-marked TaqMelt™ real-time PCR assay intended for the detection of 19 mutations in codons 12, 13 and 61 of the *KRAS* gene. The assay uses DNA extracted from FFPE tissue and is validated for use with the cobas® 4800 System.

The *KRAS* LightMix Kit from TIB MOLBIOL is a CE-marked test designed for the detection and identification of mutations in codons 12 and 13 of the *KRAS* gene. The first part of the test involves PCR amplification of the *KRAS* gene. To reduce amplification of the wild-type *KRAS* gene and therefore enrich the mutant *KRAS* gene, a wild type-specific competitor molecule is added to the reaction mix. This is called clamped mutation analysis. The second part of the test procedure involves melting curve analysis with hybridisation probes. The melting temperature is dependent on the number of mismatches between the amplification product and the probe and allows the detection and identification of a mutation within the sample. The test is run on the LightCycler® instrument (Roche).[19]

The *KRAS* StripAssay from ViennaLab is a CE-marked test for the detection of mutations in the *KRAS* gene. The test procedure involves three steps: the DNA is first isolated from the specimen; PCR amplification is then performed; and the amplification product is then hybridised to a test strip containing allele-specific probes immobilised as an array of parallel lines. Colour substrates are used to detect bound sequences, which can then be identified with the naked eye or by using a scanner and software.[20] There are two versions of the *KRAS* StripAssay: one is designed to detect 10 mutations in codons 12 and 13 of the *KRAS* gene and the other is designed to detect the same 10 mutations in codons 12 and 13 plus three mutations in codon 61.

Mutation screening tests

'In-house' laboratory-based tests are designed to detect all mutations within specific codons of the *KRAS* gene.

Pyrosequencing assays are the most commonly used method of *KRAS* mutation testing in UK laboratories (see *Table 1*). The process involves first extracting DNA from the sample and amplifying it using PCR. The PCR product is then cleaned up before the pyrosequencing reaction. The reaction involves the sequential addition of nucleotides to the mixture. A series of enzymes incorporate nucleotides into the complementary DNA strand, generate light proportional to the number of nucleotides added and degrade unincorporated nucleotides. The DNA sequence is determined from the resulting pyrogram trace.[21]

Sanger sequencing is also a commonly used method (see *Table 1*); however, there is much variation in the detail of how the method is carried out. In general, after DNA is extracted from the sample it is amplified using PCR. The PCR product is then cleaned up and sequenced in both forward and reverse directions. The sequencing reaction uses dideoxynucleotides labelled with coloured dyes that randomly terminate DNA synthesis, creating DNA fragments of various lengths. The sequencing reaction product is then cleaned up and analysed using capillary electrophoresis. The raw data are analysed using analysis software to generate the DNA sequence. All steps are performed at least in duplicate to increase confidence that an identified mutation is real. It should be noted that sequencing works well only when viable tumour cells constitute at least 25% or more of the sample.[22]

National Institute for Health and Care Excellence contact with laboratories (October/November 2012) suggested that several laboratories were planning to convert to next-generation sequencing in the coming year. As with Sanger sequencing, there is much variation in the methodology used to perform next-generation sequencing. The concept is similar to Sanger sequencing; however, the sample DNA is first fragmented into a library of small segments that can be sequenced in parallel reactions.[23]

High-resolution melt analysis assays are also commonly used by laboratories participating in the UK NEQAS scheme (see *Table 1*). For this technique the DNA is first extracted from the sample and amplified using PCR. The HRM reaction is then performed. This involves a precise warming of the DNA during which the two strands of DNA 'melt' apart. Fluorescent dye that binds only to double-stranded DNA is used to monitor the process. A region of DNA with a mutation will 'melt' at a different temperature to the same region of DNA without a mutation. These changes are documented as melt curves and the presence or absence of a mutation can be reported.[24]

Matrix-assisted laser desorption ionisation time-of-flight (MALDI-TOF) mass spectrometry is currently used by one laboratory participating in the UK NEQAS scheme. This technique involves extracting DNA and amplifying it using PCR. The PCR products are then cleaved and fragments separated based on mass by the MALDI-TOF mass spectrometer. This generates a 'fingerprint' of the DNA with each fragment represented as a peak with a certain mass. The 'fingerprint' of the test sample is compared with the 'fingerprint' of the wild-type DNA. A mutation would appear as a peak shift due to a change in the mass of a fragment caused by a base change.[25] MALDI-TOF mass spectrometry can be used to identify all mutations within selected codons in the *KRAS* oncogene and has a limit of detection of approximately 10% tumour DNA in a background of wild-type DNA.[26]

Subgroup analyses of patients tested for *KRAS* mutation status from randomised controlled trials (RCTs) have shown that treatment with the EGFR-inhibiting monoclonal antibody cetuximab in combination with standard chemotherapy can increase progression-free survival (PFS) and tumour response in patients with *KRAS* wild-type tumours compared with standard chemotherapy alone.[27,28] In contrast, patients whose tumours were positive for *KRAS* mutations had reduced PFS and tumour response when treated with cetuximab in combination with standard chemotherapy compared with standard chemotherapy alone.[27,28] These two trials formed the basis of NICE technology appraisal 176,[1] which recommends cetuximab in combination with standard chemotherapy for the first-line treatment of mCRC in patients whose tumours are *KRAS* wild type and whose metastases are confined to the liver and are unresectable. However, both of these trials used a pre-CE-marked version of the LightMix *KRAS* Kit (TIB MOLBIOL), which is not currently in use by any laboratory participating in the UK NEQAS scheme.

Care pathway

National Institute for Health and Care Excellence guidance on the diagnosis and management of CRC was updated in 2011.[29]

Diagnosis of colorectal cancer

This guideline states that patients referred to secondary care for suspected CRC should be assessed using colonoscopy, flexible sigmoidoscopy followed by barium enema or computed tomography (CT), dependent on comorbidities and local expertise and test availability. When a lesion suspicious of cancer is detected a biopsy should be performed to confirm the diagnosis.[29]

All patients with histologically confirmed CRC should be offered contrast-enhanced CT of the chest, abdomen and pelvis to estimate the stage of the disease. Further imaging [e.g. contrast-enhanced magnetic resonance imaging (MRI) or positron emission tomography/computed tomography (PET/CT)] may be considered if the CT scan shows metastatic disease only in the liver.[29] The aim of further imaging is to identify those patients who have resectable metastases, or metastases that may become resectable following response to chemotherapy. For the second group of patients, European Society for Medical Oncology (ESMO) 2010 clinical practice guidelines for the treatment of advanced CRC[30] recommend establishing *KRAS* mutation status to determine the best treatment regimen. These guidelines do not stipulate which specific mutations should be analysed or which test method should be used. The *KRAS* status of a patient's tumour is identified through analysis of a biopsy sample or, more frequently, a section of resected tumour tissue. The tissue is fixed in formalin and embedded in a block of paraffin for storage by the pathologist, who also examines the histology and evaluates the tumour content of the sample. Macro dissection may be performed before DNA is extracted and mutation analysis is carried out to determine the *KRAS* status of the tumour.

To minimise turnaround time, guidance from the Royal College of Pathologists[31] recommends that mutation testing should be ordered by the pathologist reporting on the cellular make-up of the tumour. However, this is not currently universal practice and often the decision to perform a *KRAS* mutation test is often taken at the multidisciplinary team (MDT) meeting. If a sample is stored as a FFPE specimen for a long time this can lead to DNA degradation, which can result in a higher chance of failure when testing for *KRAS* mutations. The timing of the *KRAS* test varies between patients, with some clinicians preferring to test at diagnosis, potentially before the disease becomes metastatic, and other clinicians waiting until the cancer has progressed to metastatic disease. If the *KRAS* status is tested early, then the result is then referred to if metastatic disease develops. It has been suggested that analysing multiple resection or biopsy samples from the same patient increases the chances of identifying a *KRAS* mutation because of potential heterogeneity between tumour sites. The evidence on this is conflicting, with studies reporting that testing a single site only will potentially misclassify between 2% and 10% of tumours as *KRAS* wild type.[32,33]

Treatment of colorectal cancer

In patients with unresectable liver metastases whose primary tumour has been resected or is potentially operable and who are fit enough to undergo liver surgery, the aim of chemotherapy is to induce tumour response such that resection becomes possible. The *KRAS* mutation status of a patient's tumour is used to determine the optimal chemotherapy regimen for this purpose. Evidence suggests that patients with *KRAS* wild-type tumours are more likely to benefit from treatment with an EGFR-inhibiting monoclonal antibody (cetuximab) in combination with standard chemotherapy. However, patients whose tumours are positive for *KRAS* mutations are more likely to benefit from standard chemotherapy alone. In addition, the overall health and the preferences of the patient should be taken into consideration when selecting treatment.

The choice of standard chemotherapy is covered by NICE clinical guideline 131,[29] which recommends that one of the following sequences of chemotherapy is considered:

- oxaliplatin in combination with infusional fluorouracil plus folinic acid (FOLFOX) as first-line treatment and then single-agent irinotecan as second-line treatment
- FOLFOX as first-line treatment and then irinotecan in combination with infusional fluorouracil plus folinic acid (FOLFIRI) as second-line treatment
- oxaliplatin and capecitabine (XELOX) as first-line treatment and then FOLFIRI as second-line treatment.

The guideline further states that raltitrexed should be considered only for patients who are intolerant to fluorouracil and folinic acid or for patients for whom these drugs are not suitable.[29] NICE technology appraisal 61[34] suggests that oral therapy with either capecitabine or tegafur with uracil (in combination with folinic acid) can also be considered as an option for the first-line treatment of mCRC.

With respect to the use of biological agents (EGFR inhibitors), NICE technology appraisal 176[1] recommends cetuximab in combination with FOLFOX or FOLFIRI, within its licensed indication, for the first-line treatment of mCRC:

- in patients in whom the primary colorectal tumour has been resected or is potentially operable
- in patients in whom the metastatic disease is confined to the liver and is unresectable
- when the patient is fit enough to undergo surgery to resect the primary colorectal tumour and to undergo liver surgery if the metastases become resectable after treatment with cetuximab.

The European Medicines Agency marketing authorisation for cetuximab states that it is 'indicated for the treatment of patients with EGFR-expressing, *KRAS* wild-type metastatic colorectal cancer'.[35] Therefore, *KRAS* mutation testing is an important component of the care pathway. Cetuximab (monotherapy or combination therapy) and bevacizumab (in combination with non-oxaliplatin chemotherapy) for the treatment of mCRC after first-line chemotherapy are not recommended in NICE technology appraisal 242.[36] However, these treatments may be given to some patients through the Cancer Drugs Fund. If cetuximab is considered in the third-line setting, *KRAS* status is often not retested but a decision will be made based on the result of the *KRAS* test performed earlier in the care pathway. No other biological agents are currently recommended by NICE for the first-line treatment of patients with unresectable liver metastases from CRC.

National Institute for Health and Care Excellence clinical guideline 131[29] stipulates that all patients with primary CRC undergoing treatment with curative intent should be followed up at a clinic visit 4–6 weeks after the potentially curative treatment. They should then have regular surveillance including:

- a minimum of two CT scans of the chest, abdomen and pelvis in the first 3 years and
- regular serum carcinoembryonic antigen tests (at least every 6 months in the first 3 years).

They should also have a surveillance colonoscopy at 1 year after initial treatment and, if the result is normal, further colonoscopic follow-up after 5 years and thereafter as determined by cancer networks.

Measuring response to treatment

In 1979 the World Health Organization (WHO) and the International Union Against Cancer introduced criteria for the classification of the response of solid tumours to treatment.[37] These criteria were an early attempt to standardise reporting of response outcomes and were widely adopted; however, some problems with their use have subsequently developed. There has been variation in the methods used for incorporating into response assessments the change in size of measurable lesions, as defined by WHO; the minimum lesion size and number of lesions to be recorded have also varied; the definitions of progressive disease (PD) have sometimes been related to change in a single lesion and sometimes to change in overall tumour load (sum of the measurements of all lesions); and there has been confusion around how to use three-dimensional measures from new technologies such as CT and MRI in the context of the WHO criteria.[38] The Response Evaluation Criteria in Solid Tumours (RECIST) Group is a collaborative initiative that was instigated to review the WHO criteria. The RECIST criteria use the same categories as the WHO criteria [complete response (CR), partial response (PR), stable disease (SD) and PD].[38] RECIST guidance recommends CT and MRI for measuring target lesions in response assessment and that imaging-based evaluation is generally preferable to clinical examination. It is suggested that follow-up assessments every 6–8 weeks is a 'reasonable norm'.[38] Taking into account the longest diameter only for all target lesions, the RECIST criteria, as they are applicable to this assessment, can be summarised as follows:[38]

- CR – disappearance of all target lesions and no new lesions
- PR – at least a 30% decrease in the sum of the longest diameters of target lesions, taking the sum of the baseline diameters as the reference, and no new lesions
- PD – at least a 20% increase in the sum of the longest diameters of target lesions, taking the smallest sum of the longest diameters recorded since treatment started as the reference, or the appearance of one or more new lesions
- SD – neither sufficient shrinkage to be classified as PR or sufficient increase to be classified as PD, taking the smallest sum of the longest diameters recorded since treatment started as the reference, and no new lesions.

Best overall response is defined as the best response recorded from the start of treatment to disease progression.[38]

First-line chemotherapy of unresectable colorectal liver metastases seeks to achieve a tumour response such that the tumour is judged to be resectable. For this reason, resection rate is considered the ideal reference standard for research question 2 and the optimal outcome measure for research question 3 (see *Chapter 1*). Objective response rate (ORR), defined as best overall response = CR + PR, is also of interest as there is some evidence that ORR correlates well with resection rate.[39] Tumour status following treatment/resection is defined by the residual tumour (R) classification, in which R0 = no residual tumour, R1 = microscopic residual tumour and R2 = macroscopic residual tumour.

This assessment compares the performance and cost-effectiveness of *KRAS* mutation testing options, currently available in the UK NHS, for the differentiation of adults with mCRC whose metastases are confined to the liver and are unresectable and who may benefit from first-line treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone.

Chapter 3 Assessment of clinical effectiveness

A systematic review was conducted to summarise the evidence on the clinical effectiveness of the different *KRAS* mutation testing options, currently available in the UK NHS, for differentiating adults with mCRC whose metastases are confined to the liver and are unresectable and who may benefit from first-line treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone. Systematic review methods followed the principles outlined in the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care[40] and the NICE Diagnostic Assessment Programme manual.[41] In addition to the effectiveness review, additional data were obtained from an online survey of laboratories participating in the UK NEQAS pilot scheme for *KRAS* mutation testing.

Systematic review methods

Search strategy

Search strategies were based on target condition and intervention, as recommended in the CRD guidance for undertaking reviews in health care[40] and the *Cochrane Handbook for DTA Reviews*.[42]

Candidate search terms were identified from target references, browsing database thesauri [e.g. MEDLINE medical subject headings (MeSH) and EMBASE Emtree], existing reviews identified during the rapid appraisal process and initial scoping searches. These scoping searches were used to generate test sets of target references, which informed text mining analysis of high-frequency subject indexing terms using EndNote X5 reference management software (Thomson Reuters, CA, USA). Strategy development involved an iterative approach testing candidate text and indexing terms across a sample of bibliographic databases and aimed to reach a satisfactory balance of sensitivity and specificity.

The following databases were searched for relevant studies from 2000 to January 2013:

- MEDLINE (OvidSP) (2000 to Week 2 January 2013)
- MEDLINE In-Process and Other Non-Indexed Citations and Daily Update (OvidSP) (up to 21 January 2013)
- EMBASE (OvidSP) (2000 to Week 3 2013)
- Cochrane Database of Systematic Reviews (CDSR) (Wiley) (The Cochrane Library 2000 to Issue 12, 2012)
- Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley) (The Cochrane Library 2000 to Issue 12, 2012)
- Database of Abstracts of Reviews of Effects (DARE) (Wiley) (The Cochrane Library 2000 to Issue 4, 2012)
- Health Technology Assessment (HTA) database (Wiley) (The Cochrane Library 2000 to Issue 4, 2012)
- Science Citation Index (SCI-EXPANDED) (Web of Knowledge) (2000 to 22 January 2013)
- Conference Proceedings Citation Index (CPCI-S) (Web of Knowledge) (2000 to 22 January 2013)
- Latin American and Caribbean Health Sciences Literature (LILACS) (Internet) (up to 24 January 2013), http://regional.bvsalud.org/php/index.php?lang=en
- BIOSIS Previews (Web of Knowledge) (2000 to 22 January 2013)
- National Institute for Health Research (NIHR) HTA programme (Internet) (up to 25 January 2013)
- International Prospective Register of Systematic Reviews (PROSPERO) (Internet) (up to 25 January 2013), www.crd.york.ac.uk/prospero/.

Completed and ongoing trials were identified by searches of the following resources:

- National Institutes of Health ClinicalTrials.gov (Internet) (2000 to 23 January 2013), www.clinicaltrials.gov/
- Current Controlled Trials (Internet) (2000 to 29 January 2013), www.controlled-trials.com/
- WHO International Clinical Trials Registry Platform (ICTRP) (Internet) (2000 to 25 January 2013), www.who.int/ictrp/en/.

Searches were undertaken to identify studies of *KRAS* testing for mCRC. The main EMBASE strategy for each set of searches was independently peer reviewed by a second information specialist using the Peer Review of Electronic Search Strategies Evidence-Based Checklist (PRESS-EBC).[43] Search strategies were developed specifically for each database and the keywords associated with CRC were adapted according to the configuration of each database. Searches took into account generic and other product names for the intervention. No restrictions on language or publication status were applied. Full search strategies are reported in *Appendix 1*.

Electronic searches were undertaken for the following conference abstracts:

- ASCO conference proceedings (Internet) (2007–13), www.asco.org/ASCOv2/Meetings/Abstracts
- ESMO conference proceedings (Internet) (2007–13), www.esmo.org/education-research/abstracts-virtual-meetings-and-meeting-reports.html
- American Association for Cancer Research conference proceedings (Internet) (2007–13), www.aacrmeetingabstracts.org/search.dtl
- Association for Molecular Pathology conference proceedings (Internet) (2007–13), www.amp.org/meetings/past_meetings.cfm.

Identified references were downloaded into EndNote X4 software for further assessment and handling.

References in retrieved articles were checked for additional studies. The final list of included papers was also checked on PubMed for retractions, errata and related citations.[44–46]

Inclusion and exclusion criteria

Separate inclusion criteria were developed for each of the three clinical effectiveness questions; these are summarised in *Table 2*.

Inclusion screening and data extraction

Two reviewers (MW and PW) independently screened the titles and abstracts of all reports identified by searches and any discrepancies were discussed and resolved by consensus. Full copies of all studies deemed potentially relevant were obtained and the same two reviewers independently assessed these for inclusion; any disagreements were resolved by consensus. Details of studies excluded at the full paper screening stage are presented in *Appendix 5*.

Studies cited in materials provided by the manufacturers of the Therascreen *KRAS* RGQ PCR Kit and Therascreen *KRAS* Pyro Kit (QIAGEN), the cobas *KRAS* Mutation Test Kit (Roche Molecular Systems), the *KRAS* LightMix Kit (TIB MOLBIOL) and the *KRAS* StripAssay (ViennaLab) were first checked against the project reference database, in EndNote X4; any studies not already identified by our searches were screened for inclusion following the process described above.

Data were extracted on the following: study design/details, participant details (e.g. inclusion/exclusion criteria, age, liver metastases details, criteria for unresectability, performance status, previous treatments), *KRAS* mutation test(s) and mutations targeted, intervention details, clinical outcomes, test performance outcome measures (against treatment response as reference standard), details of specific mutations identified by outcome measure (when reported), test failure rates and limits of detection. Data were

TABLE 2 Inclusion criteria

	What is the technical performance of the different *KRAS* mutation tests?	What is the accuracy of *KRAS* mutation testing, using any test, for predicting response to treatment with cetuximab in combination with standard chemotherapy?	How do outcomes from treatment with cetuximab in combination with standard chemotherapy and, when reported, from treatment with standard chemotherapy vary according to which test is used to select patients for treatment?
Participants	Adult patients (≥ 18 years) with mCRC and a resected or resectable primary tumour whose metastases are confined to the liver and are unresectable but may become resectable after response to chemotherapy	Adult patients (≥ 18 years) with mCRC and a resected or resectable primary tumour whose metastases are confined to the liver and are unresectable but may become resectable after response to chemotherapy	Adult patients (≥ 18 years) with mCRC and a resected or resectable primary tumour whose metastases are confined to the liver and are unresectable but may become resectable after response to chemotherapy Patients who have been tested for *KRAS* mutation status
Setting	Secondary or tertiary care		
Interventions (index test)	Any commercial or in-house *KRAS* mutation test listed in *Table 1*	Any commercial or in-house *KRAS* mutation test listed in *Table 1*	First-line chemotherapy with cetuximab in combination with standard chemotherapy[a]
Comparators	Not applicable	Not applicable	Standard chemotherapy[a]
Reference standard	Not applicable	Response to treatment with cetuximab in combination with standard chemotherapy (e.g. PFS, ORR, disease control rate)	Not applicable
Outcomes	Proportion of tumour cells needed, failures, limit of detection, turnaround time, costs, expertise/logistics of test	Overall survival or PFS in patients whose tumours are *KRAS* mutant vs. overall survival or PFS in patients whose tumours are *KRAS* wild type. Test accuracy – the numbers of true positives, false negatives, false positives and true negatives	PFS, overall survival, ORR, disease control rate
Study design	To be addressed by survey (see *Survey of laboratories providing* KRAS *mutation testing*); publications from UK laboratories	RCTs (CCTs and cohort studies will be considered if no RCTs are identified)	RCTs (CCTs will be considered if no RCTs are identified)

CCT, controlled clinical trial.
a Defined according to the recommendations of NICE technology appraisal 176[1] as FOLFOX or FOLFIRI.

extracted by one reviewer, using a piloted standard data extraction form, and checked by a second (MW and PW); any disagreements were resolved by consensus. Full data extraction tables are provided in *Appendix 2*.

Quality assessment

The risk of bias in included RCTs was assessed using the Cochrane Collaboration's tool for assessing risk of bias in randomised trials.[47] Studies used to derive accuracy data, for the ability of *KRAS* mutation tests to predict treatment response, were assessed using the QUADAS-2 tool.[48] Risk of bias assessments were undertaken by one reviewer and checked by a second reviewer (MW and PW) and any disagreements were resolved by consensus.

The results of the risk of bias assessments were summarised and are presented in tables and graphs in the results section of this chapter and in full, by study, in *Appendix 3*.

Survey of laboratories providing KRAS mutation testing

We conducted a web-based survey to gather data on the technical performance characteristics of *KRAS* mutation tests. We sent an e-mail invitation via NEQAS to laboratories participating in the UK NEQAS pilot scheme for *KRAS* mutation testing. We used SurveyMonkey online software to run the survey. We structured the survey into sections on:

- laboratory details
- *KRAS* testing methods
- logistics
- technical methods
- costs.

When possible we used multiple choice options with tick boxes to make the survey quick and easy to complete. A copy of the survey is provided in *Appendix 4*.

Methods of analysis/synthesis

The results of studies included in this review were summarised by research question (see *Chapter 1*), that is, studies providing technical information on *KRAS* mutation testing in NHS laboratories in the UK (see *What are the technical performance characteristics of the different* KRAS *mutation tests?*), studies providing information on the accuracy of *KRAS* mutation tests for predicting response to treatment (see *What is the accuracy of* KRAS *mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?*) and studies reporting information on how clinical outcomes may vary according to which test is used to select patients for treatment (see *How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?*). We planned to use a bivariate/hierarchical summary receiver operating characteristic (HSROC) random-effects model to generate summary estimates and a summary receiver operating characteristic (SROC) curve for test accuracy data[49–51] and a DerSimonian and Laird random-effects model to generate summary estimates of treatment effects. However, because the review identified a small number of studies with between-study variation in participant characteristics, methods used to test for *KRAS* mutations and mutations targeted, we did not consider meta-analysis to be appropriate and have provided a structured narrative synthesis.

For all studies that provided data on accuracy for the prediction of response to treatment with cetuximab in combination with standard chemotherapy, the absolute numbers of true-positive (TP), false-negative (FN), false-positive (FP) and true-negative (TN) test results, as well as sensitivity and specificity values with 95% confidence intervals (CIs), are presented in results tables, for each reference standard response (e.g. ORR or resection rate) reported. When reported, data on the numbers of failed *KRAS* mutation tests and reasons for failure were also included in the results tables. The results of individual studies were plotted in the receiver operating characteristic (ROC) plane to illustrate the trade-off between sensitivity and specificity and for ease of comparison between test methods; separate plots were provided for each reference standard response. For RCTs providing information on how clinical outcomes may vary according to which test is used to select patients for treatment with cetuximab in combination with standard chemotherapy, hazard ratios (HRs) with 95% CIs were provided for PFS and odds ratios (ORs) with 95% CIs were reported for tumour response outcomes (ORR and resection rate). The results of individual studies were illustrated in forest plots. Between-study clinical heterogeneity was assessed qualitatively. There were insufficient studies to assess heterogeneity statistically using the chi-squared test or I^2 statistic.

Results of the assessment of clinical effectiveness

The literature searches of bibliographic databases identified 7903 references. After initial screening of titles and abstracts, 100 were considered to be potentially relevant and were ordered for full paper screening. No additional papers were ordered based on screening of papers provided by test manufacturers; all studies cited in documents supplied by the test manufacturers had already been identified by the bibliographic database searches. No additional studies were identified from searches of clinical trials registries or from hand searching of conference abstracts. *Figure 1* shows the flow of studies through the review process and *Appendix 5* provides details, with reasons for exclusions, of all publications excluded at the full paper screening stage.
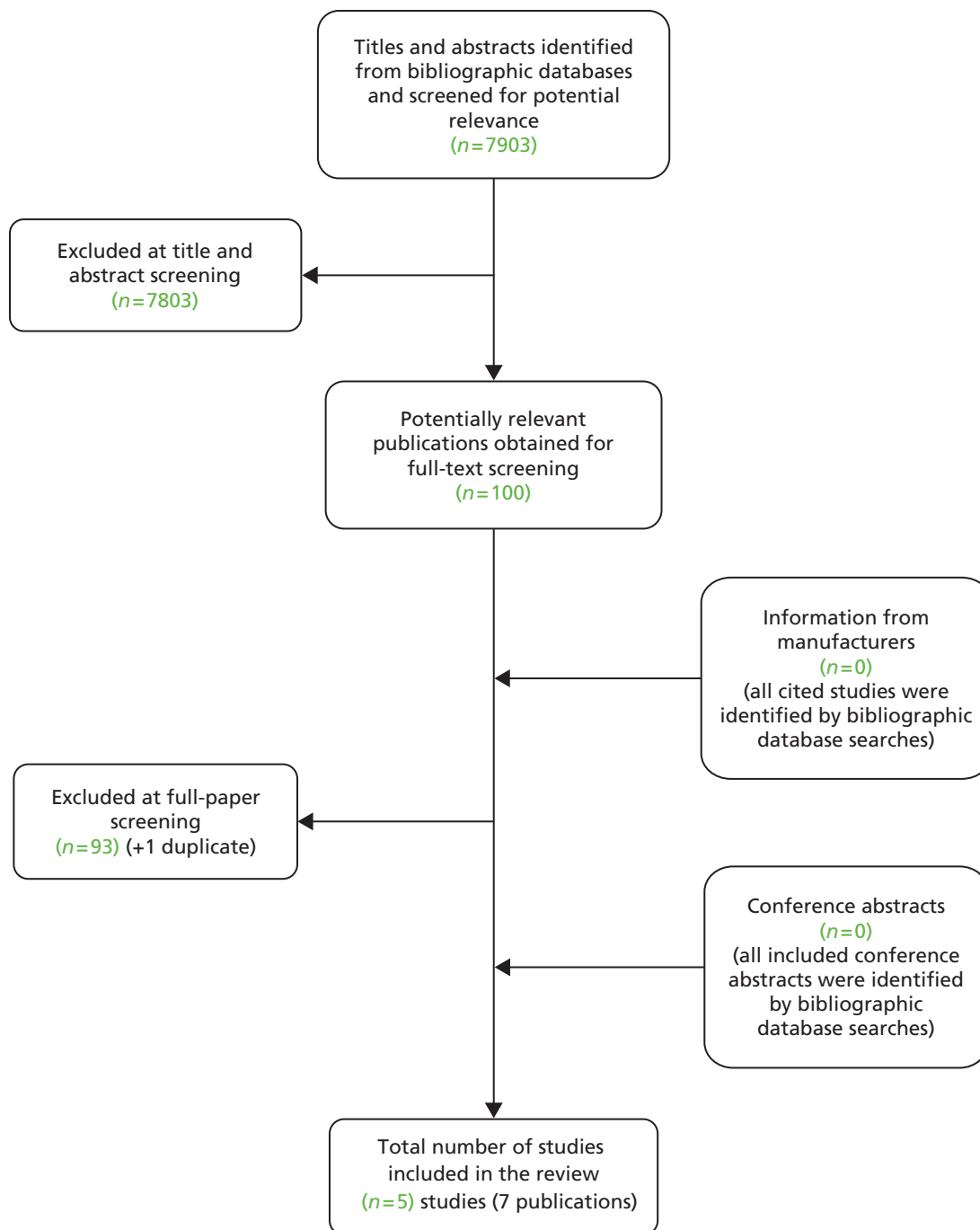
FIGURE 1 Flow of studies through the review process.

Based on the searches and inclusion screening described earlier, seven publications of five studies were included in the review.[27,28,52–56] Because data for participants with colorectal metastases and no extrahepatic metastases were frequently reported as subgroup analyses of larger trials, the authors of two additional potentially relevant trials in patients with mCRC were contacted to request subgroup data. The author of the Central European Cooperative Oncology Group (CECOG) trial[57] reported that only 23 (15%) participants had metastases that were limited to the liver and that no subgroup data were available for these participants. The authors of the NORDIC-VII trial[58] did not respond to our request.

No studies conducted in UK NHS laboratories were identified that reported information on the technical performance characteristics of *KRAS* mutation tests. One study[52] reported data on tumour response following treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI in a group of patients with unresectable colorectal liver metastases who were tested for tumour *KRAS* mutation status. This study provided information on the accuracy of the Therascreen *KRAS* PCR test for the prediction of response to treatment. Additional data, supplied by the COIN trial investigators, allowed calculation of accuracy for prediction of resection of liver metastases following treatment with cetuximab plus FOLFOX or XELOX, in which a combination of pyrosequencing and MALDI-TOF mass spectrometry was used to asses *KRAS* mutation status [D Fisher, Medical Research Council (MRC) Clinical Trials Unit, London, 19 April 2013, personal communication]. Four RCTs, reported in six publications,[27,28,53–56] compared the effectiveness of cetuximab plus standard chemotherapy with that of standard chemotherapy alone in patients whose tumours were *KRAS* wild type. Because the method used to determine mutation status varied between trials, these RCTs provide some information on how clinical outcomes may vary according to which test is used to select patients for treatment.

All included studies were published in 2009 or later. The study providing information on test accuracy was a multicentre European study funded by Merck Serono, Sanofi Aventis and Pfizer.[52] Two of the four RCTs were multicentre European studies funded by Merck Serono,[27,28,53,56] one was a multicentre study conducted in the UK and the Republic of Ireland and funded by the UK MRC[54] and one was a single-centre study conducted in China and published as an abstract only (no funding details reported).[55]

Full details of the characteristics of the study participants, study inclusion and exclusion criteria, *KRAS* mutation test used and mutations targeted, and treatment groups are reported in the data extraction tables presented in *Appendix 2*. For studies providing test accuracy data, full details of the *KRAS* mutation testing process are reported as part of the QUADAS-2 risk of bias assessment in *Appendix 3*.

What are the technical performance characteristics of the different KRAS *mutation tests?*

Literature review
No studies reporting the technical performance of *KRAS* mutation tests on clinical samples in UK laboratories were identified. Data on the technical performance characteristics of *KRAS* mutation tests, as experienced by UK laboratories, were therefore derived solely from the results of the online survey.

Laboratory survey results
A total of 31 laboratories participated in the 2012–13 UK NEQAS pilot scheme for *KRAS* mutation testing. The survey was completed by 21 laboratories; however, five of these were based outside the UK (Norway, Belgium, Switzerland, Italy and Ireland) and one was excluded as *KRAS* mutation testing was carried out for haematological malignancies only. Therefore, survey results were analysed for 15 laboratories (response rate 50%).

KRAS mutation test methods (*Figure 2*)
Fifteen laboratories stated that they used one method of *KRAS* mutation testing; one of these stated that they sometimes use a single *KRAS* mutation testing method and sometimes multiple methods (e.g. to confirm mutations).
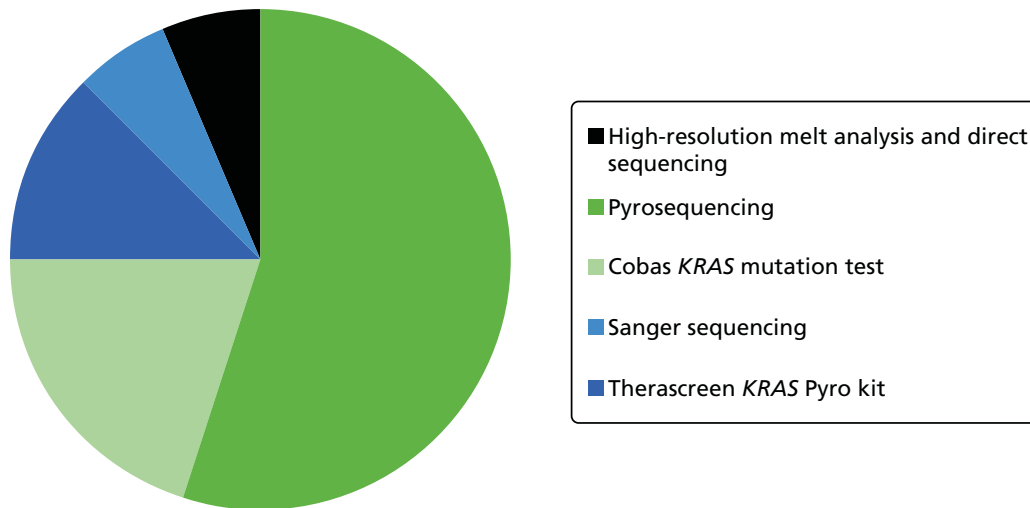
FIGURE 2 *KRAS* mutation tests used in NHS laboratories in the UK participating in the UK NEQAS pilot scheme for *KRAS* mutation testing.

Pyrosequencing, using in-house methods, was the most commonly used *KRAS* mutation test with nine laboratories using this approach, although one of the laboratories using pyrosequencing stated that it was in the process of switching to HRM analysis because of its quicker turnaround time. The cobas *KRAS* Mutation Test was used by three laboratories, Sanger sequencing was used by two laboratories and only a single laboratory used the Therascreen *KRAS* Pyro Kit. The final laboratory, which had initially reported using only a single method of *KRAS* mutation testing, stated that it used HRM analysis and direct sequencing. The laboratory that reported sometimes using multiple methods reported details for only one testing method (Sanger sequencing); however, this laboratory did state that it used Sanger sequencing in the event of an unusual pyrosequencing result. It also stated that its reason for using more than one testing method was the ability to fully characterise detected mutations. This suggests that its first choice method was in fact pyrosequencing not Sanger sequencing. This laboratory is therefore included in both methods in *Figure 2* and the numbers above. All other laboratories stated that they used the reported *KRAS* mutation testing method for 100% of the samples.

Nine laboratories reported that samples were referred to their laboratory for testing on demand (one specified that this was MDT meetings, via pathologist, or oncologist), two reported mixed referral (some centres on demand, some all CRC samples), one laboratory reported that all resected primary CRC samples were sent for testing, one reported that samples were sent through clinical trials, one reported that all resected primary CRC plus metastatic samples were sent for testing and one laboratory did not answer this question.

The main reasons cited for choice of *KRAS* mutation testing method were mutation coverage ($n = 13$, 87%), ease of use ($n = 12$, 80%) and cost ($n = 11$, 73%). Nine laboratories (60%) also selected sensitivity (proportion of tumour cells required) and seven (47%) selected turnaround time. One laboratory did not answer this question. There was no apparent association between test method and reason for choice.

Of the eight laboratories that completed the questionnaire for pyrosequencing, all reported that they targeted mutations in codons 12, 13 and 61. Two of these laboratories reported that all mutations were targeted, one using commercial primers and one using self-designed primers. Two laboratories reported that they targeted specific mutations using self-designed primers. The others all used self-designed primers but did not state whether they targeted all or specific mutations; one laboratory stated that it also targeted mutations in codon 146. Two laboratories used Sanger sequencing. One stated that it targeted specific mutations but did not provide any further details. The other stated that it targeted mutations in codons 12, 13 and 61 using self-designed primers. One laboratory stated that it used a single testing method only, HRM analysis, and subsequently stated that it used HRM analysis and direct

sequencing; mutations in codons 12, 13 and 61 and all mutations in exons 2 and 3 were targeted. Details on primers were not reported. The other four laboratories used commercial *KRAS* mutation testing kits.

KRAS mutation test logistics (*Table 3* and *Figure 3*)

The number of samples screened for *KRAS* mutations in a typical week varied by laboratory, from less than five (three laboratories) to > 20 (four laboratories). The batch size ranged from less than one to two to 15–20 samples. Only laboratories with five or less samples screened per week ran batches of three or less. Only one laboratory had a batch size of > 15 (reported as 15–20 samples per week) and this laboratory screened > 20 samples per week; most other laboratories had batch sizes between five and 10. The two laboratories using Sanger sequencing both reported screening five or less samples per week and reported batch sizes of one or two. Of the four laboratories using commercial kits, one did not report on number of samples screened or batch size, two reported screening > 20 samples per week with batch sizes of 10 and 15, and one reported screening 6–10 samples per week with a similar batch size. Only one laboratory reported that it waited until it had a certain number of samples before running the *KRAS* mutation test; this laboratory waited until it had 10 samples before running the test.

Most laboratories reported an average waiting time from requesting the sample for *KRAS* testing to receiving the sample in the laboratory of 24–48 hours (three laboratories) or 3–5 days (six laboratories), although one laboratory reported a waiting time of < 24 hours and one reported a waiting time of 6–7 days. The range in waiting times was reported by four laboratories and was 1–10 days in two and 2–30 days in one, with the fourth stating that occasionally request dates are included in referral and so the range is 1–3 weeks.

TABLE 3 Laboratory throughput by *KRAS* mutation test

KRAS mutation test	Samples per week	Batch size	Frequency of test	Wait for batch size?	Time from receiving sample to returning result to clinician
cobas *KRAS* Mutation Test Kit	6–10	6–10	Weekly	No	6–7 days
	> 20	10	Two to three times per week	Yes, 10	3–5 days
	NR	NR	NR	NR	NR
HRM analysis	6–10	4	Two to three times per week	No	3–5 days
Pyrosequencing	≤ 5	3	On demand	No	3–5 days
	6–10	6–10	Weekly	No	3–5 days
	> 20	15–20	Two to three times per week	No	3–5 days
	6–10	8	Weekly	No	6–7 days
	11–15	6–10	Two to three times per week	No	24–48 hours
	16–20	10	Two to three times per week	No	3–5 days
	6–10	5–10	Two to three times per week	No	3–5 days
	> 20	12	Daily	No	3–5 days
Sanger sequencing	≤ 5	≥ 1	Daily	No	3–5 days
	≤ 5	1–2 patients	Variable	No	3–5 days
Therascreen *KRAS* Pyro Kit	> 20	15	Two to three times per week	No	6–7 days

NR, not reported.

(a) In a typical week, how many samples do you screen for *KRAS* mutations?
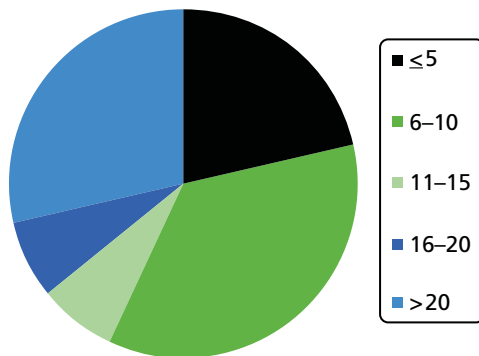
(b) What is your average batch size (number of samples)?
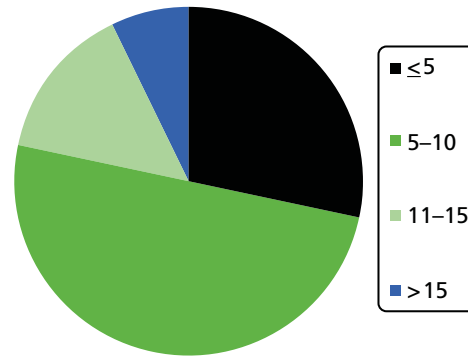
(c) How often do you run the *KRAS* mutation test?
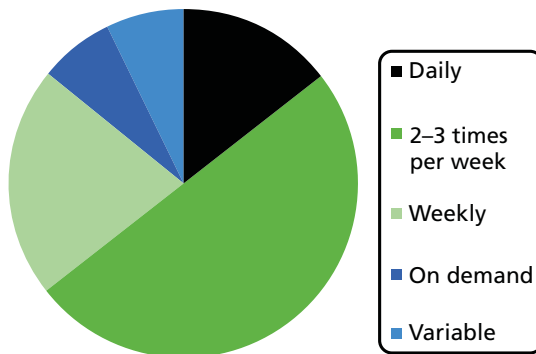
(d) On average, how long (in calendar days) does it take to receive a sample at the laboratory once it has been requested?
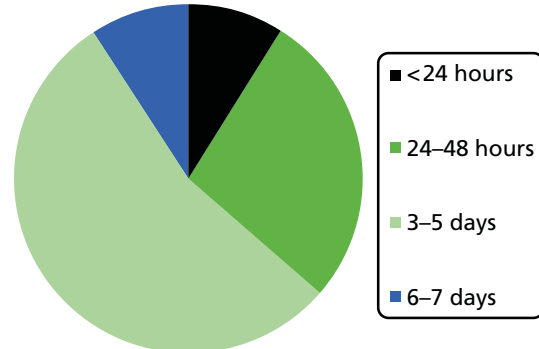
(e) On average, how long (in calendar days) does it take from receiving a sample at the laboratory to sending a result back to the clinician?
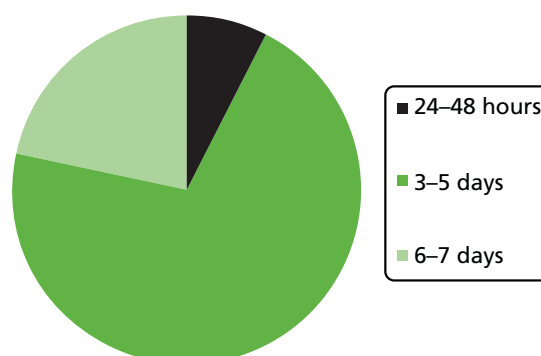
FIGURE 3 Summary of logistic information.

Four laboratories did not report data on time to receive samples at the laboratory once the sample had been requested. The majority of laboratories had a turnaround time from receiving the sample to reporting the result to the clinician of 3–5 or 6–7 days, with only one laboratory reporting a time of 24–48 hours. The laboratory with the shortest turnaround time was one that used pyrosequencing and tested 11–15 samples per week.

KRAS mutation test technical performance (Table 4)

The minimum reported percentage of tumour cells required varied between laboratories (range < 1% to > 30%), even for those using the same KRAS mutation test. All laboratories using the cobas KRAS Mutation Test reported that the limit of detection was 1–5%; the limit of detection was reported to be either 1–5% (three laboratories) or 6–10% (five laboratories) for pyrosequencing, > 10% for Sanger

TABLE 4 KRAS mutation test technical performance data

KRAS mutation test	Minimum tumour cells required (%)	Limit of detection (%)	How was limit of detection determined?	Use of microdissection?	Threshold below which microdissection used (%)
cobas KRAS Mutation Test Kit	1–5	1–5	Manufacturer guidance	Yes	10
	NR	1–5	In-house validation	No	NA
	6–10	1–5	Artificial blends of tumour DNA in normal DNA	Yes	10–15
HRM analysis	11–20	1–5	Serial dilutions of control samples	No	NA
Pyrosequencing	>30	6–10	Horizon Diagnostics reference standards	Yes	50
	11–20	6–10	Spiking of wild-type DNA with mutant DNA	Yes	20
	11–20	6–10	Dilution series of known mutations at known percentage	Yes	Always
	11–20	6–10	Dilution series of DNA from three cell lines each with a different KRAS mutation	No	NA
	6–10	1–5	Cell lines with known mutations	Yes	20; all samples that contain adenoma or when dissection would greatly improve the tumour percentage
	21–30	1–5	CE-marked kit, in-house validation conducted	Yes	20
	21–30	6–10	Internal quality control	Yes	Always
	6–10	1–5	Cell lines with set percentage tumour burden	Yes	50
Sanger sequencing	> 30	> 10	Cell line with known mutation	Yes	30
	≤ 1	> 10	Cell line control	No	NA
Therascreen KRAS Pyro Kit	NR	NR	NR	NR	NR

NA, not applicable; NR, not reported.

sequencing and 1–5% for HRM. A variety of methods were used to determine the limit of detection. Ten laboratories reported using microdissection, with two stating that they always used this technique and others using microdissection at thresholds of < 10–50%. The laboratory that used the Therascreen *KRAS* Pyro Kit did not provide any data on technical performance.

KRAS mutation test failure rates (*Table 5*)

The proportion of samples rejected before analysis was < 2% for all 13 laboratories that provided data on rejection rates. Reasons for rejection included insufficient tumour cells/tissue, sample type unsuitable for analysis and insufficient patient identifiers. The proportion of failed tests ranged from 3% to 6% for the cobas *KRAS* Mutation Test Kit and from 0.2% to 10% for pyrosequencing. The one laboratory using HRM analysis reported no failed tests and of the two laboratories using Sanger sequencing one reported no failed tests and the other did not provide information on the number of failed tests. Reasons for test failure included insufficient DNA, amplification failure, DNA degradation/quality, insufficient tumour cells and poor fixation. The laboratory that used the Therascreen *KRAS* Pyro Kit did not provide any data on failure rates.

KRAS mutation test costs (*Table 6*)

Only seven laboratories provided data on the cost of the *KRAS* mutation test. Two of these provided data on the costs of the reagents only, which were reported as £22 for Sanger sequencing and approximately £50 for pyrosequencing. A further laboratory also reported that the cost of pyrosequencing was £50. Three other laboratories reported costs for pyrosequencing, with one reporting a cost of £150, one reporting a cost of approximately £120 and the other reporting a cost of approximately £273 for a single sample, which reduced to approximately £110 per sample if running a batch of 10. The final laboratory to report cost data reported that the cost of the cobas *KRAS* Mutation Test Kit was £100–125. With the exception of two laboratories, all laboratories received some funding from Merck Serono. One laboratory did not provide any details on test costs or funding. The price charged to both the NHS and Merck Serono ranged from £99 to £150 per sample.

TABLE 5 *KRAS* mutation test failure rates

| *KRAS* mutation test | No. of samples per year[a] | | | | | |
	Submitted, *n*	Rejected, *n* (%)	Reason for rejection	Analysed, *n*	Failed, *n* (%)	Reason for failure
cobas *KRAS* Mutation Test Kit	NR	NR	NR	1000	29 (3)	Various, most commonly DNA yield too low
	1358	7 (0.5)	Sample type unsuitable for analysis, insufficient identifiers	1351	86 (6)	Insufficient extracted DNA (8%), amplification failure (92%)
	1058	5–10 (0.7)	Insufficient tumour cells	1058	28 (3)	Insufficient tumour cells, DNA degradation
HRM analysis	1000	0	NA	1000	0	NA
Pyrosequencing	9	0	NA	9	0	NA
	1000	< 10 (< 1)	Insufficient tissue left in the block	1000	100 (10)	NR
	1500	15–20 (1.5)	Insufficient tumour cells	NR	NR (1)	Assumed to be because of fixation and DNA degradation
	415	3 (0.7)	Insufficient tumour cells	412	1 (0.2)	DNA quality

TABLE 5 *KRAS* mutation test failure rates (*continued*)

| *KRAS* mutation test | No. of samples per year[a] | | | | | |
	Submitted, *n*	Rejected, *n* (%)	Reason for rejection	Analysed, *n*	Failed, *n* (%)	Reason for failure
	374	0	NA; samples preselected by laboratory	374	10–20 (4)	Poor fixation
	1000	0	NA	1000	4 (0.4)	Unknown
	1000	10 (1)	Insufficient tumour cells, unsuitable sample type	1000	50 (5)	Insufficient tumour cells, DNA degradation
	1736	~20 (1)	Insufficient tumour cells, insufficient tissue	1736	~30 (2)	Insufficient tumour cells, DNA degradation
Sanger sequencing	65	0	NA	65	0	NA
	1000	0	NA	1000	NA	Insufficient tumour cells, DNA degradation
Therascreen *KRAS* Pyro Kit	NR	NR	NR	NR	NR	NR

NA, not applicable; NR, not reported.
a Respondents were asked to provide details on the exact number of samples for their laboratory; if they did not have access to the numbers for their laboratory they were asked to provide their best estimate for a hypothetical set of 1000 samples.

TABLE 6 Summary of *KRAS* mutation test costs

KRAS mutation test	Cost to laboratory (£)	Funding	NHS price (£)	Merck Serono price (£)
cobas *KRAS* Mutation Test Kit	NR	Merck Serono	NA	NR
	NR	Merck Serono, private	NA	NR
	100–125	Merck Serono, private	NA	Unable to disclose
HRM analysis	NR	Merck Serono	NA	NR
Pyrosequencing	150	NHS, privately funded from abroad	150	NA
	NR	Merck Serono, NHS	140	100
	~120	Merck Serono, Cancer Research UK Stratified Medicine programme	99	99
	NR	Merck Serono	NA	100
	~273 for a single sample, ~110 if running a batch of 10	Merck Serono	NA	150
	~50 (reagents only)	Merck Serono	NA	100
	50	Trials unit	NA	NA
	NR	Merck Serono, NHS, private	120	120
Sanger sequencing	22 (reagents only)	Merck Serono	NA	100
	NR	Merck Serono	NR	NR
Therascreen *KRAS* Pyro Kit	NR	NR	NR	NR

NA, not applicable; NR, not reported.

What is the accuracy of KRAS mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?

One study, the CELIM trial,[52] reported sufficient data to allow calculation of the accuracy of *KRAS* mutation testing for predicting response to treatment in patients with colorectal liver metastases who are treated with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI. This study is potentially useful in that it could provide full information on the extent to which *KRAS* mutation tests are able to discriminate between patients who will have benefit from the addition of cetuximab to standard chemotherapy regimens and those who will not. The utility of the study for this assessment is limited because reporting of outcome data by mutation status was limited to objective response. Thus, we defined TPs as those patients with *KRAS* wild-type tumours who have a positive response to treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI (best observed response = CR or PR). FPs were defined as those patients with *KRAS* wild-type tumours who did not have a positive response to treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI (SD or PD). FNs were defined as those with *KRAS* mutant tumours who had a positive response to treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI. TNs were defined as those with *KRAS* mutant tumours who did not have a positive response to treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI. Full definitions of CR, PR, SD and PD are provided in *Chapter 2* (see *Measuring response to treatment*). The publication of the results of the CELIM trial included resection rates for liver metastases; however, these data were reported for all participants and by treatment group (cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI) only and not by tumour *KRAS* mutation status.[52] For all study participants, the R0 resection rate was 36/106 (34%, 95% CI 25% to 44%) and the R0/R1 resection and/or radiofrequency ablation rate was 49/106 (46%, 95% CI 36% to 56%).[52] (Commercial-in-confidence information has been removed.)[59] All participants in the CELIM trial received treatment with cetuximab in addition to standard chemotherapy; therefore, this trial could not contribute data to question 3 on how outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment.

Additional data, supplied by the COIN trial investigators,[54] allowed calculation of the accuracy of *KRAS* mutation testing, using a combination of pyrosequencing and MALDI-TOF and targeting mutations in codons 12, 13 and 61, for predicting response to treatment in patients with colorectal liver metastases who are treated with cetuximab plus FOLFOX or XELOX. These data could be viewed as being of limited applicability to this assessment because the standard chemotherapy regimen used in the COIN trial does not exactly match that in our inclusion criteria (some participants received XELOX rather than FOLFOX or FOLFIRI). However, the additional data supplied did allow the calculation of accuracy with respect to prediction of the more clinically relevant outcome of potentially curative resection. In this case we defined TPs as those patients with *KRAS* wild-type tumours who had a potentially curative resection following treatment with cetuximab plus FOLFOX or cetuximab plus XELOX. FPs were defined as those patients with *KRAS* wild-type tumours who did not have a potentially curative resection following treatment with cetuximab plus FOLFOX or cetuximab plus XELOX. FNs were defined as those with *KRAS* mutant tumours who had a potentially curative resection following treatment with cetuximab plus FOLFOX or cetuximab plus XELOX. TNs were defined as those with *KRAS* mutant tumours who did not have a potentially curative resection following treatment with cetuximab plus FOLFOX or cetuximab plus XELOX.

Study details

Participants in the CELIM trial[52] all had unresectable colorectal liver metastases with no extrahepatic metastases. Non-resectability was defined as five or more liver metastases or metastases that were viewed as technically non-resectable by a liver surgeon and a radiologist on the basis of inadequate future remnant, infiltration of all hepatic liver veins, infiltration of both hepatic arteries or infiltration of both portal veins. Study participants had a median age of 64 years [interquartile range (IQR) 56 to 71] and 64% were male. The primary tumour site was the colon in 61 (55%) participants and the rectum in 49 (44%) participants; the primary site was unknown in one participant. Most patients (83%) had a primary tumour

of stage T3/4. The primary reason for non-resectability of liver metastases was given as 'technically unresectable' for 61 (55%) participants and 'five or more metastases' for 50 (45%) participants. Full details of the study participants are reported in *Appendix 2*.

KRAS mutation testing used an older version of the Therascreen *KRAS* RGQ PCR Kit, which is identical to the current Therascreen *KRAS* RGQ PCR kit in terms of mutations targeted. Both versions of the kit detected seven mutations in codons 12 and 13 (*Table 7*) and the two versions will be treated as equivalent for the purpose of this assessment.

Tumour response was assessed according to the RECIST criteria[38] to evaluate response to EGFR inhibitor treatment; response was defined as the best response to treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI observed during treatment and was assessed every four cycles (8 weeks). Post-treatment surgical review, to assess resectability, was undertaken after eight cycles of chemotherapy by senior surgeons with experience in hepatobiliary surgery; CT and MRI scans were presented by a radiologist and the surgeons were blinded to when the scans were carried out and the participants' clinical outcome data.[52]

Details of the COIN trial[54] are provided in *'How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?'*

KRAS mutation test accuracy

Data from the CELIM trial[52] provided estimates for the accuracy of the Therascreen *KRAS* RGQ PCR Kit for discriminating between patients who are likely to benefit from addition of cetuximab to standard chemotherapy regimens and those who are not. Sensitivity for the prediction of objective response was moderate (74.6%, 95% CI 62.1% to 84.7%) and specificity was poor (35.5%, 95% CI 19.2% to 54.6%) (*Table 8* and *Figure 4*). Additional data supplied by the COIN trial investigators allowed the calculation of estimates for the accuracy of pyrosequencing and MALDI-TOF, targeting mutations in codons 12, 13 and 61, for predicting potentially curative resection following treatment with cetuximab plus FOLFOX or XELOX (D Fisher, personal communication). Sensitivity and specificity were both poor at 52.0% (95% CI 31.3% to 72.2%) and 45.6% (95% CI 37.0% to 54.3%) respectively (see *Table 8* and *Figure 4*).

TABLE 7 *KRAS* mutations detected by the Therascreen *KRAS* RGQ PCR Kit

Codon	Coding DNA	Protein/amino acid, three-letter code	Protein/amino acid, one-letter code
12	c.34G > A	p.Gly12Ser	p.G12S
	c.34G > C	p.Gly12Arg	p.G12R
	c.34G > T	p.Gly12Cys	p.G12C
	c.35G > A	p.Gly12Asp	p.G12D
	c.35G > C	p.Gly12Ala	p.G12A
	c.35G > T	p.Gly12Val	p.G12V
13	c.38G > A	p.Gly13Asp	p.G13D

TABLE 8 Accuracy of *KRAS* mutation testing for the prediction of response to treatment with cetuximab in addition to standard chemotherapy

Study	*KRAS* mutation test method and mutations targeted	Non-evaluable samples	Reference standard	TP	FP	FN	TN	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	Prevalence (%)	PPV (95% CI) (%)	NPV (95% CI) (%)
Folprecht 2010[52] (CELIM)	Therascreen *KRAS* RGQ PCR Kit	12/111 unknown mutation status (*KRAS* mutation testing not carried out successfully, no reasons reported)	Objective response	47	20	16	11	74.6 (62.1 to 84.7)	35.5 (19.2 to 54.6)	67	70.1 (58.3 to 79.8)	40.7 (24.5 to 59.3)
COIN[a]	Pyrosequencing and MALDI-TOF (codons 12, 13 and 61)		Resection rate	13	74	12	62	52.0 (31.3 to 72.2)	45.6 (37.0 to 54.3)	16	14.9 (8.9 to 23.9)	83.9 (73.8 to 90.5)

NPV, negative predictive value; PPV, positive predictive value.
a　Additional data supplied by the COIN trial investigators (D Fisher, personal communication).
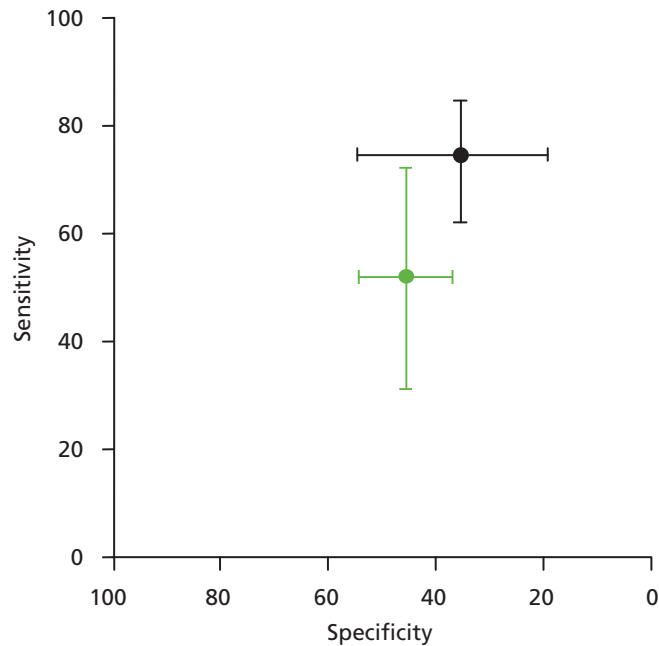
FIGURE 4 Summary receiver operating characteristic plot showing estimates of sensitivity and specificity together with 95% CIs from the CELIM trial[52] (black lines) and the COIN trial (green lines) (D FIsher, personal communication).

Quality Assessment of Diagnostic Accuracy Studies assessment

A summary of the results of the QUADAS-2 assessments for the CELIM and COIN trails are presented in *Table 9* and the full assessments are reported in *Appendix 3*. The rating of high concern regarding the applicability of the reference standard reflects the absence of data on the ability of the test (*KRAS* mutation status) to predict resection of liver metastases following treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI for the CELIM trial, and the use of standard chemotherapy in the COIN trial that did not fully match that in the inclusion criteria for this assessment. In addition, participants in the CELIM trial were described as having technically non-resectable or five or more liver metastases from CRC and it was therefore unclear whether some participants may have had potentially resectable metastases at baseline.[52] Both studies were rated as being at high risk of bias with respect to flow and timing because approximately 15% of participants were excluded from the analyses, in most cases because they were not evaluable for response.

TABLE 9 QUADAS-2 results for the studies that provided data on the accuracy of *KRAS* mutation testing for the prediction of response to treatment with cetuximab in addition to standard chemotherapy

	Risk of bias				Applicability concerns		
Study	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Folprecht 2010[52] (CELIM)	☺	☺	☺	☹	?	☺	☹
Maughan 2011[54] (COIN)	☺	☺	☺	☹	☺	☺	☹
☺, low risk; ☹, high risk; ?, unclear risk.							

How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?

Four RCTs (six publications)[27,28,53–56] provided data on the clinical effectiveness of cetuximab plus standard chemotherapy compared with standard chemotherapy alone in patients with colorectal liver metastases and no extrahepatic metastases whose tumours were *KRAS* wild type. The trials compared cetuximab in combination with standard chemotherapy (FOLFOX or FOLFIRI) with standard chemotherapy alone (see *Table 11*); in one trial[54] standard chemotherapy could be either FOLFOX or XELOX. One trial[55] included only participants with unresectable colorectal liver metastases and no extrahepatic metastases whose tumours were *KRAS* wild type. This trial was reported as a conference abstract only and some additional information on this trial was derived from the trial registry entry.[60] The remaining three trials, CRYSTAL,[27,53] OPUS[28,53,56] and COIN[54] (five publications), included participants with mCRC, conducted tumour *KRAS* mutation testing in a subgroup of these participants and reported data for a smaller subgroup of participants whose metastases were confined to the liver; in all cases outcome data on participants whose metastases were confined to the liver were reported only for those with *KRAS* wild-type tumours.

Study details

Participant characteristics varied across studies. The three studies that reported subgroup data for patients with colorectal metastases confined to the liver were multicentre studies conducted in continental Europe[27,28,53,56] or the UK and the Republic of Ireland.[54] The subgroup data taken from these studies represented between 11% and 14% of the total study population (see *Table 11*). None of the studies reported separate participant characteristics for the relevant subgroup and none reported the criteria used to define unresectable liver metastases. For the larger *KRAS* wild-type subgroup, study participants were similar across the three studies. The median age of study participants was 61–62 years and 54–68% of participants were male. More than 90% of participants in all three studies had an Eastern Cooperative Oncology Group (ECOG) or a WHO performance status of 0 or 1 and two[27,53,54] out of three studies included only participants with histologically confirmed adenocarcinoma. The trial that included only participants with unresectable colorectal liver metastases and no extrahepatic metastases whose tumours were *KRAS* wild type was reported as an abstract only and did not provide any further details of participant characteristics.[55] The trial registry entry specified histologically confirmed adenocarcinoma and ECOG performance status of 0 or 1 as inclusion criteria.[60] Full details of study participants are reported in *Appendix 2*.

The included trials used various methods to assess *KRAS* mutation status. The CRYSTAL[27] and OPUS[28] trials both used the LightMix k-ras Gly12 assay (TIB MOLBIOL). PCR reactions were performed on a LightCycler 2.0 system using a *KRAS* mutation detection-specific program. The LightMix k-ras Gly12 assay detects nine mutations in codons 12 and 13 (*Table 10*).

TABLE 10 *KRAS* mutations detected by the LightMix k-ras Gly12 assay

Codon	Coding DNA	Protein/amino acid, three-letter code	Protein/amino acid, one-letter code
12	c.34G > A	p.Gly12Ser	p.G12S
	c.34G > C	p.Gly12Arg	p.G12R
	c.34G > T	p.Gly12Cys	p.G12C
	c.35G > A	p.Gly12Asp	p.G12D
	c.35G > C	p.Gly12Ala	p.G12A
	c.35G > T	p.Gly12Val	p.G12V
	c.[34G > A; 35G > C]	p.Gly12Thr	p.G12T
13	c.37G > T	p.Gly12Cys	p.G13C
	c.38G > A	p.Gly13Asp	p.G13D

The COIN trial[54] used pyrosequencing of *KRAS* codons 12, 13 (amplification primers 5′-GGCCTGCTGAAAATGACTGA-3′ and 5′-AGAATGGTCCTGCACCAGTAATA-3′ and extension primers 5′-TGTGGTAGTTGGAGCTG-3′, 5′-TGTGGTAGTTGGAGCT-3′ and 5′-TGGTAGTTGGAGCTGGT-3′) and 61 (amplification primers 5′-CTTTGGAGCAGGAACAATGTC-3′ and 5′-CTCATGTACTGGTCCCTCATTG-3′ and extension primer 5′-ATTCTCGACACAGCAGGT-3′) together with MALDI-TOF mass spectrometry. The MALDI-TOF genotyping assay was designed using the Sequenom MassARRAY Assay Design 3.1 software (Sequenom Inc., San Diego, CA, USA) and 200 base pairs of sequence upstream and downstream of each known mutation (known mutations taken from the Catalogue of Somatic Mutations in Cancer (COSMIC) database; see www.sanger.ac.uk/genetics/CGP/cosmic). For discordant results (< 1%), Sanger sequencing of *KRAS* codons 12, 13 (primers 5′-AAAAGGTACTGGTGGAGTATTTGA-3′ and 5′-CATGAAAATGGTCAGAGAAACC-3′) and 61 (primers 5′-CTTTGGAGCAGGAACAATGTC-3′ and 5′-CTCATGTACTGGTCCCTCATTG-3) was undertaken. The final trial[55] used pyrosequencing to identify mutations in *KRAS* codons 12 and 13 (information supplied in personal communication from the study author).

All four trials reported data on R0 resection rates in patients with colorectal metastases limited to the liver and *KRAS* wild-type tumours; for the CRYSTAL and OPUS trials these data were reported only in a conference abstract.[53] Three of the four trials also reported ORR.[28,53,55] Two trials, CRYSTAL[27] and OPUS,[56] used modified WHO criteria to assess tumour response, the COIN trial[54] used RECIST criteria[38] and the final trial[55] did not specify criteria for assessing tumour response. None of the three trials that reported subgroup data for participants whose metastases were limited to the liver reported data on how resectability of liver metastases was assessed post treatment.[27,28,54] The trial registry entry for the study that included only patients with unresectable liver metastases whose metastases were confined to the liver stated that post-treatment resectability would be assessed after 4–12 cycles by a MDT of more than three liver surgeons and one radiologist, using CT and MRI images.[60] Two studies reported PFS in the relevant patient group[28,54] and some limited data were also reported for overall survival (OS).

Clinical outcomes in patients with colorectal metastases limited to the liver and *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy compared with clinical outcomes in those treated with standard chemotherapy

All studies in this section reported that the addition of cetuximab to standard chemotherapy was associated with an increase in the rate of R0 resections (*Table 11*); however, this increase reached statistical significance only in the trial by Xu *et al.*[55] (OR 4.57, 95% CI 1.56 to 13.34). All three studies that assessed ORR reported a statistically significant higher response rate for participants treated with cetuximab plus standard chemotherapy than for those treated with standard chemotherapy alone; ORs ranged from 3.00 (95% CI 1.49 to 6.03)[53] to 4.93 (95% CI 1.42 to 17.06).[28] Only the COIN trial[54] reported an improvement in PFS associated with the addition of cetuximab to standard chemotherapy (HR 0.68, 95% CI 0.48 to 0.97). The study by Xu *et al.*[55] reported a significant improvement in 3-year survival rates for participants treated with cetuximab plus standard chemotherapy compared with those treated with standard chemotherapy alone (OR 2.76, 95% CI 1.12 to 6.26). There were no clear differences in treatment effect regardless of which *KRAS* mutation test was used to identify participants whose tumours were *KRAS* wild type (*Figures 5–7*). The median PFS for participants with *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy was 11.8 months in the CRYSTAL trial and 11.9 months in the OPUS trial; the corresponding PFS values in the standard chemotherapy groups were 9.2 months and 7.9 months.[53] The median OS for participants with *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy was 27.8 months in the CRYSTAL trial and 26.3 months in the OPUS trial; the corresponding OS values in the standard chemotherapy groups were 27.7 months and 23.9 months.[53]

TABLE 11 Effectiveness of cetuximab plus standard chemotherapy compared with standard chemotherapy alone in patients with *KRAS* wild-type tumours and liver-limited metastases

Study	*KRAS* test (mutations targeted)	Participant details	Intervention	Comparator	Outcome	Effect estimate (95% CI)
Bokemeyer 2011[28,53,56] (OPUS)	LightMix k-ras Gly12 assay (*KRAS* codons 12 and 13 missense mutations)	$n = 337$ *KRAS* wild type 179; *KRAS* wild type with liver-limited metastases 48; *KRAS* mutation status unknown 22/337 (no reason reported)	Cetuximab + FOLFOX-4 ($n = 25$)	FOLFOX-4 ($n = 23$)	PFS	HR 0.64 (0.23 to 1.79)
					ORR	OR 4.93 (1.42 to 17.06)[a]
					R0R	OR 4.19 (0.43 to 40.62)[a]
Van Cutsem 2009[27,53] (CRYSTAL)	LightMix k-ras Gly12 assay (*KRAS* codons 12 and 13 missense mutations)	$n = 1198$ *KRAS* wild type 348; *KRAS* wild type with liver-limited metastases 140; *KRAS* mutation status could not be evaluated 658/1198 (no reason reported)	Cetuximab + FOLFIRI ($n = 68$)	FOLFIRI ($n = 72$)	ORR	OR 3.00 (1.49 to 6.03)[a]
					R0R	OR 2.59 (0.76 to 8.86)[a]
Maughan 2011[54] (COIN)	Pyrosequencing and MALDI-TOF mass array with Sanger sequencing for discordant samples (< 1%) (*KRAS* mutations in codons 12, 13 and 61)	$n = 1630$ *KRAS* wild type 729; *KRAS* wild type with liver-limited metastases 178; *KRAS* mutation status unknown 336/1630 (141 tumour blocks not available, 173 blocks contained insufficent tumour material for processing, 22 not successfully genotyped)	Cetuximab + FOLFOX or XELOX ($n = 87$)	FOLFOX or XELOX ($n = 91$)	PFS	HR 0.68 (0.48 to 0.97)
					R0R	OR 1.16 (0.50 to 2.70)[a]
Xu 2012[55]	Pyrosequencing (*KRAS* mutations in codons 12 and 13)	$n = 116$ *KRAS* wild type 116; *KRAS* wild type with liver-limited metastases 116	Cetuximab + FOLFIRI or FOLFOX6 ($n = 59$)	FOLFIRI or FOLFOX6 ($n = 57$)	OSR	OR 2.76 (1.12 to 6.26)[a]
					ORR	OR 3.90 (1.80 to 8.43)[a]
					R0R	OR 4.57 (1.56 to 13.34)[a]

OSR, 3-year survival rate; R0R, R0 resection rate.
a   Calculated value.
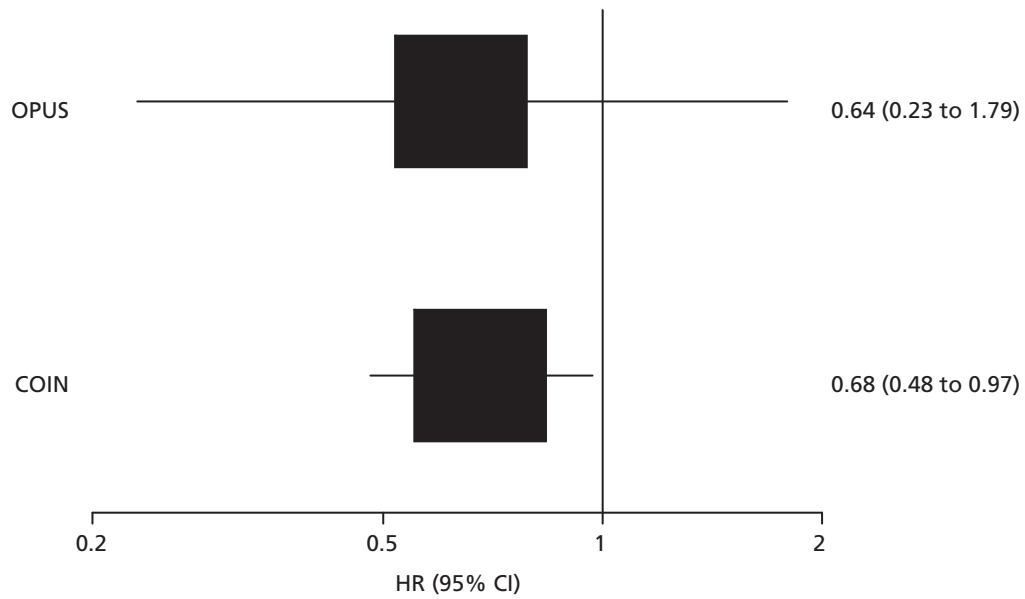
FIGURE 5 Progression-free survival in patients with colorectal metastases limited to the liver and *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy compared with those treated with standard chemotherapy.
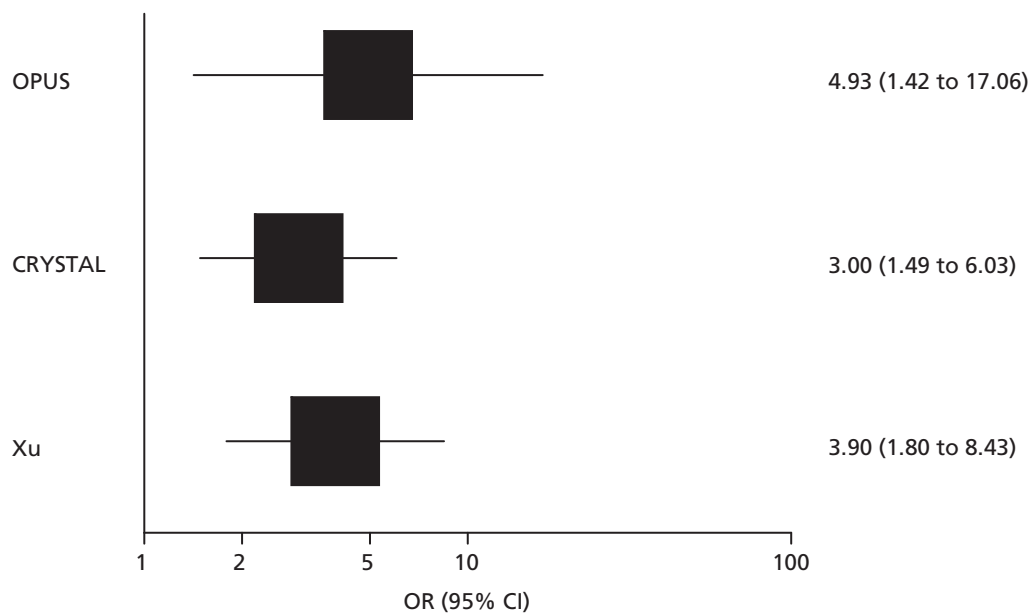
FIGURE 6 Objective response in patients with colorectal metastases limited to the liver and *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy compared with those treated with standard chemotherapy.

OPUS	4.19 (0.43 to 40.62)
CRYSTAL	2.59 (0.76 to 8.86)
COIN	1.16 (0.50 to 2.70)
Xu	4.57 (1.56 to 13.34)

OR (95% CI)
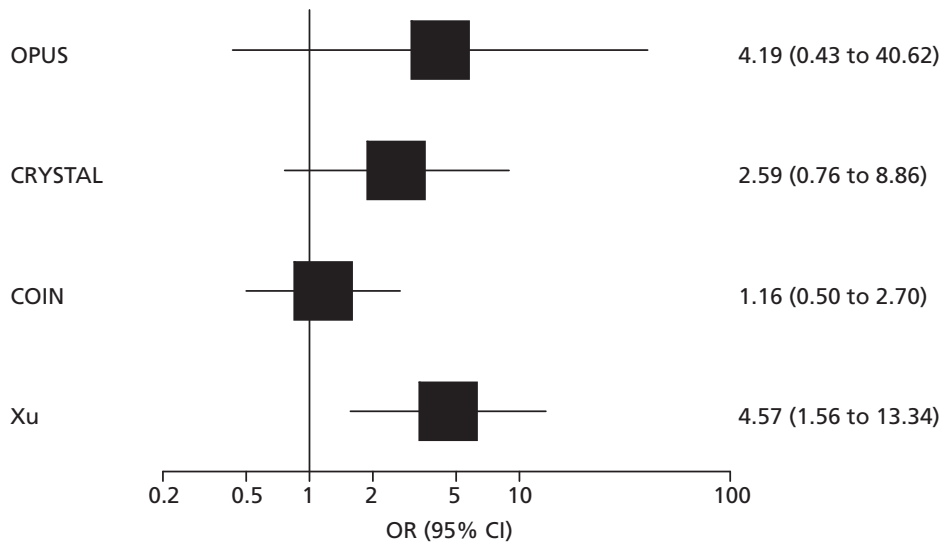
FIGURE 7 R0 resection rate in patients with colorectal metastases limited to the liver and *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy compared with those treated with standard chemotherapy.

Clinical outcome for studies that provided data for patients according to *KRAS* mutation test status

Data from the COIN trial[54] indicated that there was a slight increase in PFS for patients with initially unresectable liver metastases whose tumours were *KRAS* wild type who received cetuximab plus FOLFOX or XELOX compared with those who received FOLFOX or XELOX alone (HR 0.48, 95% CI 0.48 to 0.97). Additional data supplied by the COIN trial investigators indicated that, for patients with initially unresectable liver metastases whose tumours were *KRAS* mutant, there was no significant difference in PFS between the two treatment groups (HR 1.19, 95% CI 0.80 to 1.77). The reported rate of potentially curative resection in patients whose tumours were *KRAS* wild type was 15% (13/87) for the cetuximab plus FOLFOX or XELOX group and 13% (12/91) for the FOLFOX or XELOX only group.[54] The COIN trial investigators provided additional data for patients whose tumours were *KRAS* mutant; in these patients the potentially curative resection rate was 16% (12/74) for the cetuximab plus FOLFOX or XELOX group and 14% (6/44) for the FOLFOX or XELOX only group.

Risk of bias

All studies in this section were rated as having a low or unclear risk of bias for randomisation, incomplete outcome data and selective outcome reporting. All three of the trials that were reported as full papers stated that effectiveness analyses were conducted on an intention-to-treat (ITT) basis.[27,28,54] Details of allocation concealment were generally not reported, with the exception of the COIN trial,[54] which stated that treatment allocation was not masked. All studies were rated as having a high risk of bias for blinding of study participants and personnel; all were open-label studies. However, two studies[55,56] were rated as having a low risk of bias for blinding of outcome assessors as they reported some independent/blinded assessment of outcomes. The results of the risk of bias assessments are summarised in *Table 12* and a full risk of bias assessment for each study is provided in *Appendix 3*.

TABLE 12 Risk of bias assessments for RCTs providing data on how the effectiveness of adding cetuximab to standard chemotherapy varies according to which *KRAS* mutation test is used to select patients for treatment

Study	Risk of bias					
	Randomisation	Allocation concealment	Participant and personnel blinding	Outcome assessor blinding	Incomplete outcome data	Selective outcome reporting
Bokemeyer 2011[28,56] (OPUS)	?	?	☹	☺	☺	☺
Van Cutsem 2009[27,53] (CRYSTAL)	?	?	☹	?	☺	☺
Maughan 2011[54] (COIN)	☺	☹	☹	?	☺	☺
Xu 2012[55,60]	?	?	☹	?	?	?

☺, low risk; ☹, high risk; ?, unclear risk.

Chapter 4 Assessment of cost-effectiveness

This chapter explores the cost-effectiveness of the use of different *KRAS* mutation tests to decide between standard chemotherapy and cetuximab in combination with standard chemotherapy in adults with mCRC in whom metastases are confined to the liver and are unresectable.

Review of economic analyses of *KRAS* mutation testing

Search strategy

Searches were undertaken to identify cost-effectiveness studies of *KRAS* mutation testing in mCRC. As with the clinical effectiveness searching, the main EMBASE strategy for each set of searches was independently peer reviewed by a second information specialist using the PRESS-EBC.[43] Search strategies were developed specifically for each database and searches took into account generic and other product names for the intervention. All search strategies are reported in *Appendix 1*.

The following databases were searched for relevant studies from 2000 to January 2013:

- MEDLINE (OvidSP) (2000 to Week 3 January 2013)
- MEDLINE In-Process and Other Non-Indexed Citations and Daily Update (OvidSP) (up to 28 January 2013)
- EMBASE (OvidSP) (2000 to Week 4 2013)
- NHS Economic Evaluation Database (NHS EED) (Wiley) (The Cochrane Library 2000 to Issue 4, 2012)
- Health Economic Evaluations Database (HEED) (Wiley) (up to 30 January 2013), http://onlinelibrary.wiley.com/book/10.1002/9780470510933
- EconLit (EBSCO) (2000 to 30 January 2013)
- Science Citation Index (SCI-EXPANDED) (Web of Science) (2000 to 25 January 2013).

Inclusion criteria

Studies reporting a full economic analysis that related explicitly to the test–treat combination of *KRAS* mutation testing and treatment with cetuximab were eligible for inclusion. Specifically, one of the comparators included *KRAS* mutation testing and for this comparator the treatment decision was guided by the test result; patients whose tumour was *KRAS* mutant were also included in the treatment pathway.

Results

The search retrieved 445 references. Following title and abstract screening, 416 references were excluded. Of the remaining 29 titles, the full papers were screened, which led us to exclude another 24, leaving five references: one HTA report (from Ontario)[61] and four papers.[62–65] A summary of the included studies is provided in *Table 13* with a quality checklist based on Drummond *et al.*[66] provided in *Table 14*.

The Ontario HTA report[61] aimed to determine the cost-effectiveness of *KRAS* mutation testing for the third-line treatment of (stage IV) mCRC in Ontario. For this purpose, seven strategies were compared:

- 0 best supportive care (BSC)
- 1(a) cetuximab with *KRAS* mutation testing
- 1(b) cetuximab without *KRAS* mutation testing
- 2(a) panitumumab with *KRAS* mutation testing
- 2(b) panitumumab without *KRAS* mutation testing
- 3(a) cetuximab plus irinotecan with *KRAS* mutation testing
- 3(b) cetuximab plus irinotecan without *KRAS* mutation testing.

TABLE 13 Summary of included papers

Study details	Health Quality Ontario[61]
Population	Patients diagnosed with (stage IV) mCRC for whom cetuximab or panitumumab monotherapies or cetuximab and irinotecan combination therapy were indicated as third-line treatment
Time horizon	Lifetime
Objective	To determine the cost-effectiveness of *KRAS* mutation testing for the third-line treatment of mCRC in Ontario
Source of effectiveness information	Survival, utility weights and adverse events taken from various studies
Comparators	(0) BSC; (1a) cetuximab (perform *KRAS* mutation test); (1b) cetuximab (no *KRAS* mutation test); (2a) panitumumab (perform *KRAS* mutation test); (2b) panitumumab (no *KRAS* mutation test); (3a) cetuximab + irinotecan (perform *KRAS* mutation test); (3b) cetuximab + irinotecan (no *KRAS* mutation test)
Unit costs	Taken from the literature and the 2009 Ontario Health Insurance Plan and Ontario Case Costing Initiative administrative databases
Measure of benefit	QALYs
Study type	Cost–utility analysis: Markov model
Model assumptions	None mentioned
Perspective	Ontario Ministry of Health and Long-Term Care
Discount rate	5% for effects and costs
Uncertainty around cost-effectiveness ratio expressed	Only in text and only for the strategies for which PSA was performed. CEACs, etc., not shown as the report is very concise
Sensitivity analysis	PSA for strategies 0, 1a, 2a and 3a (i.e. does not include the 'no *KRAS* mutation testing' strategies). The PSA varied parameters only on a highly aggregated level: PFS, OS, total costs and utilities. It seems as if a uniform distribution was used for all of them?
Outcomes (cost and LYs/QALYs) per comparator	0: C$1414, 0.7455; 2a: C$12,236, 0.9719; 1a C$18,305, 1.0537; 2b C$20,424, 0.9985; 3a C$23,373, 1.2596; 1b C$29,399, 1.0447; 3b C$44,798, 1.3907
Summary of incremental analysis	For all strategies involving *KRAS* mutation testing, cetuximab with irinotecan combination therapy was the cost-effective option for increasing values of WTP. For lower WTP values, the probabilities of specific *KRAS* mutation testing strategies being cost-effective varied. At a WTP of C$50K, the probability of cetuximab monotherapy, panitumumab monotherapy and cetuximab with irinotecan combination therapy being cost-effective was approx. 14%, 44% and 42% respectively. The BSC strategy was not cost-effective (0% probability) for WTP values < C$45K
Study details	**Vijayaraghavan *et al.*[62]**
Population	Patients with mCRC in whom previous chemotherapy had failed
Time horizon	Lifetime
Objective	To assess the cost-effectiveness of testing for *KRAS* mutations before administering EGFR inhibitors such as cetuximab and panitumumab in the USA and Germany
Source of effectiveness information	Three recently published studies on the efficacy of EGFR inhibitors in patients with and without *KRAS* mutations
Comparators	(1) Combination therapy (cetuximab + irinotecan/FOLFIRI) with *KRAS* mutation testing; (2) combination therapy (cetuximab + irinotecan/FOLFIRI) without *KRAS* mutation testing; (3) cetuximab alone with *KRAS* mutation testing; (4) cetuximab alone without *KRAS* mutation testing; (5) panitumumab alone with *KRAS* mutation testing; (6) panitumumab alone without *KRAS* mutation testing
Unit costs	For the US model, costs were taken from the Medicare fee schedule. For the German model, costs were taken from published literature and expert opinion
Measure of benefit	LYs

TABLE 13 Summary of included papers (*continued*)

Study details	Vijayaraghavan *et al.*[62]
Study type	Cost-effectiveness analysis: Markov model
Model assumptions	(1) Patients with *KRAS* mutant tumours received no benefit from EGFR inhibitors; (2) patients with *KRAS* mutant tumours received some benefit from combination therapy containing FOLFIRI or irinotecan; (3) *KRAS* mutation testing has a sensitivity of 95% and a specificity of 100%
Perspective	Health-care payer perspective
Discount rate	None mentioned
Uncertainty around cost-effectiveness ratio expressed	No, only uncertainty around cost-savings presented by means of one-way sensitivity analyses
Sensitivity analysis	One-way sensitivity analyses varying percentage of patients with *KRAS* wild-type tumours, cost of cetuximab, cost of BSC and cost of *KRAS* mutation test
Outcomes (cost and LYs/QALYs) per comparator	Panitumumab with *KRAS* mutation testing: LYs 18.26, €13,787, US$19,656; panitumumab without *KRAS* mutation testing: LYs 18.26, €18,399, US$27,202; cetuximab with *KRAS* mutation testing: LYs 19.78, €13,588, US$22,893; cetuximab without *KRAS* testing: LYs 19.78, €17,444, US$30,933; combination therapy 1 with *KRAS* mutation testing: LYs 24.26, €26,292, US$35,075; combination therapy 2 with *KRAS* mutation testing: LYs 25.83, €–, US$36,148; combination therapy without *KRAS* mutation testing: LYs 25.83, €35,852, US$48,576
Summary of incremental analysis	*KRAS* mutation testing to select patients eligible for EGFR inhibitors is cost-saving at equivalent clinical outcome

Study details	Behl *et al.*[63]
Population	Patients with mCRC who are chemorefractory
Time horizon	10 years
Objective	To assess the cost-effectiveness of screening for *KRAS* and *BRAF* mutations before EGFR inhibitor treatment
Source of effectiveness information	RCTs
Comparators	(1) No anti-EGFR therapy (BSC); (2) anti-EGFR therapy without screening; (3) screening for *KRAS* mutations only (before providing anti-EGFR therapy); (4) screening for *KRAS* and *BRAF* mutations (before providing anti-EGFR therapy)
Unit costs	For cost of chemotherapy, average selling price plus median Medicare average payment for physician services for administration of the chemotherapies. Total costs also include cost of liver resection(s)
Measure of benefit	LYs
Study type	Cost-effectiveness analysis: Markov model
Model assumptions	Many small assumptions
Perspective	Not specified: probably US third-party payer
Discount rate	3% for costs and effects
Uncertainty around cost-effectiveness ratio expressed	Yes, with scatter plots, acceptability curves and frontier
Sensitivity analysis	(1) One way sensitivity analyses: conversion probability for chemotherapy is 30%; conversion probability for bevacizumab is +10%, cetuximab is +20%; cost of surgery is +50%; cost of screening is (a) +50% and (b) −50%; prognostic decrease in OS with *BRAF* mutation (regardless of treatment). (2) Cohort simulation: a cohort of 50,000 patients is analysed 10,000 times. No PSA mentioned

TABLE 13 Summary of included papers (*continued*)

Study details	Behl *et al.*[63]
Outcomes (cost and LYs/QALYs) per comparator	No anti-EGFR therapy: US$34,291, LYs 0.6686; *KRAS* and *BRAF* mutation screening with anti-EGFR therapy: US$56,324, LYs 0.7025; *KRAS* screening with anti-EGFR therapy: US$57,348, LYs 0.7029; anti-EGFR therapy without screening: US$64,841, LY 0.7055
Summary of incremental analysis	ICER (cost/LY) for *KRAS* and *BRAF* mutation screening compared with no anti-EGFR therapy was US$648,396. Other ICERS (*KRAS* mutation screening compared with *KRAS* and *BRAF* mutation screening, no screening compared with *KRAS* mutation screening) were > US$2M/LY gained

Study details	Shiroiwa *et al.*[64]
Population	Japanese patients with mCRC in whom previous chemotherapy (including fluoropyrimidine, irinotecan and oxaliplatin) had failed or who had contraindications to these drugs
Time horizon	2.5 years
Objective	To determine the cost-effectiveness of cetuximab treatment after *KRAS* mutation testing compared with BSC
Source of effectiveness information	PFS and OS were taken from the National Cancer Institute of Canada Clinical Trials Group CO.17 (NCIC CO.17) trial[67]
Comparators	(1) *KRAS* testing strategy: patients with *KRAS* wild-type tumours received cetuximab and those with *KRAS* mutations received BSC; (2) no *KRAS* mutation testing strategy: all patients received cetuximab; (3) no cetuximab: all patients received BSC
Unit costs	Costs were calculated according to the social insurance reimbursement schedule and the drug tariff based on Japanese 'fee for service'
Measure of benefit	LYs and QALYs
Study type	Cost-effectiveness and cost–utility analysis: Markov model
Model assumptions	(1) Utility of PFS was assumed to be 0.7 for all treatments (cetuximab and BSC); (2) 40% of patients were assumed to have *KRAS* mutant tumours
Perspective	Health-care payer's perspective
Discount rate	3% for both costs and effects
Uncertainty around cost-effectiveness ratio expressed	CEACs
Sensitivity analysis	One-way sensitivity analyses were performed for discount rates, body surface area, percentage of patients with *KRAS* mutant tumours, BSC costs, HR of cetuximab for wild-type patients and costs of *KRAS* mutation testing. A PSA was also performed
Outcomes (cost and LYs/QALYs) per comparator	*KRAS* mutation testing strategy: US$29,000, LYs 0.70, QALYs 0.49; no *KRAS* mutation testing strategy: US$35,000, LYs 0.69, QALYs 0.48; no cetuximab strategy: US$6800, LYs 0.52, QALYs 0.36
Summary of incremental analysis	*KRAS* testing vs. no *KRAS* mutation testing: *KRAS* mutation testing dominant; *KRAS* mutation testing vs. no cetuximab: US$180,000 per QALY gained; no *KRAS* mutation testing vs. no cetuximab: US$230,000 per QALY gained

Study details	Blank *et al.*[65]
Population	Patients with mCRC who are chemorefractory
Time horizon	Lifetime
Objective	To assess the cost-effectiveness of testing for *KRAS* and *BRAF* mutations before cetuximab treatment
Source of effectiveness information	NCIC CO.17 trial[67]
Comparators	(1) *KRAS* mutation testing; (2) *KRAS* mutation testing with subsequent *BRAF* testing of *KRAS* wild-type patients (*KRAS/BRAF*); (3) cetuximab without testing. Comparison was against a reference strategy of no cetuximab treatment. In the testing strategies, cetuximab was administered if no mutations were detected

TABLE 13 Summary of included papers (*continued*)

Study details	Blank *et al.*[65]
Unit costs	Unit costs were drawn from the official Swiss tariff list (Tarmed). Drug costs were based on official Swiss pharmacy prices
Measure of benefit	QALYs
Study type	Cost–utility analysis: Markov cohort simulation model
Model assumptions	70% of patients were assumed to have *KRAS* wild-type tumours
Perspective	Swiss health-care system
Discount rate	3% for both costs and effects
Uncertainty around cost-effectiveness ratio expressed	CEACs and cost-effectiveness acceptability frontier
Sensitivity analysis	One-way sensitivity analyses were performed for different values of utilities, sensitivity and specificity of mutation analyses, prevalence of *KRAS* and *BRAF* mutations and OS and PFS. A PSA was also performed
Outcomes (cost and LYs/QALYs) per comparator	Reference treatment (no cetuximab): €3983, QALYs 0.4430; *KRAS* and *BRAF* mutation testing: €34,771, QALYs 0.934; *KRAS* mutation testing: €35,361, QALYs 0.936; no testing: €38,662, QALYs 0.947
Summary of incremental analysis	*KRAS* and *BRAF* mutation testing compared with reference strategy: €62,653 per QALY; *KRAS* mutation testing compared with *KRAS* and *BRAF* mutation testing: €313,537 per QALY; no testing compared with *KRAS* mutation testing: €314,588 per QALY

BRAF, v-raf murine sarcoma viral oncogene homolog B; CEAC, cost-effectiveness acceptability curve; ICER, incremental cost-effectiveness ratio; LY, life-year; PSA, probabilistic sensitivity analysis; QALY, quality-adjusted life-year; WTP, willingness to pay.

TABLE 14 Checklist of study quality

	Health Quality Ontario[61]	Vijayaraghavan *et al.*[62]	Behl *et al.*[63]	Shiroiwa *et al.*[64]	Blank *et al.*[65]
Study design					
The research question is stated	✓	✓	✓	✓	✓
The economic importance of the research question is stated	✗	✓	✓	✓	✓
The viewpoint(s) of the analysis are clearly stated and justified	✓	✓	✗	✓	✓
The rationale for choosing alternative programmes or interventions compared is stated	✓	✓	✓	✓	✓
The alternatives being compared are clearly described	✓	✓	✓	✓	✓
The form of economic evaluation used is stated	✓	✓	✓	✓	✓
The choice of form of economic evaluation is justified in relation to the questions addressed	✓	✓	✓	✓	✓
Data collection					
The source(s) of effectiveness estimates used are stated	✓	✓	✓	✓	✓
Details of the design and results of the effectiveness study are given (if based on a single study)	✓	✓	✓	✗	✓
Details of the methods of synthesis or meta-analysis of estimates are given (if based on a synthesis of a number of effectiveness studies)	NA	NA	NA	NA	NA

TABLE 14 Checklist of study quality (*continued*)

	Health Quality Ontario[61]	Vijayaraghavan *et al.*[62]	Behl *et al.*[63]	Shiroiwa *et al.*[64]	Blank *et al.*[65]
The primary outcome measure(s) for the economic evaluation are clearly stated	✓	✓	✓	✓	✓
Methods to value benefits are stated	✓	✓	✓	✓	✓
Details of the subjects from whom valuations were obtained are given	✗	NA	NA	✗	✗
Productivity changes (if included) are reported separately	NA	NA	NA	NA	NA
The relevance of productivity changes to the study question is discussed	✗	✗	✗	✗	✗
Quantities of resource use are reported separately from their unit costs	✓	✗	✗	✓	✗
Methods for the estimation of quantities and unit costs are described	✓	✓	✓	✓	✓
Currency and price data are recorded	✓	✓	✓	✓	✓
Details of currency of price adjustments for inflation or currency conversion are given	✓	✓	✓	✓	✓
Details of any model used are given	✓	✓	✓	✓	✓
The choice of model used and the key parameters on which it is based are justified	✓	✓	✓	✓	✓
Analysis and interpretation of results					
Time horizon of costs and benefits is stated	✓	✓	✓	✓	✓
The discount rate(s) is stated	✓	✗	✓	✓	✓
The choice of discount rate(s) is justified	✓	✗	✓	✓	✓
An explanation is given if costs and benefits are not discounted	NA	✗	NA	NA	NA
Details of statistical tests and CIs are given for stochastic data	✗	✗	✗	✗	✗
The approach to sensitivity analysis is given	✓	✓	✓	✓	✓
The choice of variables for sensitivity analysis is justified	✗	✓	✓	✗	✓
The ranges over which the variables are varied are justified	✗	✗	✗	✗	✓
Relevant alternatives are compared	✓	✓	✓	✓	✓
Incremental analysis is reported	✓	✓	✓	✓	✓
Major outcomes are presented in a disaggregated as well as aggregated form	✓	✓	✓	✓	✓
The answer to the study question is given	✓	✓	✓	✓	✓
Conclusions follow from the data reported	✓	✓	✓	✓	✓
Conclusions are accompanied by the appropriate caveats	✗	✓	✓	✓	✓

NA, not applicable.

In the strategies with *KRAS* mutation testing, only patients with wild-type *KRAS* tumours receive the therapy in question. In the strategies without *KRAS* mutation testing, all patients receive the therapy. A cost–utility analysis was performed by means of a Markov model with a lifetime time horizon. Inputs for PFS, OS, utility weights and adverse events were obtained from various clinical studies. Although a probabilistic sensitivity analysis (PSA) was performed for the BSC strategy and all *KRAS* mutation testing strategies, the information on uncertainty around the incremental cost-effectiveness ratio (ICER) presented in the report was limited to some percentages taken from the cost-effectiveness acceptability curve (CEAC) (which was not shown). Also, it appears that the parameters that were varied within the PSA were highly aggregated (PFS, OS, utility scores and total costs) and the distribution used was not mentioned (possibly uniform, as only a range was given). The deterministic results showed that, compared with BSC, all monotherapy strategies are either dominated or extendedly dominated. The ICER for cetuximab plus irinotecan with *KRAS* mutation testing compared with BSC is C\$42,710. The ICER for cetuximab plus irinotecan without *KRAS* mutation testing compared with cetuximab plus irinotecan with *KRAS* mutation testing is C\$163,396. The authors concluded that, although *KRAS* mutation testing is cost-effective for all strategies considered, it is not equally cost-effective for all treatment options.

Vijayaraghavan *et al.*[62] developed a Markov model to compare six hypothetical strategies for the second-line treatment of patients with mCRC who have failed previous chemotherapy:

1. combination therapy (cetuximab plus irinotecan/FOLFIRI) with *KRAS* mutation testing
2. combination therapy (cetuximab plus irinotecan/FOLFIRI) without *KRAS* mutation testing
3. cetuximab alone with *KRAS* mutation testing
4. cetuximab alone without *KRAS* mutation testing
5. panitumumab alone with *KRAS* mutation testing
6. panitumumab alone without *KRAS* mutation testing.

In treatment strategies without *KRAS* mutation testing, all patients received EGFR inhibitor-based chemotherapy, as did the patients with *KRAS* wild-type tumours in the treatment strategies with *KRAS* mutation testing. Patients with *KRAS* mutant tumours received chemotherapy without EGFR inhibitors or BSC. The model results were calculated for a situation in the USA as well as in Germany, with country-specific chemotherapy regimens and associated costs. Clinical effects were assumed to be the same for the USA and Germany and were based on published studies. In the results section the treatment strategies were compared as *KRAS* mutation testing compared with no *KRAS* mutation testing within a certain treatment regimen. For all of these comparisons *KRAS* mutation testing saved costs at equivalent clinical outcomes.

The cost-effectiveness of both *KRAS* and v-raf murine sarcoma viral oncogene homolog B (*BRAF*) mutation screening in patients with mCRC who are chemorefractory was the subject of a paper by Behl *et al.*[63] A decision-analytic model was developed comparing the following strategies:

1. *KRAS* plus *BRAF* mutation screening (before providing anti-EGFR therapy)
2. *KRAS* mutation screening (before providing anti-EGFR therapy)
3. anti-EGFR therapy (no screening)
4. no anti-EGRF therapy (no screening).

Inputs for the model were estimated using observations from RCTs. The model followed each patient for a maximum of 10 years. The no cetuximab strategy was least costly (US\$34,291) but also least effective [0.6686 (LYs)], followed by the *KRAS* plus *BRAF* screening therapy, which offered more LYs (0.7025) but at a higher cost (US\$56.324). Screening for *KRAS* mutations again added more LYs (0.7029) at a higher cost (US\$57,348) but at an unfavourable ratio compared with *KRAS* plus *BRAF* mutation screening (ICER > US\$2M). Finally, the anti-EGFR strategy – providing cetuximab to all patients without screening – was the most effective (0.7055 LYs) and the most expensive (US\$64,841) strategy, which only proved cost-effective at a willingness to pay of > US\$3M. Therefore, *KRAS* plus *BRAF* mutation screening appeared to be the most cost-effective option compared with no anti-EGFR therapy, but still had an ICER of US\$648,396 per life-year gained.

Shiroiwa *et al.*[64] performed a cost-effectiveness analysis of *KRAS* testing in a Japanese population of patients with mCRC in whom previous chemotherapy had failed. The included strategies were *KRAS* mutation testing, no *KRAS* mutation testing (all patients receive cetuximab) and no cetuximab (all patients receive BSC). In the *KRAS* mutation testing strategy, patients with *KRAS* wild-type tumours received cetuximab whereas patients with *KRAS* mutant tumours received BSC. The analysis involved a three-state Markov model to estimate and extrapolate survival curves and treatment costs. Transition probabilities for disease progression and survival after disease progression were derived from the NCIC CO.17 trial.[67] The time horizon of the analysis was 2.5 years. As expected, the no cetuximab strategy was least costly (US\$6800) and least effective [0.36 quality-adjusted life-years (QALYs)]. The no *KRAS* mutation testing strategy (all patients receive cetuximab) cost US\$35,000 and resulted in 0.48 QALYs whereas the *KRAS* mutation testing strategy cost US\$29,000 and resulted in 0.49 QALYs. Therefore, *KRAS* mutation testing is considered dominant compared with no *KRAS* mutation testing. The ICER for *KRAS* mutation testing compared with no cetuximab was US\$180,000 per QALY. The authors concluded that, although *KRAS* mutation testing compared with no *KRAS* mutation testing could be considered dominant, the ICER for cetuximab treatment is too high, even if treatment is limited to patients with *KRAS* wild-type tumours.

Blank *et al.*[65] constructed a model comparing the cost-effectiveness of four strategies for chemorefractory patients with mCRC: *KRAS* mutation testing, *KRAS* mutation testing with subsequent *BRAF* mutation testing of *KRAS* wild-type tumours, cetuximab treatment without testing and the reference strategy of no cetuximab. In the testing strategies, cetuximab treatment was initiated if no mutations were detected. BSC was given to all patients. Survival times and utilities were derived from published RCTs. Costs were assessed from the perspective of the Swiss health system. Adding cetuximab to BSC increased costs considerably, but the increase in costs in the testing strategies was distinctly lower than that in the no testing strategy. The costs of mutation testing were overcompensated for by savings associated with the restriction of cetuximab administration. The least costly and least effective strategy was the reference strategy (no cetuximab). Testing for *KRAS* and *BRAF* mutations led to an ICER of €62,653 per QALY compared with the reference strategy. Testing for *KRAS* mutations only compared with testing for *KRAS* and *BRAF* mutations, as well as the no testing strategy compared with *KRAS* mutation testing, both had ICERs well above €300,000 per QALY. The authors concluded that testing for *KRAS* and *BRAF* mutations before cetuximab treatment of chemorefractory mCRC patients is clinically appropriate and economically favourable, despite high costs for predictive testing [i.e. given that cetuximab should be the next step in the treatment pathway, it is worthwhile to test for mutations first; it appears that the reference strategy (no cetuximab) was not included in this recommendation].

Based on all of these publications it can be said that, in general, although *KRAS* testing is obviously a more cost-effective option than administering cetuximab to all patients, the ICER of *KRAS* testing and treating with cetuximab only those patients with *KRAS* wild-type tumour status compared with treating all patients with standard chemotherapy alone seems rather high.

Model structure and methodology

KRAS *mutation tests considered in the model*
The health economic analysis will determine the cost-effectiveness of different methods for *KRAS* mutation testing to decide between standard chemotherapy or standard chemotherapy plus cetuximab in adults with mCRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are unresectable but may become resectable after response to chemotherapy. Standard chemotherapy regimens considered include FOLFOX and FOLFIRI. A range of methods for *KRAS* mutation testing are currently used in NHS laboratories in England and Wales. Ideally, the performance of these tests would be assessed against an objective measure of the true presence/absence of a clinically relevant *KRAS* mutation (the 'reference standard'). The comparative effectiveness of treatment (cetuximab plus chemotherapy vs. chemotherapy alone) conditional on the true or false presence/absence of the *KRAS* mutation could then be determined. However, each testing method targets a different range of mutations and has different limits of detection

(the lowest proportion of mutation detectable in tumour cells) and the exact combination of mutation type and level that will provide optimal treatment selection remains unclear. For this reason, assessment of test performance based on comparison with a conventional 'reference standard' is currently not possible. In this situation, an alternative way to determine the relative value of diagnostic methods for *KRAS* mutation testing is to use studies that report on the comparative treatment effect in patients with different *KRAS* mutation status (positive, negative or unknown), as defined using different *KRAS* mutation tests. As outlined in the previous chapter, information on the accuracy of tests (either based on ORR or tumour resection rate) for distinguishing between patients with *KRAS* wild-type tumours and patients with *KRAS* mutant tumours with metastases confined to the liver was available only for the Therascreen *KRAS* RGQ PCR Kit[52] and pyrosequencing and MALDI-TOF mass spectometry.[54] A major assumption underlying the use of these accuracy data in the health economic modelling is that differences in response rates and resection rates between the two trials are solely due to the use of different *KRAS* mutation tests.

In the COIN trial[54] patients were tested using both pyrosequencing and the MALDI-TOF mass array, with a reported concordance of > 99%. It was therefore assumed that, for the economic evaluation, MALDI-TOF and pyrosequencing are equal, that is, all results reported for pyrosequencing also apply to MALDI-TOF. However, survey data were available only for pyrosequencing and therefore pyrosequencing data are reported in the results tables.

For all other *KRAS* mutation tests listed in the scope, no accuracy data were available. As a result, for the remaining tests it was only possible to make a comparison based on differences in technical performance and test costs retrieved from the online survey of NHS laboratories in England and Wales (see *Chapter 3*, *What are the technical performance characteristics of the different* KRAS *mutation tests?*), whilst assuming a prognostic value equal to pyrosequencing across all tests. The latter assumption was not based on evidence of equality but rather on the absence of any reliable evidence to model a difference in prognostic value for these tests.

Based on the information available, two analyses were performed:

1. *'Linked evidence' analysis* – for all tests for which information on accuracy was available. In this analysis the Therascreen *KRAS* RGQ PCR Kit was compared with pyrosequencing. For the Therascreen *KRAS* RGQ PCR Kit, accuracy based on ORRs was taken from the CELIM trial.[52] Resection rates for patients with a *KRAS* wild-type test result treated with cetuximab plus chemotherapy were also based on CELIM trial data (data not reported in the published study but provided as part of the submissions that informed NICE guidance TA176[1]) whereas resection rates for patients with *KRAS* mutant and unknown test results who were treated with chemotherapy alone were taken from the Groupe Coopérateur Multidisciplinaire en Oncologie (GERCOR) study[68] as the CELIM trial did not contain a chemotherapy-only strategy. For pyrosequencing, both accuracy data (based on resection rates) and resection rates for cetuximab plus chemotherapy as well as chemotherapy alone were taken from the COIN trial[54] and from additional data supplied by the COIN triallists. PFS and OS after successful resection were assumed to be conditional on resection and treatment independent.

2. *'Assumption of equal prognostic value' analysis* – for all tests for which information on technical performance was available from the online survey. In this analysis we assessed whether the tests were likely to be cost-effective given an assumption of equal prognostic value and test-specific information on failure rate only. The equal prognostic value assigned was based on data for the pyrosequencing test (as this was the only test for which accuracy data were available on resection rates following treatment with chemotherapy, with and without cetuximab, for patients with initially inoperable liver metastases and both *KRAS* mutant and *KRAS* wild-type tumours). The following tests were included in this analysis:

    i. cobas *KRAS* Mutation Test Kit (Roche Molecular Systems)
    ii. Therascreen *KRAS* RGQ PCR Kit (QIAGEN)
    iii. Therascreen *KRAS* Pyro Kit (QIAGEN)
    iv. *KRAS* LightMix Kit (TIB MOLBIOL)

    v. *KRAS* StripAssay (ViennaLab)
   vi. HRM analysis
  vii. pyrosequencing
 viii. MALDI-TOF mass spectrometry
   ix. next-generation sequencing
    x. Sanger sequencing.

Consistency with related assessments

This assessment does not update the appraisal of cetuximab for the first-line treatment of mCRC.[1] To ensure consistency between the modelling approach used in technology appraisal TA176[1] and the assessment of the cost-effectiveness of different methods for *KRAS* mutation testing in this report, the assessment group received the electronic health economic model submitted by Merck Serono for technology appraisal TA176. This model calculates the expected cost-effectiveness of chemotherapy plus cetuximab compared with chemotherapy for the first-line treatment of mCRC patients whose metastases are confined to the liver and are unresectable and whose tumours are *KRAS* wild type as tested with a pre-CE-marked version of the *KRAS* LightMix Kit.

This model, together with the amendments suggested and made by the Evidence Review Group and NICE, was used to inform the development of a de novo model in which the long-term consequences of using different *KRAS* mutation tests were assessed not only in patients with *KRAS* wild-type tumours but also in patients with *KRAS* mutant tumours or an unknown test result.

Model structure

In the health economic model the mean expected costs and QALYs were calculated for each alternative. As specified in the protocol, this economic evaluation takes a 'no comparator' approach, which implies that the cost-effectiveness of each strategy will be presented compared with the next most cost-effective strategy.

The health economic analysis considers the long-term consequences of the technical performance and accuracy of the different tests followed by treatment with cetuximab plus standard chemotherapy or standard chemotherapy alone in patients (average starting age 60 years) with mCRC whose metastases are confined to the liver and are unresectable. For this purpose a decision tree and a Markov model were developed. The decision tree was used to model the test result (*KRAS* wild type, *KRAS* mutant or unknown) and the accompanying treatment decision. In the model, patients with a *KRAS* wild-type tumour receive cetuximab plus standard chemotherapy. It is assumed that patients with a *KRAS* mutant tumour will receive standard chemotherapy (i.e. FOLFOX). Patients with an unknown *KRAS* status are also assumed to receive standard chemotherapy as cetuximab is indicated only for patients with *KRAS* wild-type tumour status.[1] The decision tree is shown in *Figure 8*.

The long-term consequences in terms of costs and QALYs were estimated using a Markov model with a cycle time of 1 week and a lifetime time horizon (23 years were modelled using 1200 cycles). Health states in the Markov model are numbered according to NICE technology appraisal TA176:[1]

1. progression-free first line – never operated
2. PD second line – never operated
3. PD second line – unsuccessful resection
4. survival after curative resection
5. progression-free first line – unsuccessful resection
6. PD third line – never operated
7. PD third line – unsuccessful resection
8. dead.
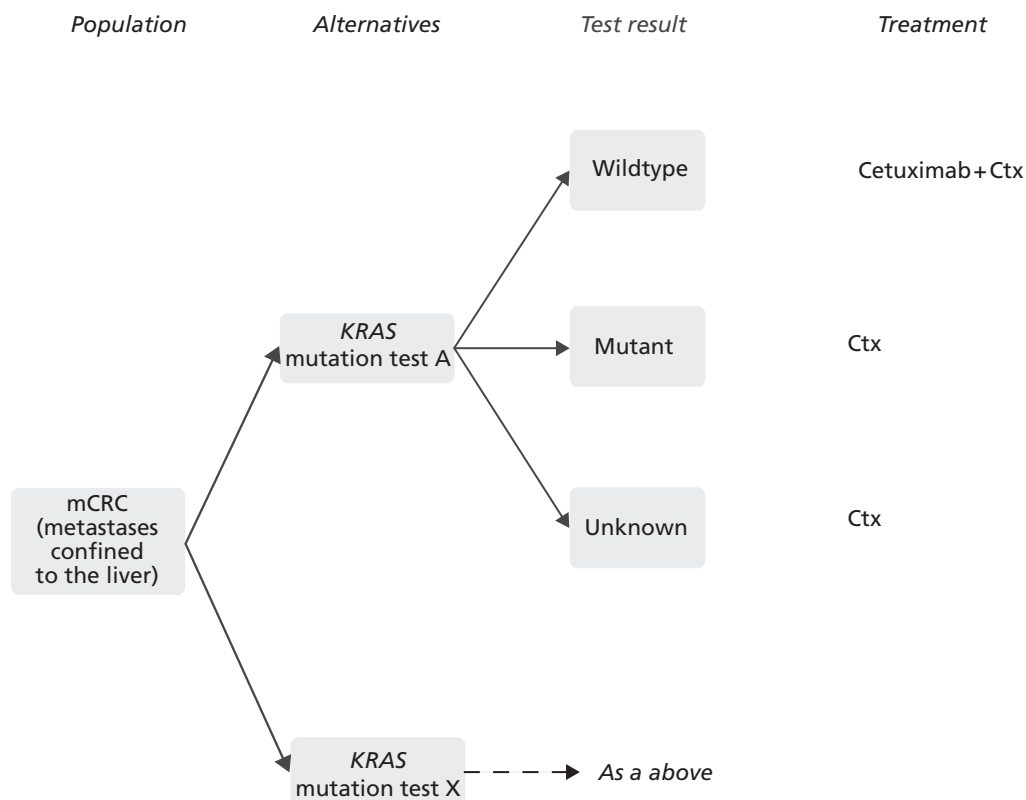
FIGURE 8 Decision tree structure. Ctx, chemotherapy.

The Markov model structure is shown in *Figure 9*. The model is described in more detail in NICE technology appraisal TA176.[1]

Model parameters

Estimates for model input parameters were retrieved from NICE technology appraisal TA176[1] and the manufacturer's submission for TA176,[59,69] the assessment of the clinical effectiveness of different *KRAS* mutation tests (see *Chapter 3*, *What is the accuracy of* KRAS *mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?*) and an online survey of NHS laboratories in England and Wales (see *Chapter 3*, *What are the technical performance characteristics of the different* KRAS *mutation tests?*).

Test result

The proportions of test failures in the laboratory for the *KRAS* mutation tests were based on the online survey of NHS laboratories in England and Wales. The proportions of *KRAS* wild-type and *KRAS* mutant test results were based on the estimated proportions of patients with *KRAS* wild-type tumours in the population (65.2% with standard error 0.8%),[70] the test accuracy (sensitivity and specificity with objective response to cetuximab or resection rate as reference standard; see *Table 8*) and the proportion of patients with an unknown test result. The proportion of patients with an unknown test result was based on the proportions of patients with unknown tumour mutation status relative to the number of patients for whom a tissue sample was available in the clinical trials. The proportion of patients with an unknown test result may be an overestimate as the clinical trials are unlikely to be representative of the true situation in current clinical practice in the trails, sample were not typically taken for the purpose of *KRAS* testing. By contrast, the results of the online survey of laboratories in England and Wales are likely to provide an underestimation of the total proportion of patients with an unknown test result as the laboratories may not have insight into the total proportion of pretest failures (samples considered inadequate by the pathologist and therefore not sent to the laboratory). In the linked evidence analysis, the proportion of patients with a unknown tumour mutation status was taken from the clinical trials. For the equal
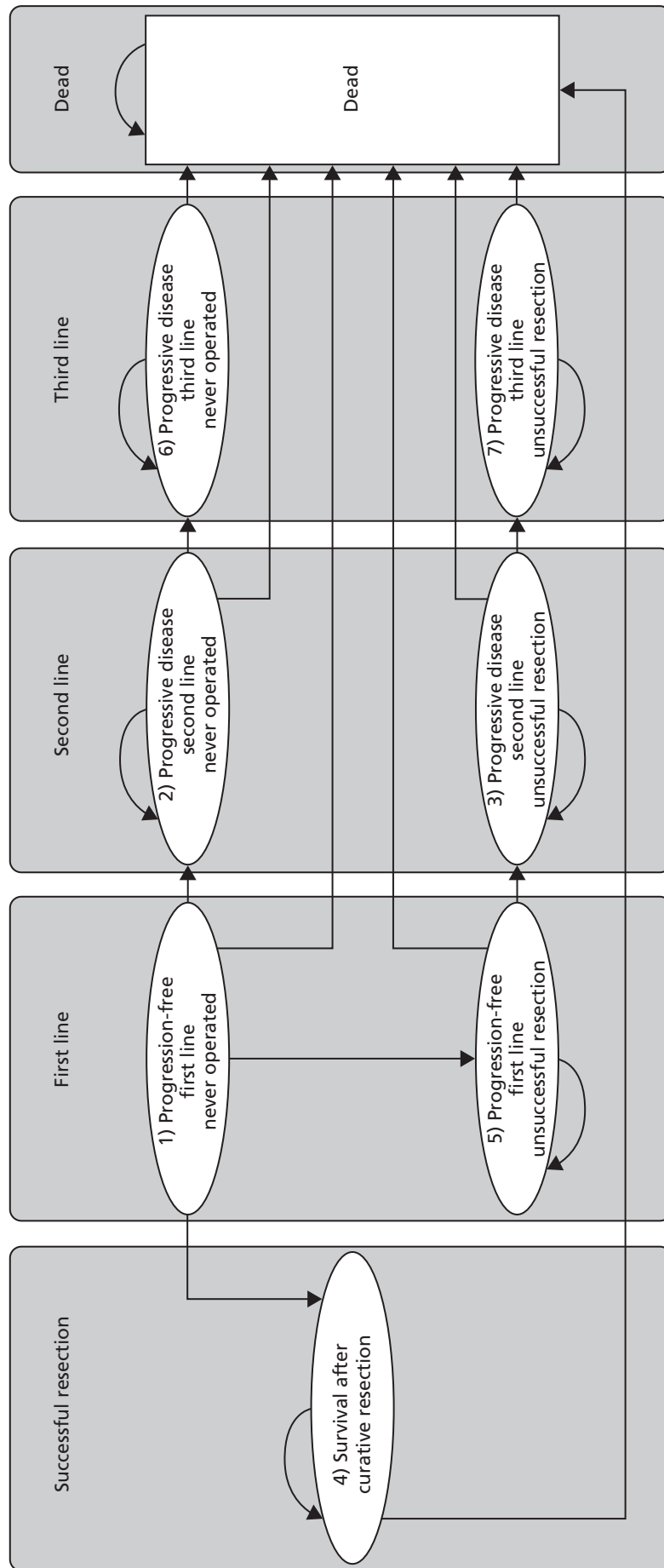
FIGURE 9 Markov model structure.

prognostic value analysis, the proportion of patients with an unknown tumour mutation status for all tests was assumed to be equal to that for the pyrosequencing test, as the COIN trial[54] (using pyrosequencing) was the only study reporting on resection rates following treatment with chemotherapy, with and without cetuximab, in patients with initially inoperable liver metastases and both *KRAS* mutant and *KRAS* wild-type tumours.

The proportions of TP (wild-type), TN (mutant), FN (mutant) and FP (wild-type) test results were calculated by:

- TP = proportion of wild-types × sensitivity × (1 – proportion of unknown tests)
- TN = (1 – proportion of wild-types) × specificity × (1 – proportion of unknown tests)
- FN = proportion of wild-types × (1 – sensitivity) × (1 – proportion of unknown tests)
- FP = (1 – proportion of wild-types) × (1 – specificity) × (1 – proportion of unknown tests).

Subsequently, the proportions of patients with a wild-type (TP + FP) and mutant (TN + FN) test result were calculated. The results are listed in *Tables 15* and *16*.

Resection rate

Patients who are in the 'progression-free first line – never operated' state can move to 'survival after curative resection', 'progression-free first line – unsuccessful resection', 'progressive/disease second line – never operated' or 'death', based on tumour resection rates, rate for failure of resection and postoperative mortality. For patients with *KRAS* wild-type tumours, the resection rate after treatment with cetuximab and chemotherapy (*Table 17*) was used; for the remaining patients (*KRAS* mutant or unknown test results), the resection rate after treatment with chemotherapy alone was used. As the CELIM trial[52] did not contain a chemotherapy-only strategy, it was not possible to use the resection rates from this trial for patients with *KRAS* mutant and unknown tumour status. Therefore, the resection rates for the Therascreen *KRAS* RGQ PCR Kit for these groups were taken from the GERCOR trial,[68] which was in line with NICE technology appraisal TA176.[1] However, the GERCOR trial also included patients with metastases outside the liver, which is not in line with the scope for this assessment. The resection rates reported and used in technology appraisal TA176[1] for the chemotherapy-only strategy were calculated based on all patients (thus including patients with metastases not confined to the liver) and are therefore probably an underestimation of the true resection rate in the population with metastases confined to the liver. In the assumption of equal prognostic value analysis, however, the resection rate used was based on the COIN trial,[54] which included a population with liver-only metastases.

The resection failure rate was set at 5%[71] and the probability of postoperative mortality was 2.8% (standard error based on PSA: 1.2%; beta PERT distribution),[72] both consistent with NICE technology appraisal TA176.[1]

Progression-free and overall survival

To ensure consistency with NICE technology appraisal TA176,[1] parametric survival models were obtained from this technology appraisal for patients without resection or patients with unsuccessful resection to estimate cycle-dependent PFS in the first and second line and OS in the first and third line. For patients with successful resection, parametric survival models were obtained from the technology appraisal to calculate cycle-dependent PFS and OS probabilities (*Table 18*).

TABLE 15 Input parameters used to calculate the proportions of patients with a *KRAS* wild-type test result, an unknown test result and a *KRAS* mutant test result

Input parameter	Estimated value (SE)	Distribution	Source
Proportion of patients with *KRAS* mutation-positive tumours in England and Wales (%)	65.2 (0.8)	Beta	Andreyev *et al.*[70]
Test accuracy			
Therascreen *KRAS* RGQ PCR Kit	Sensitivity: 74.6 (5.4); specificity: 35.5 (8.5)	Beta	CELIM[52]
Pyrosequencing	Sensitivity: 52.0 (9.8); specificity: 45.6 (4.3)	Beta	COIN[54]
Probability of unknown test result (%)			
Therascreen *KRAS* RGQ PCR Kit	10.8 (2.9)	Beta	CELIM[52]
Pyrosequencing	1.7 (0.4)	Beta	COIN[54]

SE, standard error.

TABLE 16 Probability of a *KRAS* wild-type test result, an unknown test result and a *KRAS* mutant test result

| Mutation test | Probability (SE) of test result (%) | | |
	Wild type[a]	Unknown	Mutant[a]
Therascreen *KRAS* RGQ PCR Kit	63.4 (4.7)	10.8 (2.9)	25.8 (4.4)
Pyrosequencing	52.0 (0.8)	1.7 (0.4)	46.4 (0.8)

SE, standard error.
a   Standard error is based on PSA.

TABLE 17 Resection rates

| Mutation test | Resection rate (SE)[a,b] | | | |
	Wild type	Unknown	Mutant	Source
Therascreen *KRAS* RGQ PCR Kit	0.433 (0.060)	0.092 (0.028)	0.092 (0.028)	CELIM,[52] GERCOR[68]
Pyrosequencing	0.149 (0.038)	0.132 (0.035)	0.132 (0.035)	COIN[54]

SE, standard error.
a   All resection rates were modelled using beta distributions.
b   In the equal prognostic value analysis the resection rate for pyrosequencing is used for all mutation tests.

TABLE 18 Parametric survival models

Probability of	Model distribution	Parameter	Estimated value	Standard error	Distribution	Source[a]
First line (Figure 10)						
Progression to second line	Log-normal	Cetuximab[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	OPUS, Merck Serono[59,69]
		Constant[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	OPUS, Merck Serono[59,69]
		LN(Sigma)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	OPUS, Merck Serono[59,69]
Survival	Log-normal	Cetuximab[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	CRYSTAL, Merck Serono[59,69]
		Constant[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	CRYSTAL, Merck Serono[59,69]
		LN(Sigma)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	CRYSTAL, Merck Serono[59,69]
Second line (Figure 11)						
Progression to third line	Weibull	Constant[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	GERCOR,[68] Merck Serono[59,69]
		LN(Sigma)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	GERCOR,[68] Merck Serono[59,69]
Survival	Based on age-dependent background mortality				Fixed	TA176,[1] Merck Serono[59,69]
Third line (Figure 12)						
Survival	Weibull	Constant[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	Jonker et al.,[73] Mittmann et al.,[74] Merck Serono[59,69]
		LN(Gamma)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	Jonker et al.,[73] Mittmann et al.,[74] Merck Serono[59,69]
After successful resection (Figure 13)						
Progression	Log-logistic	Constant[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	Adam et al.,[75] Merck Serono[59,69]
		LN(Gamma)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	Adam et al.,[75] Merck Serono[59,69]

continued

TABLE 18 Parametric survival models (*continued*)

Probability of	Model distribution	Parameter	Estimated value	Standard error	Distribution	Source[a]
Survival	Log-logistic	Constant[b]	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	Adam *et al.*,[75] Merck Serono[59,69]
	LN(Gamma)		(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Multivariate normal[c]	Adam *et al.*,[75] Merck Serono[59,69]

LN, natural logarithm.
a Parametric survival models were retrieved from Appendix H3, Parametric models, in the manufacturer's submission for TA176.[59,69]
b Model coefficients; for the Weibull models the exponent of these coefficients are used to calculate the lambda parameters.
c Cholesky decomposition was used to model the multivariate normal distribution.
Source: based on NICE technology appraisal TA176.[1]

Progression-free survival and OS in the first line for standard chemotherapy were based on data from the OPUS and CRYSTAL trials respectively, (Commercial-in-confidence information has been removed) and were estimated separately for patients treated with or without cetuximab. All PFS and OS probabilities for the first line are presented in *Figure 10*.

Time-dependent probabilities for PFS in the second line and OS in the third line were equal for patients who were treated with or without cetuximab and were converted to constant probabilities based on the median PFS and OS (see *Figures 11* and *12* respectively). These constant probabilities were used to prevent an unfeasible amount of tunnel states of 7200 per comparator (1200 cycles × two health states × three possible test results).

Although PFS after successful resection was not incorporated as a separate health state, the probability of progression was estimated to incorporate the utility loss and increased costs associated with progression after successful curative resection. Estimated PFS and OS are equal for patients who were treated with cetuximab and patients who were treated without cetuximab (see *Figure 13*).

Adverse events

The occurrence of adverse events was assumed to be dependent on treatment and independent of tumour *KRAS* mutation status, that is, the occurrence of adverse events for patients with *KRAS* wild-type, *KRAS* unknown and *KRAS* mutant tumours was assumed to be equal among different test strategies. Consistent with NICE technology appraisal TA176,[1] the occurrence of adverse events was included in the model only by incorporating the additional costs related to the adverse events based on the CRYSTAL[27] and OPUS[28] trials. These costs are discussed in *Resource use and costs*.

FIGURE 10 Progression-free survival and OS in the first line (Commercial-in-confidence information has been removed).

FIGURE 11 Progression-free survival in the second line (Commercial-in-confidence information has been removed).

FIGURE 12 Overall survival in the third line (Commercial-in-confidence information has been removed).

FIGURE 13 Progression-free survival and OS after successful resection (Commercial-in-confidence information has been removed).

Health state utilities

Utility scores were retrieved from NICE technology appraisal TA176[1] and are presented in *Table 19*.

If the mutation tests were to differ substantially in turnaround time, there could be a difference in process disutility associated with waiting for a test result, or even in health outcome because of the delay to the start of treatment. To investigate this, an item on turnaround time was included in the online survey. The results showed that the tests were very similar in terms of turnaround time (see *Chapter 3*, *What are the technical performance characteristics of the different* KRAS *mutation tests?*). In most laboratories the turnaround time was generally between 3 and 7 days. One laboratory (out of eight reporting on the use of pyrosequencing) had a turnaround time of 1–2 days. There was no clear association, however, between the specific test used and the turnaround time reported. Turnaround times are probably impacted most by the number of received samples and batch size. Therefore, it was assumed in the health economic analysis that the turnaround times were not test driven and that the tests did not differ with respect to process disutility or health outcomes associated with waiting for test results.

Resource use and costs

Resource use and costs were taken from NICE technology appraisal TA176,[1] with the exception of the *KRAS* test costs. These costs were based on the online survey of NHS laboratories in England and Wales. Total test costs are calculated based on *Table 20*; other costs are reported in *Table 21*.

Test costs

For patients with a *KRAS* wild-type or *KRAS* mutant test result, the full test costs were accounted for. For this purpose the NHS prices from the online survey of NHS laboratories in England and Wales (see *Table 6*) were used. As a price was reported for only one test in the online survey, it was decided to assume an equal test cost across all tests of £127.25, which was the average of the four available NHS prices from the survey for this one test (pyrosequencing). To calculate test costs for patients with an unknown tumour mutation status, it is necessary to differentiate between patients for whom the sample was considered inadequate by the pathologist before sending the specimen to the laboratory (pre-laboratory clinical failure) and patients for whom the sample was considered adequate by the pathologist but which resulted in failure once inside the laboratory (technical failures within the laboratory). In the case of an unknown mutation status because of a pre-laboratory clinical failure, no test costs were taken into account.

TABLE 19 Utility scores

Health state	Utility score	SE	Distribution	Source
Progression free (first line)	0.777	(Commercial-in-confidence information has been removed)	Beta	TA176[1]
PD (second line)	0.730	(Commercial-in-confidence information has been removed)	Beta	TA176[1]
PD (third line)	0.680	(Commercial-in-confidence information has been removed)	Beta	TA176[1]
Progression free (after successful resection)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Beta	TA176[1]
PD (after successful resection)	(Commercial-in-confidence information has been removed)	(Commercial-in-confidence information has been removed)	Beta	TA176[1]

TABLE 20 Calculation of the proportion of patients with unknown mutation status because of a technical failure in the laboratory by test

Test	Total proportion (SE) of patients with an unknown test result (%)	Distribution	Source	Proportion of technical failures in the laboratories (%)	No. of reporting laboratories	Proportion (SE) of technical failures of patients with an unknown test result (%)[a]	Distribution
Analysis 1[b]							
Therascreen *KRAS* RGQ PCR Kit	10.8 (2.9)	Beta	CELIM[52]	1.9[c]	0	15.9 (8.3)	Beta
Pyrosequencing	1.7 (0.4)	Beta	COIN[54]	3.1	7	100.0 (12.0)[d]	Beta
Analysis 2[b]							
Pyrosequencing	1.7 (0.4)	Beta	COIN[54]	3.1	7	100.0 (13.8)[d]	Beta
Therascreen *KRAS* RGQ PCR Kit	As for pyrosequencing			1.9[b]	0	100.0 (16.6)[d]	Beta
cobas *KRAS* Mutation Test	As for pyrosequencing			4.5	2	100.0 (5.6)[d]	Beta
HRM analysis	As for pyrosequencing			0.0	1	0.0 (0.0)	Beta
Sanger sequencing	As for pyrosequencing			0.0	1	0.0 (0.0)	Beta
Therascreen *KRAS* Pyro Kit[b]	NA			NR	0	NA	Beta
KRAS LightMix kit[b]	NA			NR	0	NA	Beta
KRAS StripAssay[b]	NA			NR	0	NA	Beta
MALDI-TOF mass spectrometry[b]	NA			NR	0	NA	Beta
Next-generation sequencing[b]	NA			NR	0	NA	Beta

NA, not applicable; NR, not reported; SE, standard error.
a Standard error based on PSA.
b No survey data were available for these tests. These tests were not included in the economic analysis as it was not considered informative to model these comparators because of the lack of evidence.
c Average of the technical failures reported in the survey for the other tests.
d 'IF' statements were used to ensure that this probability did not exceed 100%.

TABLE 21 Other costs[a]

Type of costs	Cost (£)	Distribution	Source
Erbitux® (cetuximab, Merck Serono) (1 mg)	1.37	Fixed	TA176[1]
Irinotecan (1 mg)	1.30	Fixed	TA176[1]
Folinic acid (1 mg)	0.39	Fixed	TA176[1]
5-Fluorouracil (1 mg)	0.01	Fixed	TA176[1]
Oxaliplatin (1 mg)	3.30	Fixed	TA176[1]
Oncology outpatient attendance	123.00	Beta PERT[b]	TA176[1]
Outpatient attendance for grade 3/4 adverse event (CRYSTAL[27])	161.51	Beta PERT[b]	TA176[1]
Outpatient attendance for grade 3/4 adverse event (OPUS[28])	(Commercial-in-confidence information has been removed)	Beta PERT[b]	TA176[1]
Outpatient attendance for serious adverse event (CRYSTAL[27])	165.91	Beta PERT[b]	TA176[1]
Outpatient attendance for serious adverse event (OPUS[28])	(Commercial-in-confidence information has been removed)	Beta PERT[b]	TA176[1]
Adverse event in second line (outpatient visit) (CRYSTAL[27])	191.27	Beta PERT[b]	TA176[1]
Adverse event in second line (outpatient visit) (OPUS[28])	(Commercial-in-confidence information has been removed)	Beta PERT[b]	TA176[1]
Serious adverse event requiring hospitalisation (CRYSTAL[27])	1170.83	Beta PERT[b]	TA176[1]
Serious adverse event requiring hospitalisation (OPUS[28])	(Commercial-in-confidence information has been removed)	Beta PERT[b]	TA176[1]
Hospitalisation for non-serious adverse event	1050.70	Beta PERT[b]	TA176[1]
Abdomen CT scan	214.00	Beta PERT[b]	TA176[1]
Chest CT scan	350.00	Beta PERT[b]	TA176[1]
Hepatic ultrasound	95.00	Beta PERT[b]	TA176[1]

a　Other cost data were commercial-in-confidence (not presented in the manufacturer's submission to TA176[1]) and are thus not reported in this table.
b　Consistent with TA176,[1] the ± 50% of the estimated costs are used as the minimum and maximum.

In the case of an unknown mutation status because of a technical failure within the laboratory, full test costs were taken into account. This proportion was calculated based on the proportion of patients with an unknown mutation status taken from the literature and the total proportion of technical failures in the laboratories reported in the online survey (see *Table 5*), using the following formula:

Proportion of technical failures within the laboratories of all patients with an unknown test result

$$
= \frac{\text{P(technical failures in laboratory)} \times (1 - \text{P(unknown)})}{1 - \text{P(technical failures in laboratory)}} \times \frac{1}{\text{P(unknown)}} \tag{1}
$$

The results of the calculations are presented in *Table 20*.

Model analyses

Expected mean costs, LYs and QALYs were estimated for all *KRAS* mutation testing methods. Long-term costs, LYs and QALYs were discounted using the UK discount rates of 3.5%. Based on the estimated outcomes (probabilistic), the ICER was calculated by dividing the incremental costs by the incremental QALYs. The ICER represents the cost of an additional QALY gained and was used to estimate the cost-effectiveness of a strategy compared with the next best alternative, as in the absence of a comparator strategy it was not possible to calculate ICERs relative to the comparator. All outcomes are based on PSA with 5000 simulations using parameter distributions as presented in this section.

Overview of the main model assumptions

The main assumptions in the health economic analysis were:

1. The differences between ORRs and resection rates for cetuximab plus chemotherapy compared with chemotherapy alone reported in the CELIM trial[52] combined with the GERCOR trial[68] and those reported in the COIN trial[54] are solely the result of the different tests used (Therascreen *KRAS* RGQ PCR Kit and pyrosequencing respectively) to distinguish between patients whose tumours are *KRAS* wild type (and who receive cetuximab) and patients whose tumours are *KRAS* mutant (and who receive chemotherapy) (linked evidence analysis).
2. To calculate the sensitivity and specificity of the tests, required to calculate the proportion of *KRAS* wild-type and *KRAS* mutant test results (see *Table 8*), patients tested as *KRAS* wild type were categorised as FP if no objective response was observed (for the Therascreen *KRAS* RGQ PCR Kit) or no liver resection was performed (for pyrosequencing) after treatment with cetuximab whereas patients were categorised as TP if an objective response was observed (Therascreen *KRAS* RGQ PCR Kit) or a liver resection was performed (pyrosequencing). Similarly, patients tested as tumour *KRAS* mutant were categorised as FN if an objective response was observed (for the Therascreen *KRAS* RGQ PCR Kit) or a liver resection was performed after treatment with cetuximab (for pyrosequencing) whereas patients were categorised as TN if no objective response was observed (Therascreen *KRAS* RGQ PCR Kit) or no liver resection was performed (pyrosequencing).
3. Test accuracy based on objective response can be compared with accuracy based on resection rates.[39]
4. The number of patients with unknown mutation status relative to the number of patients for whom a tissue sample was available in the trials[52,54] provides a realistic approximation of the proportion of patients with an unknown test result in clinical practice (both analyses).
5. As the COIN trial[54] tests for *KRAS* mutations using both pyrosequencing and MALDI-TOF, with a reported concordance of > 99%, it was assumed that the accuracy derived from this trial and also the resection rates reported apply to both pyrosequencing and MALDI-TOF, that is, all pyrosequencing results in this report also apply to MALDI-TOF.
6. The standard chemotherapy used in the COIN trial[54] (FOLFOX or XELOX) is comparable to FOLFOX6 as used in the CELIM trial.[52]

Sensitivity analyses

For both the linked evidence and the assumption of equal prognostic value analysis, the following sensitivity analyses were performed:

- mortality in the second line was based on the average of the first- and third-line mortality instead of background mortality as in NICE technology appraisal TA176.[1]
- the proportion of patients with unknown mutation status was based on the results of the online survey instead of the literature (see *Table 5*).

Results of the cost-effectiveness analyses

This section reports the results of the linked evidence analysis and the assumption of equal prognostic value analysis. As this economic evaluation takes a 'no comparator' approach, ICERs for each strategy are calculated compared with the next most cost-effective strategy.

Linked evidence analysis

The linked evidence analysis includes two tests, that is, only those tests for which evidence on test accuracy based on either resection rate or ORR was available. *Table 22* shows the probabilistic results of this analysis. It should be noted that this analysis was based on a number of substantial assumptions, which are outlined in the previous section. In short, we have only the COIN[54] and CELIM[52] trials to rely on, of which the COIN trial used pyrosequencing to test for *KRAS* mutations and the CELIM trial used the Therascreen *KRAS* RGQ PCR Kit. We assumed that the differences between the outcomes of these trials are exclusively caused by the different tests used (assumption 1). *Table 23* provides a summary of the comparability of the study populations across the COIN,[54] CELIM[52] and GERCOR[68] trials used in the linked evidence analysis. In addition, we assumed that all *KRAS* wild-type patients would respond perfectly to cetuximab – or would all have a liver resection after cetuximab – and that all *KRAS* mutant patients would not (assumption 2), and that test accuracy based on objective response can be compared with accuracy based on resection rates (assumption 3).

As is apparent from *Table 22*, pyrosequencing has the lowest total cost. The Therascreen *KRAS* RGQ PCR Kit is the more expensive but also more effective strategy, with an ICER of £17,019 per QALY gained. The CEAC in *Figure 14* shows that, for lower values of the threshold, pyrosequencing is the preferred strategy and that at thresholds of ≥ £17,000 the Therascreen *KRAS* RGQ PCR Kit is the most cost-effective option. The results of the sensitivity analyses (see *Table 22*) do not differ substantially from the base-case results in the sense that the Therascreen *KRAS* RGQ PCR Kit is consistently more expensive and more effective than pyrosequencing, with ICERs ranging from £14,860 to £20,528 per QALY gained. CEACs for the sensitivity analyses are presented in *Appendix 6*.

Assumption of equal prognostic value analysis

The assumption of equal prognostic value analysis includes all tests for which information on technical performance was available from the online survey of NHS laboratories in England and Wales. This includes the tests for which accuracy data, based on either response rates or resection rates, were not available. Therefore, this analysis assessed whether the tests were likely to be cost-effective given an assumption of equal prognostic value based on the prognostic value of testing with pyrosequencing (as this was the only test for which full data were available on resection rates following treatment with chemotherapy, with and

TABLE 22 Probabilistic results for the linked evidence analysis: base-case and sensitivity analyses

Strategy	Cost (£)	QALYs	Δ Costs (£)	Δ QALYs	ICER (£)
Base case					
Pyrosequencing[a]	30,870	1.49			
Therascreen *KRAS* RGQ PCR Kit	33,995	1.67	3125	0.18	17,019
Sensitivity analysis: mortality second line based on average of first- and third-line mortality					
Pyrosequencing[a]	29,704	1.28			
Therascreen *KRAS* RGQ PCR Kit	33,132	1.51	3428	0.23	14,860
Sensitivity analysis: proportion with unknown mutation status based on survey					
Pyrosequencing[a]	30,714	1.48			
Therascreen *KRAS* RGQ PCR Kit	34,799	1.69	4085	0.20	20,528
a Pyrosequencing results also apply to MALDI-TOF mass spectrometry.					

TABLE 23 Comparison of the study populations across the trials used in the linked evidence analysis

Study details	Participant selection	Population characteristics
Folprecht 2010[52] (CELIM) Country: Germany and Austria Study design: RCT No. randomised: 111 No. of KRAS wild-type patients randomised: 70 No. of patients with liver-limited metastases randomised: 111 Intervention: Cetuximab + FOLFOX vs. cetuximab + FOLFIRI	Inclusion criteria: Unresectable, histologically confirmed colorectal liver metastases; no extrahepatic metastases (patients with synchronous liver metastases were eligible if the primary tumour had been resected before chemotherapy); Karnofsky perfomance score ≥ 80%; adeuqate hepatic, renal and bone marrow function Exclusion criteria: Previous chemotherapy (except adjuvant chemotherapy with an interval of ≥ 6 months); previous EGFR-targeted therapy; concurrent antitumour therapy; clinically relevant coronary artery disease; inflammatory bowel disease; previous malignancy; age < 18 years	Age (years), median (range): 63 (56–71) Male, n: 71 Liver metastases, n: < 5: 30; 5–10: 58; > 10: 19; NR: 4 No. with previous liver resection: 14 Criteria for unresectability: Five or more liver metastases or metastases that were viewed as technically non-resectable by the local liver surgeon and radiologist on the basis of inadequate future liver remnant or one of the following critera: infiltration of all hepatic liver veins, infiltration of both hepatic arteries or both protal vein branches Previous treatments: 9 patients had adjuvant radiotherapy, 18 had adjuvant chemotherapy
Maughan 2011[54] (COIN) Country: UK and Republic of Ireland Study design: RCT No. randomised: 1630 No. of KRAS wild-type patients randomised: 729 No. of patients with liver-limited metastases randomised: 178 Intervention: Cetuximab + FOLFOX or XELOX vs. FOLFOX or XELOX	Inclusion criteria: Adults aged ≥ 18 years; histologically confirmed adenocarcinoma of the colon or rectum; inoperable metastatic or locoregional disease; no previous chemotherapy for metastatic disease; WHO performance status 0–2; adequate hepatic, renal and haematological function; no adjuvant chemotherapy or rectal chemoradiotherapy within 1 month of the start of the trial Exclusion criteria: Unfit for chemotherapy; severe, uncontrolled medical illness; psychiatric illness inhibitig informed consent; partial or complete bowel obstruction; pre-existing neuropathy greater than grade 1; requirement for treatment with contraindicated medication; another previous or current malignant disease that may affect treatment response; known hypersensitivity to any study treatment; brain metastases	Age (years), median (range): 64 (56–70) Male, n: 498 Liver metastases: Resection rates reported separately for patients with liver-only metastases Criteria for unresectability: NR Previous treatments: NR
Tournigand 2004[68] (GERCOR) Country: France Study design: RCT No. randomised: 226 (of whom 6 not eligible) No. of KRAS wild-type patients randomised: NA No. of patients with liver-limited metastases randomised: NR Intervention: FOLFIRI + FOLFOX (arm A) vs. FOLFOX + FOLFIRI (arm B)	Inclusion criteria: Adults aged 18–75 years; adenocarcinoma of the colon or rectum; unresectable metastases; at least one bidimensionally measurable lesion of ≥ 2 cm or a residual non-measurable lesion; adequate bone marrow, liver and renal function; WHO performance status 0–2. Previous adjuvant chemotherapy, if given, must have been completed at least 6 months before inclusion Exclusion criteria: Central nervous system metastases; second malignancies; bowel obstruction; current diarrhoea of grade 2 or higher; symptomatic angina pectoris; disease confined to previous radiation fields	Age (years), median (range): Arm A: 61 (29–75); arm B: 65 (40–75) Male, n: 142 (of 220) Metastases: Liver: 184 (84%); lung: 67 (30%); other: 98 (45%) Resection rates not reported separately for patients with liver-only metastases No. of sites of metastases: 1: 130 (59%); ≥ 2: 90 (41%) Criteria for unresectability: NR Previous treatments: 17% and 21% of arm A and arm B, respectively, had adjuvant chemotherapy

NR, not reported.

FIGURE 14 Cost-effectiveness acceptability curve for the linked evidence analysis: base case.

TABLE 24 Probabilistic results for the assumption of equal prognostic value analysis: base case

Test	Cost (95% CI) (£)	Δ Cost[a] (95% CI) (£)
HRM analysis	30,857.09 (27,079.58 to 34,736.14)	
Sanger sequencing	30,857.09 (27,079.58 to 34,736.14)	0.00 (0.00 to 0.00)[b]
Therascreen *KRAS* RGQ PCR Kit	30,857.46 (27,079.91 to 34,736.60)	0.37 (0.12 to 0.88)
Pyrosequencing[c]	30,857.70 (27,080.27 to 34,737.03)	0.61 (0.14 to 1.64)
cobas *KRAS* Mutation Test	30,857.99 (27,080.25 to 34,737.14)	0.91 (0.23 to 2.28)

a Compared with the least expensive comparator.
b Costs were equal for HRM analysis and Sanger sequencing as the proportion of failed tests in the laboratory was equal for both comparators (0%).
c Pyrosequencing results also apply to MALDI-TOF mass spectrometry.

without cetuximab, for patients with initially inoperable liver metastases and both *KRAS* mutant and *KRAS* wild-type tumours) and test-specific information on technical failures within the laboratory only (*Table 24*). In the base case and the first sensitivity analysis, the total technical failure rate (pre-laboratory plus within-laboratory technical failures) is assumed to be equal for all tests. As a result, the strategies in these analyses differ only with respect to costs (because of differences in within-laboratory technical failures). In the base case the average QALYs for all comparators were 1.48 (95% CI 1.33 to 1.64). The total costs associated with the various testing strategies (see *Table 24*) are highly similar. The same applies to the first sensitivity analysis (*Table 25*): costs are similar across strategies and average QALYs are equal by assumption at 1.28 (95% CI 1.12 to 1.44).

In the second sensitivity analysis the total technical failure rate is also test specific, which impacts on the proportion of patients with unknown (and therefore also wild-type and mutant) tumour *KRAS* status. Therefore, in this sensitivity analysis the strategies differ with respect to both effects and costs. All other input parameters, such as test costs and test accuracy, are still considered equal. The probabilistic results in *Table 26* show that the cobas *KRAS* Mutation Test is the least costly and least effective strategy. HRM analysis and Sanger sequencing have equal costs and effects and their ICER compared with the cobas *KRAS* Mutation Test is £69,815 per QALY gained. Pyrosequencing and the Therascreen *KRAS* RGQ PCR Kit are ruled out by extended dominance in this analysis. From the CEAC (*Figure 15*) it is apparent that the cobas *KRAS* Mutation Test is the preferred strategy for all threshold values of < £60,000.

TABLE 25 Probabilistic results for the assumption of equal prognostic value analysis: sensitivity analysis – mortality in the second line based on the average of first- and third-line mortality

Test	Cost (95% CI) (£)	Δ Cost[a] (95% CI) (£)
HRM analysis	29,661.10 (25,991.06 to 33,401.42)	
Sanger sequencing	29,661.10 (25,991.06 to 33,401.42)	0.00 (0.00 to 0.00)[b]
Therascreen *KRAS* RGQ PCR Kit	29,661.47 (25,991.81 to 33,401.80)	0.37 (0.12 to 0.85)
Pyrosequencing[c]	29,661.71 (25,992.12 to 33,401.81)	0.61 (0.14 to 1.59)
cobas *KRAS* Mutation Test	29,662.00 (25,993.07 to 33,402.58)	0.90 (0.23 to 2.18)

a Compared with the least expensive comparator.
b Costs were equal for HRM analysis and Sanger sequencing as the proportion of failed tests in the laboratory was equal for both comparators (0%).
c Pyrosequencing results also apply to MALDI-TOF mass spectrometry.

TABLE 26 Probabilistic results for the assumption of equal prognostic value analysis: sensitivity analysis – proportion of patients with unknown mutation status based on the survey

Test	Cost (£)	QALYs	Comparator	Δ Cost (£)	Δ QALYs	ICER (£)
cobas *KRAS* Mutation Test	30,663	1.48				
Pyrosequencing[a]	30,796	1.48	cobas *KRAS* Mutation Test	133.66	0.002	Extended dominance
Therascreen *KRAS* RGQ PCR Kit	30,876	1.48	Pyrosequencing	80.06	0.001	Extended dominance
HRM analysis	31,006	1.49	cobas *KRAS* Mutation Test	343.64	0.005	69,815[b]
Sanger sequencing	31,006	1.49	cobas *KRAS* Mutation Test	343.64	0.005	69,815[b]

a Pyrosequencing results also apply to MALDI-TOF mass spectrometry.
b HRM analysis and Sanger sequencing were equally effective and equally expensive (as the survey indicated equal failure probabilities of 0% for both comparators).
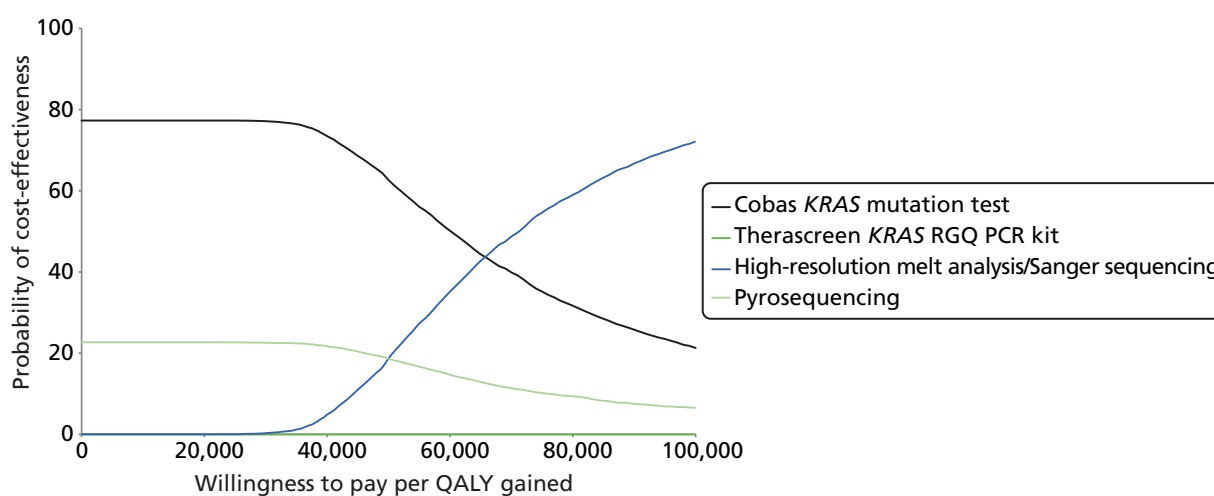
FIGURE 15 Cost-effectiveness acceptability curve for assumption of equal prognostic value sensitivity analysis: proportion of patients with unknown mutation status based on the survey.

Chapter 5 Discussion

Statement of principal findings

Clinical effectiveness

There was no clear evidence to suggest any differences between the *KRAS* mutation testing techniques for any of the measures assessed (technical performance, accuracy for predicting response to treatment with cetuximab in combination with standard chemotherapy, or variation in clinical outcomes following treatment with cetuximab in combination with standard chemotherapy depending on which method is used to classify patients as having KRAS wild-type tumours).

The survey of laboratories providing *KRAS* mutation testing indicated that in-house pyrosequencing methods, targeting *KRAS* mutations in codons 12, 13 and 61 and using self-designed primers, were the most commonly used approaches (9/15 respondents); reasons cited by respondents for their choice of this technique were the proportion of tumour cells required, ease of use, cost, mutations covered, turnaround time and experience of pyrosequencing techniques available in the laboratory. There was no apparent association between test method and reason for choice. Commercial kits used were the cobas *KRAS* Mutation Test (three laboratories) and the Therascreen *KRAS* Pyro Kit (one laboratory). More than half of the responding laboratories reported that *KRAS* mutation testing was carried out on request (e.g. from a pathologist or oncologist); only one laboratory reported routine testing of all CRC samples. In general, there was no clear indication that choice of test method was related to volume of throughput, although both of the laboratories that reported using Sanger sequencing had a low throughput (up to five samples per week). Most respondents reported turnaround times, from receipt of sample to reporting to the clinician, of between 3 and 5 days. The only laboratory to report a turnaround time of < 3 days (24–48 hours) used an in-house pyrosequencing method. Frequency of running the test did not appear to relate to laboratory throughput and only one laboratory reported waiting for a minimum batch size (10 samples) before running the test; this laboratory had a high throughput (> 20 samples per week). The minimum percentage of tumour cells required for testing varied widely across laboratories (< 1% to > 30%), even when the same test method was being used. When reported, the minimum requirement for the cobas *KRAS* Mutation Test was ≤ 10%. With the exception of those using Sanger sequencing, all laboratories reported a limit of detection for percentage mutation of ≤ 10%. The laboratory that used the Therascreen *KRAS* Pyro Kit did not provide any data on technical performance. The proportion of samples rejected before analysis was < 2% for all responding laboratories. The rate of failure for analysed samples did not appear to be dependent on test method (3–6% for the cobas *KRAS* Mutation Test and 0.2–10% for in-house pyrosequencing methods). The majority of responding laboratories reported using microdissection techniques before DNA extraction; however, there was no clear indication that non-use of this technique was associated with a higher rate of sample rejection or test failure. The laboratory that used the Therascreen *KRAS* Pyro Kit did not provide any data on failure rates. Although most respondents included cost in their reasons for choosing a particular test, it is worth noting that a relatively narrow range of costs was reported across all tests (£100–150), with one laboratory reporting a higher cost (£273) for running a single sample. Prices charged, to both Merck Serono and the NHS, ranged from £99 to £150.

When contacted by NICE in relation to a previous diagnostic assessment of EGFR mutation testing in non-small-cell lung cancer, UK NEQAS stated that '*Error rates are not always method related and it is not always possible to obtain data from all the labs committing critical genotyping errors. Therefore, any data which could be provided would be skewed with processing and reporting issues rather than being method related.*' Only one *KRAS* mutation testing method is currently approved by the US FDA; this is the Therascreen *KRAS* RGQ PCR Kit when used with the QIAamp DSP DNA FFPE Tissue Kit and the QIAGEN Rotor-Gene Q MDx (software version 2.1.0) and *KRAS* Assay Package.[16] The clinical trial[67] used to support

FDA approval was not included in this assessment as it did not match our inclusion criteria; it compared treatment with cetuximab and BSC to treatment with BSC alone in patients with mCRC who had previously failed all available chemotherapy. It should be noted that none of the laboratories participating in the UK NEQAS scheme who responded to our survey reported using the Therascreen *KRAS* RGQ PCR Kit.

Evidence to allow comparison of the accuracy of different *KRAS* mutation tests was very limited. Only one publication,[52] from the CELIM trial, provided sufficient data to allow estimation of the accuracy of a *KRAS* mutation test (version 1 of the Therascreen *KRAS* PCR Kit) for predicting response to treatment with cetuximab plus standard chemotherapy. This study reported objective response data and thus did not provide direct information on the value of the *KRAS* mutation test for predicting resection rate. Because the aim of *KRAS* mutation testing is to predict likely response to the addition of cetuximab to standard chemotherapy, test positive was defined as a *KRAS* wild-type tumour. The positive predictive value (70.1%, 95% CI 58.3% to 79.8%), reported in *Chapter 3* (see *What is the accuracy of* KRAS *mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?*), indicated that *KRAS* wild type, as determined using the Therascreen *KRAS* PCR Kit, may be moderately predictive of tumour response. If the published strong correlation between ORRs and resection rates in patients with isolated liver metastases[39] treated with various chemotherapy regimens were assumed to extrapolate to patients with *KRAS* wild-type tumours treated with standard chemotherapy plus cetuximab, then the expected R0 and R1 resection rates for these patients would be approximately 67%, based on data from the CELIM trial.[52] By contrast, the negative predictive value (40.7%, 95% CI 24.5% to 59.3%) could be interpreted as indicating that the presence of a *KRAS* mutation, as determined using the Therascreen *KRAS* PCR Kit, is a relatively poor predictor of non-response. Additional data supplied by the COIN trial investigators[54] allowed the calculation of estimates for the accuracy of pyrosequencing and MALDI-TOF (in which both tests were performed on all samples), targeting mutations in codons 12, 13 and 61, for predicting potentially curative resection following treatment with cetuximab plus FOLFOX or XELOX. The positive and negative predictive values derived from these data were 14.9% (95% CI 8.9% to 23.9%) and 83.9% (95% CI 73.8% to 90.5%) respectively; this could be interpreted as indicating that a tumour which is defined as *KRAS* wild type by this method is a poor predictor of resectability following treatment with cetuximab plus standard chemotherapy, whereas the presence of a *KRAS* mutation is a good predictor of non-response (tumour remaining unresectable after treatment). The COIN trial reported > 99% concordance on *KRAS* genotyping between pyrosequencing and MALDI-TOF; it may therefore be assumed that accuracy data from the COIN trial are also representative of the accuracy of both pyrosequencing and MALDI-TOF when used as single tests. It should be noted that any apparent differences in the ability of *KRAS* mutation tests to predict response to treatment between the CELIM trial and the COIN trial may be caused by other differences between studies (e.g. participant characteristics, in particular the definition of baseline unresectability, and treatment regimens).

Four further studies (six publications[27,28,53–56]) were included in the review; all were RCTs comparing cetuximab plus standard chemotherapy with standard chemotherapy alone in patients whose tumours were *KRAS* wild type and all reported data on patients with CRC metastases that were confined to the liver. The standard chemotherapy regimen was different in each of the four trials (FOLFOX4,[28,53,56] FOLFIRI,[27,53] FOLFIRI or FOLFOX6[55] and FOLFOX or XELOX[54]). There was no substantial evidence to indicate a significant difference in treatment effect depending on which of three *KRAS* mutation tests (LightMix k-ras Gly12, pyrosequencing and MALDI-TOF mass array for mutations in codons 12, 13 and 61, or pyrosequencing for *KRAS* mutations in codons 12 and 13) was used to identify patients with *KRAS* wild-type tumours. All three studies that assessed ORR reported a statistically significant higher response rate for participants treated with cetuximab plus standard chemotherapy than for those treated with standard chemotherapy alone; ORs ranged from 3.00 (95% CI 1.49 to 6.03)[53] to 4.93 (95% CI 1.42 to 17.06).[28] All four studies reported that the addition of cetuximab to standard chemotherapy was associated with an increase in the rate of R0 resections following treatment. However, it should be noted that the only trial to report a statistically significant treatment effect for R0 resection rate used pyrosequencing to identify *KRAS* mutations in codons 12 and 13 only.[55] This was also the only trial in which all participants had CRC metastases that were limited to the liver.

Effectiveness data from the CRYSTAL[27] and OPUS[28] trials were used to inform the technology appraisal underpinning NICE guidance TA176[1] on cetuximab for the first-line treatment of mCRC. Data from an interim analysis of the CELIM trial were used as a source of UK data for resection rates following treatment with cetuximab plus standard chemotherapy.[1] Data from the COIN trial[54] and the trial by Xu *et al.*[55] were published subsequently to TA176.

Cost-effectiveness

The review of economic analyses of different methods for *KRAS* mutation testing to decide between standard chemotherapy and cetuximab in combination with standard chemotherapy in adults with mCRC found four full papers[62–65] and one HTA report.[61] Based on all of these publications it can be said that, in general, although *KRAS* testing is obviously a more cost-effective option than administering cetuximab to all patients, the ICER of *KRAS* testing and treating only patients with *KRAS* wild-type tumours with cetuximab compared with treating all patients with standard chemotherapy alone seems rather high.

In the health economic analysis, the cost-effectiveness of different methods of *KRAS* mutation testing to decide between standard chemotherapy and cetuximab in combination with standard chemotherapy in adults with mCRC was assessed. In light of the limited amount of evidence available, two analyses were performed: 'linked evidence' and 'assumption of equal prognostic value'. All analyses took a 'no comparator' approach.

In the linked evidence analysis, the Therascreen *KRAS* RGQ PCR Kit was compared with pyrosequencing, using the available objective response and resection rate, respectively, to estimate lifetime costs and QALYs. The results of this analysis suggested that the Therascreen *KRAS* RGQ PCR Kit was more costly and more effective than pyrosequencing, with an ICER of £17,019 per QALY gained. Sensitivity analyses did not show substantial differences compared with the base case. The key driver behind the outcome was the difference in resection rate between treatment with and treatment without cetuximab and the proportions of patients with *KRAS* wild-type, *KRAS* mutant and unknown tumours. This was determined by test accuracy and therefore, for the most part, was dependent on ORR (for the Therascreen *KRAS* RGQ PCR Kit) or resection rate (for pyrosequencing).

It should be noted that this analysis was based on a number of substantial assumptions, which are outlined in *Chapter 4* (see *Overview of main model assumptions*). The following assumptions used were particularly problematic as they are open to doubt and probably have a considerable impact on the model results:

- The differences between ORRs and resection rates for cetuximab plus chemotherapy compared with chemotherapy alone as reported in the CELIM trial[52] combined with the GERCOR trial[68] and the COIN trial[54] are solely the result of the different tests used (Therascreen *KRAS* RGQ PCR Kit and pyrosequencing respectively) to distinguish between patients whose tumours are *KRAS* wild type (and who receive cetuximab) and patients whose tumours are *KRAS* mutant (and who receive chemotherapy).
- To calculate the sensitivity and specificity of the tests, required to calculate the proportion of *KRAS* wild-type and *KRAS* mutant test results (see *Table 8*), patients with a tumour tested as *KRAS* wild type were categorised as FP if no objective response was observed (for the Therascreen *KRAS* RGQ PCR Kit) or no liver resection was performed (for pyrosequencing) after treatment with cetuximab, whereas patients were categorised as TP if an objective response was observed (Therascreen *KRAS* RGQ PCR Kit) or a liver resection was performed (pyrosequencing). Similarly, patients with a tumour tested as *KRAS* mutant were categorised as FN if an objective response was observed (for the Therascreen *KRAS* RGQ PCR Kit) or a liver resection was performed (for pyrosequencing) after treatment with cetuximab, whereas patients were categorised as TN if no objective response was observed (Therascreen *KRAS* RGQ PCR Kit) or no liver resection was performed (pyrosequencing).

The results of the linked evidence analysis should therefore be interpreted on the condition that these assumptions hold. Moreover, the uncertainty presented surrounding the results is an underestimation of the true uncertainty as the uncertainty associated with the assumptions was not parameterised in the model and is therefore not reflected in the PSA.

The assumption of equal prognostic value analysis included all tests for which information on technical performance was available from the online survey of NHS laboratories in England and Wales. This includes the tests for which accuracy data based on either response rates or resection rates were not available. Therefore, this analysis assessed whether the tests were likely to be cost-effective given an assumption of equal prognostic value based on the prognostic value of testing with pyrosequencing (as this was the only test for which full data were available on resection rates following treatment with chemotherapy, with and without cetuximab, for patients with initially inoperable liver metastases and both *KRAS* mutant and *KRAS* wild-type tumours) and test-specific information on technical failures within the laboratory only, which implies that strategies can differ only with respect to costs. The results of the assumption of equal prognostic value analysis indicated that the strategies were almost equal. The first sensitivity analysis confirmed this. The second sensitivity analysis, in which the proportion of patients with unknown mutation status was taken from the survey instead of the literature, was slightly different in the sense that for this analysis the effectiveness was not assumed equal among all tests and therefore ICERs were available. The results showed that the cobas *KRAS* Mutation Test was the least expensive and least effective strategy and that Sanger sequencing and HRM analysis were equally the most costly and most effective, with an ICER of £69,815 per QALY gained compared with the cobas *KRAS* Mutation Test. The other two strategies included in this analysis, that is, the Therascreen *KRAS* RGQ PCR Kit and pyrosequencing, are ruled out by extended dominance.

Strengths and limitations of the assessment

Clinical effectiveness

Extensive literature searches were conducted in an attempt to maximise retrieval of relevant studies. These included electronic searches of a variety of bibliographic databases as well as screening of clinical trial registers and conference proceedings to identify unpublished studies. Because of the known difficulties in identifying test accuracy studies using study design-related search terms,[76] and the potential need to include non-RCTs, search strategies were developed to maximise sensitivity at the expense of specificity. Thus, large numbers of citations were identified and screened, very few of which met the inclusion criteria of the review. The specificity of searches was further reduced as it was not possible to target publications focusing on patients whose metastases were limited to the liver only; these patients were a subgroup in the majority of included studies.

The possibility of publication bias remains a potential problem for all systematic reviews. Considerations may differ for systematic reviews of test accuracy studies. It is relatively simple to define a positive result for studies of treatment, for example a significant difference between the treatment group and the control group that favours treatment. This is not the case for test accuracy studies, which measure agreement between the index test and the reference standard. It would seem likely that studies finding greater agreement (high estimates of sensitivity and specificity) will be published more often. This distinction may be less applicable to studies in this review that provided accuracy data, as these studies aimed to assess the effectiveness of treatment with cetuximab plus standard chemotherapy in different patient groups (CELIM trial[52]) or compare the effectiveness of cetuximab plus standard chemotherapy and standard chemotherapy alone (COIN trial[54]); neither study was primarily focused on test performance. Our review included a very small number of clinically heterogeneous studies, both for the accuracy of *KRAS* mutation testing to predict response to treatment with cetuximab plus standard chemotherapy and for the relative effectiveness of cetuximab plus standard chemotherapy and standard chemotherapy alone in populations with *KRAS* wild-type tumours, selected using different *KRAS* mutation test methods. We were therefore unable to undertake any meta-analyses or formal assessment of publication bias. However, our search

strategy included a variety of routes to identify unpublished studies and resulted in the inclusion of a number of conference abstracts.

Clear inclusion criteria were specified in the protocol for this review and only one protocol modification occurred during the assessment. The eligibility of studies for inclusion is therefore transparent. In addition, we have provided specific reasons for excluding any studies considered potentially relevant at initial citation screening (see *Appendix 5*). The review process followed recommended methods to minimise the potential for error and/or bias.[40] Studies were independently screened for inclusion by two reviewers and data extraction and quality assessment were carried out by one reviewer and checked by a second (MW and PW). Any disagreements were resolved by consensus.

Studies included in this review were assessed for risk of bias using published tools appropriate to the study design and/or the type of data extracted. Studies that provided data on the accuracy of *KRAS* mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy were assessed using a modification of the QUADAS-2 tool.[48] The QUADAS-2 tool is structured into four key domains covering participant selection, index test, reference standard and the flow of patients through the study (including timing of tests). Each domain is rated for risk of bias (low, high or unclear); the participant selection, index test and reference standard domains are also separately rated for concerns regarding the applicability of the study to the review question (low, high or unclear). Studies that provided data on the effectiveness of treatment with cetuximab plus standard chemotherapy compared with standard chemotherapy alone in patients with *KRAS* wild-type tumours were all RCTs or subgroup analyses from RCTs. These studies were therefore assessed using The Cochrane Collaboration's tool for assessing risk of bias in randomised trials.[42,47] The results of the risk of bias assessment are reported in full for all included studies in *Appendix 3* and in summary form in *Chapter 3* (see *What is the accuracy of* KRAS *mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?* and *How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?*). The main potential sources of bias identified were exclusion of withdrawals from the analyses (for studies providing data on the accuracy of *KRAS* mutation tests for predicting response to treatment with cetuximab plus standard chemotherapy) and blinding of participants and personnel in treatment trials. Both of the studies that provided data on the accuracy of *KRAS* mutation testing for predicting response to treatment had some limitations in their applicability to the target population for this assessment. In the case of the CELIM trial[52] data were available to calculate accuracy for the prediction of objective response only rather than for the preferred direct measure resection of liver, and in the case of the COIN trial[54] the standard chemotherapy regimen did not fully match that in the inclusion criteria for this assessment. In addition, participants in the CELIM trial[52] were described as having technically non-resectable or five or more liver metastases from CRC and it was therefore unclear whether some participants may have had potentially resectable metastases at baseline.

All of the studies included in this review have some limitations with regard to their ability to address the overall aim of comparing the clinical effectiveness of different *KRAS* mutation tests to determine which patients may benefit from the addition of cetuximab to standard chemotherapy and which should receive standard chemotherapy alone. The COIN trial[54] is likely to represent the closest approximation to the ideal study in that, when additional data supplied by the trial investigators are also considered, it provides full information on the comparative treatment effect (cetuximab plus standard chemotherapy vs. standard chemotherapy alone) for patients with both *KRAS* wild-type tumours and *KRAS* mutant tumours.
In addition, the trial was conducted in the UK and hence provides data that are likely to be directly applicable to practice in the NHS in England and Wales. However, data included in this assessment were derived from subgroup analyses of patients included in the original trial; not all patients included in the original trial had samples available for *KRAS* mutation testing and, in addition, a much smaller subgroup of patients had metastases that were limited to the liver. Further, the standard chemotherapy regimen used in the COIN trial[54] allowed a choice between FOLFOX or XELOX (depending on local hospital practice and patient preference); the use of XELOX as standard chemotherapy does not match the standard

chemotherapy described in the inclusion criteria for this assessment, as determined by the recommendations of NICE technology appraisal TA176.[1] The COIN trial used a combination of pyrosequencing and MALDI-TOF, targeting mutations in codons 12, 13 and 61, to determine *KRAS* mutation status; in common with all other studies included in this assessment, the study was not designed to assess *KRAS* mutation testing and did not provide any comparative data for other testing methods.

Because methods of testing for *KRAS* mutation status can differ in terms of both the mutations targeted and the limit of detection (the lowest proportion of tumour cells with a mutation that can be detected), the definitions of *KRAS* wild type and *KRAS* mutant vary according to which test is used. All testing methods are essentially reference standard methods for classifying mutation status, as defined by the specific test characteristics. The essential clinical question is to determine which testing method is best at classifying patients such that the maximum treatment effect is achieved both for patients whose tumours are classified as *KRAS* wild type, who receive cetuximab in addition to standard chemotherapy, and for those whose tumours are classified as *KRAS* mutant, who receive standard chemotherapy alone. To fully address this question, data of the type supplied by the COIN trial investigators (i.e. treatment effectiveness data for the addition of cetuximab to standard chemotherapy in both patients whose tumours are classified as *KRAS* wild type and those whose tumours are classified as *KRAS* mutant) would be required for each proposed *KRAS* mutation testing method. Ideally, data for all tests would be derived from the same study population, to allow meaningful comparison of the performance of tests for predicting treatment response without confounding by between-study variations in key participant characteristics. Following the recommendations made in NICE technology appraisal TA176,[1] obtaining these data may be problematic as it could be argued that a trial in which patients are randomised to receive cetuximab in addition to standard chemotherapy or standard chemotherapy alone, regardless of tumour *KRAS* mutation status, would be unethical. Although the COIN trial[54] was published after TA176,[1] more recent UK trials such as New EPOC[77] have tended to focus on determining the effectiveness of adding cetuximab to standard chemotherapy in patients with *KRAS* wild-type tumours. The recently complete, but as yet unpublished, New EPOC trial was a randomised open-label comparison between oxaliplatin/irinotecan plus fluorouracil plus cetuximab and oxaliplatin/irinotecan plus fluorouracil. The trial aimed to assess the effect on PFS of adding cetuximab to standard chemotherapy in patients with *KRAS*-wild type resectable CRC liver metastases who require chemotherapy. Trials of this type are not primarily concerned with the method used to establish mutation status. An alternative approach to this problem is provided by studies that report sufficient data to calculate the accuracy of different *KRAS* mutation tests for predicting response to treatment with cetuximab plus standard chemotherapy. These studies can potentially provide information on the extent to which *KRAS* mutation tests are able to predict resectability of liver metastases following treatment with cetuximab plus standard chemotherapy; outcome data (resection rates or ORRs) are reported for both patients with *KRAS* wild-type tumours and patients with *KRAS* mutant tumours. However, we were able to identify only one study of this type, the CELIM trial,[52] which used an older version of the Therascreen RGQ PCR Kit. Neither the CELIM trial[52] or the COIN trial[54] were intended to assess *KRAS* mutation testing and neither reported comparative data for more than one *KRAS* mutation test; hence, any apparent differences in test performance observed between the two studies may have arisen as a result of differences in study populations. Of particular note is the way in which unresectable liver metastases were defined in the two studies: participants in the CELIM trial[52] were described as having technically non-resectable or five or more liver metastases from CRC and it was therefore unclear whether some participants may have had potentially resectable metastases at baseline, whereas the COIN trial[54] explicitly excluded patients receiving combination chemotherapy before resection of operable liver metastases. This difference may partially account for the marked difference in resection rates observable between the two studies for patients with *KRAS* wild-type tumours who were treated with cetuximab plus standard chemotherapy; (Commercial-in-confidence information has been removed),[59] compared with a resection rate of 13/87 (15%) from the CELIM trial.[52] (Commercial-in-confidence information has been removed),[59] whereas the COIN trial focused on 'potentially curative liver resections',[54] and the standard chemotherapy regimens were different in the two trials.

Trials that compared the effectiveness of cetuximab plus standard chemotherapy with that of standard chemotherapy alone in patients with unresectable liver-limited metastases from CRC and whose tumours were *KRAS* wild type were also included in this review. These trials were included with the aim of providing some indication of how the favourable effects from the addition of cetuximab in these patients may vary according to how patients are selected for treatment (which *KRAS* mutation test is used). However, it should be noted that differences between these studies, other than the way in which *KRAS* wild-type mutation status is defined, particularly in relation to the baseline participant characteristics, are likely to contribute to any differences in treatment effects observed. In addition, these trials can provide no information about the relative effectiveness of cetuximab and standard chemotherapy compared with standard chemotherapy alone in patients whose tumours are classified as *KRAS* mutant.

The effectiveness data available to inform this assessment were very limited. In anticipation of this problem, our assessment included a survey of UK laboratories participating in the NEQAS scheme. This survey aimed to provide additional data on the technical performance of *KRAS* mutation tests, as seen in routine practice in the UK. We consider that data of this type are potentially more informative than data on the technical performance characteristics of tests obtained under research conditions, using non-clinical samples.

Cost-effectiveness

The review of economic analyses of different methods for *KRAS* mutation testing included only full economic analyses. Hence, economic studies that had information on test costs may be excluded from the review.

A de novo probabilistic model was developed to assess the cost-effectiveness of different methods for *KRAS* mutation testing to decide between standard chemotherapy and cetuximab in combination with standard chemotherapy in adults with mCRC in whom metastases are confined to the liver and are initially unresectable. To be consistent with related assessments/appraisals, it was first ensured that the model structure, model assumptions and input parameters in the de novo model were consistent with the manufacturer's model used in NICE technology appraisal 176.[1,59,69] Model results were also consistent for patients with *KRAS* wild-type tumours in the sense that the use of cetuximab would still be considered cost-effective according to the de novo model.

In the assessment of the economic value of different tests, a link has to be established between test accuracy, clinical value (e.g. ORR, resection rate) and relative cost-effectiveness. Ideally, the performance of *KRAS* mutation tests would be assessed against an objective measure of the true presence/absence of a clinically relevant *KRAS* mutation (the 'reference standard'), and the comparative effectiveness of treatment (chemotherapy plus cetuximab vs. chemotherapy alone) conditional on the true presence/absence of the *KRAS* mutation would be determined. However, different testing methods target different ranges of mutations and have different limits of detection (the lowest proportion of mutation detectable in tumour cells) and the optimal combination of mutation location and level for treatment selection remains unclear. For this reason, assessment of test performance based on comparison with a conventional 'reference standard' is currently not possible. An alternative way to determine the relative value of diagnostic methods for *KRAS* mutation testing is to use studies that report on the comparative treatment effect (or a substitute) in patients with both wild-type and mutant *KRAS* tumours. Thus, ORR or liver resection rate after treatment with cetuximab was assumed to correlate perfectly with the 'true' absence/presence of the *KRAS* mutation. The use of alternative outcome measures to determine test accuracy for the assessment of cost-effectiveness might impact the proportion of *KRAS* wild types to *KRAS* mutations and thus might substantially impact the assessment of cost-effectiveness (in either direction) as division of patients over the tumour mutation status categories is a major driver of cost-effectiveness. In the absence of an objective measure of the 'true' presence/absence of a clinically significant *KRAS* mutation, the current cost-effectiveness assessment is, at best, an approximation of the 'true' cost-effectiveness of test–treat combinations.

Evidence on test accuracy was available for only two tests (Therascreen *KRAS* RGQ PCR Kit and pyrosequencing); this was derived from ORR for the Therascreen *KRAS* RGQ PCR Kit and from resection rate for pyrosequencing. A major assumption underpinning our analyses was that the differences in liver resection rates observed in the two included studies from which these data were derived,[52,54] and therefore also differences in the subsequent PFS and OS, can be attributed exclusively to the specific test used. In practice, this assumption would seem unlikely to hold true. These differences could also be caused by, for instance, differences in the characteristics of the respective study populations (i.e. with respect to the type of metastases) or differences in the standard chemotherapy regimen used. In addition, if the assumption of comparability of accuracy rates based on different measures (i.e. ORR and resection rate) holds true,[39] this would reduce the likelihood that the main assumption holds.

Uncertainties

Clinical effectiveness

As noted in *Strengths and limitations*, one important consideration when selecting a *KRAS* mutation testing method is the variation between tests in the limit of detection (i.e. the minimum percentage of mutation in tumour cells required to produce a positive result). A lower limit of detection can enhance the ability of laboratories to produce results from poor-quality samples. However, it should not be assumed that a lower limit of detection will necessarily result in a more clinically effective test, as it is possible that the addition of cetuximab to standard chemotherapy may still be effective in patients with *KRAS* mutant tumours in which mutations are present at a very low level (a low proportion of tumour cells harbouring a mutation). None of the studies that met the inclusion criteria for this review reported any data on variation in treatment effect according to the limit of detection used to define a *KRAS* mutant tumour.

A further area of uncertainty concerns the clinical value of detecting rarer *KRAS* mutations. The majority of the evidence on the effectiveness of first-line treatment with cetuximab plus standard chemotherapy in patients with liver-limited colorectal metastases whose tumours are *KRAS* wild type was derived from patients selected using tests that target mutations in codons 12 and 13; only the COIN trial[54] used a test method that also targeted mutations in codon 61. Indeed, although no testing method was specified, the ASCO PCO published in 2009[15] recommended universal *KRAS* mutation testing in patients with mCRC in whom treatment with EGFR inhibitors is being considered. The recommendation also stated that testing should be carried out in an accredited laboratory and that patients whose tumours have *KRAS* mutations in codons 12 or 13 should not be treated with EGFR receptor inhibitors. The PCO also highlighted the uncertainty around the clinical relevance of detecting rare mutations in codons 61 and 146.[15] The COIN trial[54] reported detection of the following mutations in codon 61, for all samples successfully analysed: Q61H (13/1059, 1.2%), Q61L (5/1289, 0.4%) and Q61R (6/1289, 0.5%); it was not clear whether any of these mutations were detected in patients with liver-limited metastases. The additional clinical value of using tests that target a wider range of mutations remains uncertain, as the low frequency of most *KRAS* mutations makes it very difficult to adequately assess treatment effects or resistance to EGFR inhibitors in patients with these mutations. A large multicentre observational study conducted in Italy by the *KRAS* aKtive network[78] (a programme promoted by the Italian Association of Medical Oncology and the Italian Society of Surgical Pathology and Cytopathology to support the activity of oncologists and pathologists involved in the management of mCRC patients who require *KRAS* mutation testing) has collected data on a total of 7432 *KRAS* mutation analyses. The majority (77%) of testing was conducted using Sanger sequencing, and mutations other than those in codons 12 and 13 represented approximately 5% of the total detected. In addition to the issue of rare mutations, questions have been raised whether all codon 13 mutations predict lack of benefit from treatment with EGFR inhibitors; De Roock *et al.*[79] suggested that the *KRAS* G13D mutation may not predict lack of benefit. The COIN trial[54] identified 110 participants with this mutation and reported no difference in outcome with the addition of cetuximab to standard chemotherapy; the HR for PFS was 1.11 (95% CI 0.76 to 1.63) in patients with the *KRAS* G13D mutation and 1.05 (95% CI 0.87 to 1.27) for all other mutations (data for the whole trial population, not the liver-limited metastases subgroup).

As discussed in *Strengths and limitations*, when assessing the performance of different *KRAS* mutation tests for the prediction of response to treatment, it is important to have information on the relative effectiveness of different treatment options in patients whose tumours are *KRAS* mutant as well as in those whose tumours are *KRAS* wild type. This is because, even when the benefits of adding cetuximab to standard chemotherapy in patients with *KRAS* wild-type tumours have been established, it is important to determine whether there are any negative effects associated with adding cetuximab to the treatment of patients with *KRAS* mutant tumours. If there are no negative effects associated with 'overtreatment' of patients with *KRAS* mutant tumours with cetuximab, then a conservative classification of patients with rare or low-level mutations as 'wild type' for treatment purposes may be considered clinically appropriate. Similarly, the ability of a test to detect rare mutations and/or a low limit of detection may be considered less important. None of the studies included in this assessment reported any difference in OS between patients with *KRAS* mutant tumours treated with cetuximab plus standard chemotherapy and those treated with standard chemotherapy alone. The CRYSTAL trial[27] also reported no difference in ORRs or PFS, whereas the OPUS trial[28] reported a lower ORR (OR 0.46, 95% CI 0.23 to 0.92) and shorter PFS (HR 1.72, 95% CI 1.10 to 2.68) for patients with *KRAS* mutant tumours who were treated with cetuximab plus standard chemotherapy than for those treated with standard chemotherapy alone.[27] These data were for all patients in the trials with *KRAS* mutant tumours; for both the CRYSTAL and OPUS trials, data on treatment effectiveness in patients with *KRAS* mutant tumours were not available for the subgroup of patients with liver-limited metastases. Additional data supplied by the COIN trial investigators that were specific to patients with inoperable liver-limited metastases showed no significant difference in PFS or potentially curative resection rates between patients with *KRAS* mutant tumours who were treated with cetuximab plus standard chemotherapy and patients with *KRAS* mutant tumours who were treated with standard chemotherapy alone (D Fisher, personal communication).

The timing of *KRAS* mutation testing can vary, with some clinicians/hospitals undertaking routine testing of all CRC patients at diagnosis, potentially before the disease becomes metastatic, and others waiting until metastases have been detected. It should be noted that only one of the UK laboratories responding to our survey reported routine *KRAS* testing in all CRC patients. It could be argued that routine testing avoids potential delays in the start of treatment; however, clinical opinion suggested that any such delays would be unlikely to have measurable effects on clinical outcomes. Also, because cetuximab is added to standard chemotherapy in patients with *KRAS* wild-type tumours, standard chemotherapy can be commenced whilst awaiting the results of *KRAS* testing so that only the potential additional benefit of cetuximab is subject to delay. A related question is whether a stored biopsy sample from the primary tumour is adequate for *KRAS* mutation testing once metastases have been detected, or whether potential heterogeneity between tumour sites means that a sample from the metastatic site is preferable. Use of the primary tumour sample is likely to be considered preferable because all patients should have already undergone a biopsy at diagnosis for histological typing; thus, the risks and discomfort of a further invasive procedure (liver biopsy) could potentially be avoided. None of the studies included in this assessment considered the potential impact of sample site on the results of *KRAS* mutation testing. A systematic review[80] identified by our searches, which did not meet the inclusion criteria for this assessment, assessed the concordance of *KRAS* mutations between primary CRC tissue and mCRC tissue. This review included 19 publications reporting data on a total of 986 paired samples from primary tumours and distant metastases (including, but not limited to, the liver) and reported a pooled concordance rate of 94.1% (95% CI 88.3% to 95.0%). One of the primary studies included in this review specifically assessed *KRAS* mutation concordance between primary colorectal tumours and liver metastases in 305 paired samples;[32] *KRAS* mutation status was determined based on pyrosequencing of codons 12 and 13. This study reported a concordance rate of 96.4% (95% CI 93.6% to 98.2%), with clinically relevant discordance in six participants (2.0% of the study population); five primary tumours had a *KRAS* mutation with a wild-type metastasis and one primary tumour was wild type with a *KRAS* mutation in the metastasis. Although outside the scope of this assessment, these studies could be interpreted as supporting the view that *KRAS* mutation testing using stored samples from the primary tumour is a valid approach and that testing using liver biopsy samples is unlikely to produce significant clinical benefit.

A variety of *KRAS* mutation testing methods is currently used by accredited NHS laboratories in England and Wales. None of the methods reported in our survey exactly matched the methods used in any of the studies identified in our systematic review. However, because the COIN trial[54] reported > 99% concordance on *KRAS* genotyping between pyrosequencing and MALDI-TOF, it may be assumed that accuracy data from the COIN trial are also representative of the accuracy of pyrosequencing (used as a single test) for *KRAS* mutations in codons 12, 13 and 61, the method used by the majority of UK laboratories who responded to our survey. It should be noted that the performance of pyrosequencing methods may vary when different primers are used and that the potential clinical effects of using different *KRAS* mutation test methods to make decisions on first-line treatment in patients with unresectable liver-limited CRC metastases remain uncertain. The Therascreen *KRAS* RGQ PCR Kit is the only product currently approved by the FDA;[16] however, the clinical study used to support its approval was not conducted in the population specified for this assessment and none of the respondents to our survey of UK laboratories reported using this product. The Therascreen *KRAS* RGQ PCR Kit, Therascreen *KRAS* Pyro Kit, cobas *KRAS* Mutation Test, *KRAS* LightMix Kit and *KRAS* StripAssay are all CE marked. No direct data, either from our systematic review or from our survey of UK laboratories, are currently available for the following *KRAS* mutation testing methods listed in the scope: next-generation sequencing of codons 12, 13 and 61; *KRAS* StripAssay; MALDI-TOF mass spectrometry of codons 12, 13 and 61 used alone; and HRM analysis of codons 12, 13 and 61 used alone. As was the case for pyrosequencing, concordance between the two *KRAS* mutation testing methods used in the COIN trial[54] means that accuracy data derived from the trial may also be assumed to be representative of the performance of MALDI-TOF when used as a single test for the detection of *KRAS* mutations in codons 12, 13 and 61.

Cost-effectiveness

Major assumptions were made to be able to model the relative cost-effectiveness of different *KRAS* mutation tests. It was assumed that the differences in resection rates between the CELIM trial[52] and the COIN trial[54] and associated subsequent PFS and OS were exclusively attributable to the different mutation tests used (the Therascreen *KRAS* RGQ PCR Kit and pyrosequencing respectively) to distinguish between patients whose tumours are *KRAS* wild type and those whose tumours are *KRAS* mutant. As discussed previously, it is questionable whether this assumption would hold true. Furthermore, to calculate the proportions of patients with *KRAS* wild-type and *KRAS* mutant test results, patients with a *KRAS* wild-type test result were categorised as FP if no objective response was observed on cetuximab (for the Therascreen *KRAS* RGQ PCR Kit) or when no liver resection was performed (for pyrosequencing) or as TP if a objective response was observed or a resection was performed. Likewise, patients with a *KRAS* mutant test result were classified as TN when no objective response was observed on cetuximab (for the the Therascreen *KRAS* RGQ PCR Kit) or no resection was performed (for pyrosequencing) and as FN when an objective response was observed or a liver resection was performed. Ideally, the categorisation of TPs/FP and TNs/FNs should be based on an objective measure of the true presence/absence of a clinically relevant *KRAS* mutation. However, as previously described, the uncertainty around the exact definition of a clinically relevant mutation is such that, at present, there is no such thing as an objective measure or gold standard.

Moreover, as this model was partially based on the evidence and model structure used in the technology appraisal of cetuximab for the first-line treatment of mCRC,[1,59,69] the assumptions underlying that appraisal also apply to this assessment. One example, which applies only to the linked evidence analysis, is the implicit assumption in the manufacturer's model that, in the absence of a chemotherapy-only arm in the CELIM trial,[52] resection rates from the GERCOR trial[68] can be applied to patients with *KRAS* mutant and *KRAS* unknown status tumours treated with standard chemotherapy, whereas resection rates for patients with *KRAS* wild-type tumours treated with cetuximab were taken from the CELIM-trial.[52]

Finally, it should be emphasised that the uncertainty resulting from the above-mentioned assumptions was not parameterised in the model and is therefore not reflected in the PSA or in the CEACs.

Chapter 6 Conclusions

Implications for service provision

There was no strong evidence that any one method of *KRAS* mutation testing had greater accuracy than any other for predicting tumour response or potentially curative resection following treatment with cetuximab plus standard chemotherapy in patients with mCRC whose metastases were limited to the liver and were unresectable before chemotherapy. The clinical effectiveness of cetuximab plus standard chemotherapy in patients whose tumours are *KRAS* wild type did not appear to vary according to which method was used to determine tumour *KRAS* mutation status. However, it should be noted that the available data were not adequate to fully address either the comparative accuracy of different *KRAS* mutation testing methods for predicting tumour response or the comparative clinical effectiveness of cetuximab plus standard chemotherapy in patients whose tumours are *KRAS* wild type, as defined by different testing methods.

The results of the linked evidence analysis indicated that the Therascreen *KRAS* RGQ PCR Kit was more costly and more effective than pyrosequencing, with an ICER of £17,019 per QALY gained. Sensitivity analyses did not show substantial differences compared with the base case. The key driver behind the outcome was the difference in resection rate between treatment with and treatment without cetuximab and the proportions of patients with *KRAS* wild-type, *KRAS* mutant and unknown tumour status, which is determined by test accuracy and therefore, for the most part, is dependent on ORR (for Therascreen *KRAS* RGQ PCR Kit) or resection rate (for pyrosequencing). It should be noted that some problematic and substantial assumptions were necessary to arrive at the economic results, in particular the assumption that the differences in resection rates observed between the different studies are solely the result of the different tests used. This ignores all other factors that can explain variations in outcomes between the studies. Therefore, these outcomes of the assessment of cost-effectiveness should be interpreted with extreme caution.

The results of the assumption of equal prognostic value analysis (including all tests for which information on technical performance was available from the online survey of NHS laboratories in England and Wales) showed that the cobas *KRAS* Mutation Test is the least expensive and least effective strategy and that Sanger sequencing and HRM analysis are equally the most costly and most effective, with an ICER of £69,815 per QALY gained compared with the cobas *KRAS* Mutation Test. The other two strategies included in this analysis, that is, the Therascreen *KRAS* RGQ PCR Kit and pyrosequencing, are ruled out by extended dominance.

There are no data on the clinical effectiveness or cost-effectiveness of next-generation sequencing of codons 12, 13 and 61; the *KRAS* StripAssay; MALDI-TOF mass spectrometry of codons 12, 13 and 61 used alone; and HRM analysis of codons 12, 13 and 61 used alone. No published studies were identified for any of these methods and none of these methods are currently in routine clinical use in any of the NHS laboratories in England and Wales that responded to our survey.

Suggested research priorities

The available data have limitations in respect of their ability to address the overall aim of this assessment, that is, to compare the clinical effectiveness of different methods of *KRAS* mutation testing to determine which patients may benefit from the addition of cetuximab to treatment with standard chemotherapy and which should receive standard chemotherapy alone. Because each different testing method potentially selects a subtly different population, based on the targeting of a different range of mutations and different

limits of detection, the most informative studies are those that provide full information on the comparative treatment effect (cetuximab plus standard chemotherapy vs. standard chemotherapy alone) for both patients with *KRAS* wild-type tumours and patients with *KRAS* mutant tumours. No published studies of this type were identified. Additional data supplied by the COIN trial investigators meant that these data could be derived for a combination of pyrosequencing and MALDI-TOF mass spectrometry (both methods used for all samples) (D Flsher, personal communication). The very high concordance (> 99%) between the two *KRAS* mutation testing methods used in the COIN trial means that data from this trial may be assumed to also be representative of the expected values when pyrosequencing or MALDI-TOF are used as single tests to define tumour *KRAS* mutation status. However, further similar trials are unlikely as randomisation of patients to cetuximab plus standard chemotherapy or standard chemotherapy alone, regardless of tumour *KRAS* mutation status, would be against current clinical guidance and would be likely to be considered unethical. One possible solution to this problem would be to retest stored samples from previous studies in which patient outcomes are already known, using those *KRAS* mutation testing methods for which adequate data are currently unavailable. This approach could provide a 'black box' answer whereby the relative effectiveness of cetuximab plus standard chemotherapy and standard chemotherapy alone in patients with *KRAS* wild-type and *KRAS* mutant tumours could be determined for each testing method. However, it would not provide any information on the underlying reason(s) for any observed differences between tests. As they are likely to represent the most practical approach to obtaining informative data, retrospective comparative accuracy studies, using stored samples for which the patient outcome is already known, should be given priority. The application of this approach to the evaluation of next-generation sequencing might be considered a particular priority, given the likely adoption of next-generation sequencing techniques by laboratories in the near future.

Some methods of *KRAS* mutation testing, for example the Therascreen *KRAS* Pyro Kit, can provide quantitative results. Should quantitative testing become part of routine practice, longitudinal follow-up studies relating the level of mutation and/or the presence of rarer mutations to patient outcomes would become possible. Studies of this type could help to assess which features of *KRAS* mutation tests are likely to be important in determining their clinical effectiveness and should be considered going forward.

Building on information gained from the two study types described above, preliminary research to develop a multifactorial prediction model should be considered. Initially, research of this type is likely to be exploratory in nature; however, models developed could form the basis of tools that will eventually help determine more accurately which patients are most likely to benefit from the addition of cetuximab to treatment with standard chemotherapy.

As the uncertainties associated with clinical effectiveness forced the major assumptions in the economic evaluation, this type of research would also facilitate economic analyses of *KRAS* mutation testing.

Acknowledgements

The authors acknowledge the clinical advice and expert opinion provided by Dr Mark Harrison, Consultant Oncologist, Mount Vernon Cancer Centre, East and North Hertfordshire NHS Trust; Dr Newton Wong, Consultant Histopathologist, University Hospitals Bristol NHS Foundation Trust; Miss Jennie Bell, Consultant Molecular Geneticist, Birmingham Women's NHS Foundation Trust; and Dr Phil Chambers, Genomics Facility, Leeds Cancer Research UK Centre. The authors would like to thank the COIN trial investigators, in particular Dr Richard Wilson and Professor Timothy Maughan, for providing additional data. The authors would like to thank the representatives of the NHS laboratories providing *KRAS* mutation testing who kindly completed our online survey. The authors would also like to thank Merck Serono UK Ltd for providing access to the cost-effectiveness model used in NICE technology appraisal 176. Finally, the authors would like to thank the lay members of the NICE Diagnostics Advisory Committee and Assessment Subgroup for providing input on the patients' perspective at key stages of the assessment process.

Contributions of authors

References

1. National Institute for Health and Care Excellence. *Cetuximab for the First-Line Treatment of Metastatic Colorectal Cancer*. NICE technology appraisal guidance 176. London: NICE, 2009. URL: www.nice.org.uk/nicemedia/live/12216/45198/45198.pdf (accessed 30 November 2012).

2. Office for National Statistics. *Cancer Registrations in England, 2010: Number of New Cases Diagnosed By Site*. London: Office for National Statistics; 2012. URL: www.ons.gov.uk/ons/rel/vsob1/cancer-registrations-in-england/2010/2010-cancer-registrations-in-england-statistical-bulletin-2.html#tab-Number-of-new-cases-diagnosed-by-site (accessed 30 November 2012).

3. Cancer Research UK. *Bowel Cancer Incidence Statistics*. London: Cancer Research UK; 2012 URL: www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/incidence/uk-bowel-cancer-incidence-statistics (accessed 30 November 2012).

4. Cancer Research UK. *Bowel Cancer Mortality Statistics*. London: Cancer Research UK; 2012. URL: www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/mortality/uk-bowel-cancer-mortality-statistics (accessed 30 November 2012).

5. Cancer Research UK. *Bowel Cancer Survival Statistics*. London: Cancer Research UK; 2012. URL: www.cancerresearchuk.org/cancer-info/cancerstats/types/bowel/survival/bowel-cancer-survival-statistics (accessed 30 November 2012).

6. National Cancer Intelligence Network. *Cancer Incidence by Deprivation: England, 1995–2004*. London: National Cancer Intelligence Network Coordinating Centre; 2009. URL: www.ncin.org.uk/view.aspx?rid=73 (accessed 30 November 2012).

7. Finan P, Smith J, Greenaway K, Yelland A, Scott N, Henderson J, *et al. National Bowel Cancer Audit Report 2011*. London: NHS Information Centre for Health and Social Care; 2011. URL: www.ic.nhs.uk/webfiles/Services/NCASP/audits%20and%20reports/Bowel_Cancer_Audit_Report_2011_interactive.pdf (accessed 30 November 2012).

8. Neumann J, Zeindl-Eberhart E, Kirchner T, Jung A. Frequency and type of KRAS mutations in routine diagnostic analysis of metastatic colorectal cancer. *Pathol Res Pract* 2009;**205**:858–62. http://dx.doi.org/10.1016/j.prp.2009.07.010

9. Cejas P, Lopez-Gomez M, Aguayo C, Madero R, de Castro Carpeno J, Belda-Iniesta C, *et al.* KRAS mutations in primary colorectal cancer tumors and related metastases: a potential role in prediction of lung metastasis. *PLoS One* 2009;**4**:e8199. http://dx.doi.org/10.1371/journal.pone.0008199

10. Baldus SE, Schaefer KL, Engers R, Hartleb D, Stoecklein NH, Gabbert HE. Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. *Clin Cancer Res* 2010;**16**:790–9. http://dx.doi.org/10.1158/1078-0432.CCR-09-2446

11. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS. Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. *Genes Chromosomes Cancer* 2011;**50**:307–12. http://dx.doi.org/10.1002/gcc.20854

12. UK National External Quality Assessment Service for Molecular Genetics. *KRAS Pilot EQA 2012–13*. Newcastle upon Tyne: UK NEQAS for Molecular Genetics; 2012. URL: www.ukneqas-molgen.org.uk/ukneqas/index/news.html (accessed 6 December 2012).

13. Liu P, Liang H, Xue L, Yang C, Liu Y, Zhou K, *et al.* Potential clinical significance of plasma-based KRAS mutation analysis using the COLD-PCR/TaqMan((R)) – MGB probe genotyping method. *Exp Ther Med* 2012;**4**:109–12.

14. Morgan SR, Whiteley J, Donald E, Smith J, Eisenberg MT, Kallam E, *et al.* Comparison of KRAS mutation assessment in tumor DNA and circulating free DNA in plasma and serum samples. *Clin Med Insights Pathol* 2012;**5**:15–22. http://dx.doi.org/10.4137/CPath.S8798

15. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, *et al.* American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009;**27**:2091–6. http://dx.doi.org/10.1200/JCO.2009.21.9170

16. US Food and Drug Administration. *Summary of Safety and Effectiveness Data: Therascreen® KRAS RGQ PCR Kit.* 2012. URL: www.accessdata.fda.gov/cdrh_docs/pdf11/P110030b.pdf (accessed 21 May 2013).

17. QIAGEN. *Therascreen KRAS RGQ PCR Kit Handbook (Version 1).* 2011. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

18. QIAGEN. *Therascreen KRAS Pyro Kit Handbook (Version 1).* 2011. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

19. TIB MOLBIOL. *LightMix Kit K-RAS Mutation Codons 12/13 (Version 110621).* 2011. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

20. ViennaLab Diagnostics. *KRAS StripAssay Instructions for Use.* 2010. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

21. QIAGEN. *Principle of Pyrosequencing Technology.* 2012. [As cited in the NICE final scoping document, 2013. URL: www.pyrosequencing.com/DynPage.aspx?id=7454&mn1=1366&mn2=1367.]

22. Applied Biosystems. *Overview of DNA Sequencing.* 2011. [As cited in the NICE final scoping document, 2013. URL: www.appliedbiosystems.com/absite/us/en/home/applications-technologies/dna-sequencing-fragment-analysis/overview-of-dna-sequencing.html.]

23. Illumina. *An Introduction to Next Generation Sequencing Technology.* 2012. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

24. PrimerDesign. *Beginners Guide to High Resolution Melt Analysis.* 2009. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

25. Elso C, Toohey B, Reid GE, Poetter K, Simpson RJ, Foote SJ. Mutation detection using mass spectrometric separation of tiny oligonucleotide fragments. *Genome Res* 2002;**12**:1428–33. http://dx.doi.org/10.1101/gr.GR-1578R

26. Molinari F, Felicioni L, Buscarino M, De Dosso S, Buttitta F, Malatesta S, *et al.* Increased detection sensitivity for KRAS mutations enhances the prediction of anti-EGFR monoclonal antibody resistance in metastatic colorectal cancer. *Clin Cancer Res* 2011;**17**:4901–14. http://dx.doi.org/10.1158/1078-0432.CCR-10-3137

27. Van Cutsem E, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, *et al.* Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;**360**:1408–17. http://dx.doi.org/10.1056/NEJMoa0805019

28. Bokemeyer C, Bondarenko I, Hartmann JT, De Braud F, Schuch G, Zubel A, *et al.* Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol* 2011;**22**:1535–46. http://dx.doi.org/10.1093/annonc/mdq632

29. National Institute for Health and Care Excellence. *Colorectal Cancer: the Diagnosis and Management of Colorectal Cancer*. NICE clinical guideline 131. London: NICE; 2011. URL: www.nice.org.uk/nicemedia/live/13597/56957/56957.pdf (accessed 30 November 2012).

30. Labianca R, Nordlinger B, Beretta GD, Brouquet A, Cervantes A. Primary colon cancer: ESMO clinical practice guidelines for diagnosis, adjuvant treatment and follow-up. *Ann Oncol* 2010;**21**(Suppl. 5):70–7. http://dx.doi.org/10.1093/annonc/mdq168

31. Royal College of Pathologists. *The Future Provision of Molecular Diagnostic Services for Aquired Diseases in the UK*. 2010. [As cited in the NICE final scoping document, 2013. URL: www.nice.org.uk/nicemedia/live/13937/62336/62336.pdf (accessed 5 June 2014).]

32. Knijn N, Mekenkamp LJ, Klomp M, Vink-Borger ME, Tol J, Teerenstra S, *et al.* KRAS mutation analysis: a comparison between primary tumours and matched liver metastases in 305 colorectal cancer patients. *Br J Cancer* 2011;**104**:1020–6. http://dx.doi.org/10.1038/bjc.2011.26

33. Richman SD, Chambers P, Seymour MT, Daly C, Grant S, Hemmings G, *et al.* Intra-tumoral heterogeneity of KRAS and BRAF mutation status in patients with advanced colorectal cancer (aCRC) and cost-effectiveness of multiple sample testing. *Anal Cell Pathol (Amst)* 2011;**34**:61–6.

34. National Institute for Health and Care Excellence. *Guidance on the Use of Capecitabine and Tegafur with Uracil for Metastatic Colorectal Cancer*. NICE technology appraisal guidance 61. London: NICE; 2003. URL: www.nice.org.uk/nicemedia/live/11498/32624/32624.pdf (accessed 30 November 2012).

35. European Medicines Agency. *Summary of Product Characteristics: Erbitux 5 mg/ml Solution for Infusion*. London: EMEA; 2009. URL: www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000558/WC500029119.pdf (accessed 30 November 2012).

36. National Institute for Health and Care Excellence. *Cetuximab, Bevacizumab and Panitumumab for the Treatment of Metastatic Colorectal Cancer after First-Line Chemotherapy*. NICE technology appraisal guidance 242. London: NICE, 2012. URL: www.nice.org.uk/nicemedia/live/13651/57924/57924.pdf (accessed 30 November 2012).

37. World Health Organization. *WHO Handbook for Reporting Results of Cancer Treatment*. WHO Offset Publication No. 48. Geneva: WHO; 1979. URL: http://whqlibdoc.who.int/offset/WHO_OFFSET_48.pdf (accessed 4 December 2012).

38. Therasse P, Arbuck S, Eisenhauer E, Wanders J, Kaplan R, Rubinstein L, *et al.* New guidelines to evaluate the response to treatment in solid tumors. *J Natl Cancer Inst* 2000;**92**:205–16. http://dx.doi.org/10.1093/jnci/92.3.205

39. Folprecht G, Grothey A, Alberts S, Raab HR, Kohne CH. Neoadjuvant treatment of unresectable colorectal liver metastases: correlation between tumour response and resection rates. *Ann Oncol* 2005;**16**:1311–19. http://dx.doi.org/10.1093/annonc/mdi246

40. Centre for Reviews and Dissemination. *Systematic Reviews: CRD's Guidance for Undertaking Reviews in Health Care*. York: University of York; 2009. URL: www.york.ac.uk/inst/crd/SysRev/!SSL!/WebHelp/SysRev3.htm (accessed 21 May 2013).

41. National Institute for Health and Care Excellence. *Diagnostics Assessment Programme Manual*. Machester: NICE; 2011. URL: www.nice.org.uk/media/A0B/97/DAPManualFINAL.pdf (accessed 10 July 2012).

42. Cochrane Diagnostic Test Accuracy Working Group. *Handbook for DTA Reviews*. The Cochrane Collaboration, 2009. URL: http://srdta.cochrane.org/handbook-dta-reviews (accessed 29 August 2013).

43. McGowan J, Sampson M, Lefebvre C. An evidence based checklist for the peer review of electronic search strategies (PRESS EBC). *Evidence Based Libr Inform Prac* 2010;**5**:1–6.

44. Royle P, Waugh N. Should systematic reviews include searches for published errata? *Health Info Libr J* 2004;**21**:14–20. http://dx.doi.org/10.1111/j.1471-1842.2004.00459.x

45. Wright K, McDaid C. Is the retraction of journal articles in electronic journals and databases consistent and timely? A case study. 19th Cochrane Colloquium, Madrid, 19–22 October 2011.

46. Wright K, McDaid C. Reporting of article retractions in bibliographic databases and online journals. *J Med Libr Assoc* 2011;**99**:164–7. http://dx.doi.org/10.3163/1536-5050.99.2.010

47. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, *et al.* The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;**343**:d5928. http://dx.doi.org/10.1136/bmj.d5928

48. Whiting PF, Rutjes AWS, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, *et al.* QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;**155**:529–36. http://dx.doi.org/10.7326/0003-4819-155-8-201110180-00009

49. Reitsma JB, Glas AS, Rutjes AWS, Scholten RJPM, Bossuyt PMM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. *J Clin Epidemiol* 2005;**58**:982–90. http://dx.doi.org/10.1016/j.jclinepi.2005.02.022

50. Harbord RM, Deeks JJ, Egger M, Whiting P, Sterne JA. A unification of models for meta-analysis of diagnostic accuracy studies. *Biostatistics* 2007;**8**:239–51. http://dx.doi.org/10.1093/biostatistics/kxl004

51. Harbord RM, Whiting P, Sterne JA, Egger M, Deeks JJ, Shang A, *et al.* An empirical comparison of methods for meta-analysis of diagnostic accuracy showed hierarchical models are necessary. *J Clin Epidemiol* 2008;**61**:1095–103. http://dx.doi.org/10.1016/j.jclinepi.2007.09.013

52. Folprecht G, Gruenberger T, Bechstein WO, Raab HR, Lordick F, Hartmann JT, *et al.* Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. *Lancet Oncol* 2010;**11**:38–47. http://dx.doi.org/10.1016/S1470-2045(09)70330-4

53. Kohne C, Bokemeyer C, Heeger S, Sartorius U, Rougier P, Van Cutsem E. Efficacy of chemotherapy plus cetuximab according to metastatic site in KRAS wild-type metastatic colorectal cancer (mCRC): analysis of CRYSTAL and OPUS studies. *J Clin Oncol* 2011;**29**(15 Suppl.):3576.

54. Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, *et al.* Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;**377**:2103–14. http://dx.doi.org/10.1016/S0140-6736(11)60613-2

55. Xu J, Ye L, Ren L, Wei Y. A randomized, controlled trial of cetuximab plus chemotherapy for patients with kras wild-type unresectable colorectal liver-limited metastases. *Ann Oncol* 2012;**23**:190.

56. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, De Braud F, *et al.* Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J Clin Oncol* 2009;**27**:663–71. http://dx.doi.org/10.1200/JCO.2008.20.8397

57. Ocvirk J, Brodowicz T, Wrba F, Ciuleanu TE, Kurteva G, Beslija S, *et al.* Cetuximab plus FOLFOX6 or FOLFIRI in metastatic colorectal cancer: CECOG trial. *World J Gastroenterol* 2010;**16**:3133–43. http://dx.doi.org/10.3748/wjg.v16.i25.3133

58. Tveit KM, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, *et al.* Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol* 2012;**30**:1755–62. http://dx.doi.org/10.1200/JCO.2011.38.0915

59. Merck Serono Ltd. *Erbitux (Cetuximab) for the First-Line Treatment of Metastatic Colorectal Cancer: Submission to National Institute for Health and Clinical Excellence*. Single technology appraisal (STA). Merck Serono Ltd; 2008. URL: www.nice.org.uk/nicemedia/live/11918/42095/42095.pdf (accessed 21 May 2013).

60. Xu J. *Cetuximab in Combination with Chemotherapy for the Treatment of Metastatic Colorectal Cancer*. NCT01564810. Bethesda, MD: National Library of Medicine; 2012. URL: http://clinicaltrials.gov/show/NCT01564810 (accessed 21 March 2013).

61. Health Quality Ontario. KRAS testing for anti-EGFR therapy in advanced colorectal cancer: an evidence-based and economic analysis. *Ont Health Technol Assess Ser* 2010;**10**:1–49.

62. Vijayaraghavan A, Efrusy MB, Goke B, Kirchner T, Santas CC, Goldberg RM. Cost-effectiveness of KRAS testing in metastatic colorectal cancer patients in the United States and Germany. *Int J Cancer* 2012;**131**:438–45. http://dx.doi.org/10.1002/ijc.26400

63. Behl AS, Goddard KAB, Flottemesch TJ, Veenstra D, Meenan RT, Lin JS, *et al.* Cost-effectiveness analysis of screening for KRAS and BRAF mutations in metastatic colorectal cancer. *J Natl Cancer Inst* 2012;**104**:1785–95. http://dx.doi.org/10.1093/jnci/djs433

64. Shiroiwa T, Motoo Y, Tsutani K. Cost-effectiveness analysis of KRAS testing and cetuximab as last-line therapy for colorectal cancer. *Mol Diagn Ther* 2010;**14**:375–84. http://dx.doi.org/10.1007/BF03256395

65. Blank PR, Moch H, Szucs TD, Schwenkglenks M. KRAS and BRAF mutation analysis in metastatic colorectal cancer: a cost-effectiveness analysis from a Swiss perspective. *Clin Cancer Res* 2011;**17**:6338–46. http://dx.doi.org/10.1158/1078-0432.CCR-10-2267

66. Drummond MF, Jefferson TO. Guidelines for authors and peer reviewers of economic submissions to the BMJ. The BMJ Economic Evaluation Working Party. *BMJ* 1996;**313**:275–83. http://dx.doi.org/10.1136/bmj.313.7052.275

67. Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, *et al.* K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008;**359**:1757–65. http://dx.doi.org/10.1056/NEJMoa0804385

68. Tournigand C, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, *et al.* FOLFORI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004;**22**:229–37. http://dx.doi.org/10.1200/JCO.2004.05.113

69. Merck Serono Ltd. Parametric models. In *Erbitux (Cetuximab) for the First-Line Treatment of Metastatic Colorectal Cancer: Submission to National Institute for Health and Clinical Excellence*. Single technology appraisal (STA). Merck Serono Ltd; 2008. Appendix H3.

70. Andreyev HJN, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, *et al.* Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* 2001;**85**:692–6. http://dx.doi.org/10.1054/bjoc.2001.1964

71. Nordlinger B, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, *et al.* Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008;**371**:1007–16. http://dx.doi.org/10.1016/S0140-6736(08)60455-9

72. Simmonds PC, Primrose JN, Colquitt JL, Garden OJ, Poston GJ, Rees M. Surgical resection of hepatic metastases from colorectal cancer: a systematic review of published studies. *Br J Cancer* 2006;**94**:982–99. http://dx.doi.org/10.1038/sj.bjc.6603033

73. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, *et al.* Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;**357**:2040–8. http://dx.doi.org/10.1056/NEJMoa071834

74. Mittmann N, Au HJ, Tu D, O'Callaghan CJ, Karapetis CS, Moore MJ, *et al.* A prospective economic analysis of cost-effectiveness of cetuximab for metastatic colorectal cancer patients from the NCIC CTG and AGITG CO.17 trial. *J Clin Oncol* 2008;**26**(15S Suppl.):6528.

75. Adam R, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, *et al.* Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. *Ann Surg* 2004;**240**:644–57, discussion 657–8.

76. Whiting P, Westwood M, Beynon R, Burke M, Sterne JA, Glanville J. Inclusion of methodological filters in searches for diagnostic test accuracy studies misses relevant studies. *J Clin Epidemiol* 2011;**64**:602–7. http://dx.doi.org/10.1016/j.jclinepi.2010.07.006

77. University of Southampton. *Combination Chemotherapy with or without Cetuximab before and after Surgery in Treating Patients with Resectable Liver Metastases Caused by Colorectal Cancer*. NCT00482222. Bethesda, MD: National Library of Medicine; 2013. URL: http://ClinicalTrials.gov/show/NCT00482222 (accessed 14 May 2013).

78. Marchetti A, Pinto C, Taddei G, Clemente C, Troncone G, Russo A, *et al.* KRAS aKtive, an Italian network for assessment of KRAS mutations in colorectal cancer patients: results on 7,432 cases. *ASCO Meeting Abstr* 2012;**30**(Suppl. 15):e14042.

79. De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, *et al.* Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA* 2010;**304**:1812–20. http://dx.doi.org/10.1001/jama.2010.1535

80. Han CB, Li F, Ma JT, Zou HW. Concordant KRAS mutations in primary and metastatic colorectal cancer tissue specimens: a meta-analysis and systematic review. *Cancer Invest* 2012;**30**:741–7. http://dx.doi.org/10.3109/07357907.2012.732159

81. Adams R, Maughan T, Smith C, Seymour M, Wilson R, Meade A, *et al.* Addition of cetuximab to oxalplatin-based combination chemotherapy (CT) in patients with K-ras wild-type advanced colorectal cancer (ACRC): effects on overall survival. MRC COIN (CR10) trial results. *Ann Oncol* 2009;**20**:15.

82. Adams R, Wilson RH, Seymour MT, Meade AM, Madi A, Cassidy J, *et al.* Intermittent versus continuous oxaliplatin-fluoropyrimidine (Ox-Fp) chemotherapy (CT) in first-line treatment of patients (pts) with advanced colorectal cancer (aCRC): predictive factors (PF), quality of life (QL), and final efficacy results from the MRC COIN trial. *J Clin Oncol* 2010;**28**(15 Suppl.):3525.

83. Adams R, Wilson R, Seymour M, Meade A, Madi A, Cassidy J, *et al.* Intermittent vs. continuous oxaliplatinfluoropyrimidine chemotherapy in the MRC COIN trial in advanced colorectal cancer: updated efficacy results, quality of life and potential predictive factors. 12th World Congress on Gastrointestinal Cancer, ESMO Conference 2010; 30 June–3 July 2010; Barcelona, Spain. *Ann Oncol* 2010;**21**:vi19.

84. Alberts SR, Sargent DJ, Smyrk TC, Shields AF, Chan E, Goldberg RM, *et al.* Adjuvant mFOLFOX6 with or without cetuxiumab (Cmab) in KRAS wild-type (WT) patients (pts) with resected stage III colon cancer (CC): results from NCCTG Intergroup phase III trial N0147. *J Clin Oncol* 2010;**28**(18 Suppl.):CRA3507.

85. Assenat E, Desseigne F, Thezenas S, Viret F, Mineur L, Kramar A, *et al.* Cetuximab plus FOLFIRINOX (ERBIRINOX) as first-line treatment for unresectable metastatic colorectal cancer: a phase II trial. *Oncologist* 2011;**16**:1557–64. http://dx.doi.org/10.1634/theoncologist.2011-0141

86. Baker JB, Dutta D, Watson D, Maddala T, Shak S, Rowinsky EK, *et al.* Evaluation of tumor gene expression and K-RAS mutations in FFPE tumor tissue as predictors of response to cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;**26**(15 Suppl.):3512.

87. Baloglu H, Yilmaz I, Kucukodaci Z. A KRAS mutation profile in colorectal carcinomas: mutation detection technique may affect patient selection for anti-EGFR therapy. *Lab Invest* 2012;**92**:155A.

88. Bokemeyer C, Bondarenko I, Hartmann JT, De Braud FG, Volovat C, Nippgen J, *et al.* KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: the OPUS experience. *J Clin Oncol* 2008;**26**(15 Suppl.):4000.

89. Bokemeyer C, Bondarenko I, Hartmann JT, De Braud F, Schuch G, Zubel A, *et al.* Overall survival of patients with KRAS wild-type tumours treated with FOLFOX4 ± cetuximab as 1st-line treatment for metastatic colorectal cancer: the OPUS study. *Ejc Suppl* 2009;**7**:346. http://dx.doi.org/10.1016/S1359-6349(09)71174-7

90. Bokemeyer C, Kohne C, Rougier P, Stroh C, Schlichting M, Van Cutsem E. Cetuximab with chemotherapy (CT) as first-line treatment for metastatic colorectal cancer (mCRC): analysis of the CRYSTAL and OPUS studies according to KRAS and BRAF mutation status. *J Clin Oncol* 2010;**28**(15 Suppl.):3506.

91. Bokemeyer C, Van Cutsem E, Rougier P, Ciardiello F, Heeger S, Schlichting M, *et al.* Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. *Eur J Cancer* 2012;**48**:1466–75. http://dx.doi.org/10.1016/j.ejca.2012.02.057

92. Chuko J, Yeh MK, Chen BJ, Hu KY. Efficacy of cetuximab on wild-type and mutant KRAS in colorectal cancer: systematic review and meta-analysis. *J Med Sci* 2010;**30**:189–98.

93. Cohen D, Ma H, Yuan Y, Hochman T, Hochster HS. Biweekly cetuximab (C), oxaliplatin (Ox), and capecitabine (Cape) in first line therapy of metastatic colorectal cancer (mCRC): preliminary results on toxicity and K-RAS status. *J Clin Oncol* 2008;**26**(15 Suppl.):15060.

94. Colucci G, Giuliani F, Garufi C, Mattioli R, Manzione L, Russo A, *et al.* Cetuximab plus FOLFOX-4 in untreated patients with advanced colorectal cancer: a Gruppo Oncologico dell'Italia Meridionale multicenter phase II study. *Oncology* 2010;**79**:415–22. http://dx.doi.org/10.1159/000323279

95. Di Salvatore M, Inno A, Orlandi A, Martini M, Nazzicone G, Ferraro D, *et al.* KRAS and BRAF mutational status and PTEN, cMET, and IGF1R expression as predictive markers of response to cetuximab plus chemotherapy in metastatic colorectal cancer (mCRC). *J Clin Oncol* 2010;**28**(Suppl 1):e14065.

96. Dubus P, Berhouet S, Carrere N, Merlio JP. Predicting response to anti-EGFR therapy for colorectal carcinoma by KRAS mutation testing. *Cytometry Part B* 2009;**76B**:420–1.

97. Folprecht G, Kohne CH. Cetuximab in combination with 5-fluorouracil, leucovorin and irinotecan as a neoadjuvant chemotherapy in patients with initially unresectable colorectal liver metastases. *Adv Gastrointest Cancers* 2008;**6**:13–14.

98. Folprecht G, Nowacki M, Lang I, Cascinu S, Shchepotin I, Maurel J, *et al.* Cetuximab plus FOLFIRI first line in patients (pts) with metastatic colorectal cancer (mCRC): a quality-of-life (QoL) analysis of the CRYSTAL trial. *J Clin Oncol* 2009;**27**(15S Suppl.):4076.

99. Folprecht G, Kohne C, Bokemeyer C, Rougier P, Schlichting M, Heeger S, *et al.* Cetuximab and 1st-line chemotherapy in elderly and younger patients with metastatic colorectal cancer (MCRC): a pooled analysis of the crystal and opus studies. *Ann Oncol* 2010;**21**:194.

100. Gajate P, Sastre J, Bando I, Alonso T, Cillero L, Sanz J, *et al.* Influence of KRAS p.G13D mutation in patients with metastatic colorectal cancer treated with cetuximab. *Clin Colorectal Cancer* 2012;**11**:291–6. http://dx.doi.org/10.1016/j.clcc.2012.02.003

101. Gao J, Wang TT, Yu JW, Li YY, Shen L. Wild-type KRAS and BRAF could predict response to cetuximab in Chinese colorectal cancer patients. *Chinese J Cancer Res* 2011;**23**:271–5. http://dx.doi.org/10.1007/s11670-011-0271-4

102. Garufi C, Torsello A, Tumolo S, Mottolese M, Melucci E, Campanella C, *et al.* Induction of resectability of colorectal liver metastases (CLM) with cetuximab (Cmab) plus CPT-11/ 5-fluorouracil (5-FU)/leucovorin (FA)/oxaliplatin (L-OHP) (CPT-11-FFL) (POCHER trial). *Eur J Cancer* 2009;**7**:348. http://dx.doi.org/10.1016/S1359-6349(09)71181-4

103. Goldberg RM, Sargent DJ, Thibodeau SN, Mahoney MR, Shields AF, Chan E, *et al.* Adjuvant mFOLFOX6 plus or minus cetuximab (Cmab) in patients (pts) with KRAS mutant (m) resected stage III colon cancer (CC): NCCTG Intergroup Phase III Trial N0147. *J Clin Oncol* 2010;**28**(15 Suppl.):3508.

104. Griebsch I, Lang I, Sartorius U, Van Cutsem E. The effect of tumor response on quality of life (QoL) in patients with KRAS wild-type metastatic colorectal cancer (mCRC): analysis from the CRYSTAL study. *J Clin Oncol* 2011;**29**(15 Suppl.):3626.

105. Harbison CT, Horak CE, Ledeine JM, Mukhopadhyay P, Malone DP, O'Callaghan C, *et al.* Validation of companion diagnostic for detection of mutations in codons 12 and 13 of the KRAS gene in patients with metastatic colorectal cancer: analysis of the NCIC CTG CO.17 trial [published online ahead of print 3 October 2012). *Arch Pathol Lab Med* 2012. doi: 10.5858/ arpa.2012-0367-OA. http://dx.doi.org/10.5858/arpa.2012-0367-OA

106. Huang J, Sargent DJ, Mahoney MR, Thibodeau SN, Smyrk TC, Sinicrope F, *et al.* Adjuvant FOLFIRI with or without cetuximab in patients with resected stage III colon cancer: NCCTG Intergroup phase III trial N0147. *J Clin Oncol* 2011;**29**(4 Suppl.):363.

107. Ibrahim EM, Zekri JM, Bin Sadiq BM. Cetuximab-based therapy for metastatic colorectal cancer: a meta-analysis of the effect of K-RAS mutations. *Int J Colorectal Dis* 2010;**25**:713–21. http://dx.doi.org/10.1007/s00384-010-0927-4

108. Jones C, Taylor MA, McWilliams B. The role of cetuximab as first-line treatment of colorectal liver metastases. *HPB (Oxford)* 2013;**15**:11–17. http://dx.doi.org/10.1111/j.1477-2574.2012.00591.x

109. Jonker DJ, Karapetis C, Harbison C, O'Callaghan CJ, Tu D, Simes RJ, *et al.* High epiregulin (EREG) gene expression plus K-RAS wild-type (WT) status as predictors of cetuximab benefit in the treatment of advanced colorectal cancer (ACRC): results from NCIC CTG CO.17-A phase III trial of cetuximab versus best supportive care (BSC). *J Clin Oncol* 2009;**27**(15S Suppl.):4016.

110. Kimura T, Okamoto K, Miyamoto H, Kimura M, Kitamura S, Takenaka H, *et al.* Clinical benefit of high-sensitivity KRAS mutation testing in metastatic colorectal cancer treated with anti-EGFR antibody therapy. *Oncology (Switzerland)* 2012;**82**:298–304.

111. Kohne C, Stroiakovski D, Chang-chien C, Lim R, Pinter T, Bodoky G, *et al.* Predictive biomarkers to improve treatment of metastatic colorectal cancer (mCRC): outcomes with cetuximab plus FOLFIRI in the CRYSTAL trial. *J Clin Oncol* 2009;**27**(15S Suppl.):4068.

112. Ku GY, Haaland BA, de Lima Lopes G Jr. Cetuximab in the first-line treatment of K-RAS wild-type metastatic colorectal cancer: the choice and schedule of fluoropyrimidine matters. *Cancer Chemother Pharmacol* 2012;**70**:231–8. http://dx.doi.org/10.1007/s00280-012-1898-7

113. Lang I, Kohne CH, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, *et al.* Cetuximab plus FOLFIRI in 1st-line treatment of metastatic colorectal cancer: quality of life (QoL) analysis of patients (pts) with KRAS wild-type (wt) tumours in the CRYSTAL trial. *Eur J Cancer* 2009;**7**:345. http://dx.doi.org/10.1016/S1359-6349(09)71173-5

114. Lievre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, *et al.* KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;**66**:3992–5. http://dx.doi.org/10.1158/0008-5472.CAN-06-0191

115. Lin OSR. Activating KRAS mutations as an independent predictor in metastatic colorectal cancer patients treated with cetuximab. *Ann Oncol* 2010;**21**:i36.

116. Lin AY, Buckley NS, Lu A-TT, Kouzminova NB, Salpeter SR. Effect of KRAS mutational status in advanced colorectal cancer on the outcomes of anti-epidermal growth factor receptor monoclonal antibody therapy: a systematic review and meta-analysis. *Clin Colorectal Cancer* 2011;**10**:63–9. http://dx.doi.org/10.3816/CCC.2011.n.009

117. Linardou H, Dahabreh IJ, Kanaloupiti D, Siannis F, Bafaloukos D, Kosmidis P, *et al.* Assessment of somatic K-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. *Lancet Oncol* 2008;**9**:962–2. http://dx.doi.org/10.1016/S1470-2045(08)70206-7

118. Loupakis F, Cremolini C, Salvatore L, Schirripa M, Lonardi S, Vaccaro V, *et al.* Clinical impact of anti-epidermal growth factor receptor monoclonal antibodies in first-line treatment of metastatic colorectal cancer: meta-analytical estimation and implications for therapeutic strategies. *Cancer* 2012;**118**:1523–32. http://dx.doi.org/10.1002/cncr.26460

119. Malapelle U, Bellevicine C, Salatiello M, De Luca C, Rispo E, Riccio P, *et al.* Sanger sequencing in routine KRAS testing: a review of 1720 cases from a pathologist's perspective. *J Clin Pathol* 2012;**65**:940–4. http://dx.doi.org/10.1136/jclinpath-2012-200773

120. Malapelle U, Carlomagno C, Salatiello M, De Stefano A, De Luca C, Bianco R, *et al.* KRAS mutation detection by high-resolution melting analysis significantly predicts clinical benefit of cetuximab in metastatic colorectal cancer. *Br J Cancer* 2012;**107**:626–31. http://dx.doi.org/10.1038/bjc.2012.275

121. Mancuso A, Leone A, Vigna L, Calabro F, Giuliani R, De Marco S, *et al.* EGFR, DCC, and K-RAS mutations as predictive factors for cetuximab sensitivity in metastatic colorectal cancer (mCRC). *J Clin Oncol* 2008;**26**(15S Suppl.):4128.

122. Maughan T, Adams RA, Smith CG, Seymour MT, Wilson R, Meade AM, *et al.* Addition of cetuximab to oxaliplatin-based combination chemotherapy (CT) in patients with KRAS wild-type advanced colorectal cancer (ACRC): a randomised superiority trial (MRC COIN). *Eur J Cancer* 2009;**7**:4–5. http://dx.doi.org/10.1016/S1359-6349(09)72034-8

123. Maughan TS, Adams R, Smith CG, Seymour MT, Wilson RH, Meade AM, *et al.* Identification of potentially responsive subsets when cetuximab is added to oxaliplatin-fluoropyrimidine chemotherapy (CT) in first-line advanced colorectal cancer (aCRC): mature results of the MRC COIN trial. *J Clin Oncol* 2010;**28**(15 Suppl.):3502.

124. Maughan T, Adams R, Smith C, Seymour M, Wilson R, Meade A, *et al.* The addition of cetuximab to oxaliplatinfluoropyrimidine chemotherapy in first-line advanced colorectal cancer in the MRC COIN trial: identification of potentially responsive subsets of patients. *Ann Oncol* 2010;**21**:vi17–18.

125. Mayer B, Bogner A, Jauch KW, Singer T. KRAS/BRAF mutation status as a predictive biomarker for anti-EGFR antibody therapy in primary and metastatic colorectal carcinoma. *Onkologie* 2010;**33**:151.

126. Merck KGaA. *Cetuximab Combined with Irinotecan in First-Line Therapy for Metastatic Colorectal Cancer (CRYSTAL)*. NCT00154102. Bethesda, MD: National Library of Medicine; 2011. URL: http://ClinicalTrials.gov/show/NCT00154102 (accessed 23 May 2013).

127. Modest DP, Brodowicz T, Stintzing S, Jung A, Neumann J, Laubender RP, *et al.* Impact of the specific mutation in KRAS codon 12 mutated tumors on treatment efficacy in patients with metastatic colorectal cancer receiving cetuximab-based first-line therapy: a pooled analysis of three trials. *Oncology (Switzerland)* 2012;**83**:241–7.

128. Molinari FM, Felicioni LF, Buscarino MB, De Dosso SD, Buttitta FB, Malatesta SM, *et al.* High sensitive methods to identify K-RAS mutations increase the detection of non-responder metastatic colorectal cancer patients treated with anti-EGFR monoclonal antibodies. *Ann Oncol* 2010;**21**:I33–4.

129. Moosmann N, von Weikersthal LF, Vehling-Kaiser U, Stauch M, Hass HG, Dietzfelbinger H, *et al.* Cetuximab plus capecitabine and irinotecan compared with cetuximab plus capecitabine and oxaliplatin as first-line treatment for patients with metastatic colorectal cancer: AIO KRK-0104 – a randomized trial of the German AIO CRC study group. *J Clin Oncol* 2011;**29**:1050–8. http://dx.doi.org/10.1200/JCO.2010.31.1936

130. Ocvirk J, Vrbanec D, Beslija S, Koza I, Ciuleanu T, Papamichael D, *et al.* Relationship between KRAS status and efficacy of FOLFOX6 + cetuximab or FOLFIRI + cetuximab in first-line treatment of patients with metastatic colorectal cancer (MCRC): the CECOG/CORE1.2.001 trial. *Ann Oncol* 2009;**20**(Suppl. 7):17.

131. Passardi A, Ulivi P, Valgiusti M, Scarpi E, Moscati R, Chiadini E, *et al.* The role of KRAS, BRAF, and PI3K mutations as markers of resistance to cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2011;**29**(15 Suppl. 1):3603.

132. Petrelli F, Borgonovo K, Cabiddu M, Ghilardi M, Barni S. Cetuximab and panitumumab in KRAS wild-type colorectal cancer: a meta-analysis. *Int J Colorectal Dis* 2011;**26**:823–33. http://dx.doi.org/10.1007/s00384-011-1149-0

133. Petrelli F, Barni S. Resectability and outcome with anti-EGFR agents in patients with KRAS wild-type colorectal liver-limited metastases: a meta-analysis. *Int J Colorectal Dis* 2012;**27**:997–1004. http://dx.doi.org/10.1007/s00384-012-1438-2

134. Piessevaux H, Schlichting M, Heeger S, Van Cutsem E, Tejpar S. Early tumor shrinkage for the prediction of efficacy of cetuximab in metastatic colorectal cancer (MCRC): analysis from the CRYSTAL study. *Ann Oncol* 2010;**21**:viii193–4.

135. Piessevaux H, Van Cutsem E, Bokemeyer C, Schlichting M, Heeger S, Tejpar S. Early tumor shrinkage and long-term outcome in metastatic colorectal cancer (mCRC): assessment of predictive utility across treatment arms in the CRYSTAL and OPUS studies. *J Clin Oncol* 2011;**29**(15 Suppl.):3572.

136. Piessevaux H, Bokemeyer C, Schlichting M, Heeger S, Tejpar S. Impact of early tumor shrinkage on long-term outcome in metastatic colorectal cancer (mCRC) treated with FOLFOX4 with or without cetuximab: lessons from the OPUS trial. *J Clin Oncol* 2011;**29**(4 Suppl.):398

137. Qiu LX, Mao C, Zhang J, Zhu XD, Liao RY, Xue K, *et al.* Predictive and prognostic value of KRAS mutations in metastatic colorectal cancer patients treated with cetuximab: a meta-analysis of 22 studies. *Eur J Cancer* 2010;**46**:2781–7. http://dx.doi.org/10.1016/j.ejca.2010.05.022

138. Raoul JL, Van Laethem JL, Peeters M, Brezault C, Husseini F, Cals L, *et al.* Cetuximab in combination with irinotecan/5-fluorouracil/folinic acid (FOLFIRI) in the initial treatment of metastatic colorectal cancer: a multicentre two-part phase I/II study. *BMC Cancer* 2009;**9**:112. http://dx.doi.org/10.1186/1471-2407-9-112

139. Rivera F, Gravalos C, Massuti B, Puente J, Marcuello E, Valladares M, *et al.* Cetuximab plus capecitabine as first-line treatment for elderly patients (pts) with advanced colorectal cancer (mCRC). Final analysis of activity and survival according to KRAS status – the TTD-06–01 Spanish Cooperative Group trial. *Eur J Cancer* 2009;**7**:216. http://dx.doi.org/10.1016/S1359-6349(09)70738-4

140. Rose JS, Serna DS, Martin LK, Li X, Weatherby LM, Abdel-Misih S, *et al.* Influence of KRAS mutation status in metachronous and synchronous metastatic colorectal adenocarcinoma. *Cancer* 2012;**118**:6243–52. http://dx.doi.org/10.1002/cncr.27666

141. Salazar R, Mini E, Folprecht G, Subtil F, van Laethem J, Thaler J, *et al.* Adjuvant FOLFOX4 plus or minus cetuximab (CMAB) in patients with KRAS mutant (MKRAS) resected stage III colon cancer (CC). Results from the PETACC8 Intergroup trial. *Ann Oncol* 2012;**23**:178–9.

142. Schuch G, Bondarenko I, Hartmann J, De Braud F, Volovat C, Nippgen J, *et al.* The OPUS study: predictive value of KRAS status on clinical outcome in patients with metastatic colorectal cancer treated with either FOLFOX4 or FOLFOX4 plus cetuximab. *Ann Oncol* 2008;**19**:18.

143. Serna DS, Martin LK, Li X, Weatherby LM, Rose JS, Bekaii-Saab T. Influence of KRAS mutation status in metachronous and synchronous metastatic colorectal adenocarcinomas. *J Clin Oncol* 2011;**29**(4 Suppl.):388.

144. Shinozaki E, Bando H, Nishina T, Yamazaki K, Kadowaki S, Yuki S, *et al.* Clinical outcome of cetuximab for metastatic colorectal cancer patients harboring KRAS codon61, KRAS codon146, BRAF, NRAS OR PIK3CA mutations. *Ann Oncol* 2012;**23**:196–7.

145. Simon I, Tian S, Moreno V, Roepman P, Tabernero J, Snel M, *et al.* The role of activating mutations of KRAS, BRAF, and PIK3CA pathway convergence at the transcriptional level and prediction of treatment response to cetuximab in colorectal cancer. *J Clin Oncol* 2011;**29**(15 Suppl.):3534.

146. Stenger M. Cetuximab plus FOLFIRI in first-line treatment of KRAS mutation-negative, EGFR-positive metastatic colorectal cancer. *Community Oncol* 2012;**9**:304–6. http://dx.doi.org/10.1016/j.cmonc.2012.09.006

147. Stintzing S, Jung A, Vehling-Kaiser U, Stauch M, Hass H, Dietzfelbinger H, *et al.* Cetuximab plus XELIRI versus cetuximab plus XELOX as first-line treatment for patients with metastatic colorectal cancer (MCRC): analysis of the randomized trial of the German AIO CRC study group: KRK-0204. *Ann Oncol* 2010;**21**:viii189–90.

148. Taieb J, Tabernero J, Mini E, Subtil F, Folprecht G, Van Laethem JL, *et al.* Adjuvant FOLFOX4 ± cetuximab in KRAS wild-type patients with resected stage III colon cancer results from the PETACC8 Intergroup Trial. *Ann Oncol* 2012;**23**:17.

149. Tejpar S, Bokemeyer C, Celik I, Schlichting M, Sartorius U, Van Cutsem E. Influence of KRAS G13D mutations on outcome in patients with metastatic colorectal cancer (mCRC) treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol* 2011;**29**(15 Suppl.):3511.

150. Tejpar S, Bokemeyer C, Celik I, Schlichting M, Van Cutsem E. The role of the KRAS G13D mutation in patients with metastatic colorectal cancer (mCRC) treated with first-line chemotherapy plus cetuximab. *J Clin Oncol* 2011;**29**(4 Suppl.):630.

151. Tejpar S, Bokemeyer C, Celik I, Schlichting M, Heeger S, Van Cutsem E. KRAS mutations and outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *Ann Oncol* 2011;**22**:v16–17.

152. Tejpar S, Celik I, Schlichting M, Sartorius U, Bokemeyer C, Van Cutsem E. Association of KRAS G13D tumor mutations with outcome in patients with metastatic colorectal cancer treated with first-line chemotherapy with or without cetuximab. *J Clin Oncol* 2012;**30**:3570–7. http://dx.doi.org/10.1200/JCO.2012.42.2592

153. Tsoukalas N, Bagos P, Hamodrakas S. Multivariant metal-analysis of KRAS mutations's predictive value for response to cetuximab in colorectal cancer. *Ann Oncol* 2010;**21**:66.

154. Tsoukalas N, Tzovaras A, Tolia M, Papakostidi A, Kostakis I, Ardavanis A, *et al.* The efficacy of cetuximab and mutations of the KRAS gene in colorectal cancer. *Arch Hellenic Med* 2011;**28**:674–9.

155. Tsoukalas N, Tzovaras AA, Tolia M, Kostakis ID, Papakostidi A, Pistamaltzian N, *et al.* Meta-analysis of the predictive value of KRAS mutations in treatment response using cetuximab in colorectal cancer. *J BUON* 2012;**17**:73–8.

156. Tveit K, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, *et al.* Randomized phase III study of 5-fluorouracil/folinate/oxaliplatin given continuously or intermittently with or without cetuximab, as first-line treatment of metastatic colorectal cancer: the Nordic VII study (NCT00145314), by the Nordic Colorectal Cancer Biomodulation Group. *Ann Oncol* 2010;**21**:viii9.

157. Tveit K, Guren T, Glimelius B, Pfeiffer P, Sorbye H, Pyrhonen S, *et al.* Randomized phase III study of 5-fluorouracil/folinate/oxaliplatin given continuously or intermittently with or without cetuximab, as first-line treatment of metastatic colorectal cancer: the NORDIC VII study (NCT00145314), by the Nordic Colorectal Cancer Biomodulation Group. *J Clin Oncol* 2011;**29**(4 Suppl.):365.

158. Ubago R, Castillo MA, Flores S, Rodriguez R, Beltran C. Cetuximab for the first-line treatment of metastatic colorectal cancer. *Value Health* 2011;**14**:A433–4. http://dx.doi.org/10.1016/j.jval.2011.08.1104

159. Vale C, Tierney JF, Meade A, Fisher D, Kaplan R, Adams RA, *et al.* Impact of K-RAS status on the effects of EGFR-targeted monoclonal antibody (MAb) therapy in advanced colorectal cancer (ACRC): a systematic review and meta-analysis of randomized controlled trials (RCTs). *J Clin Oncol* 2009;**27**(15S Suppl.):4122.

160. Van Cutsem E, Lang I, D'Haens G, Moiseyenko V, Zaluski J, Folprecht G, *et al.* KRAS status and efficacy in the crystal study: 1st-line treatment of patients with metastatic colorectal cancer (MCRC) receiving FOLFIRI with or without cetuximab. *Ann Oncol* 2008;**19**:viii4.

161. Van Cutsem E, Rougier P, Kohne C, Stroh C, Schlichting M, Bokemeyer C. A meta-analysis of the CRYSTAL and OPUS studies combining cetuximab with chemotherapy (CT) as 1st-line treatment for patients (pts) with metastatic colorectal cancer (mCRC): results according to KRAS and BRAF mutation status. *Eur J Cancer Suppl* 2009;**7**:345. http://dx.doi.org/10.1016/S1359-6349(09)71172-3

162. Van Cutsem E, Lang I, Folprecht G, Nowacki M, Barone C, Shchepotin I, *et al.* Cetuximab plus FOLFIRI: final data from the CRYSTAL study on the association of KRAS and BRAF biomarker status with treatment outcome. *J Clin Oncol* 2010;**28**(15 Suppl.):3570.

163. Van Cutsem E, Rougier P, Lang I, Folprecht G, Nowacki M, Barone C, *et al.* The influence of KRAS and BRAF tumor mutation status on treatment outcome with cetuximab plus FOLFIRI: final data from the CRYSTAL study. *Ann Oncol* 2010;**21**:vi18.

164. Van Cutsem E, Kohne C-H, Lang I, Folprecht G, Nowacki MP, Cascinu S, *et al.* Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011;**29**:2011–19. http://dx.doi.org/10.1200/JCO.2010.33.5091

165. Wasan H, Adams RA, Wilson RH, Pugh CA, Fisher D, Madi A, *et al.* Intermittent chemotherapy (CT) plus continuous or intermittent cetuximab (C) in the first-line treatment of advanced colorectal cancer (aCRC): results of the two-arm phase II randomised MRC COIN-B trial. *Eur J Cancer* 2011;**47**:S393. http://dx.doi.org/10.1016/S0959-8049(11)71651-3

166. Whitehall V, Tran K, Umapathy A, Grieu F, Hewitt C, Evans TJ, *et al.* A multicenter blinded study to evaluate KRAS mutation testing methodologies in the clinical setting. *J Mol Diagn* 2009;**11**:543–52. http://dx.doi.org/10.2353/jmoldx.2009.090057

167. Yen L-C, Uen Y-H, Wu D-C, Lu C-Y, Yu F-J, Wu IC, *et al.* Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. *Ann Surg* 2010;**251**:254–60. http://dx.doi.org/10.1097/SLA.0b013e3181bc9d96

168. Zhang L, Ma L, Zhou Q. Overall and KRAS-specific results of combined cetuximab treatment and chemotherapy for metastatic colorectal cancer: a meta-analysis. *Int J Colorectal Dis* 2011;**26**:1025–33. http://dx.doi.org/10.1007/s00384-011-1197-5

Appendix 1 Literature search strategies

Clinical effectiveness search strategies

CRC + KRAS (limit: 2000-C)

EMBASE (OvidSP), 2000 to Week 3 2013
Searched 22 January 2013

1. exp colon cancer/ or exp rectum cancer/ or colorectal tumor/ (169,199)
2. ((colorect$ or rectal$ or rectum$ or colon$ or sigma$ or sigmo$ or rectosigm$ or bowel$ or anal or anus) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot,hw. (245,923)
3. (m-CRC or CRC).ti,ab,ot. (14,043)
4. ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (2124)
5. (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (1871)
6. (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (26)
7. or/1-6 (249,697)
8. k ras oncogene/ (4953)
9. (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS).af. (17,025)
10. (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (396)
11. (thera?screen$ or therascreen$).af. (67)
12. (Cobas adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (8)
13. (sanger sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (15)
14. (pyrosequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (25)
15. ((HRM or HRMA or dHPLC) adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (13)
16. (high resolution adj3 melt$ adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (8)
17. (SNapShot adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (5)
18. (Next generation sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)
19. high resolution melting analysis/ (691)
20. 19 and (8 or 9 or 10) (62)
21. or/8-18,20 (17,279)
22. 7 and 21 (5716)
23. limit 22 to yr="2000 -Current" (5036)
24. limit 23 to embase (4540)

MEDLINE (OvidSP), 2000 to January Week 2 2013
Searched 22 January 2013

1. exp Colorectal Neoplasms/ (134,723)
2. ((colorect$ or rectal$ or rectum$ or colon$ or sigma$ or sigmo$ or rectosigm$ or bowel$ or anal or anus) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot,hw. (165,769)
3. (m-CRC or CRC).ti,ab,ot. (8215)
4. ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (1570)
5. (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (1541)
6. (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (23)
7. or/1-6 (170,682)
8. Genes, ras/ (11,077)
9. (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS).af. (9538)
10. (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (346)
11. (thera?screen$ or therascreen$).af. (16)
12. (Cobas adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (2)
13. (sanger sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (4)
14. (pyrosequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (12)
15. ((HRM or HRMA) adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (5)
16. (high resolution adj3 melt$ adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (4)
17. (SNapShot adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (3)
18. (Next generation sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)
19. or/8-18 (16,696)
20. 7 and 19 (3083)
21. limit 20 to yr="2000 -Current" (2293)
22. remove duplicates from 21 (2278)

MEDLINE In-Process & Other Non-Indexed Citations (OvidSP) and Daily Update (OvidSP), up to 21 January 2013
Searched 22 January 2013

1. exp Colorectal Neoplasms/ (194)
2. ((colorect$ or rectal$ or rectum$ or colon$ or sigma$ or sigmo$ or rectosigm$ or bowel$ or anal or anus) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot,hw. (7747)
3. (m-CRC or CRC).ti,ab,ot. (1006)
4. ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (108)

5. (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (28)

6. (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (0)

7. or/1-6 (7930)

8. Genes, ras/ (8)

9. (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS).af. (659)

10. (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (17)

11. (thera?screen$ or therascreen$).af. (5)

12. (Cobas adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)

13. (sanger sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (2)

14. (pyrosequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)

15. ((HRM or HRMA) adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)

16. (high resolution adj3 melt$ adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)

17. (SNapShot adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)

18. (Next generation sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)

19. or/8-18 (667)

20. 7 and 19 (269)

Cochrane Database of Systematic Reviews (CDSR) (Wiley), 2000 to Issue 12, 2012; Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley), 2000 to Issue 12, 2012; Database of Abstracts of Reviews of Effects (DARE) (Wiley), 2000 to Issue 4, 2012; and Health Technology Assessment (HTA) database (Wiley), 2000 to Issue 4, 2012

Searched 22 January 2013

#1 MeSH descriptor: [Colorectal Neoplasms] explode all trees (4380)

#2 ((colorect* or rectal* or rectum* or colon* or sigma* or sigmo* or rectosigm* or bowel* or anal or anus) near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (7773)

#3 (m-CRC or CRC) (715)

#4 ((cecum or cecal or caecum or caecal or ileocecal or ileocaecal or ileocaecum or ileocecum) near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (24)

#5 (large intestin* near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor*or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (86)

#6 (lower intestin* near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (114)

#7 #1 or #2 or #3 or #4 or #5 or #6 (8053)

#8 MeSH descriptor: [Genes, ras] this term only (46)

#9 (k ras or kras or K-ras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS) (386)

#10 (Kirsten near/3 (murine or rat) near/3 sarcoma*) (7)

#11 (thera screen* or thera-screen* or therascreen*) (13)

#12 (Cobas) (115)

#13 (sanger sequencing) (7)

#14 (pyrosequencing) (18)

#15 (HRM or HRMA) (11)

#16 (high resolution near/3 melt*) (1)

#17 (SNapShot) (50)

#18 ("Next generation sequencing") (2)

#19 #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 (605)

#20 #7 and #19 from 2000 (98)

The CDSR search retrieved nine references, the CENTRAL search retrieved 65 references, the DARE search retrieved 11 references and the HTA database search retrieved nine references.

National Institute for Health Research (NIHR) Health Technology Assessment (HTA) programme (Internet), up to 25 January 2013
www.hta.ac.uk/

Searched 25 January 2013

Browsed by relevant terms; retrieved two references.

Science Citation Index (SCI-EXPANDED) (Web of Knowledge) and Conference Proceedings Citation Index (CPCI-S) (Web of Knowledge), 2000 to 22 January 2013
Searched 23 January 2013

Databases=SCI-EXPANDED, CPCI-S Timespan=2000-01-01 - 2013-01-23

#18 3597 #17 AND #6

#17 10,477 #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7

#16 26 TS=((Next SAME generation SAME sequencing) SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#15 23 TS=(SNapShot SAME (k ras or kras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#14 75 TS=((high SAME resolution SAME melt*) SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#13 59 TS=((HRM or HRMA or dHPLC) SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#12 94 TS=(pyrosequencing SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#11 49 TS=((sanger SAME sequencing) SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#10 2 TS=(Cobas SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#9 17 TS=(thera$screen* or therascreen*)

#8 96 TS=(Kirsten NEAR/3 (murine or rat) NEAR/3 sarcoma*)

#7 10,467 TS=(k ras or kras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)

#6 134,422 #1 or #2 or #3 or #4 or #5

#5 1328 TS=(lower SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#4 1113 TS=(large SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#3 484 TS=((cecum or cecal or caecum or caecal or ileocecal or ileocecum) NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#2 9622 TS=(m-CRC or CRC)

#1 130,942 TS=((colorect* or rectal* or rectum* or colon* or sigma* or sigmo* or rectosigm* or bowel* or anal or anus) NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo?r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

BIOSIS Previews (Web of Knowledge), 2000 to 22 January 2013
Searched 23 January 2013

Databases=BIOSIS Previews Timespan=2000-2013

#18 2641 #17 AND #6

#17 8621 #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7

#16 39 TS=((Next SAME generation SAME sequencing) SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#15 36 TS=(SNapShot SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#14 73 TS=((high SAME resolution SAME melt*) SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#13 55 TS=((HRM or HRMA or dHPLC) SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#12 133 TS=(pyrosequencing SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#11 95 TS=((sanger SAME sequencing) SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#10 4 TS=(Cobas SAME (k-ras or k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#9 14 TS=(thera$screen* or therascreen*)

#8 153 TS=(Kirsten NEAR/3 (murine or rat) NEAR/3 sarcoma*)

#7 8611 TS=(k-ras or k ras or kras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)

#6 97,980 #1 or #2 or #3 or #4 or #5

#5 1020 TS=(lower SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#4 805 TS=(large SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#3 424 TS=((cecum or cecal or caecum or caecal or ileocecal or ileocecum) NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#2 7235 TS=(m-CRC or CRC)

#1 95,876 TS=((colorect* or rectal* or rectum* or colon* or sigma* or sigmo* or rectosigm* or bowel* or anal or anus) NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo?r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

Latin American and Caribbean Health Sciences (LILACS) (Internet), up to 24 January 2013

Searched 23 January 2013

Terms	Records
(k-ras or "k ras" OR kras OR v-ki-ras$ OR v-k-ras OR v-ki-ras OR "v ki ras" OR c-ki-ras OR c-k-ras OR ki-ras OR "ki ras" OR kras1 OR kras2 OR kras1p OR rask OR rask1 OR rask2 OR "kirsten ras" OR therascreen$ OR thera-screen$ OR cobas OR hrm OR dhplc OR snapshot OR (high AND resolution AND melt) OR prosequencing OR (sanger AND sequencing))	213
((MH:C04.588.274.476.411.307 or MH:C06.301.371.411.307 or MH:C06.405.249.411.307 or MH:C06.405.469.158.356 or MH:C06.405.469.491.307 or MH:C06.405.469.860.180 or MH:C04.588.274.476.411.184 or colorectal neoplasms$ or "neoplasias colorrectales" or "neoplasias colorrectais" or "colorectal cancer" or CRC or m$crc) AND (k-ras or "k ras" or kras or V-Ki-ras$ or V-K-ras or V-Ki-ras or "v ki ras" or c-ki-ras or c-k-ras or ki-ras or "ki ras" or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or "Kirsten RAS" or therascreen$ or thera-screen$ or cobas or HRM or dHPLC or snapshot or (high and resolution and melt) or prosequencing or (sanger and sequencing)))	123
Total	336

International Prospective Register of Systematic Reviews (PROSPERO) (Internet), up to 25 January 2013

www.crd.york.ac.uk/prospero/

Searched 25 January 2013

Searched for terms in 'All Fields'

Terms	Records
KRAS or K-RAS	2
Colorectal Cancer	2/14 (same records as above)
Cobas	0
Therascreen	0
Thera-screen	0
Sequencing	0/3
Pyrosequencing	0
HRM or HRMA or dHPLC	0
High resolution	0
kirsten	0
Oncogene	0
RASK	0
Snapshot	0
Colon Cancer	1/3 (included in KRAS result)
Total	2

Clinicaltrials.gov (Internet), 2000 to 23 January 2013

http://clinicaltrials.gov/ct2/search/advanced

Searched 23 January 2013

Advanced search option – search terms box

Limited to results received from 1 January 2000 to 23 January 2013

Search terms	Condition	Records
(k ras OR kras OR K-ras OR V-Ki-ras* OR V-K-ras OR V-Ki-ras OR v ki ras OR c-ki-ras OR c-k-ras OR ki-ras OR ki ras OR Kras1 OR Kras2 OR KRAS1P OR RASK OR RASK1 OR RASK2 OR Kirsten RAS)	(colorect* OR rectal* OR rectum* OR colon* OR sigma* OR sigmo* OR rectosigm* OR bowel* OR anal OR anus OR CRC OR m-CRC OR cecum OR cecal OR caecum OR caecal OR ileocecal OR ileocaecal OR ileocaecum OR ileocecum OR large intestin* OR lower intestin*)	165
(Kirsten murine sarcoma* OR Kirsten rat sarcoma*)	(colorect* OR rectal* OR rectum* OR colon* OR sigma* OR sigmo* OR rectosigm* OR bowel* OR anal OR anus OR CRC OR m-CRC OR cecum OR cecal OR caecum OR caecal OR ileocecal OR ileocaecal OR ileocaecum OR ileocecum OR large intestin* OR lower intestin*)	13
thera screen OR thera-screen OR therascreen		0
Total		178

World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) (Internet), 2000 to 25 January 2013

www.who.int/ictrp/en/

Searched 25 January 2013

Advanced search option

Title	Condition	Intervention	Records
(KRAS or K-RAS or K ras)	(colon cancer or CRC or colorectal cancer or rectal cancer or rectum cancer)		67
	(colon cancer or CRC or colorectal cancer or rectal cancer or rectum cancer)	(KRAS or K-RAS or Kras)	1
(Kirsten murine sarcoma* OR Kirsten rat sarcoma*)	(colon cancer or CRC or colorectal cancer or rectal cancer or rectum cancer)		3
	(colon cancer or CRC or colorectal cancer or rectal cancer or rectum cancer)	(Kirsten murine sarcoma* OR Kirsten rat sarcoma*)	Unable to run this line because of error with results screen
thera screen OR thera-screen OR therascreen			0
		thera screen OR thera-screen OR therascreen	0
Total			71

Current Controlled Trials (metaRegister of Controlled Trials) (Internet), up to 29 January 2013

www.controlled-trials.com/

Searched 29 January 2013

Search terms	Results
(Kirsten murine sarcoma* OR Kirsten rat sarcoma*)	7
(KRAS or K-RAS or K ras)	146
(thera screen OR thera-screen OR therascreen)	0
Total	153

Conference searches

European Society for Medical Oncology conference proceedings (Internet), 2007–13

Searched 5 February 2013

2012 37th ESMO Congress, Vienna – http://annonc.oxfordjournals.org/content/23/suppl_9.

2011 ECCO 16 and 36th ESMO Multidisciplinary Congress, Brussels – www.ejcancer.info/issues.

2010 35th ESMO Congress, Milan – http://annonc.oxfordjournals.org/content/21/suppl_8.

2009 ECCO 15 and 34th ESMO Multidisciplinary Congress – www.ejcancer.info.

2008 33rd ESMO Congress, Stockholm – http://annonc.oxfordjournals.org/content/vol19/suppl_8/.

2007 ESMO Conference, Lugano – http://annonc.oxfordjournals.org/content/18/suppl_9.toc.

Intervention	2007	2008	2009[a]	2010	2011[a]	2012
KRAS	3/4	8	10	15	99	22
K-RAS	3/4	7	22	7	30	11
K RAS	22/29	30	22	34	30	44/47[b]
"Kirsten murine sarcoma"	0	0	0	0	0	0
"Kirsten rat sarcoma"	0	0	3	0	1	0
Total	28	45	57	56	160	77
Total	423					
Total after deduplication	25	31	28	36	113	50
Total after deduplication	283					

a Used 'Search within this issue' (search function not as sensitive as with other issues; may have included some additional 2011 conferences).
b Three additional references found in index/prelims that would not export.

The ESMO conference proceedings search located 423 records, with 283 remaining after deduplication.

American Association for Cancer Research conference proceedings (Internet), 2007–13

The AACR website had multiple search options retrieving different sets of results. A combination of the following was used:

Search 1: Whole website

- www.aacrmeetingabstracts.org/search.dtl
- Searched 5 February 2013
- Searched website for abstracts from 2007–10; search limited to KRAS terms in title only – retrieved 236 results

Search 2: Individual years

2009

- www.abstractsonline.com/viewer/SearchAdvanced.asp?MKey={D007B270-E8F6-492D-803B-7582CE7A0988}&AKey={728BCE9C-121B-46B9-A8EE-DC51FDFC6C15}
- Searched 6 February 2013

Keywords	Title search (advanced search)	Boolean search in presentation title
KRAS or K-RAS or K RAS		60
Kirsten AND rat AND sarcoma		0
Kirsten AND murine AND sarcoma		0
Total	60	

2010

- www.abstractsonline.com/plan/start.aspx?mkey={0591FA3B-AFEF-49D2-8E65-55F41EE8117E}
- Searched 6 February 2013

Keywords	Title search (advanced search)	Boolean search
KRAS or K-RAS or K RAS	93	
Kirsten AND rat AND sarcoma		1
Kirsten AND murine AND sarcoma		0
Total	94	

2011

- www.abstractsonline.com/plan/start.aspx?mkey={507D311A-B6EC-436A-BD67-6D14ED39622C}
- Searched 6 February 2013

Keywords	Title search (advanced search)	Boolean search
KRAS or K-RAS or K RAS	82	
Kirsten AND rat AND sarcoma		1
Kirsten AND murine AND sarcoma		0
Total	83	

2012

- www.abstractsonline.com/plan/start.aspx?mkey={2D8C569E-B72C-4E7D-AB3B-070BEC7EB280}
- Searched 6 February 2013

Keywords	Title (advanced search)	Boolean search
KRAS or K-RAS or K RAS	93	
Kirsten AND rat AND sarcoma		1
Kirsten AND murine AND sarcoma		0
Total	94	

The combined American Association for Cancer Research conference proceedings search located 567 records in total.

American Society of Clinical Oncology conference proceedings (Internet), 2007–13

www.asco.org/ASCOv2/Meetings/Abstracts

Searched 5 February 2013

Searched 2007–12 Annual Meetings

Keywords	Searched in title	Searched in abstract	Total
KRAS	204		204
K-RAS	46		46
K RAS	46 (same results as K-RAS)		
Kirsten rat sarcoma	0	1	1
Kirsten murine sarcoma	0	1 (same result as Kirsten rat sarcoma)	
Total			251

The ASCO conference proceedings search located 251 records.

Association for Molecular Pathology conference proceedings, 2007–13

Searched 6 February 2013

2012 AMP Abstracts; Long Beach, CA, 25–27 October 2012 – http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157812002115.pdf.

2011 AMP Abstracts; Grapevine, TX, 17–19 November 2011 – http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157811002546.pdf.

2010 AMP Abstracts; San Jose, CA, 18–20 November 2010 – http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810601365.pdf.

2009 AMP Abstracts; Kissimmee, FL, 19–22 November 2009 – http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810602851.pdf.

2008 AMP Abstracts; Grapevine, TX, 29 October–2 November 2008 – http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810602000.pdf.

2007 AMP Abstracts; Los Angeles, CA, 7–10 November 2007 – http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810604424.pdf.

Intervention	2007	2008	2009	2010	2011	2012
KRAS	4	5	23	32	32	38
K-RAS	1/2	0	2/4	0/1	0	0
K RAS	0/2	0	0/3	0/1	0/1	0
Kirsten murine sarcoma	0	0	0	0	0	0
Kirsten rat sarcoma	0	0	0	0	0	0
Total per year	5	5	25	32	32	38
Total	137					

The AMP conference proceedings search located 137 records.

Cost-effectiveness search strategies

CRC + KRAS + Economics filter (limit: 2000-C)

EMBASE (OvidSP), 2000 to Week 4 2013
Searched 29 January 2013

1. exp colon cancer/ or exp rectum cancer/ or colorectal tumor/ (169,460)
2. ((colorect$ or rectal$ or rectum$ or colon$ or sigma$ or sigmo$ or rectosigm$ or bowel$ or anal or anus) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot, hw. (246,253)
3. (m-CRC or CRC).ti,ab,ot. (14,068)
4. ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (2125)
5. (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (1871)
6. (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (26)
7. or/1-6 (250,031)
8. k ras oncogene/ (4967)
9. (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS).af. (17,128)
10. (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (399)
11. (thera?screen$ or therascreen$).af. (67)

12. (Cobas adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (8)

13. (sanger sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (15)

14. (pyrosequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (25)

15. ((HRM or HRMA or dHPLC) adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (13)

16. (high resolution adj3 melt$ adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (8)

17. (SNapShot adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (5)

18. (Next generation sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)

19. high resolution melting analysis/ (701)

20. 19 and (8 or 9 or 10) (62)

21. or/8-18,20 (17,382)

22. 7 and 21 (5731)

23. health-economics/ (32,282)

24. exp economic-evaluation/ (194,421)

25. exp health-care-cost/ (186,276)

26. exp pharmacoeconomics/ (160,882)

27. or/23-26 (445,930)

28. (econom$ or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic$).ti, ab. (542,934)

29. (expenditure$ not energy).ti,ab. (21,678)

30. (value adj2 money).ti,ab. (1191)

31. budget$.ti,ab. (22,178)

32. or/28-31 (565,244)

33. 27 or 32 (823,889)

34. letter.pt. (811,274)

35. editorial.pt. (424,059)

36. note.pt. (543,769)

37. or/34-36 (1,779,102)

38. 33 not 37 (742,302)

39. (metabolic adj cost).ti,ab. (800)

40. ((energy or oxygen) adj cost).ti,ab. (3005)

41. ((energy or oxygen) adj expenditure).ti,ab. (18,652)

42. or/39-41 (21,682)

43. 38 not 42 (737,540)

44. 22 and 43 (310)

45. limit 44 to yr="2000 -Current" (303)

46. limit 45 to embase (285)

Costs filter: Centre for Reviews and Dissemination. *NHS EED Economics Filter: EMBASE (Ovid) Weekly Search*. York: CRD; 2010. URL: www.york.ac.uk/inst/crd/intertasc/nhs_eed_strategies.html (accessed 17 March 2011).

MEDLINE (OvidSP), 2000 to January Week 3 2013

Searched 29 January 2013

1. exp Colorectal Neoplasms/ (134,899)
2. ((colorect$ or rectal$ or rectum$ or colon$ or sigma$ or sigmo$ or rectosigm$ or bowel$ or anal or anus) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot, hw. (165,996)
3. (m-CRC or CRC).ti,ab,ot. (8243)
4. ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (1571)
5. (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (1542)
6. (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (23)
7. or/1-6 (170,912)
8. Genes, ras/ (11,084)
9. (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS).af. (9558)
10. (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (346)
11. (thera?screen$ or therascreen$).af. (16)
12. (Cobas adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (2)
13. (sanger sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (5)
14. (pyrosequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (12)
15. ((HRM or HRMA) adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (5)
16. (high resolution adj3 melt$ adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (4)
17. (SNapShot adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (3)
18. (Next generation sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)
19. or/8-18 (16,720)
20. 7 and 19 (3092)
21. economics/ (26,342)
22. exp "costs and cost analysis"/ (168,037)
23. economics, dental/ (1847)
24. exp "economics, hospital"/ (18,317)
25. economics, medical/ (8474)
26. economics, nursing/ (3868)
27. economics, pharmaceutical/ (2383)
28. (economic$ or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic$).ti, ab. (375,308)
29. (expenditure$ not energy).ti,ab. (15,563)
30. (value adj1 money).ti,ab. (18)
31. budget$.ti,ab. (15,762)

32. or/21-31 (493,254)
33. ((energy or oxygen) adj cost).ti,ab. (2454)
34. (metabolic adj cost).ti,ab. (667)
35. ((energy or oxygen) adj expenditure).ti,ab. (14,408)
36. or/33-35 (16,880)
37. 32 not 36 (489,444)
38. letter.pt. (758,034)
39. editorial.pt. (307,072)
40. historical article.pt. (288,506)
41. or/38-40 (1,339,895)
42. 37 not 41 (463,260)
43. 20 and 42 (74)
44. limit 43 to yr="2000 -Current" (69)

Costs filter: Centre for Reviews and Dissemination. *NHS EED Economics Filter: MEDLINE (Ovid) Monthly Search*. York: CRD; 2010. URL: www.york.ac.uk/inst/crd/intertasc/nhs_eed_strategies.html (accessed 28 September 2010).

MEDLINE In-Process & Other Non-Indexed Citations (OvidSP) and MEDLINE Daily Update (OvidSP), up to 28 January 2013
Searched 29 January 2013

1. exp Colorectal Neoplasms/ (132)
2. ((colorect$ or rectal$ or rectum$ or colon$ or sigma$ or sigmo$ or rectosigm$ or bowel$ or anal or anus) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot,hw. (7699)
3. (m-CRC or CRC).ti,ab,ot. (1022)
4. ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (111)
5. (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (28)
6. (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$ or sarcoma$ or adenom$ or lesion$)).ti,ab,ot. (0)
7. or/1-6 (7889)
8. Genes, ras/ (6)
9. (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS).af. (666)
10. (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (17)
11. (thera?screen$ or therascreen$).af. (5)
12. (Cobas adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)
13. (sanger sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)
14. (pyrosequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)
15. ((HRM or HRMA) adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)
16. (high resolution adj3 melt$ adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)

17. (SNapShot adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (1)
18. (Next generation sequencing adj3 (k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)).af. (0)
19. or/8-18 (671)
20. 7 and 19 (270)
21. economics/ (1)
22. exp "costs and cost analysis"/ (143)
23. economics, dental/ (0)
24. exp "economics, hospital"/ (8)
25. economics, medical/ (0)
26. economics, nursing/ (0)
27. economics, pharmaceutical/ (1)
28. (economic$ or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic$).ti, ab. (33,295)
29. (expenditure$ not energy).ti,ab. (992)
30. (value adj1 money).ti,ab. (3)
31. budget$.ti,ab. (1659)
32. or/21-31 (35,017)
33. ((energy or oxygen) adj cost).ti,ab. (186)
34. (metabolic adj cost).ti,ab. (46)
35. ((energy or oxygen) adj expenditure).ti,ab. (681)
36. or/33-35 (890)
37. 32 not 36 (34,752)
38. letter.pt. (19,083)
39. editorial.pt. (12,353)
40. historical article.pt. (144)
41. or/38-40 (31,562)
42. 37 not 41 (34,345)
43. 20 and 42 (17)

Costs filter:

Costs filter: Centre for Reviews and Dissemination. *NHS EED Economics Filter: MEDLINE (Ovid) Monthly Search*. York: CRD; 2010. URL: www.york.ac.uk/inst/crd/intertasc/nhs_eed_strategies.html (accessed 28 September 2010).

NHS Economic Evaluation Database (NHS EED) (Wiley), 2000 to Issue 4, 2012
Searched 22 January 2013

#1 MeSH descriptor: [Colorectal Neoplasms] explode all trees (4380)

#2 ((colorect* or rectal* or rectum* or colon* or sigma* or sigmo* or rectosigm* or bowel* or anal or anus) near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (7773)

#3 (m-CRC or CRC) (715)

#4 ((cecum or cecal or caecum or caecal or ileocecal or ileocaecal or ileocaecum or ileocecum) near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (24)

#5 (large intestin* near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor*or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (86)

#6 (lower intestin* near/3 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (114)

#7 #1 or #2 or #3 or #4 or #5 or #6 (8053)

#8 MeSH descriptor: [Genes, ras] this term only (46)

#9 (k ras or kras or K-ras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS) (386)

#10 (Kirsten near/3 (murine or rat) near/3 sarcoma*) (7)

#11 (thera screen* or thera-screen* or therascreen*) (13)

#12 (Cobas) (115)

#13 (sanger sequencing) (7)

#14 (pyrosequencing) (18)

#15 (HRM or HRMA) (11)

#16 (high resolution near/3 melt*) (1)

#17 (SNapShot) (50)

#18 ("Next generation sequencing") (2)

#19 #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 (605)

#20 #7 and #19 from 2000 (98)

The NHS EED search retrieved three references.

Science Citation Index (SCI-EXPANDED) (Web of Knowledge), 2000 to 25 January 2013
Searched 30 January 2013

Databases=SCI-EXPANDED Timespan=2000-01-01 - 2013-01-30.

#27 117 #6 and #17 and #26

#26 532,023 #21 not #25

#25 33,411 #22 or #23 or #24

#24 15,970 TS=((energy or oxygen) NEAR expenditure)

#23 2095 TS=(metabolic NEAR cost)

#22 17,130 TS=((energy or oxygen) NEAR cost)

#21 551,568 #18 or #19 or #20

#20 909 TS=(value NEAR money)

#19 10,944 TS=(expenditure* not energy)

#18 546,907 TS=(economic* or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic* or budget*)

#17 10,434 #16 OR #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 OR #8 OR #7

#16 27 TS=((Next SAME generation SAME sequencing) SAME (k ras or kras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or k-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#15 23 TS=(SNapShot SAME (k ras or kras or k-rasor V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#14 76 TS=((high SAME resolution SAME melt*) SAME (k ras or kras or k-ras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#13 59 TS=((HRM or HRMA or dHPLC) SAME (k ras or kras or k-ras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#12 95 TS=(pyrosequencing SAME (k ras or kras or k-ras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#11 50 TS=((sanger SAME sequencing) SAME (k ras or kras or k-ras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#10 3 TS=(Cobas SAME (k ras or kras or k-ras or V-Ki-ras* or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS))

#9 17 TS=(thera$screen* or therascreen*)

#8 97 TS=(Kirsten NEAR/3 (murine or rat) NEAR/3 sarcoma*)

#7 10,424 TS=(k ras or kras or K-ras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)

#6 132,645 #1 or #2 or #3 or #4 or #5

#5 1321 TS=(lower SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#4 1097 TS=(large SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#3 481 TS=((cecum or cecal or caecum or caecal or ileocecal or ileocecum) NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

#2 8999 TS=(m-CRC or CRC)

#1 129,783 TS=((colorect* or rectal* or rectum* or colon* or sigma* or sigmo* or rectosigm* or bowel* or anal or anus) NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo?r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))

EconLit (EBSCO), 2000 to 30 January 2013
Searched 30 January 2013

Search modes – Boolean/phrase

S6 s1 or s2 or s3 or s4 Limiters - Published Date from: 20000101- (104)
S5 s1 or s2 or s3 or s4 (135)
S4 TX ((colorect* or rectal* or rectum* or colon* or sigma* or sigmo* or rectosigm* or bowel* or anal or anus) N4 (cancer* or neoplas* or oncolog* or malignan* or tumour* or tumor* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*)) (90)
S3 TX(thera screen* or thera-screen* or therascreen*) (0)
S2 TX(Kirsten murine sarcoma* or Kirsten rat sarcoma*) (0)
S1 TX(k ras or kras or k-ras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS) (45)

Health Economic Evaluations Database (HEED) (Internet), up to 30 January 2013
http://onlinelibrary.wiley.com/book/10.1002/9780470510933

Searched 30 January 2013

Compound search (all data), unable to limit by date

Keywords	Results
k ras or kras or k-ras or V-Ki-ras$ or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS	11
Kirsten murine sarcoma OR Kirsten rat sarcoma	2
thera screen OR thera-screen OR therascreen	0
Total	13

The HEED search retrieved 13 records.

Appendix 2 Data extraction tables

Study details	Selection	Population	Intervention details			KRAS test details	Other
			Detail	EGFR inhibitor	Standard chemotherapy		
Bokemeyer 2011[28,53,56] (OPUS) Country: Germany, Spain, Italy, Ukraine, Russian Federation, Romania and Poland Study design: RCT Funding: Merck Serono Recruitment: August 2006 Total no. randomised: 337 No. of KRAS wild-type patients randomised: 179 No. with liver-limited metastases randomised: 48	Inclusion criteria: Adults (aged ≥ 18 years); histologically confirmed first occurrence of non-resectable, EGFR-expressing mCRC with at least one radiologically measurable lesion; life expectancy of at least 12 weeks; maximum ECOG performance status 2; adequate hepatic, renal and bone function. KRAS wild-type population data only extracted Exclusion criteria: Pregnancy; previous EGFR-targeted therapy or previous chemotherapy (excluding adjuvant treatment) for mCRC; uncontrolled severe organ or metabolic dysfunction	Age (years), median (range): 61 (24–82) Male, n: 97 Criteria for unresectability: NR Performance status: ECOG 0: 70; 1: 93; 2: 16	Intervention Dose Median (IQR) duration (weeks) No. with KRAS wild type treated No. with liver metastases treated	FOLFOX-4 + cetuximab As standard + cetuximab, initial dose 400 mg/m², subsequent doses 250 mg/m² As standard + weekly Cetuximab 24 (13–38); oxaliplatin 23 (14–31); 5-FU 24 (14–36) 82 25	FOLFOX-4 Oxaliplatin 85 mg/m²; folinic acid 200 mg/m² followed by 5-FU as a 400 mg/m² bolus then a 600 mg/m² infusion over 22 hours Days 1 and 2 of a 14-day cycle Oxaliplatin 24 (16–30); 5-FU 24 (16–32) 97 23	KRAS test: one-step LightCycler PCR reaction (LightMix k-ras Gly12 assay) Manufacturer: TIB MOLBIOL Analysis software: LightCycler 2.0 system Mutations targeted: KRAS codons 12 and 13 missense mutations	Data for KRAS wild-type population and liver metastases-only subgroup are post-hoc analyses. Baseline data are for entire wild-type population

Study details	Selection	Population	Intervention details			KRAS test details	Other
			Detail	EGFR inhibitor	Standard chemotherapy		
Folprecht 2010[52] (CELIM)	Inclusion criteria: Unresectable, histologically confirmed colorectal liver metastases; no extrahepatic metastases (patients with synchronous liver metastases were eligible if the primary tumour had been resected before chemotherapy); Karnofsky performance score ≥ 80%; adequate hepatic, renal and bone marrow function	Age (years), median (range): 63 (56–71)	Intervention	Cetuximab + FOLFOX6	Cetuximab + FOLFIRI	KRAS test: Direct sequencing and DxS KRAS Mutation Test Kit	
Country: Germany and Austria		Male, n: 71	Dose	(Cetuximab 400 mg/m² initially then 250 mg/m² subsequently) + (100 mg/ m² oxaliplatin, 400 mg/ m² folinic acid and 400 mg/m² bolus 5-FU followed by 2400 mg/m² over 46 hours)	(Cetuximab 400 mg/m² initially then 250 mg/m² subsequently) + (180 mg/m² irinotecan, 400 mg/m² folinic acid and 400 mg/m² bolus 5-FU followed by 2400 mg/m² over 46 hours)	Manufacturer: DxS, Manchester, UK	
Study design: RCT		Liver metastases: < 5 metastases: 30; 5–10 metastases: 58; > 10 metastases: 19; NR: 4				Analysis software: NR	
Funding: Merck Serono, Sanofi-Aventis and Pfizer		No. with previous liver resection: 14				Mutations targeted: KRAS mutations in codons 12 and 13	
Recruitment: December 2004–March 2008		Criteria for unresectability: Five or more liver		(Day 1 of a weekly cycle) + (day 1 of a 2-weekly cycle)	(Day 1 of a weekly cycle) + (day 1 of a 2-weekly cycle)		
No. randomised: 111		metastases or metastases that were viewed as technically non-resectable by the local liver surgeon and radiologist on the basis of inadequate future liver remnant, or one of the following criteria: infiltration of all hepatic liver veins, infiltration of both hepatic arteries or both portal vein branches	No. with KRAS wild type treated	56	55		
No. of KRAS wild-type patients randomised: 70	Exclusion criteria: Previous chemotherapy (except adjuvant chemotherapy with an interval of ≥ 6 months); previous EGFR-targeted therapy; concurrent antitumour therapy; clinically relevant coronary artery disease; inflammatory bowel disease; previous malignancy; age < 18 years		No. with liver metastases treated	56	55		
No. with liver-limited metastases randomised: 111		Performance status: NR					
		Previous treatments: nine patients had adjuvant radiotherapy, 18 had adjuvant chemotherapy					

Study details	Selection	Population	Intervention details			KRAS test details	Other
			Detail	EGFR inhibitor	Standard chemotherapy		
Kohne 2011[27,53] (CRYSTAL)[a]

Country: Germany, Russian Federation, Poland, Singapore, South Korea, South Africa, France and Belgium (201 centres)

Study design: RCT

Funding: Meck Serono

Recruitment: July 2004–November 2005

No. randomised: 1202

No. of KRAS wild-type patients randomised: 666

No. with liver-limited metastases randomised: 140 | Inclusion criteria: Adults (aged $\geq$ 18 years); histologically confirmed adenocarcinoma of the colon or rectum; first occurrence of metastatic disease that could not be resected for curative purposes; EGFR expressing; maximum ECOG performance status 2; adequate hepatic, renal and haematological function

Exclusion criteria: Previous anti-EGFR therapy or irinotecan-based therapy; previous chemotherapy for mCRC; adjuvant treatment within 6 months of the start of the trial; radiotherapy, surgery (excluding previous diagnostic biopsy) or any investigational drug with 30 days of the start of the trial | Age (years), median (range): 61 (22–79)

Male, n: 201

Criteria for unresectability: NR

Performance status: ECOG 0: 203; ECOG 1: 131; ECOG 2: 14

Previous treatments: NR | Intervention

Dose

Median (IQR) duration (weeks)

No. treated

No. with liver metastases treated | Cetuximab + FOLFORI

As standard + initial 120-minute infusion of cetuximab 400 mg/m^2 on day 1 followed by weekly 60-minute infusions of cetuximab 250 mg/m^2

Weekly

Cetuximab 25.0 (12.9–40.4); irinotecan 26.0 (14.0–40.3); 5-FU 26.0 (13.8–40.4)

172

68 | FOLFORI

30- to 90-minute infusion of irinotecan 180 mg/m^2, 120-minute infusion of racemic leucovorin 400 mg/m^2 or L-leucovorin 200 mg/m^2, 5-FU 400 mg/m^2 bolus then 46-hour infusion of 2400 mg/m^2

Day 1 of a 14-day cycle

Irinotecan 25.7 (15.1–39.5); 5-FU 25.7 (14.9–36.0)

176

72 | KRAS test: PCR clamping and melting curve method (LightMix k-ras Gly12 assay)

Manufacturer: TIB MOLBIOL

Analysis software: LightCycler 2.0 system

Mutations targeted: KRAS codons 12 and 13 missense mutations | Data for KRAS wild-type population and liver metastases-only subgroup are post-hoc analyses. Treatment continued until disease progression, toxic effects or withdrawal of consent. No definition of unresectable liver metastases was reported |

Study details	Selection	Population	Intervention details			KRAS test details	Other
			Detail	**EGFR inhibitor**	**Standard chemotherapy**		
Maughan 2011[54] (COIN)	Inclusion criteria: Adults (aged ≥ 18 years); histologically confirmed adenocarcinoma of the colon or rectum; inoperable metastatic or locoregional disease; no previous chemotherapy for metastatic disease; WHO performance status 0–2; adequate hepatic, renal and haematological function; no adjuvant chemotherapy or rectal chemoradiotherapy within 1 month of the start of the trial	Age (years), median (range): 64 (56–70)	Intervention	Cetuximab + standard chemotherapy (oxaliplatin + 5-FU and folinic acid, or oxaliplatin + capecitabine)	Standard chemotherapy (oxaliplatin + 5-FU and folinic acid, or oxaliplatin + capecitabine)	KRAS test: Pyrosequencing and MALDI-TOF mass array, with Sanger sequencing for discordant samples (< 1%)	Data for KRAS wild-type population and liver metastases-only subgroup are post-hoc analyses. These participants were also BRAF and NRAS wild type.
Country: UK and Republic of Ireland		Male, n: 498					
Study design: RCT		Criteria for unresectability: NR	Dose	As standard + initial 2-hour infusion of 400 mg/m² cetuximab and 250 mg/m² over 1 hour subsequently	2-hour infusion of L-folinic acid 175 mg or racemic folinic acid 350 mg, 2-hour infusion of oxaliplatin 85 mg/m², followed by 400 mg/m² bolus 5-FU and then 46-hour infusion of 2400 mg/m² 5-FU, or 2-hour infusion of 130 mg/m² oxaliplatin followed by oral capecitabine (1000 mg/m²) twice a day for 2 weeks, reduced to 850 mg/m² in later patients	Manufacturer: In house	Unavailable tumour blocks = 141; insufficient tumour material for processing = 163; not successfully genotyped = 22
Funding: UK MRC		Performance status: WHO 0: 348; WHO 1: 337; WHO 2: 44; WHO 3: 0				Analysis software: Sequenom, San Diego, CA	
Recruitment: March 2005–May 2008						Mutations targeted: KRAS mutations in codons 12, 13 and 61	Standard chemotherapy was FOLFOX for some patients (approximately one-third of the whole trial) and XELOX for some patients (approximately two-thirds of the whole trial).
No. randomised: 1630		Previous treatments: NR					
No. of KRAS wild-type patients randomised: 729	Exclusion criteria: Unfit for chemotherapy; severe uncontrolled medical illness; psychiatric illness inhibiting informed consent; partial or complete bowel obstruction; pre-existing neuropathy of grade 1 or higher; requirement for treatment with contraindicated		Median (IQR) duration (weeks)	28.1 (15.4–42.0)	29.3 (15.7–40.1)		Proportions unknown for the subgroup with liver metastases
No. with liver-limited metastases randomised: 178			No. treated	357	358		
			No. with liver metastases treated	87	91		

Study details	Selection	Population	Detail	EGFR inhibitor	Standard chemotherapy	KRAS test details	Other
			Intervention details				
Xu 2012[55]	Inclusion criteria: Resected primary colorectal tumour; non-resectable synchronous liver-limited metastases; KRAS wild type	Age: NR	Intervention	Cetuximab + FOLFIRI or FOLFOX6	FOLFIRI or FOLFOX6	KRAS test: Pyrosequencing	178 KRAS wild-type patients had liver metastases only; 153 were included in the analysis (25 patients missing)
Country: China		Male, n: NR	Dose	NR	NR	Analysis software: NR	
Study design: RCT		Criteria for unresectability: NR	Frequency	NR	NR	Manufacturer: NR	
Funding: NR			Mean number of cycles	NR	NR	Mutations targeted: NR	
Recruitment: June 2008–December 2011	Exclusion criteria: None reported	Performance status: NR	Duration	NR	NR		
No. eligible: NR		Previous treatments: NR	No. with KRAS wild type treated	59	57		
No. randomised: 116			No. with liver metastases treated	59	57		
No. of KRAS wild-type patients randomised: 116							
No. with liver-limited metastases randomised: 116							

medication; another previous or current malignant disease that may affect treatment response; known hypersensitivity to any study treatment; brain metastases

5-FU, fluorouracil; NR, not reported; NRAS, neuroblastoma RAS viral oncogene homolog.
a Population data come from full paper[27] and relate to a smaller sample of 348 patients with wild-type KRAS mutation status; the full paper is an earlier report than the abstract[53] from which the results data are taken.

Appendix 3 Risk of bias assessments

QUADAS-2 assessments

Folprecht et al.[52] *(CELIM)*

Domain 1: Patient selection	
A. Risk of bias	
Describe methods of patient selection: Phase II RCT comparing FOLFOX6 + cetuximab with FOLFIRI + cetuximab in patients with unresectable liver metastases from CRC. In total, 111 participants were included, of whom 94 received *KRAS* testing and were included in this assessment. There were no inappropriate exclusions from the trial	
Was a consecutive or random sample of patients enrolled?	Yes
Was a case–control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Yes
Could the selection of patients have introduced bias?	*Risk: low*
B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test and setting): Study participants were described as having technically non-resectable or five or more liver metastases from CRC; it was unclear whether some participants may have had potentially resectable metastases at baseline	
Is there concern that the included patients do not match the review question?	*Concern: unclear*
Domain 2: Index test(s)	
A. Risk of bias	
Describe the index test and how it was conducted and interpreted: Tumour *KRAS* mutation status (index test) was determined before the clinical outcome (reference standard) was known	
Were the index test results interpreted without knowledge of the results of the reference standard?	Yes
Could the conduct or interpretation of the index test have introduced bias?	*Risk: low*
B. Concerns regarding applicability	
Is there concern that the target condition as defined by the reference standard does not match the review question? Tumour KRAS *mutation status was determined using the Therascreen* KRAS *PCR kit*	*Concern: low*
Domain 3: Reference standard	
A. Risk of bias	
Describe the reference standard and how it was conducted and interpreted: Clinical outcome (objective response) was used as the reference standard; data on resection rates were not reported by tumour *KRAS* mutation status. Analysis of objective response by tumour *KRAS* mutation status was retrospective	
Is the reference standard likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear
Could the reference standard, its conduct or its interpretation have introduced bias?	*Risk: low*
B. Concerns regarding applicability	
Is there concern that the included patients do not match the review question?	*Concern: high*

Domain 4: Flow and timing

A. Risk of bias

Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2 × 2 table: 17 (15%) participants were not included in the analysis. It was not clear whether this was because tumour *KRAS* mutation status was unknown or because follow-up data were not available

Describe the time interval and any interventions between the index test(s) and the reference standard: Tumour response was assessed every four cycles (8 weeks) for a maximum of 2 years

Was there an appropriate interval between the index test(s) and the reference standard?	Yes
Did all patients receive a reference standard?	Unclear
Did patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	*Risk: high*

Maughan et al.[54] (COIN)

Domain 1: Patient selection

A. Risk of bias

Describe methods of patient selection: RCT comparing cetuximab + FOLFOX or XELOX with FOLFOX or XELOX. All patients received *KRAS* mutation testing but only the subgroup of patients with unresectable liver metastases were included in this assessment. There were no inappropriate exclusions from the trial

Was a consecutive or random sample of patients enrolled?	Yes
Was a case–control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Yes
Could the selection of patients have introduced bias?	*Risk: low*

B. Concerns regarding applicability

Describe included patients (prior testing, presentation, intended use of index test and setting): All study participants included in this assessment had inoperable liver metastases from CRC and no extrahepatic metastases or previous chemotherapy. Patients receiving combination chemotherapy before resection of operable liver metastases were explicitly excluded

Is there concern that the included patients do not match the review question?	*Concern: low*

Domain 2: Index test(s)

A. Risk of bias

Describe the index test and how it was conducted and interpreted: Tumour *KRAS* mutation status (index test) was determined before the clinical outcome (reference standard) was known

Were the index test results interpreted without knowledge of the results of the reference standard?	Yes
Could the conduct or interpretation of the index test have introduced bias?	*Risk: low*

B. Concerns regarding applicability

Is there concern that the target condition as defined by the reference standard does not match the review question? Tumour KRAS *mutation status was determined using pyrosequencing and MALDI-TOF, targeting mutations in codons 12, 13 and 61*	*Concern: low*

Domain 3: Reference standard

A. Risk of bias

Describe the reference standard and how it was conducted and interpreted: Clinical outcome (resection) was used as the reference standard. It was not clear whether investigators assessing resectability were aware of tumour *KRAS* mutation status. Treatment arms included FOLFOX or XELOX as standard chemotherapy and XELOX was not specified as standard chemotherapy for this review

Is the reference standard likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the results of the index test?	Unclear
Could the reference standard, its conduct or its interpretation have introduced bias?	*Risk: low*

B. Concerns regarding applicability	
Is there concern that the included patients do not match the review question?	*Concern: high*
Domain 4: Flow and timing *A. Risk of bias*	

Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2 × 2 table: Tumour response was assessed every 12 weeks. In total, 19% of the original participants did not receive *KRAS* mutation testing because no sample was available; testing failed in a further 1%. However, it was not clear how many, if any, participants in the unresectable liver metastases subgroup did not receive testing. A total of 153 participants with *KRAS* wild-type tumours were included in the analysis [25 (14%) missing]

Was there an appropriate interval between the index test(s) and the reference standard?	Yes
Did all patients receive a reference standard?	Unclear
Did patients receive the same reference standard?	Yes
Were all patients included in the analysis?	No
Could the patient flow have introduced bias?	*Risk: high*

Cochrane risk of bias assessments

Bokemeyer et al.[28,53,56] *(OPUS)*

	Support for judgement	Risk of bias
Random sequence generation	1 : 1 randomisation was carried out using a stratified permuted-block procedure, with ECOG performance status as a stratification factor	Unclear
Allocation concealment	No details reported	Unclear
Participant/personnel blinding	Open-label design	High
Outcome assessor blinding	Outcomes assessed by a blinded independent review committee	Low
Incomplete outcome data	Outcomes for the whole study polulation were analysed using ITT analysis and outcome data appeared to be reported for all patients with liver-limited metasteses, however, details were limited	Low
Selective outcome reporting	All specified outcomes appear to be reported	Low

Van Cutsem et al.[27,53] *(CRYSTAL)*

	Support for judgement	Risk of bias
Random sequence generation	1 : 1 randomisation was carried out using a stratified permuted-block procedure, with ECOG performance status as a stratification factor	Unclear
Allocation concealment	No details reported	Unclear
Participant/personnel blinding	Open-label design	High
Outcome assessor blinding	No details reported	Unclear
Incomplete outcome data	Outcomes for the whole study polulation were analysed using ITT analysis and outcome data appeared to be reported for all patients with liver-limited metasteses; however, data for these patients were reported only in an abstract and details were limited	Low
Selective outcome reporting	All specified outcomes appear to be reported	Low

Maughan et al.[54] *(COIN)*

	Support for judgement	Risk of bias
Random sequence generation	Patients were randomly assigned with minimisation by the MRC Clinical Trials Unit by telephone	Low
Allocation concealment	Treatment allocation was not masked	High
Participant/personnel blinding	Open-label design	High
Outcome assessor blinding	No details reported	Unclear
Incomplete outcome data	Outcomes for the whole study polulation were analysed using ITT analysis and outcome data appeared to be reported for all patients with liver-limited metasteses; however, data for these patients were reported only in an abstract and details were limited	Low
Selective outcome reporting	All specified outcomes appear to be reported	Low

Xu et al.[55]

	Support for judgement	Risk of bias
Random sequence generation	No details reported	Unclear
Allocation concealment	No details reported	Unclear
Participant/personnel blinding	Open-label design	High
Outcome assessor blinding	No details reported	Unclear
Incomplete outcome data	No details reported	Unclear
Selective outcome reporting	No details reported	Unclear

Appendix 4 Survey of NHS laboratories participating in the UK National External Quality Assurance Scheme pilot for *KRAS* mutation testing

LABORATORY DETAILS

This questionnaire has been designed to collect information to inform a NICE diagnostic assessment review on KRAS *testing in samples collected from patients with liver metastases from colorectal cancer.*

1. At which laboratory are you based?

KRAS TESTING METHODS

2. What is the *KRAS* mutation testing strategy in your laboratory?

N.B. If your laboratory uses different KRAS *mutation testing methods for different samples (options c or d), please complete the relevant sections of the survey for each method used (to minimise time taken, some questions will be automatically skipped on second and subsequent completions).*

    (a) We only use one method of *KRAS* mutation testing

    (b) We use more than one *KRAS* mutation testing method in combination on all samples

    (c) We use different *KRAS* mutation testing methods depending on sample quality (e.g. % tumour cells)

    (d) We sometimes use a single *KRAS* mutation testing method and sometimes multiple methods (e.g. to confirm mutations)

    (e) Other (please specify)

3. Which *KRAS* mutation testing method(s) do you currently use in your laboratory?

NB: If you selected options (a) or (b) above, please select all tests used in your laboratory.

If you selected options (c) or (d) above, please select only one test and complete the relevant sections of the survey again for additional tests.

- Sanger sequencing
- Cobas *KRAS* Mutation Test (Roche Molecular Systems)
- Therascreen *KRAS* RGQ PCR Kit (Qiagen)
- Therascreen *KRAS* Pyro Kit (Qiagen)
- *KRAS* LightMix® Kit (TIB MolBiol)
- *KRAS* StripAssay® (ViennaLab)
- High resolution melt analysis
- Pyrosequencing
- MALDI-TOF mass spectrometry
- Next generation sequencing
- Other (please specify)/Comments:

4. What proportion of samples are tested using the indicated method(s)?Cost

- 100%
- Other (please specify)

5. Are you completing this survey for a second or subsequent time?

- Yes
- No

6. How are samples referred to your laboratory for *KRAS* mutation testing?

- All resected primary CRC
- On demand
- Not known
- Other (please specify)

7. Why have you chosen the *KRAS* mutation testing method(s) that you have (please select all that apply):

- Cost
- Sensitivity (Proportion of tumour cells required)
- Mutation coverage
- Ease of use
- Turnaround time
- Other (please specify)

8. If your *KRAS* mutation testing strategy uses more than one method, what is the reason for this? (Please select all that apply)

- NA, we only use one method
- Sensitivity (proportion of tumour cells required)
- Verification of mutations
- Ability to fully characterise detected mutation
- Other (please specify)

9. In which codons does your *KRAS* mutation testing strategy aim to detect mutations and does the strategy aim to detect all mutations or does it target specific mutations? (Please select all that apply)

- Codon 12
- Codon 13
- Codon 61
- All mutations
- Targeted mutations
- Other (please specify)

10. If you use pyrosequencing, which primers do you use?

- Commercial primers
- Self-designed primers
- Details

LOGISTICS

11. In a typical week, how many samples do you screen for *KRAS* mutations?
    • ≤5
    • 6-10
    • 11-15
    • 16-20
    • >20
 	
12. What is your average batch size for *KRAS* mutation testing?
 	
13. How often do you run *KRAS* mutation testing?
    • Daily
    • 2-3 times per week
    • Weekly
    • Other (please specify)
 	
14. Do you wait until you have certain number of samples before running *KRAS* mutation testing?
    • No
    • Yes
    • If yes, how many?
 	
15. On average, how long (in calender days) does it take to receive a sample at the lab once it has been requested?
    • <24-hours
    • 24-48 hours
    • 3-5 days
    • 6-7 days
    • 8-10 days
    • >10 days
    Please describe the range of waits experienced by your laboratory (shortest to longest)

16. On average, how long (in calender days) does it take from receiving a sample at the lab to sending a result back to the clinician?
 	
    • <24-hours
 	
    • 24-48 hours
 	
    • 3-5 days
 	
    • 6-7 days
 	
    • 8-10 days
 	
    • >10 days

TECHNICAL PERFORMANCE

Please complete this page only for *KRAS* mutation testing in samples from patients with liver metastases from colorectal cancer.

17. What is the minimum sample requirement of the *KRAS* mutation test in terms of the % tumour cells?

- ≤1%
- 1-5%
- 6-10%
- 11-20%
- 21-30%
- >30%

18. What is the limit of detection of the *KRAS* mutation test in terms of % mutation in extracted DNA?

- ≤1%
- 1-5%
- 6-10%
- >10%

19. How was the limit of detection determined in your laboratory?

20. Do you use microdissection techniques to process samples prior to DNA isolation?

- Yes, always
- No
- Yes, only when tumour content is below a minimum threshold (please specify)

21. We would like to get an idea of the number of samples which could not be analysed and reasons for this. If possible please provide details on the exact number of samples submitted to your laboratory last year with number of rejected samples and reasons for rejection. If you do not have access to the numbers for your lab please provide your best estimate for a hypothetical set of 1000 samples seen in your lab:

Total number of samples submitted to your laboratory for *KRAS* mutation testing (type 1000 if providing an estimate):

22. Number of samples rejected prior to analysis

23. What are the reasons for sample rejection? (Please select all that apply)

- Insufficient tumour cells
- Sample type unsuitable for analysis

Other (please specify)

24. We would also like to get an idea of the number of *KRAS* mutation tests for which no result could be provided (test failures) and reasons for this. If possible please provide details on the exact number of *KRAS* tests undertaken last year with number of failed samples and reasons for failure. If you do not have access to the numbers for your lab please provide your best estimate for a hypothetical set of 1000 samples seen in your lab:

Total number of *KRAS* mutation tests undertaken (type 1000 if providing an estimate):

25. Total number of test failures

26. What are the reasons for failed tests? (Please select all that apply)Insufficient tumour cells

- Insufficient tumour cells in sample
- DNA degradation
- Fixative type
- Other (please specify)

COSTS

27. What is the cost of the test (including purchase costs, personnel, material and overheads)?

28. If you do not have this information, please provide any information on cost that you have available

29. How is *KRAS* mutation testing in your laboratory funded? (please select all that apply)

- NHS
- Merck Serono
- Other (please specify)

30. If applicable, what is the price that you charge to the NHS for the test?

31. If applicable, what is the price that you charge to Merck Serono for the test?

32. Do you have any final comments?

Thank you for taking the time to complete the survey. If you use more than one *KRAS* testing method in your laboratory please could you complete the relevant sections of the survey again for additional testing methods.

# Appendix 5 Table of excluded studies with rationale

Study	Reason for exclusion (once a study had failed on one criterion it was not assessed further)			
	1. Not a primary study	2. Did not include patients with mCRC and a resected or resectable primary tumour whose metastases are confined to the liver and are unresectable	3. *KRAS* mutation test not performed and/or test and mutation not specified or deducible	4. Study did not report on response to treatment, survival or PFS
Adams 2009[81]	✓			
Adams 2010[82]		✓		
Adams 2010[83]				✓
Alberts 2010[84]		✓		
Assenat 2011[85]		✓		
Baker 2008[86]		✓		
Baloglu 2012[87]	✓			
Bokemeyer 2008[88]		✓		
Bokemeyer 2009[56]		✓		
Bokemeyer 2009[89]		✓		
Bokemeyer 2010[90]	✓			
Bokemeyer 2012[91]	✓			
Chuko 2010[92]	✓			
Cohen 2008[93]			✓	
Colucci 2010[94]		✓		
Di Salvatore 2010[95]		✓		
Dubus 2009[96]	✓			
Folprecht 2008[97]	✓			
Folprecht 2009[98]		✓		
Folprecht 2010[99]	✓			
Gajate 2012[100]		✓		
Gao 2011[101]		✓		
Garufi 2009[102]			✓	
Goldberg 2010[103]		✓		
Griebsch 2011[104]		✓		
Harbison 2012[105]		✓		
Huang 2011[106]		✓		
Ibrahim 2010[107]	✓			
Jones 2013[108]	✓			
Jonker 2009[109]		✓		

|	Reason for exclusion (once a study had failed on one criterion it was not assessed further)			
Study	1. Not a primary study	2. Did not include patients with mCRC and a resected or resectable primary tumour whose metastases are confined to the liver and are unresectable	3. *KRAS* mutation test not performed and/or test and mutation not specified or deducible	4. Study did not report on response to treatment, survival or PFS
Kimura 2012[110]		✓		
Kohne 2009[111]		✓		
Ku 2012[112]	✓			
Lang 2009[113]		✓		
Lievre 2006[114]		✓		
Lin 2010[115]		✓		
Lin 2011[116]	✓			
Linardou 2008[117]	✓			
Loupakis 2012[118]	✓			
Malapelle 2012[119]		✓		
Malapelle 2012[120]		✓		
Mancuso 2008[121]		✓		
Maughan 2009[122]		✓		
Maughan 2010[123]		✓		
Maughan 2010[124]		✓		
Mayer 2010[125]		✓		
Merck KGaA 2011[126]	✓			
Modest 2012[127]	✓			
Molinari 2010[128]		✓		
Moosmann 2011[129]		✓		
Ocvirk 2009[130]	✓			
Ocvirk 2010[57]	✓			
Passardi 2011[131]		✓		
Petrelli 2011[132]	✓			
Petrelli 2012[133]	✓			
Piessevaux 2010[134]	✓			
Piessevaux 2011[135]	✓			
Piessevaux 2011[136]		✓		
Qiu 2010[137]	✓			
Raoul 2009[138]		✓		
Rivera 2009[139]		✓		
Rose 2012[140]		✓		
Salazar 2012[141]		✓		
Schuch 2008[142]		✓		
Serna 2011[143]		✓		

| Study | Reason for exclusion (once a study had failed on one criterion it was not assessed further) | | | |
	1. Not a primary study	2. Did not include patients with mCRC and a resected or resectable primary tumour whose metastases are confined to the liver and are unresectable	3. *KRAS* mutation test not performed and/or test and mutation not specified or deducible	4. Study did not report on response to treatment, survival or PFS
Shinozaki 2012[144]		✓		
Simon 2011[145]		✓		
Stenger 2012[146]	✓			
Stintzing 2010[147]		✓		
Taieb 2012[148]		✓		
Tejpar 2011[149]	✓			
Tejpar 2011[150]	✓			
Tejpar 2011[151]	✓			
Tejpar 2012[152]	✓			
Tsoukalas 2010[153]	✓			
Tsoukalas 2011[154]	✓			
Tsoukalas 2012[155]	✓			
Tveit 2010[156]		✓		
Tveit 2011[157]		✓		
Tveit 2012[58]		✓		
Ubago 2011[158]	✓			
Vale 2009[159]	✓			
Van Cutsem 2008[160]		✓		
Van Cutsem 2009[27]		✓		
Van Cutsem 2009[161]	✓			
Van Cutsem 2010[162]		✓		
Van Cutsem 2010[163]		✓		
Van Cutsem 2011[164]		✓		
Wasan 2011[165]		✓		
Whitehall 2009[166]		✓		
Yen 2010[167]		✓		
Zhang 2011[168]	✓			

Appendix 6 Cost-effectiveness acceptability curves for sensitivity analyses
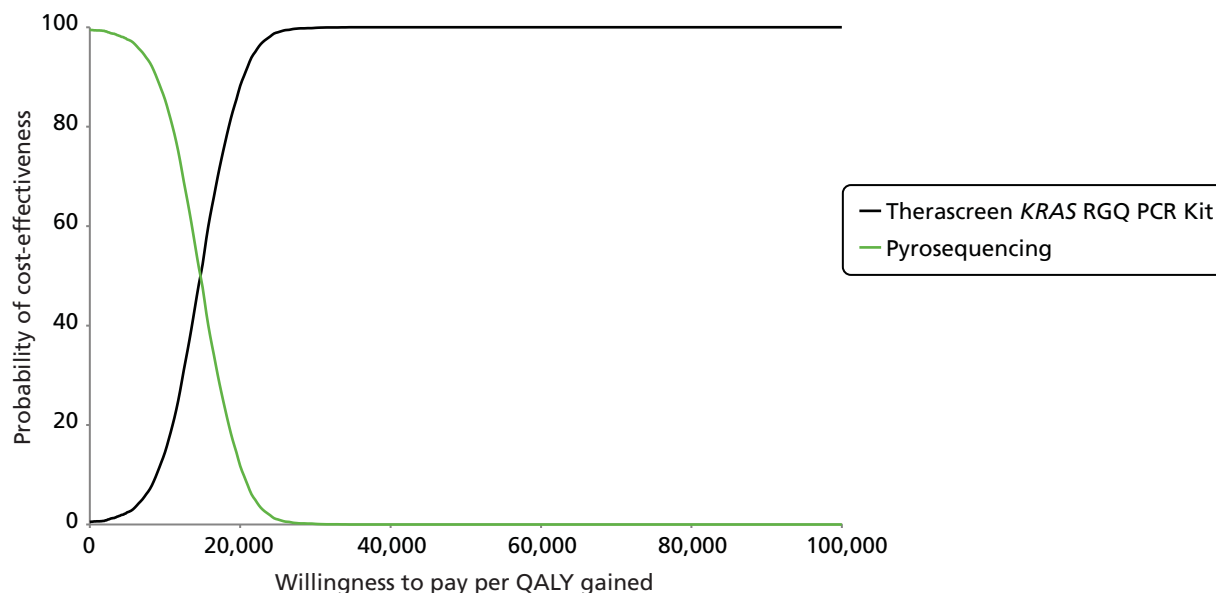
Cost-effectiveness acceptability curve for the linked evidence analysis sensitivity analysis: mortality second line based on average of first- and third-line mortality.
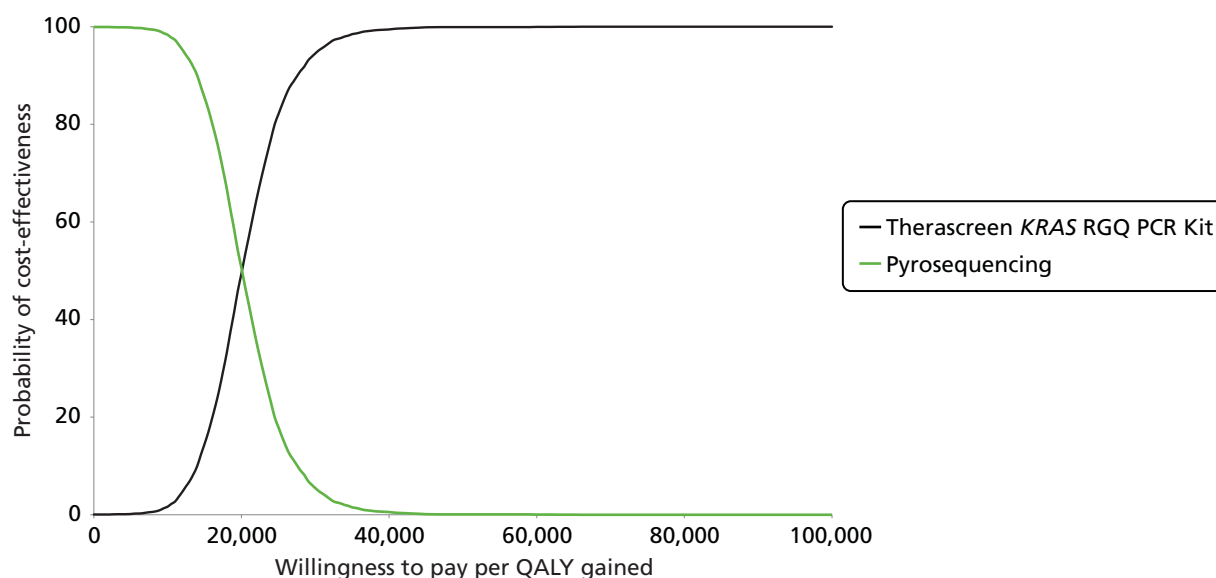
Cost-effectiveness acceptability curve for linked evidence analysis sensitivity analysis: proportion of patients with unknown mutation status based on the survey.

Appendix 7 National Institute for Health and Care Excellence guidance relevant to the management of metastatic colorectal cancer

Cancer service guidance

National Institute for Clinical Excellence. *Improving Outcomes in Colorectal Cancer: Manual Update*. Cancer service guidance. London: NICE; June 2004. URL: http://guidance.nice.org.uk/CSGCC (accessed 14 May 2013).

Clinical guidelines

National Institute for Health and Clinical Excellence. *Colorectal Cancer – Capecitabine and Tegafur Uracil*. NICE technology appraisal 61. London: NICE; May 2005. URL: http://guidance.nice.org.uk/TA61 (accessed 14 May 2013).

National Institute for Health and Clinical Excellence. *Colorectal Cancer (Advanced) – Irinotecan, Oxaliplatin and Raltitrexed.* NICE technology appraisal 93. London: NICE; August 2005. URL: http://guidance.nice.org.uk/TA93 (accessed 14 May 2013).

National Institute for Health and Clinical Excellence. *Colon Cancer (Adjuvant) – Capecitabine and Oxaliplatin*. NICE technology appraisal 100. London: NICE; April 2006. URL: http://guidance.nice.org.uk/TA100 (accessed 14 May 2013).

National Institute for Health and Clinical Excellence. *Colorectal Cancer – Laparoscopic Surgery*. NICE technology appraisal 105. London: NICE; August 2006. URL: http://guidance.nice.org.uk/TA105 (accessed 14 May 2013).

National Institute for Health and Clinical Excellence. *Colorectal Cancer: the Diagnosis and Management of Colorectal Cancer.* Clinical guideline CG131. London: NICE; November 2011. URL: http://guidance.nice.org.uk/CG131 (accessed 14 May 2013). [Date of review: to be confirmed.] [Clinical guideline CG131 updates and replaces TA93 and incorporates TA61, TA100 and TA105.]

Technology appraisals

National Institute for Health and Clinical Excellence. *Colorectal Cancer (Metastatic) – Bevacizumab and Cetuximab*. NICE technology appraisal 118. London: NICE; January 2007. URL: http://guidance.nice.org.uk/TA118 (accessed 14 May 2013).

National Institute for Health and Clinical Excellence. *Colorectal Cancer (Metastatic) – Cetuximab (Terminated Appraisal)*. NICE technology appraisal 150. London: NICE; June 2008. URL: http://guidance.nice.org.uk/TA150 (accessed 14 May 2013).

National Institute for Health and Clinical Excellence. *Cetuximab for the First-Line Treatment of Metastatic Colorectal Cancer*. NICE technology appraisal 176. London: NICE; August 2009. URL: http://guidance.nice.org.uk/TA176 (accessed 14 May 2013). [Date of review: August 2012. The last review decision was in June 2011, when it was agreed that TA176 would be cross-referenced with CG131. The reason given for not

incorporating TA176 into CG131 was 'as the results of the further subgroup analyses of the COIN study could potentially lead to the need to update the recommendations in the future'.]

National Institute for Health and Clinical Excellence. *Bevacizumab in Combination with Oxaliplatin and either Fluorouracil Plus Folinic Acid or Capecitabine for the Treatment of Metastatic Colorectal Cancer*. NICE technology appraisal 212. London: NICE; December 2010. URL: http://guidance.nice.org.uk/TA212 (accessed 13 May 2013). [Date of review: to be confirmed.]

National Institute for Health and Clinical Excellence. *Colorectal Cancer (Metastatic) 2nd Line: Cetuximab, Bevacizumab and Panitumumab*. NICE technology appraisal 242. London: NICE; January 2012. URL: http://guidance.nice.org.uk/TA242 (accessed 14 May 2013). [Date of review: January 2015.] [Technology appraisal 242 replaces TA150 and partially updated TA118.]

National Institute for Health and Care Excellence. *Aflibercept in Combination with Irinotecan and Fluorouracil-Based Therapy for Treating Metastatic Colorectal Cancer that has Progressed Following Prior Oxaliplatin-Based Chemotherapy*. NICE technology appraisal guidance 307. London: NICE; 2014. URL: http://publications.nice.org.uk/aflibercept-in-combination-with-irinotecan-and-fluorouracil-based-therapy-for-treating-metastatic-ta307 (accessed 10 June 2014). [Date for review: August 2016.]

National Institute for Health and Clinical Excellence pathways

National Institute for Health and Clinical Excellence. *NICE Pathways: Colorectal Cancer Overview*. London: NICE; November 2011. URL: http://pathways.nice.org.uk/pathways/colorectal-cancer (accessed 14 May 2013).

Quality standards

National Institute for Health and Clinical Excellence. *Colorectal Cancer (QS20).* London: NICE; August 2012. URL: http://guidance.nice.org.uk/QS20 (accessed 14 May 2013).

Terminated

National Institute for Health and Clinical Excellence. *Panitumumab in Combination with Chemotherapy for the Treatment of Metastatic Colorectal Cancer (Terminated Appraisal).* NICE technology appraisal 240. London: NICE; December 2011. URL: http://guidance.nice.org.uk/TA240 (accessed 14 May 2013). ['NICE is unable to recommend the use in the NHS of panitumumab in combination with chemotherapy for the treatment of metastatic colorectal cancer because no evidence submission was received from the manufacturer or sponsor of the technology'.]

Appendix 8 Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist

Section/topic	No.	Checklist item	Reported on page no.
Title			
Title	1	Identify the report as a systematic review, meta-analysis or both	p. i
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number	Executive summary – pp. xix–xxii; PROSPERO registration – p. xxii
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	*Chapter 2*, background – pp. 3–9
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes and study design (PICOS)	Objectives – p. 1; inclusion criteria – *Table 2*, p. 13
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g. web address) and, if available, provide registration information including registration number	Protocol (see www.metaxis.com/prospero/full_doc.asp?RecordID=3663); PROSPERO registration number: CRD42013003663
Eligibility criteria	6	Specify study characteristics (e.g. PICOS, length of follow-up) and report characteristics (e.g. years considered, language, publication status) used as criteria for eligibility, giving rationale	*Table 2* – p. 13
Information sources	7	Describe all information sources (e.g. databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	*Chapter 3*, *Search strategy* – pp. 11–12
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	*Appendix 1* – pp. 85–103
Study selection	9	State the process for selecting studies (i.e. screening, eligibility, included in systematic review and, if applicable, included in the meta-analysis)	*Chapter 3*, *Inclusion screening and data extraction* – p. 12
Data collection process	10	Describe method of data extraction from reports (e.g. piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	*Chapter 3*, *Inclusion screening and data extraction* – pp. 12–13

Section/topic	No.	Checklist item	Reported on page no.
Data items	11	List and define all variables for which data were sought (e.g. PICOS, funding sources) and any assumptions and simplifications made	*Chapter 3, Data extraction* – pp. 12–13; *Table 2* – p. 13
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was carried out at the study or outcome level) and how this information is to be used in any data synthesis	*Chapter 3, Quality assessment* – p. 13
Summary measures	13	State the principal summary measures (e.g. risk ratio, difference in means)	*Chapter 3, Methods of analysis/ synthesis* – p. 14
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if carried out, including measures of consistency (e.g. I^2) for each meta-analysis	*Chapter 3, Methods of analysis/ synthesis* – p. 14
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g. publication bias, selective reporting within studies)	NA
Additional analyses	16	Describe methods of additional analyses (e.g. sensitivity or subgroup analyses, meta-regression), if carried out, indicating which were prespecified	NA
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	*Chapter 3, Results of the assessment of clinical effectiveness* – pp. 15–16; *Figure 1* – p. 15
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g. study size, PICOS, follow-up period) and provide the citations	*Chapter 3, What is the accuracy of KRAS mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?* – pp. 23–24; *Chapter 3, How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?* – pp. 27–28; *Appendix 2* – pp. 105–110
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12)	*Chapter 3, What is the accuracy of KRAS mutation testing for predicting response to treatment with cetuximab plus standard chemotherapy and subsequent resection rates?* – pp. 23–26; *Table 9* – p. 26; *Chapter 3, How do outcomes from treatment with cetuximab plus standard chemotherapy vary according to which test is used to select patients for treatment?* – pp. 31–32; *Table 12* – p. 32; *Appendix 3* – pp. 111–114
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot	*Table 8* – p. 25; *Figure 4* – p. 26; *Table 11* – p. 29; *Figures 5–7* – pp. 30–31
Synthesis of results	21	Present results of each meta-analysis carried out, including confidence intervals and measures of consistency	NA

Section/topic	No.	Checklist item	Reported on page no.
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)	NA
Additional analysis	23	Give results of additional analyses, if carried out (e.g. sensitivity or subgroup analyses, meta-regression; see item 16)	NA
Discussion			
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g. health-care providers, users and policy-makers)	*Chapter 5*, *Statement of principal findings* – pp. 57–60
Limitations	25	Discuss limitations at study and outcome level (e.g. risk of bias) and at review level (e.g. incomplete retrieval of identified research, reporting bias)	*Chapter 5*, *Strengths and limitations of the assessment* and *Uncertainties* – pp. 60–64
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	*Chapter 6* – pp. 67–68
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g. supply of data) and the role of funders for the systematic review	NIHR HTA programme, project number 12/75/01 – p. xxii

NA, not applicable; PICOS, participants, interventions, comparisons, outcomes and study design.

**EME
HS&DR
HTA
PGfAR
PHR**

Part of the NIHR Journals Library
www.journalslibrary.nihr.ac.uk

Published by the NIHR Journals Library