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

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Maastricht University

Kleijnen Systematic Reviews Ltd in collaboration with Erasmus University Rotterdam and Maastricht University

Authors Marie Westwood, Review Manager, Kleijnen Systematic Reviews Ltd, UK
Thea van Asselt, Senior Health Economist, Department of Clinical Epidemiology and Medical Technology Assessment, Maastricht University Medical Centre, The Netherlands
Bram Ramaekers, Health Economist, Department of Clinical Epidemiology and Medical Technology Assessment, Maastricht University Medical Centre, The Netherlands
Penny Whiting, Review Manager, Kleijnen Systematic Reviews Ltd, UK
Manuela Joore, Associate Professor Health Economics, Department of Clinical Epidemiology and Medical Technology Assessment, Maastricht University Medical Centre, the Netherlands
Nigel Armstrong, Senior Health Economist, Kleijnen Systematic Reviews Ltd, UK
Caro Noake, Information Specialist, Kleijnen Systematic Reviews Ltd, UK
Janine Ross, Information Specialist, Kleijnen Systematic Reviews Ltd, UK
Johan Severens, Professor of Evaluation in Healthcare, Institute of Health Policy and Management, Erasmus university Rotterdam, the Netherlands
Jos Kleijnen, Professor of Systematic Reviews in Health Care, School for Public Health and Primary Care (CAPHRI), Maastricht University, the Netherlands

Correspondence to Marie Westwood
Kleijnen Systematic Reviews Ltd
Unit 6, Escrick Business Park
Riccall Road
Escrick
York YO19 6FD
Tel: 01904 727983
Email: marie@systematic-reviews.com

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Contributions of authors

Marie Westwood and Penny Whiting planned and performed the systematic review and interpretation of evidence. Manuela Joore, Thea van Asselt and Bram Ramaekers planned and performed the cost-effectiveness analyses and interpreted results. Nigel Armstrong contributed to planning and interpretation of cost-effectiveness analyses and acquisition of input data for modelling. Caro Noake and Janine Ross devised and performed the literature searches and provided information support to the project. Jos Kleijnen and Johan Severens provided senior advice and support to the systematic review and cost-effectiveness analyses, respectively. All parties were involved in drafting and/or commenting on the report.

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LIST OF ABBREVIATIONS

Technical terms and abbreviations are used throughout this report. The meaning is usually clear from the context, but a glossary is provided for the non-specialist reader.

ARMS	amplification refractory mutation system
ASCO	American Society of Clinical Oncology
BSC	best supportive care
CEAC	cost-effectiveness acceptability curve
CT	computed tomography
CCT	controlled clinical trial
CI	confidence interval
CLIA	Clinical Laboratory Improvement Amendments
CR	complete response
CRC	colorectal cancer
DC	disease control
DNA	deoxyribonucleic acid
DTA	diagnostic test accuracy
ECOG	Eastern Cooperative Oncology Group
EGFR	epidermal growth factor receptor
FDA	Food and Drug Administration
FFPE	formalin fixed paraffin embedded
FN	false negative
FNA	fine needle aspiration
FNB	fine-needle biopsy
FP	false positive
HR	hazard ratio
HRM	high resolution melt analysis
HRQoL	Health-Related Quality of Life
HSROC	hierarchical summary receiver operating characteristic
HTA	Health technology Assessment
IC	incremental cost
ICER	Incremental Cost-Effectiveness Ratio
IQR	interquartile range
ITT	intention-to-treat
KRAS	Kirsten rat sarcoma viral oncogene
LY	life year
MALDI-TOF	Matrix Assisted Laser Desorption ionisation Time-of-Flight
mCRC	metastatic colorectal cancer
MDT	multi-disciplinary team
MRC	Medical Research Council
MRI	magnetic resonance imaging
NA	not applicable
NEQAS	National External Quality Assurance Scheme
NHS	National Health Service
NICE	National Institute for Health and Clinical Excellence

NPV	negative predictive value
NR	not reported
OR	odds ratio
ORR	objective response rate
OS	overall survival
PCO	Provisional Clinical Opinion
PCR	polymerase chain reaction
PD	progressive disease
PET	positron emission tomography
PET/CT	positron emission tomography/computed tomography
PFS	progression-free survival
PPV	positive predictive value
PR	partial response
PRESS EBC	Peer Review of Electronic Search Strategies
PS	performance status
PSA	probabilistic sensitivity analysis
PSSRU	Personal Social Services Research Unit
QALY	Quality-Adjusted Life Year
RCT	randomised controlled trial
RECIST	Response Evaluation Criteria in Solid Tumours
R0R	R0 resection rate
ROC	receiver operating characteristic
SD	stable disease
SROC	summary receiver operating characteristic
TN	true negative
TP	true positive
WHO	World Health Organisation

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 healthcare 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 (ICER)	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 whose 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 of life	An individual's emotional, social and physical well-being and their ability to perform the ordinary tasks of living.
Quality-adjusted life year (QALY)	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.
Receiver Operating Characteristic (ROC) curve	A graph which illustrates the trade-offs between sensitivity and specificity which 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-diseases persons with a negative test result.
True positive	Correct positive test result – number of diseased persons with a positive test result.

EXECUTIVE 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 one year after diagnosis is around 73%, and of surviving five years is around 55%. Most bowel cancers are initially treated with surgery, but around 1 in 6 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. 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 which 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) to differentiate adults with metastatic CRC, whose metastases are confined to the liver and are un-resectable, 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 to January 2013. A web-based survey gathered data on technical performance of KRAS mutation tests. 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 QUADAS-2. 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 (HRs) and tumour response data as relative risks (RRs) 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 only be presented as compared to 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 one 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.

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 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 Cobas® KRAS Mutation Test (three laboratories), Sanger sequencing was used by two laboratories, one laboratory used the Therascreen® KRAS Pyro Kit, and one used high resolution melt analysis and direct sequencing. More than half of responding laboratories reported that KRAS mutation testing was one 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 ≤ 10%.

What is the accuracy of KRAS mutation testing for predicting response to treatment with cetuximab + 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 ® PCR Kit for predicting objective response (OR) 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 MALDI-TOF 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 to standard chemotherapy. Two trials used the LightMix k-ras Gly12 assay (TIB MolBiol), one used pyrosequencing together with MALDI-TOF mass spectrometry, and one used pyrosequencing alone.

All studies reported improvements in OR for patients with KRAS wild-type tumours who were treated with cetuximab plus standard chemotherapy compared to 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 the different KRAS mutation tests to decide between standard chemotherapy or cetuximab plus standard chemotherapy?

Linked evidence' analysis

The 'linked evidence' analysis included two tests, i.e. only those tests for which evidence on test accuracy for prediction of either resection rate or objective response was available. We only have data from the COIN and CELIM trials; 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, at an ICER of £17,019 per QALY gained. The cost-effectiveness acceptability curve indicates that for lower values of the threshold, pyrosequencing is to be preferred, and that the Therascreen® KRAS RGQ PCR Kit is the most cost-effective option at thresholds of £17,000 and higher. The results of the sensitivity analyses do not differ substantially from the base case, 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 equal for all tests. As a result, the strategies in these analyses only differ 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 highly 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 - 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 high resolution melt analysis and Sanger sequencing have equal costs and effects and their ICER compared to 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 cost-effectiveness acceptability curve it is apparent that the Cobas® KRAS Mutation test is the preferred strategy for all threshold values below £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 at an ICER of £17,019 per QALY gained; sensitivity analyses did not show substantial differences compared to the base case. 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) 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 as 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

Re-testing of stored samples from previous studies, where 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, where mutation status is 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 or 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.

1. OBJECTIVE

The overall objective of this project was to summarise the evidence on the clinical- and cost-effectiveness of Kirsten rat sarcoma viral ongogene (KRAS) mutation tests (commercial or in-house) to differentiate adults with metastatic CRC, whose metastases are confined to the liver and are un-resectable, 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 NICE Technology Appraisal TA176.[1] In order 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 both in terms of the mutations targeted and limit of detection (the lowest proportion of tumour cells with a mutation that can be detected), the definition of KRAS mutant and KRAS wild-type varies 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 tumour cells needed, limit of detection (minimum percentage mutation detectable against a background of wild-type 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, where reported, from treatment with standard chemotherapy 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.

2. BACKGROUND AND DEFINITION OF THE DECISION PROBLEM(S)

2.1 Population

The indication for this assessment is the detection of mutations in the KRAS oncogene in adults with metastatic colorectal cancer (CRC), where metastases are confined to the liver and are un-resectable. The presence or absence of KRAS (Kirsten rat sarcoma viral oncogene) 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, London showed that 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 five year survival rates for CRC in England (2005-2009) 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 and peaking in the over 80s; in the UK (2007-2009) 72% of new cases were diagnosed in people over 65 years.[3] There is some evidence of an association between incidence of CRC and deprivation in UK males; 2000-2004 data show incidence rates approximately 11% higher for men living in more deprived areas compared with the least deprived.[6] The National Bowel Cancer Audit (NBCA) data for 2011 included 28,260 new cases for England and Wales, of which 21,306 (75.4%) were surgically treated and 3,425 (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 metastatic CRC 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 inhibiting monoclonal antibodies, the potential effects of mutations in codons 61 and 146 of KRAS have also been investigated. A US study, which found KRAS codon 12 or 13 mutations in 900/2121 (42.4%) of CRC patients, conducted further analysis of the 513 wild-type samples and

found 19 additional mutations at KRAS codon 61 and 17 at KRAS codon 146; these additional mutations represent less than 2% of the total study population.[11]

2.2 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 grouped into two subgroups: mutation screening and targeted mutation detection. Mutation screening tests screen samples for all KRAS mutations (known and novel) whilst 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 mutation detection at as little as 1% against a background of wild-type DNA (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.[12, 13] 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 which should not be included in this assessment.

A Provisional Clinical Opinion (PCO) from the American Society of Clinical Oncology (ASCO), published in 2009, recommended that "all patients with metastatic CRC who are candidates for anti-epidermal growth factor receptor (EGFR) antibody therapy should have their tumour tested for KRAS mutations in a Clinical Laboratory Improvement Amendments (CLIA)-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic CRC should not receive anti-EGFR antibody therapy as part of their treatment."[14] At the time that this guidance was published, there were no 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, area of interest selected by the pathologist.[14] Acceptable assay types were listed as: Real-time PCR, using probes specific for the most common mutations in codons 12 and 13; direct sequencing of exon 1 in the KRAS gene; the Therascreen® commercial kit (at that time manufactured by DxS, UK).[14]

Subsequently, the QIAGEN Therascreen® KRAS RGQ PCR Kit has been approved by the FDA, when used with the QIAGEN QIAamp® DSP DNA FFPE Tissue Kit and the QIAGEN Rotor-Gene Q MDx, Software version 2.1.0, and KRAS Assay Package.[15]

2.2.1 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 polymerase chain reaction (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 Food and Drug Administration (FDA) for the application covered by this assessment, i.e. the selection of patients with metastatic colorectal cancer 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 bi-functional molecules containing a polymerase chain reaction (PCR) primer covalently linked to a fluorescently labelled probe. A real-time PCR instrument (Rotor-Gene Q 5-Plex HRM for consistency with CE-marking) is used to perform the amplification and to measure fluorescence.[16] There is an earlier version of the Therascreen® KRAS PCR Kit which also uses Amplification Refractory Mutation System (ARMS) and Scorpions for the detection of KRAS mutations and is designed to detect the same KRAS mutations as the current, re-formulated and re-validated 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, then amplified DNA is immobilised on Steptavidin Sepharose High Performance beads. Single-stranded DNA is prepared and sequencing primers added. The samples are then analysed on the PyroMark Q24 System. The KRAS Plug-in Report is recommended by the manufacturer for the analysis of results, however, the analysis tool within the pyrosequencer can also be used.[17]

The Cobas® KRAS Mutation Test (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 formalin-fixed paraffin-embedded tissue and is validated for use with the Cobas® 4800 System.

The KRAS LightMix® Kit (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. In order 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).[18]

The KRAS StripAssay® (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; 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.[19] There are two versions of the KRAS StripAssay®: one is designed to detect 10 mutations in codons 12 and 13 of the KRAS gene; a second is designed to detect the same 10 mutations in codons 12 and 13 plus three mutations in codon 61 of the KRAS gene.

2.2.2 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 (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.[20]

Sanger sequencing is a commonly used method (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 which 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 only works well when viable tumour cells constitute at least 25% or more of the sample.[21]

NICE 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.[22]

High resolution melt (HRM) analysis assays are also commonly used by laboratories participating in the UK NEQAS scheme (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 which only binds 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.[23]

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 where each fragment is represented as a peak with a certain mass. The 'fingerprint' of the test sample is compared to 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.[24] MALDI-TOF 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.[25]

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*	Lab contact†
Commercial tests				
Therascreen® KRAS RGQ PCR Kit (Qiagen)	Targeted (7 mutations: 6 codon 12 and 1 codon 13)	0.77-6.43%	3	1
Therascreen® KRAS Pyro Kit (Qiagen)	Targeted (12mutations: 6 codon 12, 1 codon 13 and 5 codon 61)	1.0-3.5%		2
Cobas® KRAS Mutation Test (Roche Molecular Systems)	Targeted (19 mutations: 6 codon 12, 6 codon 13 and7 codon 61)	1.6-6.3% depending on mutation	4	4
KRAS LightMix® kit (TIB MolBiol)	Targeted (9 mutations: 7 codon 12, 2 codon 13)	unclear	0	0
KRAS StripAssay® (ViennaLab)	Targeted (13 mutations: 8 codon 12, 2 codon 13 and 3 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%†	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%†	2	2
Next generation sequencing	All mutations within specific codons of the KRAS gene	~5%†	0	0
MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) Mass spectrometry	All mutations within selected codons in the KRAS oncogene	~10%	1	0

* NEQAS pilot scheme 2012-2013, run 2.[26] Thirty UK based laboratories participated in the scheme; some laboratories used more than one method

† 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.

Subgroup analyses of patients tested for KRAS mutation status, from randomised controlled trials, have shown that treatment with the epidermal growth factor 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 to standard chemotherapy alone.[27, 28] Whereas patients whose tumours were positive for KRAS mutations had reduced (PFS) and tumour response when treated with cetuximab in combination with standard chemotherapy compared to standard chemotherapy alone.[27, 28] These two trials formed the basis of NICE Technology Appraisal 176, which recommends cetuximab in combination with standard chemotherapy for the first line treatment of metastatic colorectal cancer in patients whose tumours are KRAS wild-type and whose metastases are confined to the liver and are unresectable.[1] 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.

2.3 Care pathway

NICE guidance on the diagnosis and management of colorectal cancer was updated in 2012.[29]

2.3.1 Diagnosis of CRC

This guideline states that patients referred to secondary care for suspected colorectal cancer should be assessed using colonoscopy, flexible sigmoidoscopy followed by barium enema, or computed tomography (CT), dependent upon comorbidities and local expertise and test availability. Where 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-CT (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 which may become resectable following response to chemotherapy. For the second group of patients, European Society for Medical Oncology clinical practice guidelines for the treatment of advanced colorectal cancer (2010) recommend establishing KRAS mutation status in order to determine the best treatment regimen. These guidelines do not stipulate which specific mutations should be analysed, or which test method should be used.[30] 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 (FFPE) 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 recommends that mutation testing should be ordered by the pathologist reporting on the cellular make-up of the tumour.[31] However, this is not currently universal practice and often the decision to perform a KRAS mutation test is often taken at the multidisciplinary team meeting. If a sample is stored as an 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 due to 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]

2.3.2 Treatment of CRC

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

The guideline further states that raltitrexed should only be considered for patients who are intolerant to fluorouracil and folinic acid, or for whom these drugs are not suitable.[29] NICE

Technology Appraisal 61 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 metastatic colorectal cancer.[34]

With respect to the use of biological agents (epidermal growth factor receptor inhibitors), NICE Technology Appraisal Guidance 176 recommends cetuximab in combination with FOLFOX or FOLFIRI, within its licensed indication, for the first-line treatment of metastatic colorectal cancer in whom:

- The primary colorectal tumour has been resected or is potentially operable.
- The metastatic disease is confined to the liver and is unresectable.
- The patient is fit enough to undergo surgery to respect the primary colorectal tumour and to undergo liver surgery if the metastases become resectable after treatment with cetuximab.[1]

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 metastatic colorectal cancer 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 live metastases from CRC.

NICE Guideline 131 stipulates that all patients with primary colorectal cancer undergoing treatment with curative intent should have follow-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's of the chest, abdomen and pelvis in the first three years and
- Regular serum carcinoembryonic antigen tests (at least every six months in the first three years).

They should also have a surveillance colonoscopy at one year after initial treatment and, if the result is normal, further colonoscopic follow-up after five years, and thereafter as determined by cancer networks.[29]

2.3.3 Measuring response to treatment

In 1979 the World Health Organisation (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 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); there has been confusion around how to use three dimensional measures from new technologies, such as CT and MRI, in the context of WHO criteria.[38] The Response Evaluation in Solid Tumours (RECIST) Group is a collaborative initiative which was initiated to review the WHO criteria. The RECIST criteria use the same categories (complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD)).[38] RECIST guidance states that "CT and MRI are the best currently available and most reproducible methods for measuring target lesions selected for 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 30% decrease in the sum of the longest diameter 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 diameter of target lesions, taking the smallest sum of the longest diameters recorded since treatment started as the reference, or 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 question 2 and the optimal outcome measure for

question 3. 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, where 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, to differentiate adults with metastatic CRC, 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.

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, to differentiate adults with metastatic CRC, 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 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.

3.1 Systematic review methods

3.1.1 Search strategy

Search strategies were based on target condition and intervention, as recommended in the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care and the Cochrane Handbook for Diagnostic Test Accuracy Reviews.[40, 42]

Candidate search terms were identified from target references, browsing database thesauri (e.g. Medline 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 reference management software. 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) (2000-2013/01/wk2)
- MEDLINE In-Process Citations and Daily Update (OvidSP) (up to 2013/01/21)
- EMBASE (OvidSP) (2000-2013/wk3)
- Cochrane Database of Systematic Reviews (CDSR) (Wiley): Cochrane Library Issue 2000-2012/Issue12
- Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley): Cochrane Library Issue 2000-2012/Issue12

- Database of Abstracts of Reviews of Effects (DARE) (Wiley): Cochrane Library Issue 2000-2012/Issue 4
- Health Technology Assessment Database (HTA) (Wiley): Cochrane Library Issue 2000-2012/Issue 4
- Science Citation Index (SCI) (Web of Science) (2000-2013/01/22)
- Conference Proceedings Citation Index (CPCI)) (Web of Science) (2000-2013/01/22)
- LILACS (Latin American and Caribbean Health Sciences Literature) (Internet) (2000-2013/01/24)
  http://regional.bvsalud.org/php/index.php?lang=en
- Biosis Previews (Web of Knowledge) (2000-2013/01/22)
- NIHR Health Technology Assessment Programme (Internet) (2000-2013/01/25)
- PROSPERO (International Prospective Register of Systematic Reviews) (Internet) (up to 2013/01/25)
  http://www.crd.york.ac.uk/prospero/

Completed and on-going trials were identified by searches of the following resources:

- NIH ClinicalTrials.gov (2000-2013/01/23) (Internet)
  http://www.clinicaltrials.gov/
- Current Controlled Trials (2000-2013/01/29) (Internet)
  http://www.controlled-trials.com/
- WHO International Clinical Trials Registry Platform (ICTRP) (2000-2013/01/25) (Internet)
  http://www.who.int/ictrp/en/

Searches were undertaken to identify studies of KRAS testing for metastatic colorectal cancer. The main Embase strategy for each set of searches was independently peer reviewed by a second Information Specialist, using the PRESS-EBC checklist.[43] Search strategies were developed specifically for each database and the keywords associated with colorectal cancer 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 (American Society of Clinical Oncology) (2007-2013) (Internet): http://www.asco.org/ASCOv2/Meetings/Abstracts

- ESMO Conference Proceedings (European Society of Medical Oncology) (2007-2013) (Internet): http://www.esmo.org/education-research/abstracts-virtual-meetings-and-meeting-reports.html
- AACR Conference Proceedings (American Association for Cancer Research): 2007-2013 (Internet): http://www.aacrmeetingabstracts.org/search.dtl
- AMP Conference Proceedings (Association for Molecular Pathology): 2007-2013 (Internet): http://www.amp.org/meetings/past_meetings.cfm

Identified references were downloaded in 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]

3.1.2 Inclusion and exclusion criteria

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

Table 2: Inclusion criteria

Question	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, where reported, from treatment with standard chemotherapy vary according to which test is used to select patients for treatment?
Participants:	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy.	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy.	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable 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
Comparators:	Not applicable	Not applicable	Standard chemotherapy
Reference standard:	Not applicable	Response to treatment with cetuximab in combination with standard chemotherapy (e.g. progression free survival, objective response rate, disease control rate)	Not applicable
Outcomes:	Proportion tumour cells needed, failures, limit of detection, turnaround time, costs, expertise/logistics of test	Overall survival or progression free survival in patients whose tumours are KRAS mutant versus wild-type. Test accuracy – the number of true positive, false negative, false positive and true negative.	Progression free survival, overall survival, objective response rate, disease control rate
Study design:	To be addressed by survey; see below 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)

3.1.3 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 and Therascreen® KRAS Pyro kits (Qiagen), the Cobas® KRAS Mutation Test Kit (Roche Diagnostics), 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, pervious 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 (where reported), test failure rates and limits of detection. Data were 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.

3.1.4 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 QUADAS-2.[48] Risk of bias assessments 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 presented in tables and graphs in the results of the systematic review and were presented in full, by study, in Appendix 3.

3.1.5 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 email invitation via NEQAS to laboratories participating in the UK NEQAS pilot scheme for KRAS mutation testing. We used the Survey Monkey online software to run the survey. We structured the survey into sections on:

- Laboratory details
- KRAS testing methods
- Logistics
- Technical Methods
- Costs

Where 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.

3.1.6 Methods of analysis/synthesis

The results of studies included in this review were summarised by research question (see Section 1), i.e. studies providing technical information on KRAS mutation testing in NHS laboratories in the UK (Section 3.2.1), studies providing information on the accuracy of KRAS mutation tests for predicting response to treatment (Section 3.2.2), and studies reporting information on how clinical outcomes may vary according to which test is used to select patients for treatment (Section 3.2.3). We planned to use a bivariate/hierarchical summary receiver operating characteristic (HSROC) random effects model to generate summary estimates and an 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-analyses 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, false negative, false positive and true negative 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. objective response rate (ORR), or resection rate) reported. Where 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 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 progression-free survival (PFS) and odds ratios (OR), 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 such as the chi-squared test and I^2 statistic.

3.2 Results of the assessment of clinical effectiveness

The literature searches of bibliographic databases identified 7,903 references. After initial screening of titles and abstracts, 100 were considered to be potentially relevant and ordered for full paper screening. No additional papers were ordered based on screening of papers provided by test manufacturers; all studies submitted cited in documents supplied by the test manufacturers had already been identified by bibliographic database searches. No additional studies were identified from searches of clinical trials registries, or 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.

Based on the searches and inclusion screening described above, seven publications of five studies were included in the review.[27, 28, 52-55] Hand searching of conference proceedings did not identify any additional publications. Because data for participants with colorectal metastases and no extra-hepatic metastases were frequently reported as sub-groups of larger trials, the authors of two additional potentially relevant trials in patients with metastatic colorectal cancer were contacted to request subgroup data. The author of the CECOG trial[56] reported that only 23 (15%) of participants had metastases which were limited to the liver, and that no subgroup data were available for these participants. The author of the NORDIC-VII trial[57] did not respond to our request.

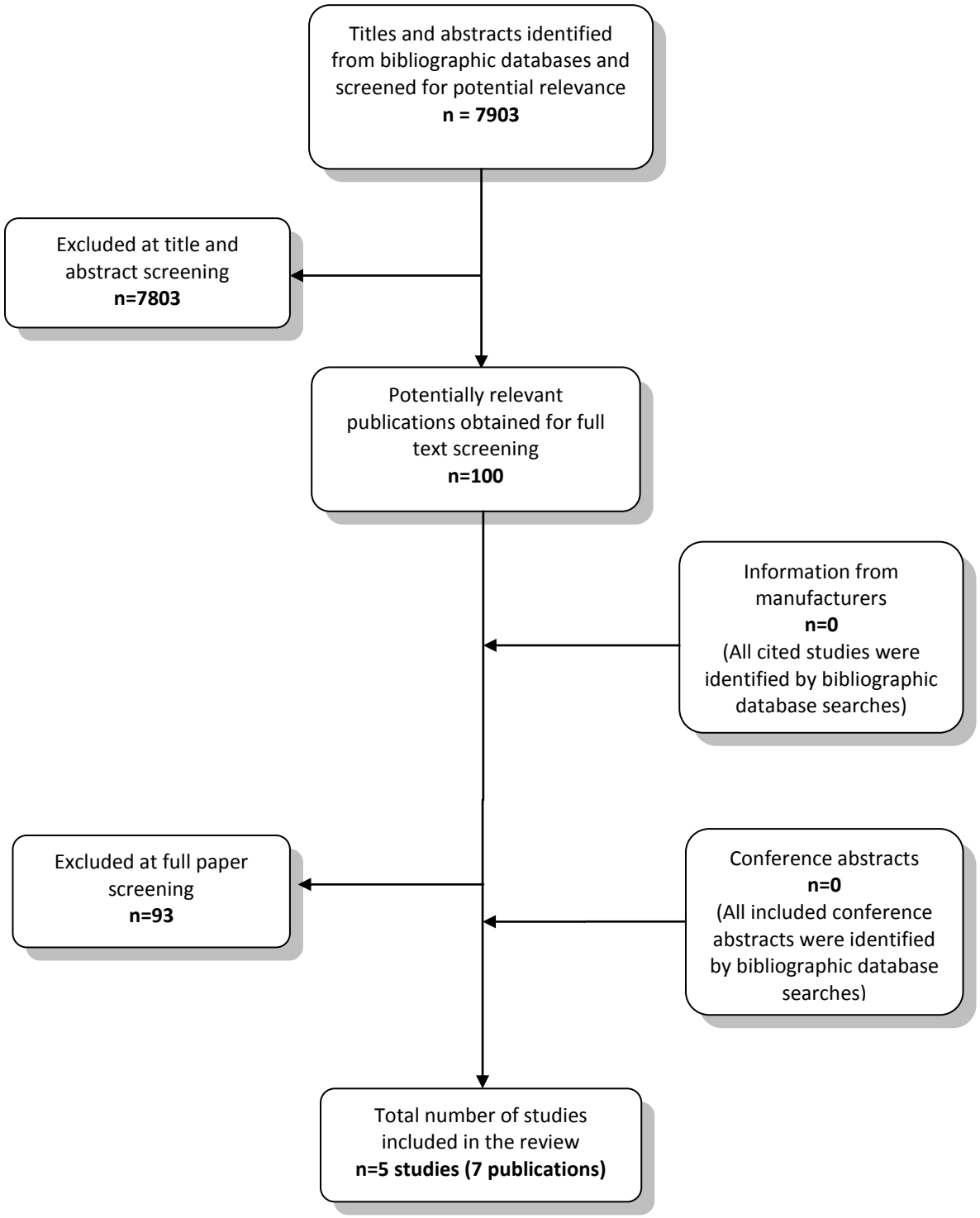
No studies, conducted in UK NHS laboratories, were identified which reported information on the technical performance characteristics of KRAS mutation tests. One study reported data on tumour response following treatment with cetuximab plus FOLFOX6 or cetuximab

plus FOLFIRI in a group of patients with unresectable colorectal liver metastases who were tested for tumour KRAS mutation status.[52] 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, where a combination of pyrosequencing and MALDI-TOF was used to asses KRAS mutation status. Four RCTs, reported in six publications, compared the effectiveness of cetuximab plus standard chemotherapy with that of standard chemotherapy alone in patients whose tumours were KRAS wild-type.[27, 28, 53-55, 58] 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 multi-centre European study, funded by Merck Serono, Sanofi Aventis and Pfizer.[52] Two of the four RCTs were multi-centre European studies, funded by Merck Serono,[27,28,53,58] one was a multi-centre study conducted in the UK and the Republic of Ireland and funded by the UK Medical Research Council (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 study participants, study inclusion and exclusion criteria, KRAS mutation test used and mutations targeted, and treatment groups are reported in the data the 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.

Figure 1: Flow of studies through the review process



3.2.1 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

There were 31 laboratories participating in the 2012-2013 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%).

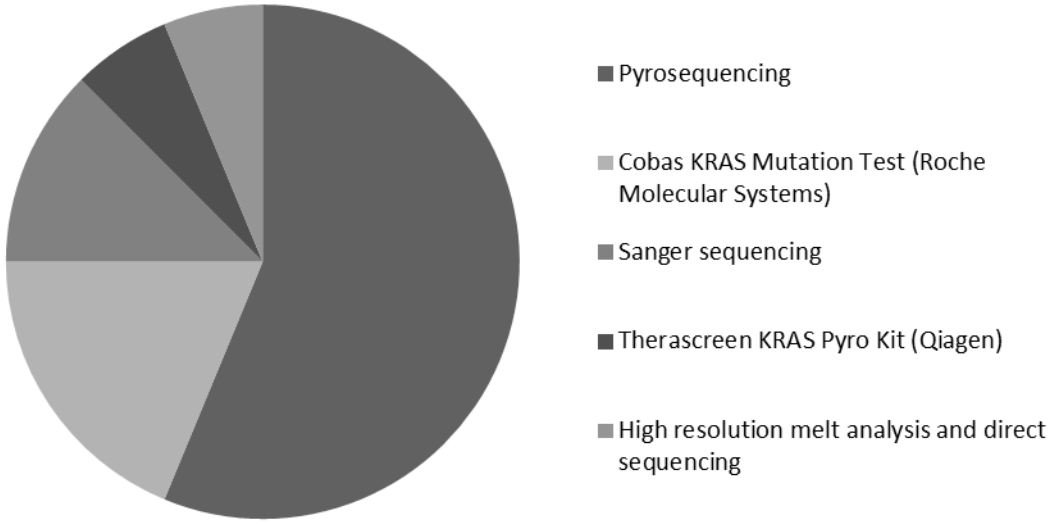
KRAS mutation test methods (Figure 2)

Fifteen laboratories stated that they used one method of KRAS mutation testing, one laboratory stated that they sometimes use a single KRAS mutation testing method and sometimes multiple methods (e.g. to confirm mutations).

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 high resolution melt analysis due to its quicker turnaround time. Cobas® KRAS Mutation Test was used by three laboratories, Sanger sequencing was used by two laboratories with only a single laboratory using the Therascreen® KRAS Pyro Kit. The final laboratory, which had initially reported only using a single method of KRAS mutation testing stated that it used high resolution melt analysis and direct sequencing. The laboratory which reported sometimes using multiple methods only reported details for one testing method (Sanger sequencing), however, this laboratory did state that they used Sanger sequencing in the event of an unusual pyrosequencing result. They also stated that their reason for using more than one testing method was the ability to fully characterise detected mutations. This suggests that their 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 samples.

Nine laboratories reported that samples were referred to their laboratory for testing on demand (one specified that this was multi-disciplinary team (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 and one laboratory did not answer this question.

Figure 2: KRAS mutations tests used in NHS Laboratories in the UK participating in the UK NEQAS pilot scheme for KRAS mutation testing



*Please note that this figure cannot be adequately displayed in black and white; categories listed from top to bottom appear in clockwise order on the chart, starting from the top with pyrosequencing as the largest part of the figure.

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 stated that they also targeted mutations in codon 146. Two

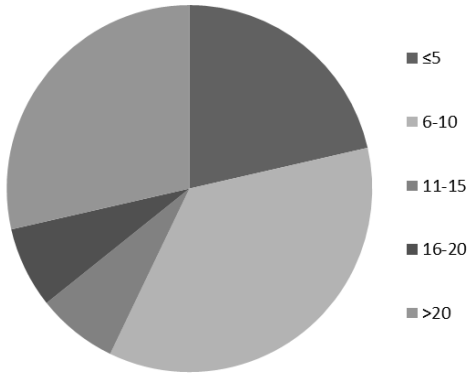
laboratories used Sanger sequencing. One stated that they targeted specific mutations but did not provide any further details. The other stated that they targeted mutations in codons 12, 13, and 61 using self-designed primers. One laboratory stated that they only used a single testing method, high resolution melt analysis, and subsequently stated that they used high resolution melt 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, Figure 3)

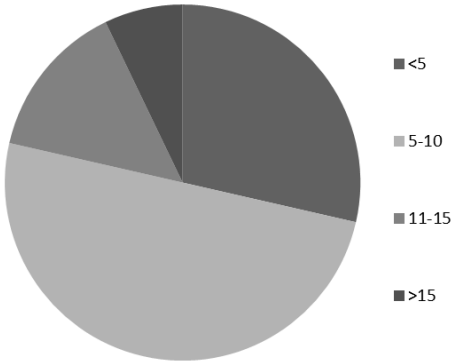
The number of samples screened for KRAS mutations in a typical week varied by laboratory from less than five (three laboratories) to more than 20 (four laboratories). The batch size ranged from less than 1-2 to 15-20 samples (Figure 3). Only laboratories with five or less samples screened per week ran 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 more than 20 samples per week; most other laboratories had batch sizes between 5 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 more than 20 samples per week with batch size of 10 and 15, and one reported screening 6-10 samples per week with a similar batch size. Only one laboratory reported that they waited until they had a certain number of samples before running the KRAS mutation test, this laboratory waited until they had 10 samples before running the test.

Figure 3: Summary of logistic information

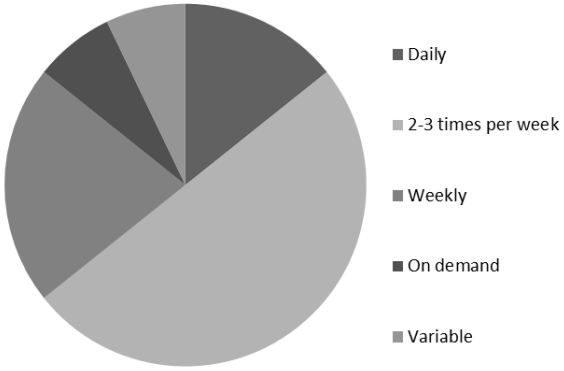
a. In a typical week, how many samples do you screen for KRAS mutations?



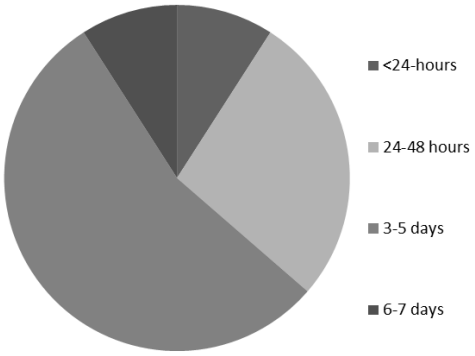
b. What is you average batch size (number of samples)?



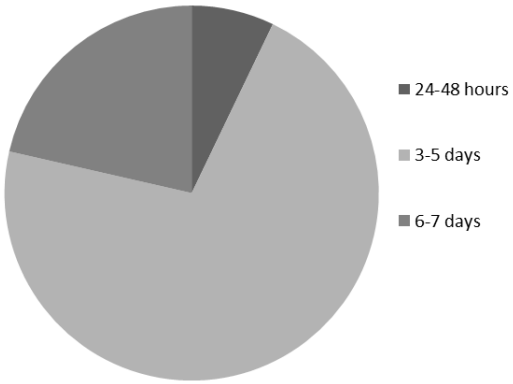
c. How often do you run the KRAS mutation test?



d. On average, how long (in calender days) does it take to receive a sample at the lab once it has been requested?



e. 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?



*Please note that this figure cannot be adequately displayed in black and white; categories listed from top to bottom appear in clockwise order on the charts.

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 ranged from 1-10 days in three, from 2-30 days in one, and one stated that occasionally request dates are included in referral and so the range is 1-3 weeks. 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 having a time of 24-48 hours. The laboratory with the shortest turnaround time was one which used pyrosequencing and tested 11-15 samples per week.

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 test to returning result to clinician
Cobas® KRAS Mutation Test	6-10	6-10	Weekly	No	6-7 days
	>20	10	2-3 times per week	Yes, 10	3-5 days
	NR	NR	NR	NR	NR
HRM	6-10	4	2-3 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	2-3 times per week	No	3-5 days
	6-10	8	Weekly	No	6-7 days
	11-15	6-10	2-3 times per week	No	24-48 hours
	16-20	10	2-3 times per week	No	3-5 days
	6-10	5-10	2-3 times per week	No	3-5 days
	>20	12	Daily	No	3-5 days
Sanger	≤5	1 or more	Daily	No	3-5 days
	≤5	1-2 patients	Variable	No	3-5 days
Therascreen® KRAS Pyro kit	>20	15	2-3 times per week	No	6-7 days

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 kit reported that the limit of detection was 1-5%, the limit of detection for pyrosequencing was reported to be either 1-5% (three laboratories) or 6-10% (five laboratories), for Sanger sequencing was >10%, and for HRM was 1-5%. A variety of methods were used to determine the limit of detection (Table 5). Ten laboratories reported using microdissection, two stated that they always used this technique others used microdissection at thresholds below 10-50%. The laboratory that used the Therascreen® KRAS Pyro kit did not provide any data on technical performance.

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 micro-dissection ?	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	11-20%	1-5%	Serial dilutions of controls	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 %	Yes	Always
	11-20%	6-10%	Dilution series of DNA from 3 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 where dissection would greatly improve the tumour percent
	21-30%	1-5%	CE marked kit, in-house validation conducted	Yes	20%
	21-30%	6-10%	Internal QC	Yes	Always
	6-10%	1-5%	Cell-lines with set % tumour burden	Yes	50%
Sanger	>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

KRAS mutation test failure rates (Table 5)

The proportion of samples rejected prior to analysis was less than 2% for all 13 laboratories that provided data on rejection rates. Reasons for rejection included insufficient tumour

cells/tissue, sample types unsuitable for analysis, and insufficient patient identifiers. The proportion of failed tests ranged from 3-6% for the Cobas® KRAS Mutation Test kit and from 0.2-10% for pyrosequencing. The one study assessing HRM reported no failed tests, of the two studies of 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.

Table 5: KRAS mutation test failure rates

KRAS mutation test	Number of samples per year*					
	Submitted	Rejected (%)	Reasons for rejection	Analysed	Failed (%)	Reasons 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	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 due to fixation and DNA degradation
	415	3 (0.7)	Insufficient tumour cells	412	1 (0.2)	DNA quality
	374	0	NA; samples pre-selected 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	65	0	NA	65	0	NA
	1000	0	NA	1000	NA	Insufficient tumour cells, DNA degradation
Therascreen®	NR	NR	NR	NR	NR	NR

KRAS mutation test	Number of samples per year*					
	Submitted	Rejected (%)	Reasons for rejection	Analysed	Failed (%)	Reasons for failure
KRAS Pyro kit						
*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						

KRAS mutation test costs (Table 6)

Only six laboratories provided data on the cost of the KRAS mutation test. Two of these only provided data on the costs of the reagents which were reported as £22 for Sanger sequencing and £50 for pyrosequencing. A further laboratory reported that the cost of pyrosequencing was £50. Two other laboratories reported costs for pyrosequencing one reported a cost of approximately £120 and the other reported a cost of £273 for a single sample but that this reduced to approximately £110 per sample if running a batch of ten. The final laboratory to report cost data reported that the cost of the Cobas® KRAS Mutation Test was £100 to £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 6: Summary of KRAS mutation test costs

KRAS mutation test	Cost to laboratory	Funding	NHS Price	Merck Serono Price
Cobas® KRAS Mutation Test kit	NA	Merck Serono	NR	NR
	NR	Merck Serono, Private	NA	NR
	£100-125	Merck Serono, Private	NA	Unable to disclose
HRM	NR	Merck Serono	NA	NR
Pyrosequencing	£150	NHS, Privately funded from abroad	£150	NA
	NR	Merck Serono, NHS	£140	£100
	~£120	Merck Serono, CR-UK Strat Med 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	FOC	£100
	£50	Trials unit	NA	NA
	NA	Merck Serono, NHS, Private	£120	120
Sanger	£22 (reagents only)	Merck Serono	NA	£100
	NR	Merck Serono	NR	NR

KRAS mutation test	Cost to laboratory	Funding	NHS Price	Merck Serono Price
Therascreen® KRAS Pyro kit	NR	**NR**	**NR**	**NR**

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

One study, the CELIM trial, 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.[52] 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 to this assessment is limited because reporting of outcome data by mutation status was limited to objective response (OR). Thus, we defined true positives 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 = complete response (CR) or partial response (PR)). False positives 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 (stable disease (SD) or progressive disease (PD)). False negatives were defined as those with KRAS mutant tumours who had a positive response to treatment with cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI. True negatives 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 section 2.3.3. The publication of the results of the CELIM trial reported resection rates for liver metastases, however, these data were only reported for all participants and by treatment group (cetuximab plus FOLFOX6 or cetuximab plus FOLFIRI) **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] [redacted].[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 2, “how do 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, allowed calculation of the accuracy of KRAS mutation, using a combination of pyrosequencing and MALDI-TOF and targeting mutations in codons 12, 13 and 61, testing 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 of limited applicability to this assessment, because the standard chemotherapy regimen used in the COIN trial does not exactly match our inclusion criteria (some participants received XELOX). 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 true positives as those patients with KRAS wild-type tumours who had a potentially curative resection following treatment with cetuximab plus FOLFOX or cetuximab plus XELOX. False positives 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. False negatives were defined as those with KRAS mutant tumours who had a potentially curative resection following treatment with cetuximab plus FOLFOX or cetuximab plus XELOX. True negatives 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 all had unresectable colorectal liver metastases with no extra-hepatic metastases.[52] 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 (IQR 56 to 71) and 64 % were male. The primary tumour site was the colon in 61 (55%) of participants and the rectum in 49 (44%) of participants; the primary site was unknown in one participant. Most patients (83%) had primary tumour stage T3/4. The primary criterion for non-resectability of liver metastases was classified as “technically unresectable,” n = 61 (55%), or “≥ 5 metastases,” n = 50 (45%). Full details of 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 detect seven mutations in codons 12 and 13 (Table 7) and the two versions will be treated as equivalent for the purpose of this assessment.

Table 7: KRAS mutations detected by the Therascreen® KRAS RGQ PCR kit

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-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

Tumour response was assessed according to the Response Evaluation Criteria In Solid Tumours (RECIST) criteria[38] to evaluate response to TKI 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 (eight weeks). Post-treatment surgical review, to assess respectability, 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 scan was taken and the participants' clinical outcome data.[52]

Details of the COIN trial are provided in section 3.2.3.[54]

KRAS mutation test accuracy

Data from the CELIM trial provided estimates for the accuracy of the Therascreen® 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 OR was moderate, 74.6% (95% CI: 62.1 to 84.5%), and specificity was poor, 35.5% (95% CI: 19.2 to 54.6%), (Table 8 and Figure 4).[52] 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. Sensitivity and specificity were both poor, 52.0% (95% CI: 31.3 to 72.2%) and 45.6% (95% CI: 37.0 to 54.3%), respectively, (Table 8 and Figure 4).

QUADAS-2 Assessment

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 reflects the use of standard chemotherapy which did not fully match the inclusion criteria for this assessment in the COIN trial. In addition, participants in the CELIM trial were described as having technically non-resectable or ≥ five liver metastases from CRC and it was therefore unclear whether some participants may have had potentially resectable metastases at baseline.[54] Both studies were rated as 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 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(CELIM)(2010) [52]	Therascreen® PCR kit	12/111 unknown mutation status (KRAS mutation testing not done successfully, no reasons reported)	Objective Response	47	20	16	11	74.6 (62.1, 84.7)	35.5 (19.2, 54.6)	67%	70.1 (58.3, 79.8)	40.7 (24.5, 59.3)
COIN*	Pyrosequencing and MADI-TOF (codons 12, 13 and 61)		Resection Rate	13	74	12	62	52.0 (31.3, 72.2)	45.6 (37.0, 54.3)	16%	14.9 (8.9, 23.9)	83.9 (73.8, 90.5)

CI: confidence interval; FN: false negative; FP: false positive; NPV: negative predictive value; PPV: positive predictive value; TN: true negative; TP: true positive

*: additional data supplied by the COIN trial investigators

Figure 4: SROC plot showing estimates of sensitivity and specificity together with 95% confidence intervals from the CELIM (black lines) and COIN (grey lines) trials

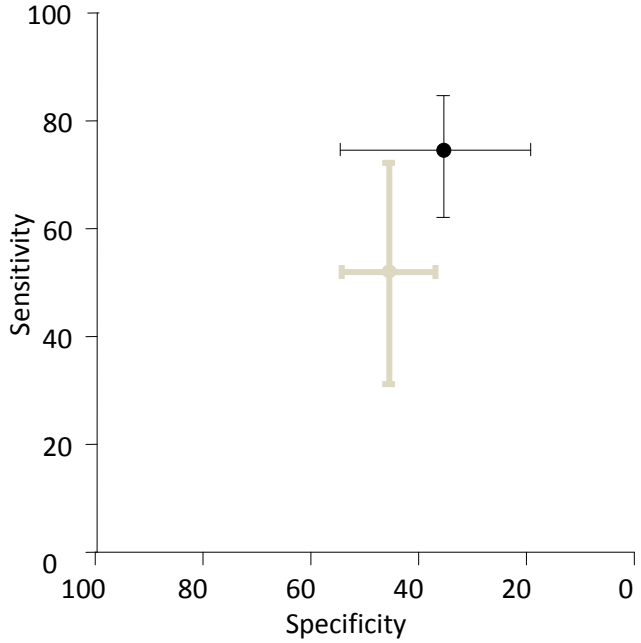


Table 9: QUADAS-2 results for the study which provided data on the accuracy of KRAS mutation testing for the prediction of response to treatment with cetuximab in addition to standard chemotherapy

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Folprecht (CELIM)(2010) [52]	☺	☺	☺	☹	?	☺	☹
Maughan (COIN)(2011)[54]	☺	☺	☺	☹	☺	☺	☹

☺Low Risk ☹High Risk ? Unclear Risk

3.2.3 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) provided data on the clinical effectiveness of cetuximab plus standard chemotherapy compared to standard chemotherapy alone in patients with colorectal liver metastases and no extra-hepatic metastases whose tumours were KRAS wild-type.[27, 28, 53-55, 58] The trials compared cetuximab in combination with standard chemotherapy (FOLFOX or FOLFIRI) with standard chemotherapy alone (see Table 11); in one trial standard chemotherapy could be either FOLFOX or XELOX.[54] One trial included only participants with unresectable colorectal liver metastases and no extra-hepatic metastases, whose tumours were KRAS wild-type.[55] 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, OPUS and COIN (five publications) included participants with metastatic colorectal cancer, 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 outcomes data on participants whose metastases were confined to the liver were only reported for those with KRAS wild-type tumours.[27, 28, 53, 54, 58]

Study details

Participant characteristics varied across studies. The three studies which reported subgroup data for patients with colorectal metastases confined to the liver were multi-centre studies conducted in continental Europe,[27, 28, 53, 58] 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 Eastern Cooperative Oncology Group (ECOG) or WHO performance status of 0 or 1 and two out of three studies included only participants with histologically confirmed adenocarcinoma.[27, 53, 54] The trial which included only participants with unresectable colorectal liver metastases and no extra-hepatic 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 and OPUS trials both the he LightMix® k-ras Gly12 assay (TIB MolBiol).[27, 28] 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: KRAS mutations detected by the LightMix® k-ras Gly12 assay

Codon	Coding DNA	Protein/amino acid, 3-letter code	Protein/amino acid, 1-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 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 and 200 base pairs of sequence upstream and downstream of each known mutation (known mutations taken from Catalogue of Somatic Mutations in Cancer (COSMIC) database (http://www.sanger.ac.uk/genetics/CGP/cosmic)).{Maughan, 2011 #53} 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.[54] 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 only reported in a conference abstract.[53] Three of the four trials also reported ORR.[28, 53, 55] Two trials, CRYSTAL and OPUS used modified WHO criteria to assess tumour response,[27, 58] the COIN trial[54] used RECIST criteria,[38] and the final trial did not specify criteria for assessing tumour response.[55] None of the three trials which reported subgroup data for participants whose metastases were limited to the liver reported data on how respectability

of liver metastases was assessed post-treatment.[27, 28, 54] The trial registry for the study which included only people with unresectable liver metastases whose metastases were confined to the liver stated that post-treatment respectability would be assessed after, 4-12 cycles, by a multi-disciplinary team of more than three liver surgeons and one radiologist, using CT and MRI images.[60] Two reported PFS in the relevant patient group,[28, 54] and some limited data were also 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 to those treated with standard chemotherapy

All studies in this section reported the addition of cetuximab to standard chemotherapy was associated with an increase in the rate of R0 resections (Table 11), however, this increase only reached statistical significance in the trial by Xu et al. (OR 4.57 (95% CI: 1.56 to 13.34)).[55] All three studies which assessed objective response rate reported a statistically significant higher response rate for participants treated with cetuximab plus standard chemotherapy compared to those treated with standard chemotherapy alone; ORs ranged from 3.00 (95% CI: 1.49, 6.03)[53] to 4.93 (95% CI: 1.42 to 17.06).[28] No study reported an improvement in PFS associated with the addition of Cetuximab to standard chemotherapy. The study by Xu et al reported a significant improvement in three year survival rates for participants treated with cetuximab plus standard chemotherapy compared to those treated with standard chemotherapy alone (OR 2.76 (95% CI: 1.12 to 6.26)).[55] 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 (see Figures 5, 6 and 7). Where reported 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]

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 to those treated with standard chemotherapy

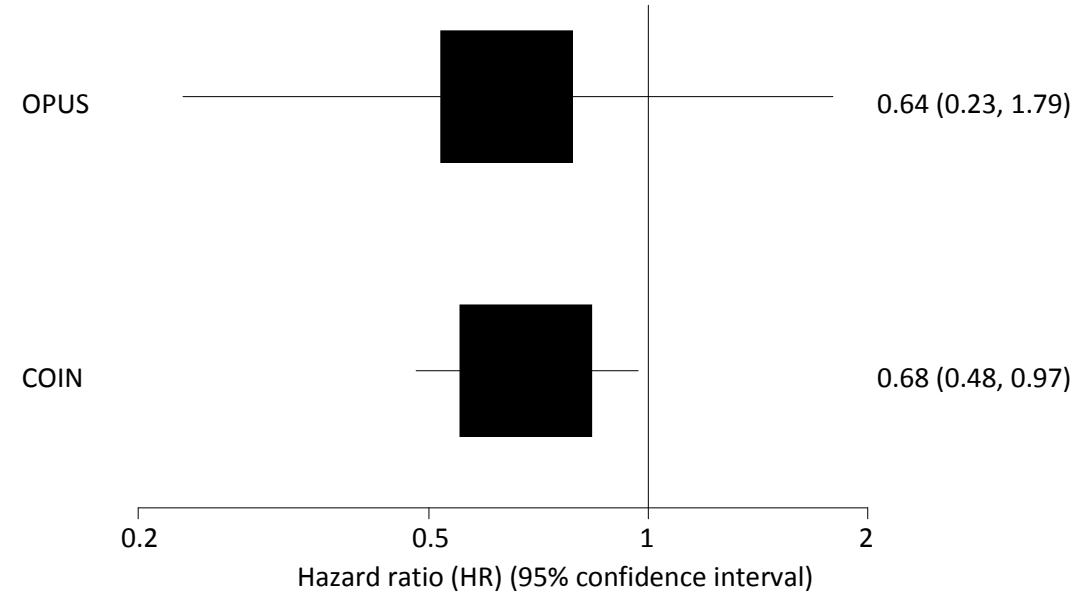


Figure 6: Objective Response in patients with colorectal metastases limited to the liver and KRAS wild-type tumours who were treated with cetuximab plus standard chemotherapy compared to those treated with standard chemotherapy

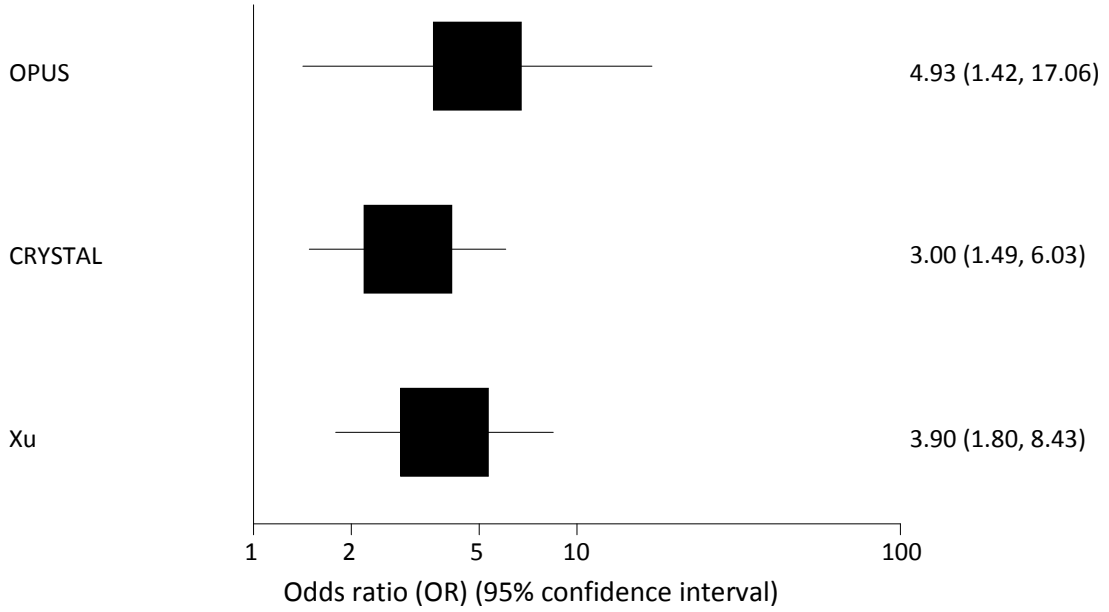
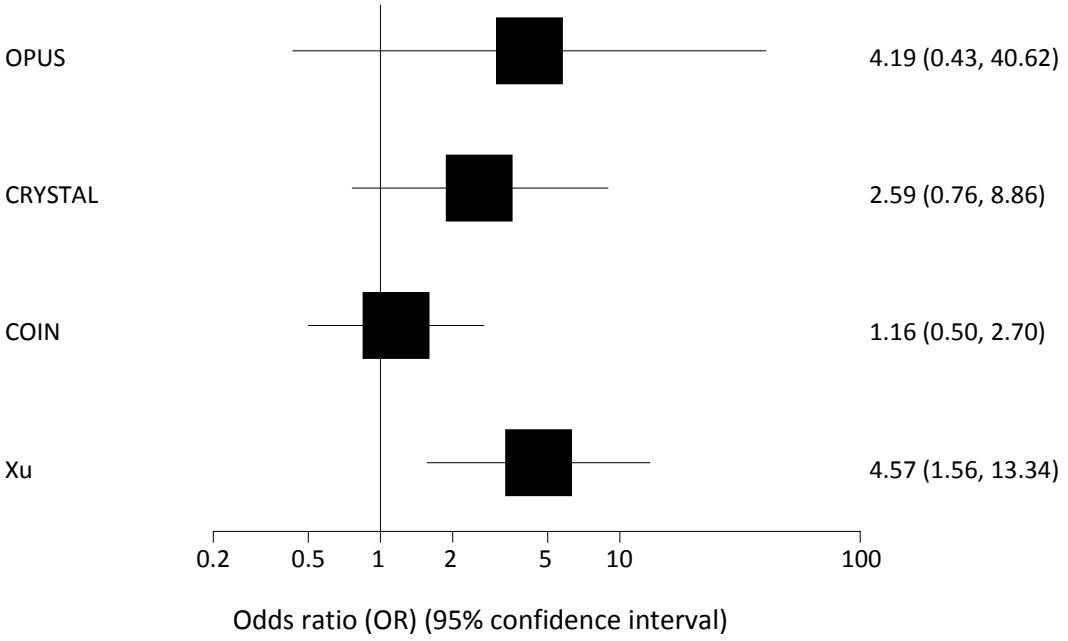


Figure 7: R0 resection rate in patients with colorectal metastases limited to the liver and KRAS wild-type tumours who were treated with cetuximab plus standard chemotherapy compared to those treated with standard chemotherapy



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

Data from the COIN trial indicated that a slight increase in PFS for patients with initially unresectable liver metastases, whose tumours were KRAS wild-type and who received cetuximab plus FOLFOX or XELOX compared to those who received FOLFOX or XELOX alone (HR 0.48 (95% CI: 0.48, 0.97)).[54] 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, 1.77)). The reported rates of potentially curative resection, in patients whose tumours were KRAS wild type, were 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 addition data for patients whose tumours were KRAS mutant; in these patients the potentially curative resection rates were 16% (12/74) for the cetuximab plus FOLFOX or XELOX group and 14% (6/44) for the FOLFOX or XELOX only group.

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 (OPUS)(2011) [28] Bokemeyer (OPUS)(2009) [58] Kohne (OPUS)(2011)[53]	LightMix® k-ras Gly12 Test Kit (KRAS codon 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, 1.79)
					ORR	OR 4.93 (1.42, 17.06)[a]
					R0R	OR 4.19 (0.43, 40.62)[a]
Kohne (CRYSTAL)(2011)[53] VanCutsem (CRYSTAL)(2009)[27]	LightMix® k-ras Gly12 Test Kit (KRAS codon 12 and 13 missense mutations)	n = 1,198 KRAS wild type 348 KRAS wild type with liver limited metastases 140 KRAS mutation status could not be evaluated 658/1,198 (no reason reported)	Cetuximab + FOLFIRI (n = 68)	FOLFIRI (n = 72)	ORR	OR 3.00 (1.49, 6.03)[a]
					R0R	OR 2.59 (0.76, 8.86)[a]
Maughan (COIN)(2011)[54]	Pyrosequencing and MALDI-TOF mass array, with Sanger sequencing for discordant samples (<1%) (KRAS mutations in codons 12, 13 and 61)	n = 1,630 KRAS wild type 729 KRAS wild type with liver limited metastases 178 KRAS mutation status unknown 336/1,630 (141 tumour blocks not available; 163 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, 0.97)
					R0R	OR 1.16 (0.50, 2.70)[a]
Xu(2010) [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, 6.26)[a]
					ORR	OR 3.90 (1.80, 8.43)[a]
					R0R	OR 4.57 (1.56, 13.34)[a]

CI: confidence interval; OR: odds ratio; ORR: objective response rate; OSR: 3 year survival rate; PFS: progression-free survival; R0R: R0 resection rate [a]: calculated value

Risk of Bias

All studies in this section were rated as 'low' or 'unclear' risk of bias for randomisation, incomplete outcome data and selective outcome reporting. All three of the trials which 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, which stated that treatment allocation was not masked.[54] All studies were rated as 'high' risk of bias for blinding of study participants and personnel; all were open label studies. However, two studies were rated as 'low' risk of bias for blinding of outcome assessors, as they reported some independent/blinded assessment of outcomes.[55, 58] The results of risk of bias assessments are summarised in Table 12 below and full risk of bias assessments for each study are 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 (OPUS)(2011) [28] Bokemeyer (OPUS)(2009)[58]	?	?	☹	☺	☺	☺
Kohne (CRYSTAL)(2011)[53] VanCutsem (CRYSTAL)(2009)[27]	?	?	☹	?	☺	☺
Maughan (COIN)(2011)[54]	☺	☹	☹	?	☺	☺
Xu(2010) [55, 60]	?	?	☹	?	?	?

☺Low Risk ☹High Risk ? Unclear Risk

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 metastatic colorectal cancer, where metastases are confined to the liver and are unresectable.

4.1 Review of economic analyses of KRAS mutation testing

4.1.1 Search strategy

Searches were undertaken to identify cost-effectiveness studies of KRAS mutation testing in metastatic colorectal cancer. 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 Peer Review of Electronic Search Strategies (PRESS-EBC) checklist.[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) (2000-2013/01/wk3)
- MEDLINE In-Process Citations and Daily Update (OvidSP) (2000-2013/01/28)
- EMBASE (OvidSP) (2000-2013/wk4)
- NHS Economic Evaluation Database (NHS EED) (Wiley): Cochrane Library Issue 2000-2012/Issue 4)
- Health Economic Evaluation Database (HEED) (Wiley) (2000-2013/01/30)
- http://onlinelibrary.wiley.com/book/10.1002/9780470510933
- EconLit (EBSCO) (2000-2013/01/30)
- Science Citation Index (SCI) (Web of Science) (2000-2013/01/25)

4.1.2 Inclusion criteria

Studies reporting a full economic analysis, which 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.

4.1.3 Results

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

The Ontario HTA report[62] aimed to determine the cost-effectiveness of KRAS mutation testing for the third line treatment of (stage IV) metastatic colorectal cancer (mCRC) in Ontario. For this purpose, seven strategies were compared:

0) Best supportive care
1a) cetuximab (with KRAS testing)
1b) cetuximab (without KRAS mutation testing)
2a) panitumumab (with KRAS mutation testing)
2b) panitumumab (without KRAS mutation testing)
3a) cetuximab plus irinotecan (with KRAS mutation testing)
3b) cetuximab plus irinotecan (without KRAS mutation testing)

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 on progression free survival, overall survival, utility weights and adverse events were obtained from various clinical studies. Although a probabilistic sensitivity analysis (PSA) was performed for the best supportive care (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 CEAC (which was not shown). Also, it appears that the parameters that were varied within the PSA were highly aggregated (PFS, OS, utility, score and total costs) and the distribution used was not mentioned (possibly uniform, since only a range was given). The deterministic results showed that, compared to BSC, all monotherapy strategies are either dominated or extended dominated. The ICER of cetuximab plus irinotecan with KRAS mutation testing compared to BSC is $42,710. The ICER of cetuximab plus irinotecan without KRAS mutation testing compared to cetuximab plus irinotecan with KRAS mutation testing is $163,396. The authors concluded that whilst KRAS mutation testing is cost-effective for all strategies considered, it is not equally cost-effective for all treatment options.

Vijayaraghavan et al[63] developed a Markov model to compare six hypothetical strategies for second line treatment for patients with mCRC who have failed prior 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 USA and Germany and were based on published studies. In the results section, the treatment strategies were compared as KRAS mutation testing versus no KRAS mutation testing, within a certain treatment regimen. For all these comparisons, KRAS mutation testing saved costs at equivalent clinical outcomes.

The cost-effectiveness of both KRAS and BRAF mutation screening in patients with mCRC who are chemorefractory was the subject of a paper by Behl et al.[64] 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 randomised controlled trials. The model followed each patient for a maximum of 10 years. The no-cetuximab strategy was least costly ($34,291), but also least effective (0.6686 lifeyears), followed by the KRAS plus BRAF screening therapy, which offered more lifeyears (0.7025) but at a higher cost ($56.324). Screening for KRAS mutations again added lifeyears (0.7029) at a cost ($57,348) but at an unfavourable ratio as compared to KRAS plus BRAF mutation screening (ICER > $2

million). Finally, the anti-EGFR strategy – providing cetuximab to all patients without screening, was the most effective (0.7055 lifeyears) and most expensive ($64,841) strategy, which only proved cost-effective at a willingness to pay of more than US$3 million. Therefore, KRAS plus BRAF mutation screening appeared the most cost-effective option compared to no anti-EGFR therapy, but still at an ICER of $648,396 per lifeyear gained.

Shiroiwa et al.[65] 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 CO.17 trial.[66] The time horizon of the analysis was two and a half years. As expected, the no cetuximab strategy was least costly and least effective, with $6,800 and 0.36 Quality-Adjusted Life Years (QALYs). The no KRAS mutation testing strategy (all patients receive cetuximab) cost $35,000 and resulted in 0.48 QALYs. The KRAS mutation testing strategy ended up in between, with $29,000 and 0.48 QALYs. Although there was a small difference in QALYs to the advantage of no KRAS mutation testing, the ICER of KRAS mutation testing versus no KRAS mutation testing was reported as dominant. The ICER of KRAS mutation testing versus no cetuximab was $180,000 per QALY. The authors concluded that although KRAS mutation testing versus 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[67] 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-types, 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 randomised clinical trials. 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 in the no-testing strategy. The costs of mutation testing were overcompensated 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 gained compared to the reference strategy. Testing for KRAS mutations only as compared to testing for KRAS and BRAF mutations, as well as the no testing strategy compared to KRAS mutation testing, both had ICERs well above €300,000 per QALY gained. The authors concluded that testing for KRAS and BRAF mutations *prior to cetuximab* treatment of chemorefractory mCRC patients is clinically appropriate and economically favourable, despite high costs for predictive testing. (That is: 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 only patients with KRAS wildtype tumour status with cetuximab as compared to standard chemotherapy alone for all patients seems rather high.

Table 13: Summary of included papers

Study details	Ontario HTA report[62]
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	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 AEs taken from various studies
Comparators	0 Best Supportive Care 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 literature, 2009 OHIP and OCCI administrative databases
Measure of benefit	QALY
Study type	Cost-utility analysis: Markov model
Model assumptions	None mentioned
Perspective	Ontario Ministry of Health and Long-Term Care (MOHLTC)
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, 3a (i.e. does not include the 'no KRAS mutation testing' strategies)

Study details	Ontario HTA report[62]
	The PSA varied parameters only on a highly aggregated level: PFS, OS, Total costs and utility. It seems like a uniform distribution was used for all of them?
Outcome (cost and Lys/QALYs) per comparator	0: BSC (No KRAS mutation test; No treatment) $1,414 0.7455 2a: Panitumumab (Perform KRAS mutation test) $12,236 0.9719 1a: Cetuximab (Perform KRAS mutation test) $18,305 1.0537 2b: Panitumumab (No KRAS mutation test) $20,424 0.9985 3a: Cetuximab + Irinotecan (Perform KRAS mutation test) $23,373 1.2596 1b: Cetuximab (No KRAS mutation test) $29,399 1.0447 3b: Cetuximab + Irinotecan (No KRAS mutation test) $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 $50K, the probabilities of cetuximab monotherapy, panitumumab monotherapy and cetuximab with irinotecan combination therapy being cost-effective were approx. 14%, 44% and 42% respectively. The BSC strategy was not cost-effective (0% probability) for WTP values below $45K.

Study details	Vijayaraghavan et al.[63]
Population	Patients with mCRC in whom prior 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 USA 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	Life Years
Study type	Cost-effectiveness analysis: Markov model
Model assumptions	- Patients with KRAS mutant tumours received no benefit from EGFR-inhibitors - Patients with KRAS mutant tumours received some benefit from combination therapy containing FOLFIRI or Irinotecan - KRAS mutation testing has a sensitivity of 95% and a

Study details	Vijayaraghavan et al.[63]
	specificity of 100%
Perspective	Healthcare 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 with varying percentage of patients with KRAS wild-type tumours, cost of Cetuximab, cost of BSC, and cost of KRAS mutation test.
Outcome (cost and Lys/QALYs) per comparator	Panitumumab with KRAS mutation testing: LY 18.26 €13,787 $19,656 Panitumumab without KRAS mutation testing: LY 18.26 €18,399 $27,202 Cetuximab with KRAS mutation testing: LY 19.78 €13,588 $22,893 Cetuximab without KRAS testing: LY 19.78 €17,444 $30,933 Combination therapy 1 with KRAS mutation testing: LY 24.26 €26,292 $35,075 Combination therapy 2 with KRAS mutation testing: LY 25.83 €- $36,148 Combination therapy without KRAS mutation testing: LY 25.83 €35,852 $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.[64]
Population	Patients with mCRC who are chemorefractory
Time horizon	10 yrs
Objective	Assess the cost-effectiveness of screening for KRAS and BRAF mutations prior to EGFR-inhibitor treatment
Source of effectiveness information	RCTs
Comparators	1) no anti-EGFR therapy (best supp care) 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	Lifeyears
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	One way sensitivity analyses: 1) conversion probability for chemotherapy is 30% 2) conversion probability for bevacizumab is +10%, cetuximab is +20% 3) cost of surgery is +50%

Study details	Behl et al.[64]
	4) cost of screening is a) +50% and b) -50% 5) prognostic decrease in overall survival with BRAF mutation (regardless of treatment) Cohort simulation: A cohort of 50,000 patients is analyzed 10,000 times No PSA mentioned
Outcome (cost and Lys/QALYs) per comparator	No anti-EGFR therapy: $ 34,291 LY 0.6686 KRAS and BRAF mutation screening with anti-EGFR therapy $ 56,324 LY 0.7025 KRAS screening with anti-EGFR therapy $ 57,348 LY 0.7029 Anti-EGFR therapy without screening $ 64,841 LY 0.7055
Summary of incremental analysis	ICER (cost/LY) for KRAS and BRAF mutation screening as compared to no anti-EGFR therapy was $ 648,396 Other ICERS (KRAS screening compared to KRAS and BRAF mutation screening, no screening compared to KRAS mutation screening) were over 2 million dollar per LY gained.

Study details	Shiroiwa et al.[65]
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 yrs
Objective	Determine the cost-effectiveness of cetuximab treatment after KRAS mutation testing compared with best supportive care (BSC)
Source of effectiveness information	Progression free survival and overall survival were taken from the NCIC CO.17 trial
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	- Utility of progression free survival was assumed 0.7 for all treatments (cetuximab and BSC) - 40% of patients were assumed to have KRAS mutant tumours
Perspective	Healthcare payer's perspective
Discount rate	3% for both costs and effects
Uncertainty around cost-effectiveness ratio expressed	Cost-effectiveness acceptability curves
Sensitivity analysis	One way sensitivity analyses were performed for: discount rates, body surface area, % of patients with KRAS mutant tumours, BSC costs, hazard ratio of cetuximab for wild-type patients, and costs of KRAS mutation testing Also, a PSA was performed
Outcome (cost and Lys/QALYs) per comparator	KRAS mutation testing strategy: $ 29,000 LY 0.70 QALY 0.49 No-KRAS mutation testing strategy: $ 35,000 LY 0.69 QALY 0.48 No-cetuximab strategy: $ 6,800 LY 0.52 QALY 0.36

Study details	Shiroiwa et al.[65]
Summary of incremental analysis	KRAS testing versus no KRAS mutation testing: KRAS mutation testing dominant KRAS mutation testing versus no cetuximab: $180,000/QALY gained No KRAS mutation testing versus no cetuximab: $230,000/QALY gained

Study details	Blank et al.[67]
Population	Patients with mCRC who are chemorefractory
Time horizon	Lifetime
Objective	Assess cost-effectiveness of testing for KRAS and BRAF mutations prior to cetuximab treatment
Source of effectiveness information	CO.17 trial[66]
Comparators	1)KRAS mutation testing 2)KRAS mutation testing with subsequent BRAF testing of KRAS wild-types (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
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	Cost-effectiveness acceptability curves and CEA 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 overall and progression-free survival. PSA was performed as well
Outcome (cost and Lys/QALYs) per comparator	Reference treatment (no cetuximab): € 3,983 QALY 0.4430 KRAS and BRAF mutation testing: € 34,771 QALY 0.934 KRAS mutation testing: € 35,361 QALY 0.936 No testing: € 38,662 QALY 0.947
Summary of incremental analysis	KRAS and BRAF mutation testing compared to reference strategy: € 62,653/QALY KRAS mutation testing compared to KRAS and BRAF mutation testing: €313,537/QALY No testing compared to KRAS mutation testing: €314,588/QALY

Table 14: Checklist of study quality

	Ontario HTA report[62]	Vijayaraghavan et al.[63]	Behl et al.[64]	Shiroiwa et al.[65]	Blank et al.[67]
Study design					
The research question is stated	√	√	√	√	√
The economic importance of the research question is stated	x	√	√	√	√
The viewpoint(s) of the analysis are clearly stated and justified	√	√	x	√	√
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 effectiveness study are given (if based on a single study)	√	√	√	x	√
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
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 were given	x	NA	NA	x	x
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	√	√	√	√	√

	Ontario HTA report[62]	Vijayaraghavan et al.[63]	Behl et al.[64]	Shiroiwa et al.[65]	Blank et al.[67]
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 confidence intervals are given for stochastic data	x	×	×	×	×
The approach to sensitivity analysis is given	√	√	√	√	√
The choice of variables for sensitivity analysis is justified	x	√	√	×	√
The ranges over which the variables are varied are justified	x	×	×	×	√
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	x	√	√	√	√

4.2 Model structure and methodology

4.2.1 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 metastatic colorectal cancer 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'). Comparative effectiveness of treatment (cetuximab plus chemotherapy versus chemotherapy alone) conditional upon the true or false presence/absence of the KRAS mutation could then be determined. However, each different testing method targets a different range of mutations and has different limits of detection (lowest proportion of mutation detectable in tumour cells) and the exact combination of mutation type and level which 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 accuracy of tests (either based on objective response rate or tumour resection rate) to distinguish between patients with KRAS wild-type tumours and patients with KRAS mutant tumours with metastases confined to the liver, was only available for the Therascreen® KRAS RGQ PCR Kit[52] and pyrosequencing and MALDI-TOF.[54] A major assumption underlying the use of these accuracy data in the health economic modelling is, however, that differences in response rate and resection rate between the two trials are solely due to the use of different KRAS mutation tests.

In the COIN trial,[54] patients were tested with both pyrosequencing and 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 only available for pyrosequencing, therefore pyrosequencing is 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 (Section 3.2.1), whilst assuming a prognostic value equal to pyrosequencing across all tests. The latter assumption was not based on evidence of equality, but rather on absence of any reliable evidence to model a difference in prognostic value for these tests.

Based on the information available to us, two analyses were performed:

- '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 objective response rates 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, whereas resection rates for patients with KRAS mutant and unknown test results, who were treated with chemotherapy alone, were taken from the 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 trialists. PFS and OS after successful resection were assumed to be conditional on resection and treatment-independent.
- 'Assumption of equal prognostic value' analysis: for all tests for which information on technical performance were 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:
    - 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 (Matrix Assisted Laser Desorption Ionization Time-of-Flight) Mass spectrometry
- Next generation sequencing
- Sanger sequencing

4.2.2 Consistency with related assessments

This assessment does not update the appraisal of cetuximab for the first line treatment of metastatic colorectal cancer.[1] In order to ensure consistency between the modelling approach used in Technology Appraisal 176 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 176. This model calculates the expected cost-effectiveness of cetuximab compared to chemotherapy for the first line treatment of metastatic colorectal cancer 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 LightMix KRAS Kit (TIB MolBiol).

This model, together with the amendments suggested and made by the ERG 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.

4.2.3 Model structure

In the health economic model the mean expected costs and quality adjusted life years (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 as compared to the next cost-effective strategy.

The health economic analysis considers the long-term consequences of technical performance and accuracy of the different tests followed by treatment with cetuximab plus standard chemotherapy or standard chemotherapy alone in patients with metastatic colorectal cancer 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, since cetuximab is only indicated 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 one week, and a lifetime time horizon (23 years were modelled using 1,200 cycles). Health states in the Markov model are (numbered according to NICE Technology Appraisal 176[1]:

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

The Markov model structure is shown in Figure 9. The model is described in more detail in NICE Technology Appraisal 176.[1]

4.2.4 Model parameters

Estimates for model input parameters were retrieved from NICE Technology Appraisal 176 and the manufacturer's submission for TA176,[1,59,69] the assessment of the clinical effectiveness of different KRAS mutation tests (Sections 3.2.2), and an online survey of NHS laboratories in England and Wales (Section 3.2.1).

Figure 8: Decision tree structure

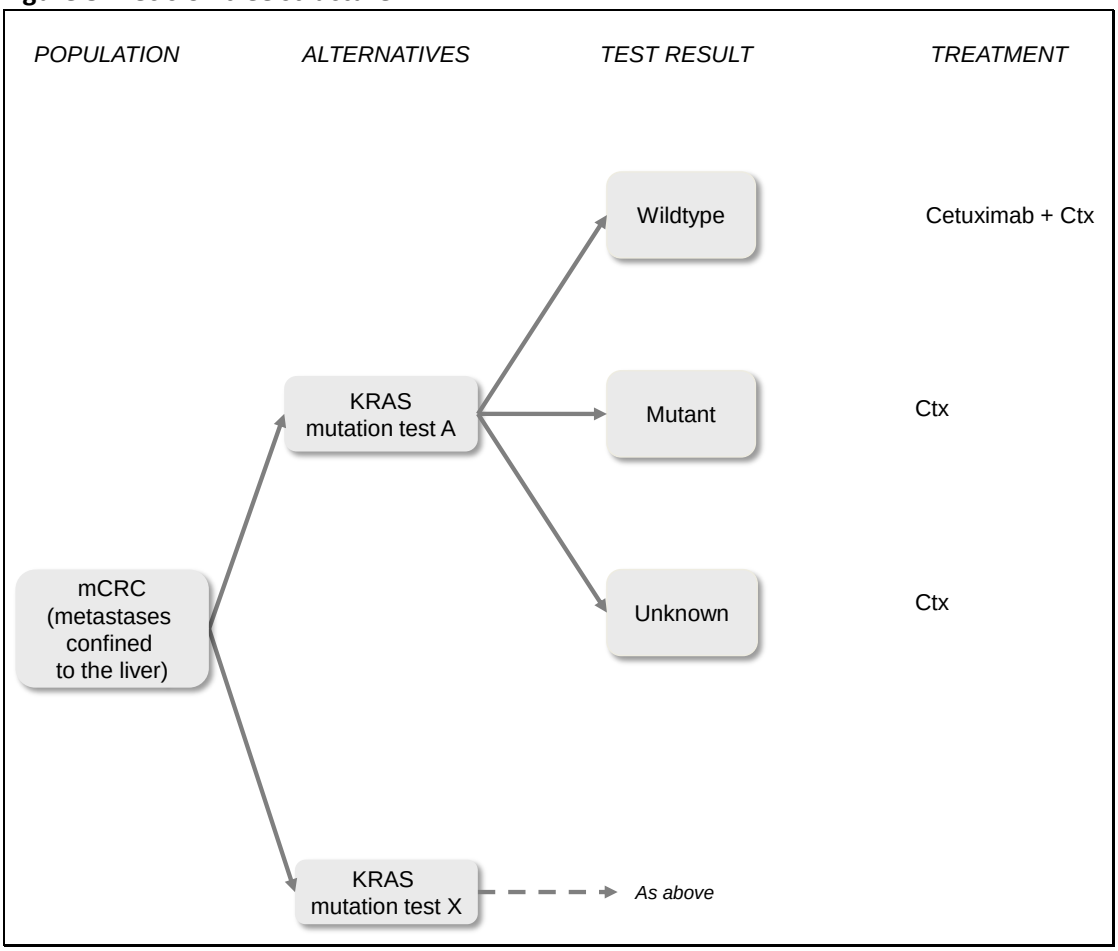
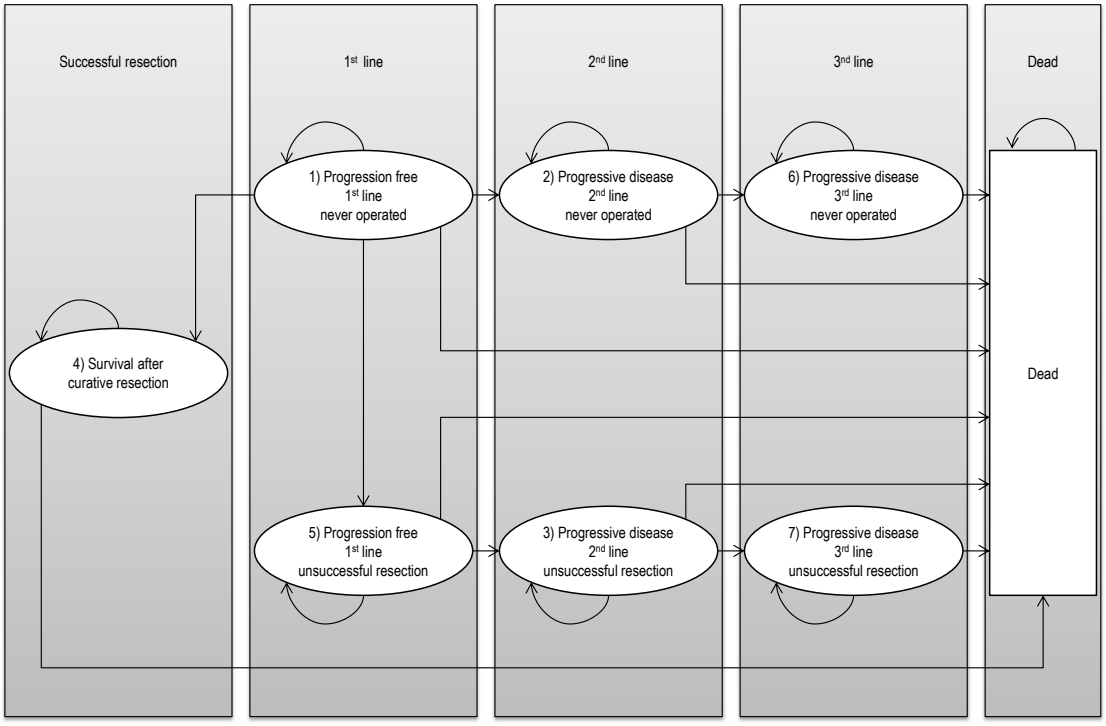


Figure 9: Markov model structure



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 Section 3.2.2) and the proportion of patients with an unknown test result. The proportion of patients with an unknown test result were 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 over estimate as the clinical trials are unlikely to be representative of the true situation in current clinical practice. 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 in the total proportion of pre-test failures (samples considered inadequate by the pathologist and therefore not sent to the laboratory). In the 'linked evidence' analysis, the proportion of unknowns was taken from the clinical trials. For the 'equal prognostic value' analysis, the proportion of unknowns for all tests was assumed to be equal to the pyrosequencing test, as the COIN trial (using pyrosequencing) was the only study reporting 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 proportion of true (wild-type) positives (TP), true (mutant) negatives (TN), false (mutant) negative (FN) and false (wild-type) positive (FP) 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.

Table 15: Input parameters used to calculate the proportion of patients with KRAS wild-type test result, unknown test result and KRAS mutant test result

Input parameter (Estimated value (se))			Distribution	Source
Proportion of patients with KRAS mutation positive tumours in England and Wales				
Proportion of mutation positives	65.2% (0.8%)		Beta	Andreyev 2001[70]
Test accuracy	**Sensitivity**	**Specificity**		
Therascreen® KRAS RGQ PCR Kit	74.6% (5.4%)	35.5% (8.5%)	Beta	Folprecht 2010[52]
Pyrosequencing	52.0% (9.8%)	45.6% (4.3%)	Beta	Maughan 2011[54]
Probability of unknown test result				
Therascreen® KRAS RGQ PCR Kit	10.8% (2.9%)		Beta	Folprecht 2010[52]
Pyrosequencing	1.7% (0.4%)		Beta	Maughan 2011[54]

Table 16: Probability of KRAS wild-type test result, unknown test result and 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 probabilistic sensitivity analysis.

Resection rate

Patients who are in the 'progression-free first line – never operated' state can move to 'survival after successful resection', 'progression free first line – unsuccessful resection', progression free 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 and the resection rate after treatment with chemotherapy alone was used for the remaining patients (KRAS mutant or unknown test results). As the CELIM trial did not contain a chemotherapy-only strategy, it was not possible to use resection rates from this trial for patients with KRAS mutant and unknown tumour status. Therefore, the resection rates for Therascreen® KRAS RGQ PCR Kit for these groups were taken from the GERCOR trial,[68] which was in line with STA 176.[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 STA 176[1] for the chemotherapy-only strategy were calculated based on

all patients (thus including patients with metastases not confined to the liver), and therefore probably are 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 was a population with liver-only metastases.

The resection failure rate was set at 5%[71] and the probability of postoperative mortality was 2.8% (se based on PSA: 1.2%; Beta PERT distribution),[72] both consistent with STA176.

Table 17: Resection rates

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

a: All resection rates were modelled using beta distributions.
b: In the 'equal prognostic value' analysis the response rate for pyrosequencing is used for all mutation tests.

Progression free and overall survival

To ensure consistency with NICE Technology Appraisal 176,[1] parametric survival models were obtained for patients without resection or with unsuccessful resection from this Technology Appraisal to estimate cycle-dependent progression free survival in the first and second line and overall survival in the first and third line. For patients with successful resection, parametric survival models were obtained from NICE Technology Appraisal 176[1] to calculate cycle-dependent progression free survival and overall survival probabilities (Table 18).

Progression free and overall survival in the first line for standard chemotherapy were based on data from the OPUS and CRYSTAL trials, respectively, [redacted] and were estimated separately for patients treated with or without cetuximab. All progression free and overall survival probabilities for the first line are presented in Figure 10.

Figure 10: Progression free survival and overall survival in first line

Table 18: Parametric survival models (based on NICE Technology Appraisal 176)

Probability for	Model distribution	Parameter	Estimated value	Standard error	Distribution	Source[c]
First line (Figure 10)						
						CRYSTAL[59, 69]
						CRYSTAL[59, 69]
						CRYSTAL[59, 69]
Progression to second line	Log normal	Cetuximab[a]	[redacted]	[redacted]	Multivariate normal[b]	OPUS[59, 69]
		Constant[a]			Multivariate normal[b]	OPUS[59, 69]
		LN(Sigma)			Multivariate normal[b]	OPUS[59, 69]
Survival	Log normal	Cetuximab[a]			Multivariate normal[b]	CRYSTAL[59, 69]
		Constant[a]			Multivariate normal[b]	CRYSTAL[59, 69]
		LN(Sigma)			Multivariate normal[b]	CRYSTAL[59, 69]
Second line (Figure 11)						
Progression to third line	Weibull	Constant[a]	[redacted]	[redacted]	Multivariate normal[b]	Tournigand[59, 68, 69]
		LN(Sigma)			Multivariate normal[b]	Tournigand[59, 68, 69]
Survival	Based on age dependent background mortality				Fixed	STA 176[1, 59, 69]
Third line (Figure 12)						
Survival	Weibull	Constant[a]	[redacted]	[redacted]	Multivariate normal[b]	Mittmann[59, 69]
		LN(Gamma)			Multivariate normal[b]	Mittmann[59, 69]
After successful resection (Figure 13)						
Progression	Log logistic	Constant[a]	[redacted]	[redacted]	Multivariate normal[b]	Adam[59, 69, 73]
		LN(Gamma)			Multivariate normal[b]	Adam[59, 69, 73]
Survival	Log logistic	Constant[a]			Multivariate normal[b]	Adam[59, 69, 73]
		LN(Gamma)			Multivariate normal[b]	Adam[59, 69, 73]

Abbreviations: LN = natural logarithm; a: Model coefficients; for the Weibull models the exponent of these coeffecients are used to calculate the lambda parameters; b: Cholesky decomposition was used to model the multivariate normal distribution; c: Parametric survival models were retrieved from Appendix H3 'parametric models' in the manufacturer's submission for TA176[59]

Time-dependent probabilities for (progression free) survival in the second line and third line were equal for patients who were treated with or without cetuximab and were converted to constant probabilities based on the median (progression free) survival (see Figures 11 and 12). These constant probabilities were used to prevent an unfeasible amount of tunnel states of 7,200 per comparator (1,200 cycles × 2 health states × 3 possible test results).

Figure 11: Progression free survival in second line

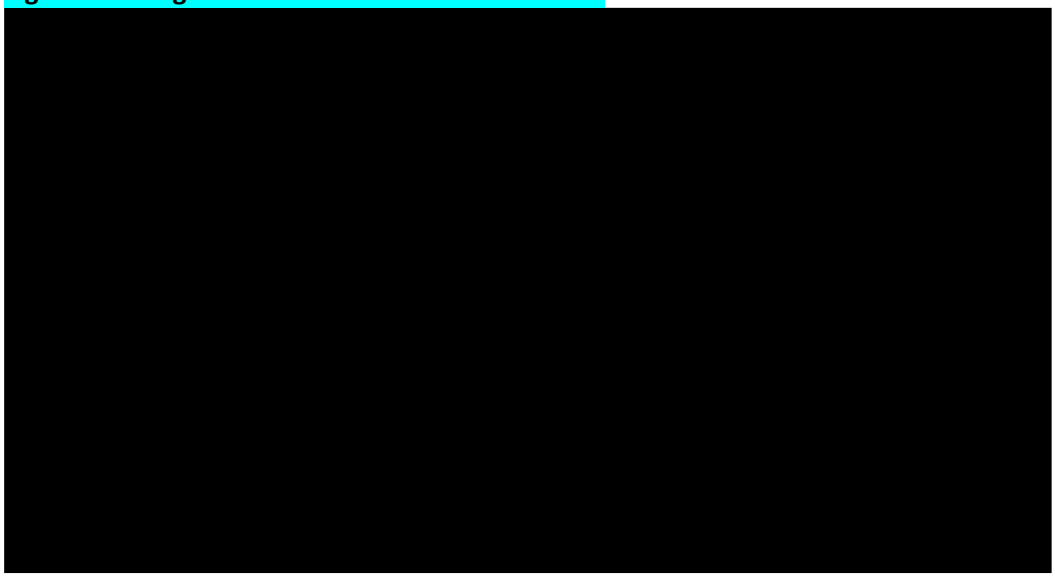
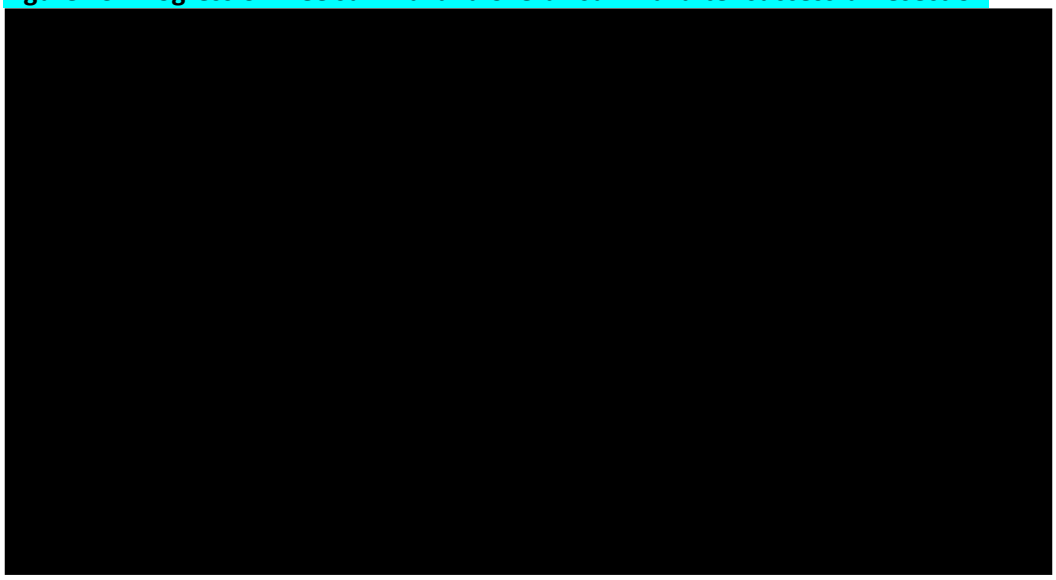


Figure 12: Overall survival in third line

Although progression free survival after successful resection was not incorporated as a separate health state, the probability of progression was estimated in order to incorporate the utility loss and increased costs associated with progression after successful curative resection. Estimated (progression free) survival is equal for patients who were treated with or without cetuximab (Figure 13).

Figure 13: Progression free survival and overall survival after successful resection



Adverse events

The occurrence of adverse events was assumed to be dependent on treatment and independent of tumour KRAS mutation status, i.e. occurrence of adverse events for patients with KRAS wild-type, KRAS unknown and KRAS mutant tumours were assumed to be equal among different test strategies. Consistent with STA 176, the occurrence of adverse events was only included in the model by incorporating the additional costs related to the adverse events based on the CRYSTAL and OPUS trials. These costs are discussed below in the section resource use and costs.

Health state utilities

Utility scores were retrieved from NICE Technology Appraisal 176 and presented in Table 19.

Table 19: Utility scores

Health state	Utility score	Se	Distribution	Source
Progression free (first line)	0.777	[redacted]	Beta	STA 176
Progressive disease (second line)	0.730	[redacted]	Beta	STA 176
Progressive disease (third line)	0.680	[redacted]	Beta	STA 176
Progression free (after successful resection)	[redacted]	[redacted]	Beta	STA 176
Progressive disease (after successful resection)	[redacted]	[redacted]	Beta	STA 176

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 health outcome due to delayed start of treatment. To investigate this, an item on turnaround time was included in the online survey. The results (Section 3.2.1) showed that the tests were very similar. In most laboratories, the turnaround times were generally between 3 and 7 days. One laboratory (out of eight reporting on the use of pyrosequencing) had a turnaround time of 1 to 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 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 the tests did not differ with respect to process disutility or health outcomes associated as a result of waiting for the test results.

Resource use and costs

Resource use and costs were taken from NICE Technology Appraisal 176,[29] with the exception of the KRAS test costs. These costs were based on the online survey of NHS laboratories in England and Wales.

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 (Table 6, Section 3.2.1) were used. As a price was reported for only one test in the online survey, it was decided to assume equal test costs across all tests at £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 with an unknown tumour mutation because the sample was considered inadequate by the pathologist before sending the specimen to the laboratory (pre-laboratory clinical failure), and patients with a sample considered adequate by the pathologist that results in a failure once inside the laboratory (technical failures within the laboratory). In the case of an unknown mutation status due to

a pre-laboratory clinical failure, no test costs were taken into account. In the case of an unknown mutation status due to a technical failure within the laboratory full test costs were taken into account. This proportion was calculated based from the proportion of patients with an unknown mutation status as taken from the literature and the total proportion of technical failures in the laboratories as reported in the online survey (Table 5, section 3.2.1), using the following formula:

Proportion of technical failures within the laboratory of all patients with an unknown test results =

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

The results of the calculations are presented in Table 20.

Table 20: Explanation of calculation of proportion of patients with unknown mutations status due to a technical failure in the laboratory per test

Test	Total proportion of patients with unknown test result (se)	Distribution	Source	Proportion of technical failures in laboratory	Number of reporting laboratories	Proportion of technical failures of patients with unknown test results (se)[c]	Distribution
Analysis 1[a]							
Therascreen® KRAS RGQ PCR Kit	10.8% (2.9%)	Beta	Folprecht 2010[52]	1.9%[b]	0	15.9% (8.3%)	Beta
Pyrosequencing	1.7% (0.4%)	Beta	Maughan 2011[54]	3.1%	7	100.0% (12.0%)[d]	Beta
Analysis 2[a]							
Pyrosequencing	1.7% (0.4%)	Beta	Maughan 2011[54]	3.1%	7	100.0% (13.8%)[d]	Beta
Therascreen® KRAS RGQ PCR Kit	As for Pyrosequencing			1.9%[a]	0	100.0% (16.6%)[d]	Beta
Cobas® KRAS Mutation Test (Roche Molecular Systems)	As for Pyrosequencing			4.5%	2	100.0% (5.6%)[d]	Beta
High resolution melt 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 (Qiagen)[a]	NA			NR	0	NA	Beta
KRAS LightMix kit (TIB MolBiol)[a]	NA			NR	0	NA	Beta
KRAS StripAssay (ViennaLab)[a]	NA			NR	0	NA	Beta
MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) Mass spectrometry[a]	NA			NR	0	NA	Beta
Next generation sequencing[a]	NA			NR	0	NA	Beta

Se = standard error, NA = not applicable, NR = not reported.

[a] 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 lacking evidence

[b] Average of the technical failures reported in the survey for the other tests

[c] Standard error based on probabilistic sensitivity analysis

[d] 'IF' statements were used to ensure this probability did not exceed 100%

Table 21: Other costs used

		Distribution	Source
Erbitux (1 mg)	£1.37	Fixed	STA 176
Irinotecan (1 mg)	£1.30	Fixed	STA 176
Folinic acid (1 mg)	£0.39	Fixed	STA 176
5Fluorouracil (1 mg)	£0.01	Fixed	STA 176
Oxaliplatin (1 mg)	£3.30	Fixed	STA 176
Cost of oncology outpatient attendance	£123.00	Beta PERT**	STA 176
Oncology outpatient attendance	£123.00	Beta PERT**	STA 176
Outpatient attendance for grade 3/4 adverse event (CRYSTAL)	£161.51	Beta PERT**	STA 176
Outpatient attendance for grade 3/4 adverse event (OPUS)	[redacted]	Beta PERT**	STA 176
Outpatient attendance for serious adverse event (CRYSTAL)	£165.91	Beta PERT**	STA 176
Outpatient attendance for serious adverse event (OPUS)	[redacted]	Beta PERT**	STA 176
Adverse event in 2nd line (outpatient visit) (CRYSTAL)	£191.27	Beta PERT**	STA 176
Adverse event in 2nd line (outpatient visit) (OPUS)	[redacted]	Beta PERT**	STA 176
Serious adverse event requiring hosptalization (CRYSTAL)	£1,170.83	Beta PERT**	STA 176
Serious adverse event requiring hosptalisation (OPUS)	[redacted]	Beta PERT**	STA 176
Hosptalisation for non-serious adverse event	£1,050.70	Beta PERT**	STA 176
Abdomen CT scan	£214.00	Beta PERT**	STA 176
Chest CT scan	£350.00	Beta PERT**	STA 176
Hepatic ultrasound	£95.00	Beta PERT**	STA 176

* Other cost data were commercial in confidence (not presented in manufacturer submission of STA 176) and thus not reported in this table

** Consistent with STA 176 the ± 50% of the estimated costs are used as minimum and maximum

4.3 Model analyses

Expected mean costs, life years (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% for both costs and effects. Based on the estimated outcomes (probabilistic), the incremental cost-effectiveness ratio (ICER) was calculated by dividing the incremental costs by the incremental QALYs. The ICER represents the costs of an additional QALY gained and was used to estimate the cost-effectiveness of a strategy opposed to 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 probabilistic sensitivity analyses with 5,000 simulations using parameter distributions as presented in this section.

4.3.1 Overview of main model assumptions

The main assumptions in the health economic analyses were:

1. The differences between objective response and resection rates for cetuximab plus chemotherapy versus chemotherapy alone reported in the CELIM trial combined with the GERCOR trial[52, 68] and those reported in the COIN trial[54] are solely due to the different tests used (Therascreen® KRAS RGQ PCR Kit and pyrosequencing, respectively) to distinguish between patients whose tumours are KRAS wild-type (and receive cetuximab) and patients whose tumours are KRAS mutant (and 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 (Table 8), patients tested as tumour KRAS wild-type were categorised as false positive if no objective response was observed (for Therascreen® KRAS RGQ PCR Kit) or no liver resection was performed (for pyrosequencing) after treatment with cetuximab, while patients were categorised as true positive if objective response was observed, or a liver resection was performed, respectively. Similarly, patients tested as tumour KRAS mutant were categorised as false negative if an objective response was observed (for Therascreen® KRAS RGQ PCR Kit) or a liver resection was performed after treatment with cetuximab (for pyrosequencing) while patients were categorised as true negative if no objective response was observed or no liver resection was performed (both analyses).
3. Test accuracy based on objective response can be compared with accuracy based on resection rates.[39]
4. The proportion 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 with both pyrosequencing and MALDI-TOF with a reported concordance of >99%, it was assumed that the accuracy as derived from this trial and also the resection rates reported here apply to both pyrosequencing and MALDI-TOF. That is, all pyrosequencing results in this report also apply to MALDI-TOF.
6. The standard chemotherapy applied in the COIN-trial[54] (FOLFOX or XELOX) is comparable to FOLFOX6 as used in the CELIM trial.[52]

4.3.2 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 average of first and third line mortality instead of background mortality as in STA 176.
- the proportion of unknown patients was based on the results of the online survey instead of the literature (Table 5, Section 3.2.1).

4.4 Results of cost-effectiveness analyses

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

4.4.1 'Linked evidence' analysis

The 'linked evidence' analysis includes two tests, i.e. only those tests for which evidence on test accuracy based on either resection rate or objective response 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 section 4.3.1. In short, we have only the COIN and CELIM trials to rely on, of which COIN[54] used pyrosequencing to test for KRAS mutations and CELIM[52] 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; section 4.3.1); 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 assume that all KRAS wild-type patients would respond perfectly to cetuximab - or would all have a liver resection after cetuximab - and all KRAS mutant patients would not (assumption 2; section 4.3.1), and that test accuracy based on objective response can be compared with accuracy based on resection rates (assumption 3; section 4.3.1).

As is apparent from Table 22, pyrosequencing results in the lowest total cost. The Therascreen® KRAS RGQ PCR Kit is the more expensive but also more effective strategy, at an ICER of £17,019 per QALY gained. The cost-effectiveness acceptability curve in Figure 14 shows that for lower values of the threshold, pyrosequencing is to be preferred, and that the Therascreen® KRAS RGQ PCR Kit is the most cost-effective option at thresholds of £17,000 and higher. The results of the sensitivity analyses (Table 22) do not differ substantially from the base case, 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. Cost-effectiveness acceptability curves for the sensitivity analyses are presented in Appendix 6.

Table 22: Probabilistic results for 'linked evidence' analysis: base case and sensitivity analyses

Strategy	Costs	QALYs	Δ Costs	Δ QALYs	ICER
Base case					
Pyrosequencing*	£30,870	1.49			
Therascreen® KRAS RGQ PCR Kit	£33,995	1.67	£3,125	0.18	£17,019
Sensitivity analysis: mortality 2nd line based on average of 1st and 3rd line mortality					
Pyrosequencing*	£29,704	1.28			
Therascreen® KRAS RGQ PCR Kit	£33,132	1.51	£3,428	0.23	£14,860
Sensitivity analysis: unknowns from survey					
Pyrosequencing*	£30,714	1.48			
Therascreen® KRAS RGQ PCR Kit	£34,799	1.69	£4,085	0.20	£20,528

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry

Figure 14: Cost-effectiveness acceptability curve for 'linked evidence' analysis, base case

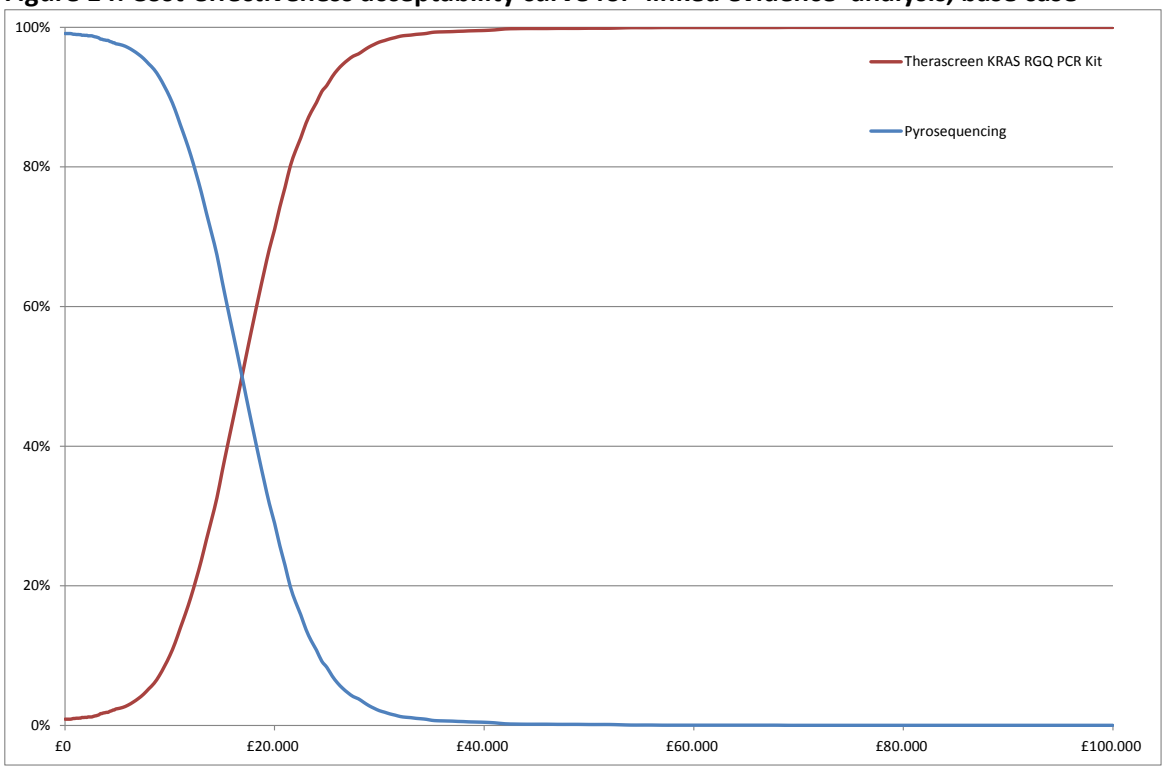


Table 23: Comparison of the study populations across the trials used in the 'linked evidence' analysis

Study details	Participant selection	Population characteristics
Study Details Folprecht (CELIM)(2010)[52] **Country** Germany and Austria **Study Design** RCT **Number randomised:** 111 **Number KRAS wild-type randomised:** 70 **Number with liver limited metastases randomised:** 111 **Intervention:** Cetuximab + FOLFOX versus Cetuximab + FOLFIRI	**Inclusion criteria** Unresectable, histologically confirmed colorectal liver metastases; no extra-hepatic 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 anti-tumour therapy, clinically relevant coronary artery disease, infl ammatory bowel disease, previous malignancy, and age < 18 years.	**Median Age (range):** 63(56-71) **Number Male:** 71 **Liver Metastases:** Number with <5 metastases:30 Number with 5-10 metastases:58 Number with >10 metastases:19 Number with NR metastases:4 Number 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

Study details	Participant selection	Population characteristics
Study Details Maughan (COIN)(2011)[54] **Country** UK and Republic of Ireland **Study Design** RCT **Number randomised:** 1630 **Number KRAS wild-type randomised:** 729 **Number with liver limited metastases randomised:** 178 **Intervention:** Cetuximab + standard chemotherapy versus standard chemotherapy	**Inclusion criteria** Adults (18 years or older); histologically confirmed adenocarcinoma of the colon or rectum; inoperable metastatic or locoregional disease; no previous chemotherapy fro metastatis 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 > grade 1; requirement for treatment with contra-indicated medication; another previous or current malignant disease which may affect treatment response; known hypersensitivity to any tudy treatment; brain metastases.	**Median Age (range):** 64(56-70) **Number Male:** 498 **Liver metastases:** Resection rates reported separately for patients with liver-only metastases **Criteria for unresectability:** NR **Previous treatments:** NR

Study details	Participant selection	Population characteristics
Study Details Tournigand (GERCOR)(2004)[68] **Country** France **Study Design** RCT **Number randomised:** 226 (of whom 6 not eligible) **Number KRAS wild-type randomised:** NA **Number with liver limited metastases randomised:** NR **Intervention:** FOLFIRI + FOLFOX (arm A) vs FOLFOX + FOLFIRI (arm B)	**Inclusion criteria** Adults (age 18-75); Adenocarcinoma of the colon or rectum; unresectable metastases; at least one bidimensionally measurable lesion of ≥ 2 cm or a residual nonmeasurable 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** CNS metastases, second malignancies, bowel obstruction, current diarrhea ≥ grade 2, symptomatic angina pectoris, or disease confined to previous radiation fields	**Median Age (range):** Arm A: 61 (29-75) Arm B: 65 (40-75) **Number Male:** 142 (of 220) **Metastases:** Liver: 184 (84%) Lung: 67 (30%) Other: 98 (45%) Resection rates not reported separately for patients with liver-only metastases **Number 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

4.4.2 '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 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 (Table 24). In the base case and in the first sensitivity analysis, the total technical failure rate (pre-laboratory plus within laboratory technical failures) is assumed equal for all tests. As a result, the strategies in these analyses only differ with respect to costs (due to differences in within-laboratory technical failures). In the base case, the average QALYs for all comparators were 1.48 (95% CI: 1.33 - 1.64). The total costs associated with the various testing strategies (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 - 1.44).

Table 24: Probabilistic results for 'assumption of equal prognostic value' analysis, base case

	Costs (95% CI)	**Δ Costs** (95% CI)**
High resolution melt analysis	£30,857.09 (£27,079.58 - £34,736.14)	
Sanger sequencing	£30,857.09 (£27,079.58 - £34,736.14)	£0.00 (£0.00 - £0.00)***
Therascreen® KRAS RGQ PCR Kit	£30,857.46 (£27,079.91 - £34,736.60)	£0.37 (£0.12 - £0.88)
Pyrosequencing*	£30,857.70 (£27,080.27 - £34,737.03)	£0.61 (£0.14 - £1.64)
Cobas® KRAS Mutation Test	£30,857.99 (£27,080.25 - £34,737.14)	£0.91 (£0.23 - £2.28)

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry
**Compared to least expensive comparator
***Costs were equal for High resolution melt analysis and Sanger sequencing as the proportion of failed tests in the laboratory was equal for both comparators (0%).

Table 25: Probabilistic results for 'assumption of equal prognostic value', sensitivity analysis: mortality in second line based on average of first and thirdline

	Costs (95% CI)	Δ Costs** (95% CI)
High resolution melt analysis	£29,661.10 (£25,991.06 - £33,401.42)	
Sanger sequencing	£29,661.10 (£25,991.06 - £33,401.42)	£0.00 (£0.00 - £0.00)***
Therascreen® KRAS RGQ PCR Kit	£29,661.47 (£25,991.81 - £33,401.80)	£0.37 (£0.12 - £0.85)
Pyrosequencing*	£29,661.71 (£25,992.12 - £33,401.81)	£0.61 (£0.14 - £1.59)
Cobas® KRAS Mutation Test	£29,662.00 (£25,993.07 - £33,402.58)	£0.90 (£0.23 - £2.18)

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry
**Compared to least expensive comparator
***Costs were equal for High resolution melt analysis and Sanger sequencing as the proportion of failed tests in the laboratory was equal for both comparators (0%).

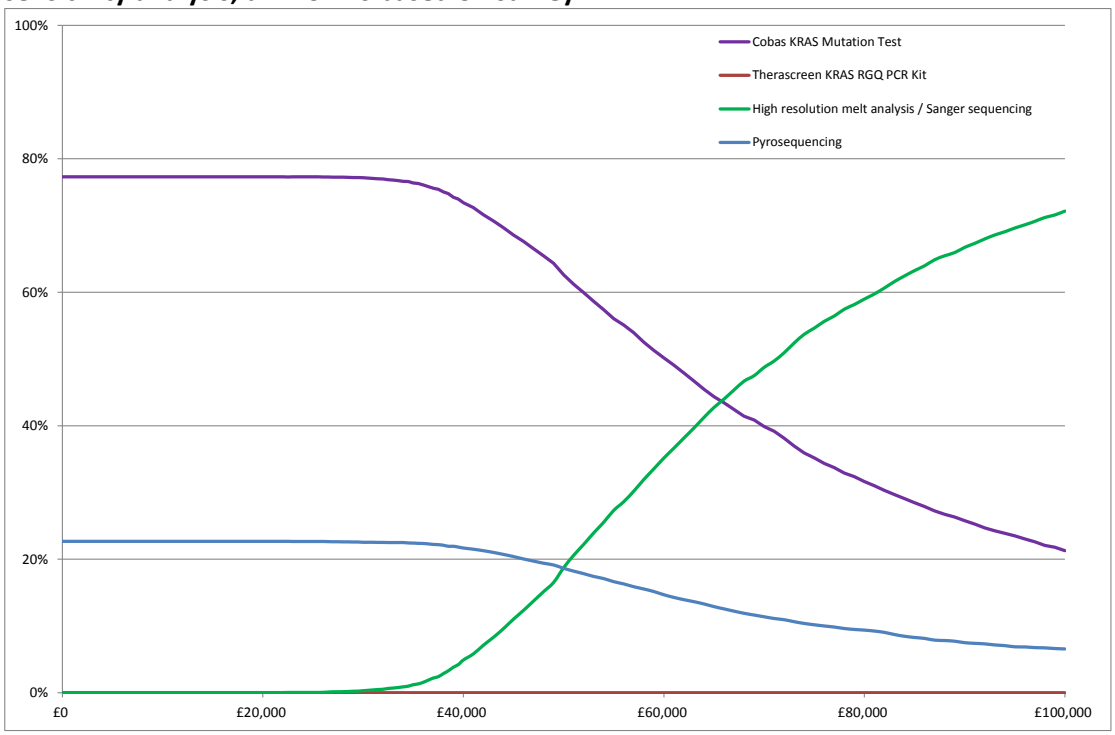
In the second sensitivity analysis the total technical failure rate is also test specific, which impacts the proportion of patients with unknown (and therefore also wild-type and mutant) tumour KRAS status. Therefore, in this sensitivity analysis, the strategies differ with respect to both effects and 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. High resolution melt analysis and Sanger sequencing have equal costs and effects and their ICER compared to 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 cost-effectiveness acceptability curve (Figure 15) it is apparent that the Cobas® KRAS Mutation test is the preferred strategy for all threshold values below £60,000.

Table 26: Probabilistic results for 'assumption of equal prognostic value' sensitivity analysis, unknowns based on survey

	Costs	QALYs	Comparator	Δ Costs	Δ QALYs	iCER
Cobas® KRAS Mutation Test	£30,663	1.48				
Pyrosequencing*	£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
High resolution melt analysis	£31,006	1.49	Cobas® KRAS Mutation Test	£343.64	0.005	£69,815**
Sanger sequencing	£31,006	1.49	Cobas® KRAS Mutation Test	£343.64	0.005	£69,815**

* Pyrosequencing results also apply to MALDI-TOF Mass spectrometry
** High resolution melt 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, unknowns based on survey



5. DISCUSSION

5.1 Statement of principal findings

5.1.1 Clinical effectiveness

There was no clear evidence to suggest any differences between 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 upon 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 approach (9 out of 15 respondents); reasons cited by respondents for their choice of this technique were: proportion of tumour cells required; ease of use; cost; mutations covered; turnaround time; 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 responding laboratories reported that KRAS mutation testing was one 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 (≤ 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 less than three 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 where the same test method was being used. Where 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 prior to analysis was < 2% for all responding laboratories.

The rate of failures for analysed samples did not appear to be dependent upon 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 micro-dissection techniques prior to DNA extraction, however, there was no clear indication that none use of this technique was associated with higher rates 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 costs 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 to £150), with one laboratory reporting a higher cost (£273) for running a single sample. Prices charged, to both Merck Serono and the NHS, ranged for £99 to £150.

When contacted by NICE in relation to a previous diagnostic assessment on 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 USA 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.[15] The clinical trial 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 best supportive care to best supportive care alone in patients with metastatic CRC who had previously failed all available chemotherapy.[74] 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, 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.[52] This study reported data for 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, reported in section 3.2.2 of the results, (70.2% (95% CI: 57.7 to 80.7%)) 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 objective response rates 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 rate 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: 22.4 to 61.2%)) 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 allowed the calculation of estimates for the accuracy of pyrosequencing and MALDI-TOF (where 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 respectability following treatment with cetuximab plus standard chemotherapy, where as 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;[54] 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 and COIN trials, 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) 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 which were confined to the liver.[27, 28, 53-55, 58] The standard chemotherapy regimen was different in each of the four trials: FOLFOX4;[28, 53, 58] FOLFIRI;[27, 53] FOLFIRI or FOLFOX6;[55] 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 used (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 which assessed objective response rate reported a statistically significant higher response rate for participants treated with cetiximab plus standard chemotherapy compared to those treated with standard chemotherapy alone; ORs ranged from 3.00 (95% CI: 1.49, 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 which 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 on cetuximab for the first line treatment of metastatic colorectal cancer.[1] 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 Xu trial[55] were published subsequently to TA176.

5.1.2 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 metastatic colorectal cancer found four full papers and one HTA report. 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 as compared to standard chemotherapy alone for all patients seems rather high.

In the health economic analysis, 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 metastatic colorectal cancer was assessed. In light of the scarce evidence that was 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 to pyrosequencing, using the available objective response and resection rate, respectively, in order 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 at an ICER of £17,019 per QALY gained. Sensitivity analyses did not show substantial differences compared to the base case. The key driver behind the outcome was the difference in resection rate between treatment with and without cetuximab and the proportion 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 objective response rate (for 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 section 4.3.1. The following assumptions used were particularly problematic since they are open to doubt and probably have a considerable impact on the model results:

- The differences between objective response and resection rates for cetuximab plus chemotherapy versus chemotherapy alone as reported in the CELIM trial[52] combined with the GERCOR trial[68] and those reported in the COIN trial[54] are solely due to the different tests used (Therascreen® KRAS RGQ PCR Kit and pyrosequencing, respectively) to distinguish between patients whose tumours are KRAS wild-type (and receive cetuximab) and patients whose tumours are KRAS mutant (and 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 (Table 8), patients tested as KRAS wild-type tumour were categorised as false positive if no objective response was observed (for Therascreen® KRAS RGQ PCR Kit) or no liver resection was performed (for pyrosequencing) after treatment with cetuximab, while patients were categorised as true positive if objective response was observed, or a liver resection was performed, respectively. Similarly, patients tested as KRAS mutant tumour were categorised as false negative if an objective response was observed (for Therascreen® KRAS RGQ PCR Kit) or a liver resection was performed after treatment with cetuximab while patients were categorised as true negative if no objective response was observed or no liver resection was performed.

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 probabilistic sensitivity analysis.

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 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 only differ 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, for which the rate of unknowns 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 high resolution melt analysis share a position in being most costly and most effective at an ICER of £69,815 per QALY gained compared to the Cobas® KRAS Mutation Test. The other two strategies included in this analysis, i.e. the Therascreen® KRAS RGQ PCR Kit and pyrosequencing, are ruled out by extended dominance.

5.2 Strengths and limitations of assessment

5.2.1 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 trials registers and conference abstracts to identify unpublished studies. Because of the known difficulties in identifying test accuracy studies using study design-related search terms,[75] and potential need to include non-randomised controlled trials, search strategies were developed to maximise sensitivity at the expense of reduced

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, e.g. a significant difference between the treatment and control groups which favours treatment. This is not the case for test accuracy studies, which measure agreement between index test and 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 which provided accuracy data, as these studies either aimed to assess the effectiveness of treatment with cetuximab plus standard chemotherapy in different patient groups, or to compare the effectiveness of cetuximab plus standard chemotherapy compared to standard chemotherapy alone; neither study was primarily focussed upon test performance. Our review included very small numbers 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 compared to 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 the one protocol modification that occurred during the assessment is noted in Appendix 9. The eligibility of studies for inclusion is therefore transparent. In addition, we have provided specific reasons for excluding all of the studies considered potentially relevant at initial citation screening (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 done 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 study design and/or the type of data extracted. Studies which provided data

on the accuracy of KRAS mutation testing to predict response to treatment with cetuximab plus standard chemotherapy were assessed using a modification of the QUADAS-2 tool. [48] QUADAS-2 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 domain are also, separately rated for concerns regarding the applicability of the study to the review question (low, high, or unclear). Studies which 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 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 (Appendix 3) and in summary in the results (sections 3.2.2 and 3.3.3). 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 to predict response to treatment with cetuximab plus standard chemotherapy) and blinding of participants and personnel in treatment trials. Both of the studies which provided data on the accuracy of KRAS mutation testing to predict response to treatment had some limitations in their applicability to the target population for this assessment. In the case of the CELIM trial data were only available to calculate accuracy for prediction of objective response, rather than for the preferred direct measure, resection of liver,[52] and in the case of the COIN trial the standard chemotherapy regimen did not fully match the inclusion criteria for this assessment.[54] In addition, participants in the CELIM trial were described as having technically non-resectable or ≥ five liver metastases from CRC and it was therefore unclear whether some participants may have had potentially resectable metastases at baseline.[54]

All of the studies included in this review have some limitations in respect of their ability to address the overall aim of comparing the clinical effectiveness of different KRAS mutation tests to determine which patients are may benefit from addition of cetuximab to standard chemotherapy and which should receive standard chemotherapy alone. The COIN trial 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 versus standard chemotherapy alone) for both patients with KRAS wild-type and KRAS mutant tumours. In addition, the trial was conducted in the UK and hence provides data which 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.[54] Further, the standard chemotherapy regimen used in the COIN trial allowed a choice between FOLFOX or XELOX (depending upon local hospital practice and patient preference);[54] the use of XELOX as standard chemotherapy does not match the inclusion criteria for this assessment, as determined by the recommendations of TA176.[1] Data from the COIN trial were for KRAS mutation testing using a combination of pyrosequencing and MALDI-TOF and targeting mutations in codons 12, 13 and 61; 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 KRAS mutation status can differ both in terms of the mutations targeted and limit of detection (the lowest proportion of tumour cells with a mutation that can be detected), the definition of KRAS wild-type versus mutant varies 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 '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 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 would be required (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 positive) 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 TA176,[1] obtaining these data may be problematic, since it could be argued that a trial where 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 was published after TA176, more recent UK trials such as New EPOC have tended to focus on determining the

effectiveness of adding cetuximab to standard chemotherapy in patients with KRAS wild-type tumours.[76] The recently complete, but as yet un-published, New EPOC trial was a randomised open-label comparison of oxaliplatin/irinotecan plus fluorouracil plus cetuximab with 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.[76] 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 which 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 different KRAS mutation tests are able to respectability of liver metastases following treatment with cetuximab plus standard chemotherapy; outcome data (resection rates or objective response) are reported for both patients with KRAS wild-type and KRAS mutant tumours. However, we were only able to identify one of this type, the CELIM trial, which all used an older version of the Therascreen® RGQ PCR Kit.[52] Neither the CELIM or COIN trials 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 were described as having technically non-resectable or ≥ five liver metastases from CRC and it was therefore unclear whether some participants may have had potentially resectable metastases at baseline,[52] where as the COIN trial explicitly excluded patients receiving combination chemotherapy prior to resection of operable liver metastases.[54] This difference may partially account for the marked difference in resection rates, for patients with KRAS wild-type tumours who were treated with cetuximab plus standard chemotherapy, observable between the two studies;

[redacted],[59] compared with a resection rate of 13/87 (15%) from the CELIM trial. [redacted],[59] where as the COIN trial focused on "potentially curative liver resections,"[54] and the standard chemotherapy regimens were different in the two trials.

Trials which compared the effectiveness of cetuximab plus standard chemotherapy with that of standard chemotherapy alone in patients with unresectable liver-limited metastases from CRC, whose tumours were KRAS wild-type, were also included in this review. These trials were included with the aim of providing some indication on how the favourable effects for 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 versus 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.

5.2.2 Cost-effectiveness

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 metastatic colorectal cancer, where metastases are confined to the liver and are initially unresectable. In order 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. objective response rate, 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 comparative effectiveness of treatment

(chemotherapy plus cetuximab versus chemotherapy alone) conditional upon the true presence/absence of the KRAS mutation would be determined. However, different testing method targets different ranges of mutations and have different limits of detection (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, objective response rate 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 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 only available for two tests (the Therascreen® KRAS RGQ PCR Kit and pyrosequencing); this was derived from objective response rate 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 as observed in the two included studies from which these data were derived,[52, 54] and therefore also differences in the subsequent progression free and overall survival, 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 characteristics of the respective study populations (i.e. with respect to the type of metastases) or differences in the standard chemotherapy regimen. In addition, if the assumption of comparability of accuracy rates based on different measures (i.e. objective response rate and resection rate) holds true,[39] this would reduce the likelihood that the main assumption holds.

5.3 Uncertainties

5.3.1 Clinical effectiveness

As noted in section 5.2.1 'Strengths and Limitations', one important consideration when selecting an KRAS mutation testing method is the variation between tests in 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, where mutations are present at a very low level (a low proportion of tumour cells harbouring mutation). None of the studies which met the inclusion criteria for this review reported any data on variation in treatment effect with 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-types, was derived from patients selected using tests which target mutations in codons 12 and 13; only the COIN trial used a test method which also targeted mutations in codon 61.[54] Indeed, although no testing method was specified, the ASCO PCO published in 2009 stated that "all patients with metastatic CRC who are candidates for anti-EGFR antibody therapy should have their tumour tested for KRAS mutations in a Clinical Laboratory Improvement Amendments (CLIA)-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients with metastatic CRC should not receive anti-EGFR antibody therapy as part of their treatment."[14] The PCO also highlighted the uncertainty around the clinical relevance of detecting rare mutations in codons 61 and 146.[14] The COIN trial 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.[54] The additional clinical value of using tests which target a wider range of mutations remains uncertain, since the low frequency of most KRAS mutations makes it very difficult to adequately assess treatment effects or resistance to EGFR inhibitors in patients with mutations these mutations. A large, multi-centre observational study conducted in Italy by the KRAS aKtive network (a program 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 metastatic CRC patients who require KRAS mutation testing) has collected data on a total of 7,432 KRAS mutation analyses.[77] 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.[77] In addition to the issue of rare mutations, questions have been raised as to whether all codon 13 mutations predict lack of benefit from treatment with EGFR inhibitors; De Roock et al suggested that the KRAS Gly13Asp may not predict lack of benefit.[78] The COIN trial 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 Gly13Asp 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).[54]

As discussed in section 5.2.1 '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 where 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 'over treatment' 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 overall survival between patients with KRAS mutant tumours treated with cetuximab plus standard chemotherapy and those treated with standard chemotherapy alone. The CRYSTAL trial also reported no difference in objective response rates or PFS,[28] where as the OPUS trial reported a lower objective response rate, 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 compared with those treated with standard chemotherapy alone.[27] These data were for all patients in the trials with KRAS mutant tumours; for both the CYSTAL 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 compared and those treated with standard chemotherapy alone.

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. Routine testing could be argued to avoid 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 that of whether a stored biopsy sample form 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 metastasis site is preferable. Use of the primary tumour sample is likely to be considered preferable since all patients should have already undergone biopsy at diagnosis for histological typing, thus the risks and discomfort of further invasive procedures (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 (Han et al.) identified by our searches, which did not meet the inclusion criteria for this assessment, assessed the concordance of KRAS mutations between primary colorectal cancer tissue and metastatic colorectal cancer tissue.[79] 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 concordancy rate of 94.1% (95% CI: 88.3 to 95.0%).[79] One of the primary studies included in the Han review specifically assessed KRAS mutation concordancy between primary colorectal tumours and liver metastases in 305 paired samples; KRAS mutation status was determined based on pyrosequencing of codons 12 and 13.[32] This study reported a concordancy 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.[32] Though 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 testing using liver biopsy samples is unlikely to produce significant clinical benefit.

A variety of KRAS mutation testing methods are 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 reported >99% concordance on KRAS genotyping between pyrosequencing and MALDI-TOF,[54] 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 where 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 unresectable liver-limited CRC metastases remains uncertain. The Therascreen® KRAS RGQ PCR Kit is the only product currently approved by the FDA, 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.[15] 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 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 (ViennaLab); MALDI-TOF mass spectrometry of codons 12, 13 and 61 used alone; high resolution melt analysis of codons 12, 13 and 61 used alone. As was the case for pyrosequencing, concordance between the two KRAS mutation testing methods used means that accuracy data derived from the COIN 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.

5.3.2 Cost-effectiveness

Major assumptions were made in order 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 in the previous section, it is questionable whether this assumption would hold true. Furthermore, in order to calculate the proportion of patients with a KRAS wild-type and KRAS mutant test result, patients with a KRAS wild-type test result were categorised as false positive if no objective response was observed on cetuximab (for the Therascreen® KRAS RGQ PCR Kit) or when no liver resection was performed (for pyrosequencing), while patients were categorised as true positive if a objective response was observed or a resection was performed. Likewise, patients with a KRAS mutant test result were classified as true negative when no objective response was observed on cetuximab (for the the Therascreen® KRAS RGQ PCR Kit) or no resection was performed (for pyrosequencing), while an objective response or a liver resection would imply a classification as false negative. Ideally, the categorisation of true/false positives/negatives 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 current, 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 appraisal of cetuximab for the first line treatment of mCRC (NICE Technology Appraisal 176,[1, 59, 69] the assumptions underlying that appraisal also apply to this assessment. An example, which only applies 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 tumours treated with standard chemotherapy, while 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 probabilistic sensitivity analyses or in the cost-effectiveness acceptability curves.

6. CONCLUSIONS

6.1 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 at an ICER of £17,019 per QALY gained. Sensitivity analyses did not show substantial differences compared to the base case. The key driver behind the outcome was the difference in resection rate between treatment with and without cetuximab and the proportion of patients with KRAS wild-type, KRAS mutant, and unknown tumour status, which is determined by test accuracy and therefore, for the most part, dependent on objective response rate (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 as 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.

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 high resolution melt analysis share a position in being most costly and most effective at an ICER of £69,815 per QALY gained compared to the Cobas® KRAS Mutation Test. The other two strategies included in this analysis, i.e. the Therascreen® KRAS RGQ PCR Kit and pyrosequencing, are ruled out by extended dominance.

There are no data on the clinical or cost-effectiveness of next generation sequencing of codons 12, 13 and 61; KRAS stripAssay (ViennaLab); MALDI-TOF mass spectrometry of codons 12, 13 and 61 used alone; high resolution melt analysis of codons 12, 13 and 61 used

alone. No published studies were identified for any of these methods and neither method is currently in routine clinical use in any of NHS laboratories in England and Wales who responded to our survey.

6.2 Suggested research priorities

The available data have limitations in respect of their ability to address the overall aim of this assessment, 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 which provide full information on the comparative treatment effect (cetuximab plus standard chemotherapy versus standard chemotherapy alone) for both patients with KRAS wild-type and 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 (both methods used for all samples). 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 where 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 re-test stored samples from previous studies, where 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, where by 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.

Some methods of KRAS mutation testing, e.g. 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 or 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 upon information gained from the two study types described above, preliminary research to develop a multi-factorial 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 which will eventually help determine more accurately which patients are most likely to benefit from the addition of treatment with cetuximab to 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.

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APPENDIX 1: LITERATURE SEARCH STRATEGIES

Clinical effectiveness search strategies

CRC + KRAS (limit: 2000-C)

Embase (OVIDSP): 2000-2013/wk3
Searched: 22.1.13

1 exp colon cancer/ or exp rectum cancer/ or colorectal tumor/ (169199)
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$)).ti,ab,ot,hw. (245923)
3 (m-CRC or CRC).ti,ab,ot. (14043)
4 ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ 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$)).ti,ab,ot. (26)
7 or/1-6 (249697)
8 k ras oncogene/ (4953)
9 (k ras or kras or k-ras or V-Ki-ras$ 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. (17025)
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 (17279)
22 7 and 21 (5716)
23 limit 22 to yr="2000 -Current" (5036)
24 limit 23 to embase (4540)

Medline (OVIDSP): 2000-2013/1/wk2
Searched: 22.1.13

1 exp Colorectal Neoplasms/ (134723)
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$)).ti,ab,ot,hw. (165769)
3 (m-CRC or CRC).ti,ab,ot. (8215)
4 ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ 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$)).ti,ab,ot. (23)
7 or/1-6 (170682)
8 Genes, ras/ (11077)
9 (k ras or kras or k-ras or V-Ki-ras$ 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 (16696)
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 Citation (OvidSP): up to 2013/01/21
Medline Daily Update (OvidSP): up to 2013/01/21
Searched 22.1.13

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): 2000-2012/ Issue 12
Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley): 2000-2012/ Issue 12
Database of Abstracts of Reviews of Effects (DARE) (Wiley): 2000-2012/ Issue 4
Health Technology Assessment Database (HTA) (Wiley): 2000-2012/ Issue 4
Searched 22.1.13

#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

CDSR search retrieved 9 references.
CENTRAL search retrieved 65 references.
DARE search retrieved 11 references.
HTA search retrieved 9 references.

NIHR Health Technology Assessment (HTA) (Internet): up to 2013/01/25
http://www.hta.ac.uk/
Searched 25.1.13

Browsed by relevant terms found 2 references

Science Citation Index (SCI-EXPANDED) (Web of Knowledge): 2000-2013/01/22
Conference Proceedings Citation Index (CPCI-S) (Web of Knowledge): 2000-2013/01/22
Searched 23.1.13

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

 # 18 3,597 #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$screen* or therascreen*)
8 96 TS=(Kirsten NEAR/3 (murine or rat) NEAR/3 sarcoma*)
7 10,467 TS=(k ras or kras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)
6 134,422 #1 or #2 or #3 or #4 or #5
5 1,328 TS=(lower SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))
4 1,113 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 9,622 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): 2000-2013/01/22
Searched 23.1.13

Databases=BIOSIS Previews Timespan=2000-2013

 # 18 2,641 #17 AND #6
17 8,621 #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$screen* or therascreen*)
8 153 TS=(Kirsten NEAR/3 (murine or rat) NEAR/3 sarcoma*)
7 8,611 TS=(k-ras or k ras or kras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)
6 97,980 #1 or #2 or #3 or #4 or #5
5 1,020 TS=(lower SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$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 7,235 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*))

LILACS (Latin American and Caribbean Health Sciences) (Internet): up to 2013/01/23
Searched: 24.1.13

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	213

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))	
((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**

PROSPERO (International Prospective Register of Systematic Reviews) (Internet): up to 2013/01/25
http://www.crd.york.ac.uk/prospero/
Searched 25.1.13

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
Ongogene	0
RASK	0
Snapshot	0
Colon Cancer	1/3 (included in KRAS result)
Total	**2**

Clinicaltrials.gov (Internet): 2000-2013/01/23
http://clinicaltrials.gov/ct2/search/advanced
Searched 23.1.13

Advanced search option – search terms box
Limited to results received from 01/01/2000 to 01/23/2013

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**

WHO International Clinical Trials Registry Platform (ICTRP) (Internet): 2000-2013/01/25
http://www.who.int/ictrp/en/
Searched 25.1.13

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	(Kirsten murine sarcoma* OR Kirsten rat sarcoma*)	Unable to run this line due to error with

	cancer)		results screen
thera screen OR thera-screen OR therascreen			**0**
		thera screen OR thera-screen OR therascreen	**0**
Total			**71**

Current Controlled Trials (mRCT – metaRegister of Controlled Trials) (Internet): Up to 29/01/2013
http://www.controlled-trials.com/
Searched 29.1.13

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

ESMO Conference Proceedings (European Society of Medical Oncology) (Internet): 2007-2013
Searched 5.2.13

2012 37th ESMO Congress, Vienna:
http://annonc.oxfordjournals.org/content/23/suppl_9
2011 ECCO 16 and 36th ESMO Multidisciplinary Congress, Brussels:
http://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:
http://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±**	**2010**	**2011±**	**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*
"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 deplication	**283**					

*3 additional refs found in index/prelims, would not export
± Used "Search within this issue" (search function not as sensitive as with other issues, may have included some additional 2011 conferences)

ESMO conference search located 423 records, 283 after deduplication.

AACR Conference Proceedings (American Association for Cancer Research)(Internet): 2007-2013
Searched 5.2.13

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

Whole Website:

2007-2010: http://www.aacrmeetingabstracts.org/search.dtl

Searched website above for abstracts from 2007-2010, Search limited to KRAS terms in title only **– retrieved 236 results**

Individual Years:

2009:
http://www.abstractsonline.com/viewer/SearchAdvanced.asp?MKey={D007B270-E8F6-492D-803B-7582CE7A0988}&AKey={728BCE9C-121B-46B9-A8EE-DC51FDFC6C15}
Searched: 6.2.13

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:http://www.abstractsonline.com/plan/start.aspx?mkey={0591FA3B-AFEF-49D2-8E65-55F41EE8117E}
Searched: 6.2.13

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: http://www.abstractsonline.com/plan/start.aspx?mkey={507D311A-B6EC-436A-BD67-6D14ED39622C}
Searched 6.2.13

Keywords	**Title search (advanced search)**	**Boolean search**	
KRAS or K-RAS or K RAS	**82**		
Kirsten AND rat AND		**1**	

sarcoma			
Kirsten AND murine AND sarcoma"		**0**	
Total			**83**

2012: http://www.abstractsonline.com/plan/start.aspx?mkey={2D8C569E-B72C-4E7D-AB3B-070BEC7EB280}
Searched 6.2.13

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**

Combined AACR conference search located 567 records in total

ASCO Conference Proceedings (American Society of Clinical Oncology): 2007-2013
http://www.asco.org/ASCOv2/Meetings/Abstracts
Searched 5.2.13

Searched 2007-2012 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**

ASCO conference search located 251 records.

AMP Conference Proceedings (Association for Molecular Pathology): 2007-2013 Searched 6.2.13

2012 AMP Abstracts; 25-27 Oct 2012; Long Beach, CA:
http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157812002115.pdf
2011 AMP Abstracts; 17-19 Nov 2011; Grapevine, TX:
http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157811002546.pdf
2010 AMP Abstracts; 18-20 Nov 2010; San Jose, CA:
http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810601365.pdf
2009 AMP Abstracts; 19-22 Nov 2009; Kissimmee, FL:
http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810602851.pdf
2008 AMP Abstracts; 29 Oct- 2 Nov 2008; Grapevine, TX:
http://download.journals.elsevierhealth.com/pdfs/journals/1525-1578/PIIS1525157810602000.pdf
2007 AMP Abstracts; 7-10 Nov 2007; Los Angeles, CA:
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**					

AMP conference search located 137 records.

Cost effectiveness searches

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

Embase (OVIDSP): 2000-2013/wk4
Searched: 29.1.13

1 exp colon cancer/ or exp rectum cancer/ or colorectal tumor/ (169460)
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$)).ti,ab,ot,hw. (246253)
3 (m-CRC or CRC).ti,ab,ot. (14068)
4 ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ 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$)).ti,ab,ot. (26)
7 or/1-6 (250031)
8 k ras oncogene/ (4967)
9 (k ras or kras or k-ras or V-Ki-ras$ 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. (17128)
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 (17382)
22 7 and 21 (5731)
23 health-economics/ (32282)
24 exp economic-evaluation/ (194421)
25 exp health-care-cost/ (186276)
26 exp pharmacoeconomics/ (160882)
27 or/23-26 (445930)
28 (econom$ or cost or costs or costly or costing or price or prices or pricing or pharmacoeconomic$).ti,ab. (542934)
29 (expenditure$ not energy).ti,ab. (21678)
30 (value adj2 money).ti,ab. (1191)
31 budget$.ti,ab. (22178)
32 or/28-31 (565244)
33 27 or 32 (823889)
34 letter.pt. (811274)
35 editorial.pt. (424059)
36 note.pt. (543769)
37 or/34-36 (1779102)
38 33 not 37 (742302)
39 (metabolic adj cost).ti,ab. (800)
40 ((energy or oxygen) adj cost).ti,ab. (3005)
41 ((energy or oxygen) adj expenditure).ti,ab. (18652)
42 or/39-41 (21682)
43 38 not 42 (737540)
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 [Internet]. York: Centre for Reviews and Dissemination; 2010 [cited 17.3.11]. Available from:
http://www.york.ac.uk/inst/crd/intertasc/nhs_eed_strategies.html

Medline (OVIDSP): 2000-2013/1/wk3
Searched: 29.1.13

1 exp Colorectal Neoplasms/ (134899)

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$)).ti,ab,ot,hw. (165996)
3 (m-CRC or CRC).ti,ab,ot. (8243)
4 ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ 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$)).ti,ab,ot. (23)
7 or/1-6 (170912)
8 Genes, ras/ (11084)
9 (k ras or kras or k-ras or V-Ki-ras$ 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 (16720)
20 7 and 19 (3092)
21 economics/ (26342)
22 exp "costs and cost analysis"/ (168037)

23 economics, dental/ (1847)
24 exp "economics, hospital"/ (18317)
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$).ti,ab. (375308)
29 (expenditure$ not energy).ti,ab. (15563)
30 (value adj1 money).ti,ab. (18)
31 budget$.ti,ab. (15762)
32 or/21-31 (493254)
33 ((energy or oxygen) adj cost).ti,ab. (2454)
34 (metabolic adj cost).ti,ab. (667)
35 ((energy or oxygen) adj expenditure).ti,ab. (14408)
36 or/33-35 (16880)
37 32 not 36 (489444)
38 letter.pt. (758034)
39 editorial.pt. (307072)
40 historical article.pt. (288506)
41 or/38-40 (1339895)
42 37 not 41 (463260)
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 [Internet]. York: Centre for Reviews and Dissemination; 2010 [cited 28.9.10]. Available from:
http://www.york.ac.uk/inst/crd/intertasc/nhs_eed_strategies.html

Medline In-Process & Other Non-Indexed Citations (OVIDSP): up to 2013/1/28
Medline Daily Update (OVIDSP): up to 2013/1/28
Searched: 29.1.13

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$).ti,ab. (33295)
29 (expenditure$ not energy).ti,ab. (992)
30 (value adj1 money).ti,ab. (3)

31 budget$.ti,ab. (1659)
32 or/21-31 (35017)
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 (34752)
38 letter.pt. (19083)
39 editorial.pt. (12353)
40 historical article.pt. (144)
41 or/38-40 (31562)
42 37 not 41 (34345)
43 20 and 42 (17)

Costs filter:
Centre for Reviews and Dissemination. NHS EED Economics Filter: Medline (Ovid) monthly search [Internet]. York: Centre for Reviews and Dissemination; 2010 [cited 28.9.10]. Available from:
http://www.york.ac.uk/inst/crd/intertasc/nhs_eed_strategies.html

NHS Economic Evaluation Database (NHS EED)(Wiley): Issue 4:2012
Searched 22.1.13

#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

NHS EED search retrieved 3 references

Science Citation Index (SCI-EXPANDED) (Web of Knowledge): 2000-2013/01/25 Searched 30.01.2013

 # 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 2,095 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$screen* or therascreen*)
8 97 TS=(Kirsten NEAR/3 (murine or rat) NEAR/3 sarcoma*)
7 10,424 TS=(k ras or kras or K-ras or V-Ki-ras* or V-K-ras or V-Ki-ras or v ki ras or c-ki-ras or c-k-ras or ki-ras or ki ras or Kras1 or Kras2 or KRAS1P or RASK or RASK1 or RASK2 or Kirsten RAS)
6 132,645 #1 or #2 or #3 or #4 or #5
5 1,321 TS=(lower SAME intestin* NEAR/3 (cancer* or neoplas* or oncolog* or malignan* or tumo$r* or carcinoma* or adenocarcinoma* or metasta* or meta-sta* or sarcoma* or adenom* or lesion*))
4 1,097 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 8,999 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*))

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

EconLit (EBSCO): 2000-2013/01/30
Searched: 30.1.13

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*))
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 Evaluation Database (HEED) (Internet): up to 2013/01/30
http://onlinelibrary.wiley.com/book/10.1002/9780470510933
Searched 30.1.13

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**

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**		
Study Details Bokemeyer (OPUS)(2011)[28, 53, 58] **Country** Germany, Spain, Italy, Ukraine, Russian Federation, Romania, Poland **Study Design** RCT **Funding** Merck Serono, Germany **Recruitment** - August 2006 **Total Number randomised: 337** **Number wild type randomised:** 179 **Number with liver metastases only:** 48	**Inclusion criteria** Adults (18 years or older); 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 only extracted. **Exclusion criteria** Pregnancy; previous EGFR-targeted therapy or previous chemotherapy (excluding adjuvant treatment) for mCRC; uncontrolled severe organ or metabolic dysfunction	**Median Age (range):** 61(24-82) **Number Male:** 97 **Criteria for unresectability:** NR **Performance status: ECOG** 0: 70 1: 93 2: 16 **Previous treatments:** 153 had previous surgery, 34 had previous adjuvant chemotherapy, and 24 had radiotherapy	**Intervention**	FOLFOX-4 + Cetuximab	FOLFOX-4	**KRAS Test** one-step Lightcycler PCR reaction (LightMIX, k-ras Gly12 assay) **Manufacturer** TIB MOLBIOL, Berlin, Germany **Analysis Software** LightCycler 2.0 system **Mutations Targeted** KRAS codon 12 and 13 missense mutations	**Withdrawals** Data for KRAS wild type population and liver metastases only subgroup are post-hoc analyses. Baseline data are for entire wild type population
			Dose	As standard + cetuximab initial dose 400 mg/m^2, subsequent doses 250 mg/m^2	oxaliplatin 85 mg/m^2; folinic acid 200 mg/m^2, followed by 5-FU as a 400 mg/m^2 bolus then a 600 mg/m^2 infusion over 22 h		
				As standard + weekly	days 1 and 2 of a 14 day cycle		
			Median Duration (IQR)	cetuximab 24 (13-38); oxaliplatin 23 (14-31); 5-FU 24 (14-36)	oxaliplatin 24 (16-30); 5-FU 24 (16-32)		
			Number with wild-type treated	82	97		
			Number with liver metastases treated	25	23		

Study details	Selection	Population	Intervention Details			KRAS Test Details	Other
			Detail	EGFR Inhibitor	Standard Chemotherapy		
Study Details Folprecht (CELIM)(2010)[52] **Country** Germany and Austria **Study Design** RCT **Funding** Merck- Serono, Sanofi-Aventis, and Pfizer **Recruitment** December 2004 - March 2008 **Number randomised:** 111 Number KRAS wild-type randomised: 70 Number with liver limited metastases randomised: 111	**Inclusion criteria** Unresectable, histologically confirmed colorectal liver metastases; no extra-hepatic 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 anti-tumour therapy, clinically relevant coronary artery	**Median Age (range):** 63(56-71) **Number Male:** 71 **Liver Metastases:** Number with <5 metastases:30 Number with 5-10 metastases:58 Number with >10 metastases:19 Number with NR metastases:4 Number 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. **Performance status:** NR	**Intervention**	Cetuximab + FOLFOX6	Cetuximab + FOLFIRI	**KRAS Test** Direct sequencing and DxS KRAS Mutation Test Kit **Manufacturer** DxS, Manchester, UK **Analysis Software** **NR** **Mutations Targeted** KRAS mutations in codins 12 and 13	
			Dose	(400 mg/m^2 initially then 250 mg/m^2 subsequently) + (100 mg/m^2 oxaliplatin, 400 mg/m^2 folinic acid and 400 mg/m^2 bolus 5-FU followed by 2400 mg/m^2 over 46 hrs)	(400 mg/m^2 initially then 250 mg/m^2 subsequently) + (irinotecan 180 mg/m2, 400 mg/m^2 folinic acid and 400 mg/m^2 bolus 5-FU followed by 2400 mg/m^2 over 46 hrs)		
				(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)		
			Number with KRAS wild type treated	56	55		
			Number with liver metastases treated	56	55		

Study details	Selection	Population	Intervention Details			KRAS Test Details	Other
			Detail	EGFR Inhibitor	Standard Chemotherapy		
	disease, infl ammatory bowel disease, previous malignancy, and age < 18 years.	**Previous treatments:** 9 patients had adjuvant radiotherapy, 18 had adjuvant chemotherapy					

Study details	Selection	Population*	Intervention Details			KRAS Test Details	Other
			Detail	EGFR Inhibitor	Standard Chemotherapy		
Study Details Kohne (CRYSTAL)(2011)[27, 53] **Country** Germany, Russian Federation, Poland, Singapore, South Korea, South Africa, France, Belgium (201 centres) **Study Design** RCT **Funding** Meck Serono **Recruitment** July 2004 -	**Inclusion criteria** Adults (18 years or older); 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	**Median Age (range):** 61(22-79) **Number Male:** 201 **Criteria for unresectability:** NR **Performance status: ECOG** 0: 203 1: 131 2: 14 **Previous treatments:** NR	**Intervention**	Cetuximab + FOLFORI	FOLFORI	**KRAS Test** PCR clamping and melting curve method (LightMIX, k-ras Gly12 assay) **Manufacturer** TIB MOLBIOL, Germany **Analysis Software** LightCycler 2.0 system **Mutations Targeted** KRAS codon 12 and 13 missense mutations	**Withdrawals** 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. **Comments** No definition of un-resectable
			Dose	As standard + initial 120 min infusion cetuximab 400 mg/m^2, 60 min infusions of 250 mg/m^2	30-90 min infusion irinotecan 180 mg/m^2, 120 min infusion racemic leucovorin 400 mg/m^2 or L-leucovorin 200mg/m^2, 5-fluorouracil 400 mg/m^2 bolus then 46 hour infusion 2400 mg/ m^2		
				weekly	day 1 of a 14 day cycle		
			Median Duration (IQR)	cetuximab 25.0 (12.9-40.4); irinotecan 26.0 (14.0-40.3); 5-FU 26.0 (13.8-40.4)	irinotecan 25.7 (15.1-39.5); 5-FU 25.7 (14.9-36.0)		
			Number treated	172	176		

Study details	Selection	Population*	Intervention Details			KRAS Test Details	Other
			Detail	EGFR Inhibitor	Standard Chemotherapy		
November 2005 **Number randomised:** 1202 Number KRAS wild-type randomised: 666 Number with liver limited metastases randomised: 140	therapy or irinotecan-based therapy; previous chemotherapy for metastatic colorectal cancer; 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.		**Number with liver metastases treated**	68	72		liver metastases was reported.

*Population data come from full paper report and relate to smaller sample of 348 patients with wild-type KRAS mutation status; full paper is earlier report than abstract from which results data are taken

Study details	Selection	Population	Intervention Details			KRAS Test Details	Other
			Detail	EGFR Inhibitor	Standard Chemotherapy		
Study Details Maughan (COIN)(2011)[54] **Country** UK and Republic of Ireland	**Inclusion criteria** Adults (18 years or older); histologically confirmed adenocarcinoma of the colon or rectum; inoperable metastatic or	**Age** Median(range):64(56-70) **Number Male:** 498 **Criteria for unresectability:** NR	**Intervention**	Cetuximab + standard chemotherapy (xaliplatin + fluorouracil and folinic acid, or oxaliplatin + capecitabine)	Standard chemotherapy (xaliplatin + fluorouracil and folinic acid, or oxaliplatin + capecitabine)	**KRAS Test** Pyrosequencing and MALDI-TOF mass array, with Sanger sequencing for discordant samples (<1%)	**Withdrawals** Data for KRAS wild type population and liver metastases only subgroup are post-hoc analyses. These

Study details	Selection	Population	Intervention Details			KRAS Test Details	Other
			Detail	**EGFR Inhibitor**	**Standard Chemotherapy**		
Study Design RCT **Funding** UK Medical Research Council **Recruitment** March 2005 - May 2008 **Number randomised:** 1630 Number KRAS wild-type randomised: 729 Number with liver limited metastases randomised: 178	locoregional disease; no previous chemotherapy fro metastatis 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 > grade 1; requirement for treatment with contra-indicated medication; another	**Performance status: WHO** 0: 348 1: 337 2: 44 3: 0 **Previous treatments:** NR	**Dose**	As standard + initial 2hr infusion cetuximab 400 mg/m^2 and 250 mg/m^2 over 1h subsequently	2 hr infusion L-folinic acid (175 mg) or racemic folinic acid (350 mg) 2 hr infusion oxaliplatin (85 mg/m^2), followed by bolus 5-FU (400 mg/m^2) then 46 hr infusion 5-FU (2400 mg/m^2) or 2 hr infusion of oxaliplatin 130 mg/m^2 , followed by oral capecitabine twice a day for 2 weeks (1000 mg/m^2 orally twice daily in most patients, reduced to 850 mg/m2 in later patients).	**Manufacturer** **In house** **Analysis Software** Sequenom, USA **Mutations Targeted** KRAS mutations in codons 12, 13 and 61	participants were also BRAF and NRAS wild type. ; unavailable tumour blocks = 141; insufficient tumour material for processing = 163; not successfully genotyped = 22 Standard chemotherapy was FOLFOX for some patients (approximately 1/3 of whole trial) and XELOX for some patients (approximately 2/3 of whole trial). Proportions unknown for the sub-group with liver
				weekly	2 weekly for folonic acid, 3 weekly for capecitabine		
			Median Duration (IQR)	28.1 (15.4-42.0)	29.3 (15.7-40.1)		
			Number treated	357	358		

Study details	Selection	Population	Intervention Details			KRAS Test Details	Other
			Detail	EGFR Inhibitor	Standard Chemotherapy		
	previous or current malignant disease which may affect treatment response; known hypersensitivity to any tudy treatment; brain metastases.		**Number with liver metastases treated**	87	91		metastases. 178 KRAS wild type patients had liver metastases only, 153 were included in the analysis (25 patients missing)

Study details	Selection	Population	Intervention Details			KRAS Test Details
			Detail	EGFR Inhibitor	Standard Chemotherapy	
Study Details Xu(2012)[55] **Country** China **Study Design** RCT **Funding** Not reported **Recruitment** June 2008 - December 2011	**Inclusion criteria** Resected primary colorectal tumour; non-resectable synchronous liver-limited metastases; KRAS wild type **Exclusion criteria** None reported	**Age:** NR **Number Male:** NR **Criteria for unresectability:** NR **Performance status:** NR	**Intervention**	Cetuximab + FOLFIRI or FOLFOX6	FOLFIRI or FOLFOX6	**KRAS Test** Pyrosequencing **Manufacturer** NR **Analysis Software** NR **Mutations Targeted** NR
			Dose	NR	NR	
			Frequency	NR	NR	
			Mean number of cycles	NR	NR	
			Duration	NR	NR	
			Number with KRAS wild type treated	59	57	

Study details	Selection	Population	Intervention Details			KRAS Test Details
			Detail	EGFR Inhibitor	Standard Chemotherapy	
Number eligible: NR **Number randomised:** 116 Number KRAS wild-type randomised: 116 Number with liver limited metastases randomised: 116		**Previous treatments: NR**	**Number with liver metastases treated**	59	57	

APPENDIX 3: RISK OF BIAS ASSESSMENTS

a. QUADAS-2 assessments

Study name: Folprecht (CELIM)(2010)[52]

DOMAIN 1: PATIENT SELECTION	
A. Risk of Bias	
Describe methods of patient selection: Phase 2 RCT comparing FOLFOX6 + cetuximab with FOLFIRI + cetuximab in patients with unresectable liver metastases from CRC. 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 ≥ 5 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 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 2x2 table: 17 (15%) of 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 index test(s) and reference standard:* Tumour response was assessed every four cycles (eight weeks) for a maximum of two years.	
Was there an appropriate interval between index test(s) and 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**

Study name: Maughan (COIN)(2011)[54]

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 sub-group 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 extra-hepatic metastases or previous chemotherapy. Patients receiving combination chemotherapy prior to 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 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 MADI-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 respectability were aware of tumour KRAS mutation status. Treatment arms included FOLFOX or XELOX as the standard chemotherapy and XELOX was not specified as the standard chemotherapy for this review.	
Is the reference standard likely to correctly classify the target	yes

condition?	
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 2x2 table: Tumour response was assessed every 12 weeks. 19% of participants in the original trial did not receive KRAS mutation testing because no sample was available and 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. 153 Participants with KRAS wild type tumours were included in the analysis (25 (14%) missing).	
Was there an appropriate interval between index test(s) and 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**

b. Cochrane Risk of Bias Assessments

Study Name: Bokemeyer (OPUS)(2011)[28, 53, 58]

	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 ITT and outcomes 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

Study Name: Van Cutsem (CRYSTAL)(2009)[27])[53]

	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 ITT and outcomes data appeared to be reported for all patients with liver limited metasteses, however, data for these patients were only reported in an abstract and details were limited	Low
Selective outcome reporting	All specified outcomes appear to be reported	Low

Study Name: Maughan (COIN)(2011)[54]

	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 ITT and outcomes data appeared to be reported for all patients with liver limited metasteses, however, data for these patients were only reported in an abstract and details were limited	Low
Selective outcome reporting	All specified outcomes appear to be reported	Low

Study Name: Xu(2012)[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 NEQAS PILOT SCHEME 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 included patients with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable.	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 progression free survival
(2012)[80]	✓			
Adams (2010)[81]		✓		
Adams (2010)[82]				✓
Adams (2010)[83]		✓		
Adams(2009)[84]	✓			
Alberts(2010)[85]		✓		
Assenat (2011)[86]		✓		
Baker(2008)[87]		✓		
Baloglu(2012)[88]	✓			
Bokemeyer (2009)[58]		✓		
Bokemeyer (2009)[89]		✓		
Bokemeyer (2010)[90]	✓			
Bokemeyer 2008)[91]		✓		
Bokemeyer(2012)[92]	✓			
Chuko(2010)[93]	✓			
Cohen(2008)[94]			✓	
Colucci (2010)[95]		✓		
Di Salvatore(2010)[96]		✓		
Dubus(2009)[97]	✓			
Folprecht (2010)[98]	✓			
Folprecht(2008)[99]	✓			
Folprecht(2009)[100]		✓		
Gajate(2012)[101]		✓		
Gao(2011)[102]		✓		
Garufi(2009)[103]			✓	

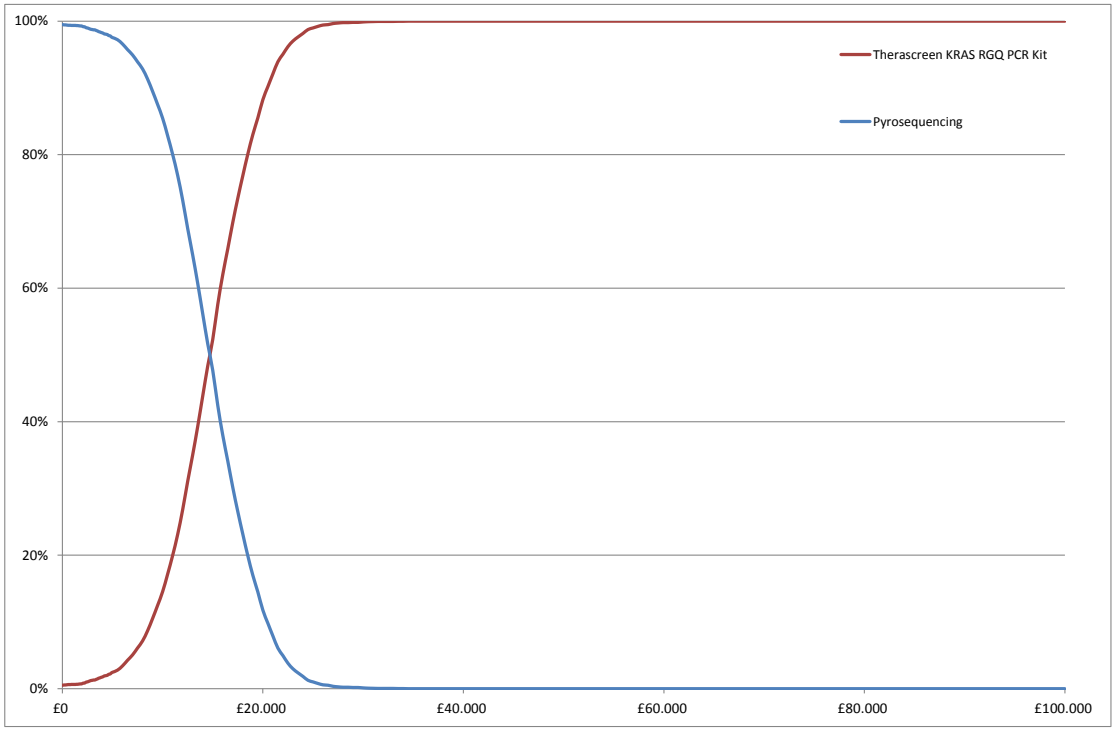
Study	Reason for exclusion (once a study had failed on one criterion it was not assessed further)			
	1. Not a primary study	2. Did not included patients with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable.	3. KRAS mutation test not performed and/or test and mutation not specified or deducible	4. Study did not report on response to treatment, survival or progression free survival
Goldberg(2010)[104]		✓		
Griebsch(2011)[105]		✓		
Harbison(2012)[106]		✓		
Huang(2011)[107]		✓		
Ibrahim(2010)[108]	✓			
Jones(2013)[109]	✓			
Jonker(2009)[110]		✓		
Kimura(2012)[111]		✓		
Kohne(2009)[112]		✓		
Ku (2012)[113]	✓			
Lang (2009)[114]		✓		
Lievre(2006)[115]		✓		
Lin(2010)[116]		✓		
Lin(2011)[117]	✓			
Linardou(2008)[118]	✓			
Loupakis (2012)[119]	✓			
Malapelle(2012)[120]		✓		
Malapelle(2012)[121]		✓		
Mancuso(2008)[122]		✓		
Maughan(2009)[123]		✓		
Maughan(2010)[124]		✓		
Maughan(2010)[125]		✓		
Mayer(2010)[126]		✓		
Merck KgaA(2011)[127]	✓			
Modest(2012)[128]	✓			
Molinari(2010)[129]		✓		
Moosmann (2011)[130]		✓		
Ocvirk(2009)[131]	✓			

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 included patients with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable.	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 progression free survival
Ocvirk(2010)[56]	✓			
Passardi(2011)[132]		✓		
Petrelli (2011)[133]	✓			
Petrelli(2012)[134]	✓			
Piessevaux(2010)[135]	✓			
Piessevaux(2011)[136]	✓			
Piessevaux(2011)[137]		✓		
Qiu(2010)[138]	✓			
Raoul (2009)[139]		✓		
Rivera(2009)[140]		✓		
Rose (2012)[141]		✓		
Salazar(2012)[142]		✓		
Schuch(2008)[143]		✓		
Serna(2011)[144]		✓		
Shinozaki (2012)[145]		✓		
Simon(2011)[146]		✓		
Stintzing(2010)[147]		✓		
Taieb(2012)[148]		✓		
Tejpar(2011)[149]	✓			
Tejpar(2011)[150]	✓			
Tejpar(2011)[151]	✓			
Tejpar(2012)[152]	✓			
Tsoukalas (2011)[153]	✓			
Tsoukalas (2012)[154]	✓			
Tsoukalas(2010)[155]	✓			
Tveit (2012)[57]		✓		
Tveit(2010)[156]		✓		
Tveit(2011)[157]		✓		

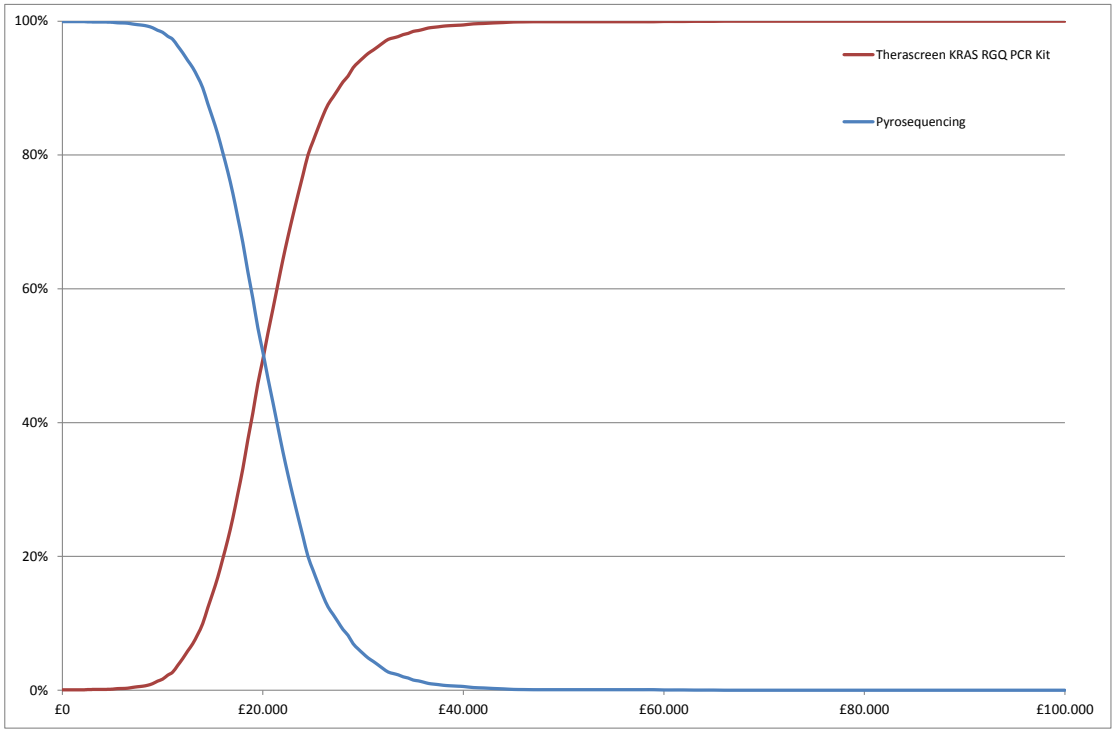
Study	Reason for exclusion (once a study had failed on one criterion it was not assessed further)			
	1. Not a primary study	**2. Did not included patients with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable.**	**3. KRAS mutation test not performed and/or test and mutation not specified or deducible**	**4. Study did not report on response to treatment, survival or progression free survival**
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, L.-C.U (2010)[167]		✓		
Zhang(2011)[168]	✓			

APPENDIX 6: COST EFFECTIVENESS ACCEPTABILITY CURVES FOR SENSITIVITY ANALYSES

Cost-effectiveness acceptability curve for '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 unknowns from survey



APPENDIX 7: NICE 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 [Internet]. London: NICE, June 2004 [accessed 14.5.13]. 136p. Available from: http://guidance.nice.org.uk/CSGCC

Clinical guideline

- National Institute for Health and Clinical Excellence. Colorectal cancer: the diagnosis and management of colorectal cancer (CG131) [Internet]. London: NICE, November 2011 [accessed 14.5.13]. 37p. Available from http://guidance.nice.org.uk/CG131 Date of Review: TBC.

CG131 updates and replaces TA93, and incorporates TA100, TA105 and TA61.

- National Institute for Health and Clinical Excellence. Colorectal cancer (advanced) - irinotecan, oxaliplatin and raltitrexed. NICE technology appraisal 93 [Internet]. London: NICE, August 2005 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA93
- National Institute for Health and Clinical Excellence. Colon cancer (adjuvant) - capecitabine and oxaliplatin. NICE technology appraisal 100 [Internet]. London: NICE, April 2006 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA100
- National Institute for Health and Clinical Excellence. Colorectal cancer - laparoscopic surgery. NICE technology appraisal 105 [Internet]. London: NICE, August 2006 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA105
- National Institute for Health and Clinical Excellence. Colorectal cancer - capecitabine and tegafur uracil. NICE technology appraisal 61 [Internet]. London: NICE, May 2005 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA61

Technology appraisals

- National Institute for Health and Clinical Excellence. Colorectal cancer (metastatic) 2nd line: cetuximab, bevacizumab and panitumumab. NICE technology appraisal 242 [Internet]. London: NICE, January 2012 [accessed 14.5.13]. 54p. Available from: http://guidance.nice.org.uk/TA242 Date for review: January 2015.

TA242 replaces TA150 and partially updated TA118.

- National Institute for Health and Clinical Excellence. Colorectal cancer (metastatic) - cetuximab (terminated appraisal). NICE technology appraisal 150 [Internet]. London: NICE, June 2008 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA150

- National Institute for Health and Clinical Excellence. Colorectal cancer (metastatic) - bevacizumab and cetuximab. NICE technology appraisal 118 [Internet]. London: NICE, January 2007 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA118
- 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 [Internet]. London: NICE, December 2010 [accessed 13.5.13]. Available from: http://guidance.nice.org.uk/TA212 Date for review: TBC.
- National Institute for Health and Clinical Excellence. Cetuximab for the first-line treatment of metastatic colorectal cancer. NICE technology appraisal 176 [Internet]. London: NICE, August 2009 [accessed 14.5.13]. 37p. Available from: http://guidance.nice.org.uk/TA176 Date for 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."

NICE pathways

- National Institute for Health and Clinical Excellence. NICE Pathway Colorectal cancer [Internet]. London: NICE, November 2011 [accessed 14.5.13]. Available from: http://pathways.nice.org.uk/pathways/colorectal-cancer

Quality standards

- National Institute for Health and Clinical Excellence. Colorectal cancer (QS20) [Internet]. London: NICE, August 2012 [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/QS20

Under development

- National Institute for Health and Care Excellence. Colorectal cancer (metastatic) - aflibercept [ID514] [Internet]. London: NICE [accessed 14.5.13]. Available from: http://guidance.nice.org.uk/TA/Wave0/617 (publication expected October 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 [Internet]. London: NICE, December 2011 [accessed 14.5.13]. 7p. Available from: http://guidance.nice.org.uk/TA240

"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: PRISMA CHECK LIST

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	page 1
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 – page 11 to 16 PROSPERO registration – page 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	section 2, background – page 17 to 27
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	objectives – page 16 inclusion criteria – table 2, page 31
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 – appendix 9, page 181 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 – page 31
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.	section 3.1.1 – page 28 to 30
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	appendix 1 – page 127 to 150

Section/topic	#	Checklist item	Reported on page #
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).	section 3.1.3 – page 32
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.	section 3.1.3 – page 32
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	section 3.1.3 – page 32 table 2 – page 31
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	section 3.1.4 – page 32
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	section 3.1.6 – page 33 to 34
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	section 3.1.6 – page 33 to 34
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 done, indicating which were pre-specified.	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.	section 3.2 – page 34 to 35 figure 1 – page 36
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.	section 3.2.2 – page 45 to 48 section 3.2.3 – page 50 to 53 appendix 2 – page 151 to 157
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).	section 3.2.2 – page 48 table 9 – page 50 section 3.2.3 – page 57 table 12 – page 57

Section/topic	#	Checklist item	Reported on page #
			appendix 3 – page 158 to 170
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	table 8 – page 49 figure 4 – page 50 table 11 – page 56 figures 5, 6 and 7 – page 54 to 55
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
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 done (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., healthcare providers, users, and policy makers).	section 5.1 – page 94 to 98
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).	sections 5.2 and 5.3 – page 99 to 110
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	section 6 – page 111 to 113
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NIHR HTA programme, project number 12/75/01 – page 2

APPENDIX 9: PROTOCOL

Diagnostic Assessment Report commissioned by the NIHR HTA Programme on behalf of the National Institute for Health and Clinical Excellence – Protocol

Title of project

KRAS mutation testing in tumours for adults with metastatic colorectal cancer.

Name of External Assessment Group (EAG) and project lead

Kleijnen Systematic Reviews Ltd. Assessment Group

Project lead: Marie Westwood

Second Contact: Penny Whiting

Kleijnen Systematic Reviews Ltd

Unit 6, Escrick Business Park

Riccall Road

Escrick

York YO19 6FD

Tel: 01904 727983

Email: marie@systematic-reviews.com; penny@systematic-reviews.com

Health economics lead: Manuela Joore

Department of Clinical Epidemiology and Medical Technology Assessment

Maastricht University Medical Centre & CAPHRI School for Public Health and Primary Care

Department of Health Services Research

Maastricht University

P.O. Box 5800

6200 AZ Maastricht

The Netherlands

Tel: +31-43-3875434

Email: m.joore@mumc.nl

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. The likelihood of surviving 1 year after diagnosis is around 73%, and the likelihood of surviving 5 years after diagnosis is lower at around 55% and continues to fall after 5 years.

Bowel cancer occurs when uncontrolled cell growth begins in the bowel. Rather than growing into normal healthy bowel cells the abnormal cells form lumps or masses of tissue called tumours which may interfere with normal bowel function; early symptoms of bowel cancer often include altered bowel habit and/or blood in the stool. Around three quarters of bowel cancers are initially treated with surgery, but around 1 in 6 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.

Certain mutations within tumour cells can make them more or less receptive to specific types of chemotherapy. KRAS mutations make some tumours less responsive to treatment with biological therapies, such as cetuximab. Before deciding on which treatment to offer patients with bowel cancer that has spread to the liver patients are therefore tested to see if their tumour has a mutation in the KRAS gene. There are a variety of tests available to detect these specific mutations but it is not known which test is the best test to use. The different tests vary in the specific mutations which 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 projects aims to evaluate KRAS mutation tests to determine which should be the recommended test or tests for use in the NHS in England and Wales. The assessment will consider both clinical effectiveness (improvement in patients' symptoms associated with the test) and cost effectiveness (cost of different testing strategies).

Decision problem

Population

The indication for this assessment is the detection of mutations in the KRAS oncogene in adults with metastatic colorectal cancer (CRC), where metastases are confined to the liver and are un-resectable. The presence or absence of KRAS (Kirsten rat sarcoma viral oncogene homolog) 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, London showed that 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 ten 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 five year survival rates for CRC in England (2005-2009) 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 and peaking in the over 80s; in the UK (2007-2009) 72% of new cases were diagnosed in people over 65 years.[3] There is some evidence of an association between incidence of CRC and deprivation in UK males; 2000-2004 data show incidence rates approximately 11% higher for men living in more deprived areas compared with the least deprived.[6] The National Bowel Cancer Audit (NBCA) data for 2011 included 28,260 new cases for England and Wales, of which 21,306 (75.4%) were surgically treated and 3,425 (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 metastatic CRC 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 inhibiting monoclonal antibodies, the potential effects of mutations in codons 61 and 146 of KRAS have also been investigated. A US study, which found KRAS codon 12 or 13 mutations in 900/2121 (42.4%) of CRC patients, conducted further analysis of the 513 wild-type samples and found 19 additional mutations at KRAS codon 61 and 17 at KRAS codon 146; these additional mutations represent <2% of the total study population.[11]

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 grouped into two subgroups: mutation screening and targeted mutation detection. Mutation screening tests screen samples for all KRAS mutations (known and novel) whilst 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 mutation detection at as little as 1% against a background of wild-type DNA (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 mutation positive 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.[12, 13] 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 which should not be included in this assessment.

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 Food and Drug Administration (FDA) for the application covered by this assessment, i.e. the selection of patients with metastatic colorectal cancer 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 bi-functional molecules containing a polymerase chain reaction (PCR) primer covalently linked to a fluorescently labelled probe. A real-time PCR instrument (Rotor-Gene Q 5-Plex

HRM for consistency with CE-marking) is used to perform the amplification and to measure fluorescence.[16] There is an earlier version of the Therascreen® KRAS PCR Kit which also uses ARMS and Scorpions for the detection of KRAS mutations and is designed to detect the same KRAS mutations as the current, re-formulated and re-validated 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 twelve 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, then amplified DNA is immobilised on Steptavidin Sepharose High Performance beads. Single-stranded DNA is prepared and sequencing primers added. The samples are then analysed on the PyroMark Q24 System. The KRAS Plug-in Report is recommended by the manufacturer for the analysis of results, however, the analysis tool within the pyrosequencer can also be used.[17]

The cobas® KRAS Mutation Test (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 formalin-fixed paraffin-embedded tissue and is validated for use with the cobas® 4800 System.

The KRAS LightMix® Kit (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. In order 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).[18]

The KRAS StripAssay® (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; 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.[19] There are two versions of the KRAS StripAssay®: one is designed to detect 10 mutations in codons 12 and 13 of the KRAS gene; a second is designed to detect the same 10 mutations in codons 12 and 13 plus 3 mutations in codon 61 of the KRAS gene.

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 (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.[20]

Sanger sequencing is a commonly used method (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 which 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 only works well when viable tumour cells constitute at least 25% or more of the sample.[21]

NICE 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.[22]

High resolution melt (HRM) analysis assays are also commonly used by laboratories participating in the UK NEQAS scheme (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 which only binds 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.[23]

MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) 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 where each fragment is represented as a peak with a certain mass. The 'fingerprint' of the test sample is compared to 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.[24] MALDI-TOF 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.[25]

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*	Lab contact†
Commercial tests				
Therascreen® KRAS Kit (PCR) (Qiagen)	Targeted (7 mutations: 6 codon 12 and 1 codon 13)	0.77-6.43%	3	1
Therascreen® KRAS Kit (Pyro) (Qiagen)	Targeted (12mutations: 6 codon 12, 1 codon 13 and 5 codon 61)	1.0-3.5%		2
cobas® KRAS mutation test (Roche Molecular Systems)	Targeted (19 mutations: 6 codon 12, 6 codon 13 and7 codon 61)	1.6-6.3% depending on mutation	4	4
KRAS LightMix® kit (TIB MolBiol)	Targeted (9 mutations: 7 codon 12, 2 codon 13)	unclear	0	0
KRAS StripAssay® (ViennaLab)	Targeted (13 mutations: 8 codon 12, 2 codon 13 and 3 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%†	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%†	2	2
Next generation sequencing	All mutations within specific codons of the KRAS gene	~5%†	0	0
MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight) Mass spectrometry	All mutations within selected codons in the KRAS oncogene	~10%	1	0

* NEQAS pilot scheme 2012-2013, run 2.[26] Thirty UK based laboratories participated in the scheme; some laboratories used more than one method

† 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.

Subgroup analyses of patients tested for KRAS mutation status, from randomised controlled trials, have shown that treatment with the epidermal growth factor 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 to standard chemotherapy alone.[27, 28] Whereas patients whose tumours were positive for KRAS mutations had reduced (PFS) and tumour response when treated with cetuximab in combination with standard chemotherapy compared to standard chemotherapy alone.[27, 28] These two trials formed the basis of NICE Technology Appraisal 176, which recommends cetuximab in combination with standard chemotherapy for the first-line treatment of metastatic colorectal cancer in patients whose tumours are KRAS wild-type and whose metastases are confined to the liver and are un-resectable.[1] 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

NICE guidance on the diagnosis and management of colorectal cancer was updated in 2012.[29]

Diagnosis of CRC

This guideline states that patients referred to secondary care for suspected colorectal cancer should be assessed using colonoscopy, flexible sigmoidoscopy followed by barium enema, or computed tomography (CT), dependent upon comorbidities and local expertise and test availability. Where 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 MRI or 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 which may become resectable following response to chemotherapy. For the second group of patients, European Society for Medical Oncology clinical practice guidelines for the treatment of advanced colorectal cancer (2010) recommend establishing KRAS mutation status in order to determine the best treatment regimen. These guidelines do not stipulate which specific mutations should be analysed, or which test method should be used.[30] 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 (FFPE) for storage by the pathologist who also examines the histology and evaluates the tumour content of the sample. Macrodissection 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 recommends that mutation testing should be ordered by the pathologist reporting on the cellular make-up of the tumour.[31] However, this is not currently universal practice and often the decision to perform a KRAS mutation test is often taken at the multidisciplinary team meeting. If a sample is stored as a formalin fixed and paraffin embedded (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 due to 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 CRC

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

The guideline further states that raltitrexed should only be considered for patients who are intolerant to fluorouracil and folinic acid, or for whom these drugs are not suitable.[29] NICE technology appraisal 61 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 metastatic colorectal cancer.[34]

With respect to the use of biological agents (epidermal growth factor receptor inhibitors), NICE technology appraisal guidance 176 recommends cetuximab in combination with FOLFOX or FOLFIRI, within its licensed indication, for the first-line treatment of metastatic colorectal cancer in whom:

- The primary colorectal tumour has been resected or is potentially operable.
- The metastatic disease is confined to the liver and is unresectable.
- The patient is fit enough to undergo surgery to respect the primary colorectal tumour and to undergo liver surgery if the metastases become resectable after treatment with cetuximab.[1]

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 metastatic colorectal cancer 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 live metastases from CRC.

NICE guideline 131 stipulates that all patients with primary colorectal cancer undergoing treatment with curative intent should have follow-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's 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 five years, and thereafter as determined by cancer networks.[29]

Objectives

The overall objective of this project is to summarise the evidence on the clinical- and cost-effectiveness of KRAS mutation tests (commercial or in-house) to differentiate adults with metastatic CRC, whose metastases are confined to the liver and are un-resectable, 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 NICE Technology Appraisal TA176.[1] In order to address the clinical-effectiveness we would ideally like data on the analytical validity of the different KRAS mutation tests (sensitivity/specificity for detection mutations known to be linked to be treatment effectiveness). However, there is no gold standard for KRAS mutation testing and the exact mutations, and level of mutation, linked to the effectiveness of different treatment options is not known. We therefore defined the following research questions to address the review objectives:

- What is the technical performance of the different KRAS mutation tests (e.g. proportion tumour cells needed, limit of detection (minimum percentage mutation detectable against a background of wild-type DNA), failures, costs, turnaround time)?
- 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?
- How do clinical outcomes from treatment with cetuximab in combination with standard chemotherapy and, where reported, from treatment with standard chemotherapy vary according to which test is used to select patients for treatment?
- 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?

Methods for assessing clinical effectiveness

Systematic review methods will follow the principles outlined in the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care[40] and NICE Diagnostic Assessment Programme manual.[41] In addition to the effectiveness review additional data will be obtained by contacting those reference laboratories in England and Wales known to perform KRAS mutation testing.

Inclusion and exclusion criteria

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

Table 2: Inclusion criteria

Question	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, where reported, from treatment with standard chemotherapy vary according to which test is used to select patients for treatment?
Participants:	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy.	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy.	Adult patients (≥18 years) with metastatic CRC and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable 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
Comparators:	Not applicable	Not applicable	Standard chemotherapy
Reference standard:	Not applicable	Response to treatment with cetuximab in combination with standard chemotherapy (e.g. progression free survival, objective response rate, disease control rate)	Not applicable
Outcomes:	Proportion tumour cells needed, failures, limit of detection, turnaround time, costs, expertise/logistics of test	Overall survival or progression free survival in patients whose tumours are KRAS mutation positive versus wild-type. Test accuracy – the number of true positive, false negative, false positive and true negative.	Progression free survival, overall survival, objective response rate, disease control rate
Study design:	To be addressed by survey; see below 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)

Questionnaire

To address the research question on the technical performance of the different KRAS mutation tests, we will need to collect data from sources other than the systematic review. This section provides a brief description of these data and will be expanded as necessary to inform the economic model. A web-based questionnaire will be developed to gather information from laboratories in England and Wales offering KRAS testing that participate in the UK NEQAS scheme. Questions will cover, but will not be limited to:

1. Assay method used
2. Is the method targeted or screening?
3. If targeted method, mutations targeted
4. In your institution is KRAS mutation testing performed on initial diagnosis of CRC or later in the point of disease?
5. If later, at what time point is the test carried out?
6. If screening, which codons are screened?
7. Limit of detection (minimum % mutation)
8. Sample requirements (minimum % tumour cells required to run the test)
9. Definition and proportion of inadequate sample
10. Definition and proportion of failed tests (for reasons other than inadequate sample)
11. Number of samples processed
12. Batching size – do you wait until you have certain number of samples before running the test
13. Costs of the test (fixed and variable costs, i.e. what is cost of a full batch and what is the cost of e.g. 50% full batch if partial batches are routinely run)
14. Turnaround time, including definition
15. Any logistic / other issues related to the use of the test?

Information obtained from this survey will be used to provide information on tests that have not been evaluated in studies included in the systematic review. If any published reports on technical performance, from NHS laboratories in England and Wales, are identified by the systematic review searches, these will be summarised alongside the survey data.

Search strategy

Search strategies will be based on target condition and intervention, as recommended in the Centre for Reviews and Dissemination (CRD) guidance for undertaking reviews in health care and the Cochrane Handbook for Diagnostic Test Accuracy Reviews.[40] Additional supplementary searches will

be carried out as necessary. Searches for studies for cost and quality of life will be developed separately.

Candidate search terms will be identified from target references, browsing database thesauri (e.g. Medline MeSH and Embase Emtree), existing reviews identified during the rapid appraisal process and initial scoping searches. These scoping searches will be used to generate test sets of target references, which will inform text mining analysis of high-frequency subject indexing terms using Endnote reference management software. Strategy development will involve an iterative approach testing candidate text and indexing terms across a sample of bibliographic databases, aiming to reach a satisfactory balance of sensitivity and specificity.

The following databases will be searched for relevant studies from 2000 to the present:

- MEDLINE (OvidSP)
- MEDLINE In-Process Citations and Daily Update (OvidSP)
- EMBASE (OvidSP)
- Cochrane Database of Systematic Reviews (CDSR) (Internet)
- Cochrane Central Register of Controlled Trials (CENTRAL) (Internet)
- Database of Abstracts of Reviews of Effects (DARE) (Internet)
- Health Technology Assessment Database (HTA) (Internet)
- Science Citation Index (SCI) (Web of Science)
- LILACS (Latin American and Caribbean Health Sciences Literature) (Internet) http://regional.bvsalud.org/php/index.php?lang=en
- Biosis Previews (Web of Science)
- NIHR Health Technology Assessment Programme (Internet)
- PROSPERO (International Prospective Register of Systematic Reviews) (Internet) http://www.crd.york.ac.uk/prospero/

Completed and ongoing trials will be identified by searches of the following resources (2000-present):

- NIH ClinicalTrials.gov (http://www.clinicaltrials.gov/)
- Current Controlled Trials (http://www.controlled-trials.com/)
- WHO International Clinical Trials Registry Platform (ICTRP) (http://www.who.int/ictrp/en/)

Key conference proceedings, to be identified in consultation with clinical experts, will be screened for the last five years. References in retrieved articles and relevant systematic reviews will be checked. Search strategies will be developed specifically for each database and the keywords associated with colorectal cancer will be adapted according to the configuration of each database.

No restrictions on language or publication status will be applied. Searches will take into account generic and other product names for the intervention. Examples of the search strategies to be used are presented in Appendix 1; these will be adapted as necessary following consultation with clinical experts. The main Embase strategy for each search will be independently peer reviewed by a second Information Specialist, using the PRESS-EBC checklist.[43] Identified references will be downloaded in Endnote X4 software for further assessment and handling. References in retrieved articles will be checked for additional studies. The final list of included papers will also checked on PubMed for retractions and errata.[44-46]

Review strategy

Two reviewers will independently screen titles and abstracts of all reports identified by the searches and discrepancies will be discussed. Full copies of all studies deemed potentially relevant, after discussion, will be obtained and two reviewers will independently assess these for inclusion; any disagreements will be resolved by consensus or discussion with a third reviewer.

Where available, data will be extracted on the following: study design/details, participants, KRAS mutation test(s), clinical outcomes, and test performance outcome measures (against treatment response as reference standard), test failure rates, limit of detection. Data will be extracted by one reviewer, using a piloted, standard data extraction form. A second reviewer will check data extraction and any disagreements will be resolved by consensus or discussion with a third reviewer.

Quality assessment strategy

The methodological quality of included RCTs will be assessed using the Cochrane Risk of Bias Tool.[47] Diagnostic accuracy studies will be assessed using QUADAS-2. [48] The results of the quality assessment will be used for descriptive purposes to provide an evaluation of the overall quality of the included studies and to provide a transparent method of recommendation for design of any future studies. Quality assessment will be undertaken by one reviewer and checked by a second reviewer, any disagreements will be resolved by consensus or discussion with a third reviewer.

Methods of analysis/synthesis

If sufficient data are available summary estimates of the sensitivity and specificity together with 95% confidence intervals (CIs) and prediction regions of each mutation test for the prediction of response to treatment will be calculated. We will use the bivariate/hierarchical summary receiver operating characteristic (HSROC) random effects model to generate summary estimates and an SROC curve.[49-

[51] If more than one RCT evaluates treatment effect in patients who were tested with the same KRAS mutation test, then data will be pooled on treatment effect (e.g. hazard ratios, odds ratio, relative risks) within the test positive and, where available test negative arms. The DerSimonian and Laird random effects model will be used to generate summary estimates together with 95% CIs.

Where meta-analysis is considered unsuitable for some or all of the data identified (e.g. due to the heterogeneity and/or small numbers of studies), we will employ a narrative synthesis. Typically, this will involve the use of text and tables to summarise data. These will allow the reader to consider any outcomes in the light of differences in study designs and potential sources of bias for each of the studies being reviewed. Studies will be organised by research question addressed and by KRAS mutation test. A detailed commentary on the major methodological problems or biases that affected the studies will also be included, together with a description of how this may have affected the individual study results. Recommendations for further research will be made based on any gaps in the evidence or methodological flaws.

Methods for synthesising evidence of cost-effectiveness

Identifying and reviewing published cost-effectiveness studies

Exploration of the literature will be focused on published economic evaluations, utility studies and cost studies relevant to the use of KRAS mutation tests (commercial or in-house) to differentiate adults with metastatic CRC, whose metastases are confined to the liver and are un-resectable, and who may benefit from first-line treatment with cetuximab in combination with standard chemotherapy from those who should receive standard chemotherapy alone. The literature search will be performed in the literature databases listed above. In addition, specific health economic databases will be searched (e.g. NHSEED (NHS Economic Evaluation Database), and HEED (Health Economic Evaluation Database). Searches will focus on original papers that report on cost, cost-accuracy, cost-effectiveness or cost-utility analyses.

The results and the methodological quality of the studies selected will be summarised. Assessment of methodological quality will follow the criteria for economic evaluations in health care as described in the NICE methodological guidance.[41, 169] Data extraction will focus on technologies compared, indicated population, main results in terms of costs and consequences of the alternatives compared, and the incremental cost-effectiveness, but also on methods of modelling used (if applicable), analytical methods and robustness of the study findings.

Evaluation of costs, quality of life and cost-effectiveness

Decision analytic modelling will be undertaken to determine the cost-effectiveness of different KRAS mutation tests to decide between standard chemotherapy or standard chemotherapy plus cetuximab in adults with metastatic colorectal cancer and a resected or resectable primary tumour, whose metastases are confined to the liver and are un-resectable but may become resectable after response to chemotherapy. Standard chemotherapy regimens considered include FOLFOX and FOLFIRI.[1]

Diagnosis and treatment strategies

The analysis will consider the long term consequences of technical performance, clinical validity and prognostic value (i.e. prediction of relative response to treatment with cetuximab in combination with standard chemotherapy and from treatment with standard chemotherapy alone) of the different tests.

For tests for which technical performance, clinical validity and/or prognostic value is unclear, when feasible, assumptions will be made to provide some indication of the (range) of cost-effectiveness outcomes.

Model structure

Published studies that report on the value of KRAS mutation testing from initial diagnosis through to intermediate (curative resection rate) and final (progression free and overall survival) health outcomes are likely to be very scarce. During the scoping phase, one end-to-end study of the Therascreen® KRAS RGQ PCR kit was identified,[106] but since this study only included patients that had failed previous chemotherapy it is not directly relevant to the population included in the scope. There are two studies using the LightMix® KRAS assay,[27, 28] but the LightMix® test is currently not in use in laboratories in the UK. The COIN study, finally, uses both pyrosequencing and MADLI-TOF mass array.[54] In order to be able to report on tests listed in the scope for which no data on relative effectiveness (curative resection rate, progression free and overall survival) is available, an alternative scenario analysis will be performed assuming equal prognostic value of the tests. Necessary choices and definitions regarding the structure of the model will depend on the findings from the literature review and consultation with clinical experts.

In order to be consistent with earlier related assessments, the economic model used in STA 176 for Cetuximab in KRAS wild type patients[1] will be used as starting point to model treatment pathways. First, consistency with STA176 will be ensured by replicating the outcomes with the de novo model. Next, the model will be expanded with the test phase and non KRAS wild type patients. In addition,

the existence/availability of any other electronic models that reflect the cost-effectiveness of diagnosis and treatment pathways for these patients, and are representative of current care within the NHS, will be determined.

Issues relevant to analyses:

- Longer term costs and consequences will be discounted using the UK discount rates of 3.5% of both costs and effects.
- One way sensitivity analyses will be performed for all key parameters, especially for parameters in the models which are based on expert opinion.
- Probabilistic sensitivity analyses will be performed using parameter distributions instead of fixed values.
- Decision uncertainty regarding mutually exclusive alternatives will be reflected using cost-effectiveness planes and cost-effectiveness acceptability curves.

A simple draft model structure is presented (Appendix 3); this may be developed/expanded as indicated and as available data allow.

Protocol modification: In the absence of a formal comparator, the strategies will be presented from least to most expensive, and ICERs will be calculated compared to the next cost-effective alternative.

The potential impact of KRAS mutation testing on initial presentation with CRC, rather than testing of stored samples following diagnosis of un-resectable liver metastases (as recommended in NICE Technology Appraisal TA176[1]), will not be formally investigated in the cost-effectiveness analyses. A summary of the arguments for and against testing on presentation will be included in the discussion section of the Diagnostic Assessment Report.

Health outcomes

Utility values, based on literature or other sources, will be incorporated in the economic model. QALYs will be calculated from the economic modelling.

Costs

Resource utilisation will be estimated for the diagnostic tests and treatments. Data for the cost analyses will be drawn from routine NHS sources (e.g. NHS reference costs, Personal Social Services Research Unit (PSSRU), British National Formulary (BNF)), discussions with individual hospitals and with the manufacturers of the comparators, and the online survey.

Handling of information from the companies

All data submitted by the manufacturers/sponsors will be considered if received by the EAG no later than 09/04/2013. Data arriving after this date will not be considered. If the data meet the inclusion criteria for the review they will be extracted and quality assessed in accordance with the procedures outlined in this protocol.

Any 'commercial in confidence' data provided by manufacturers, and specified as such, will be highlighted in [redacted] in the assessment report (followed by company name in parentheses). Any 'academic in confidence' data provided by manufacturers, and specified as such, will be highlighted in [redacted] in the assessment report. Any confidential data used in the cost-effectiveness models will also be highlighted.

Competing interests of authors

None

Timetable/milestones

Milestones	**Completion data**
Draft protocol	10/12/2012
Final protocol	09/01/2013
Progress report	09/04/2013
Draft assessment report	06/06/2013
Final assessment report	04/07/2013

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Appendix 1: Clinical effectiveness search

Embase (OvidSP): 2000-2012/wk 48
Searched 4.12.12

(Colorectal Cancer + KRAS) Limits = 2000-2012

1 exp colon cancer/ or exp rectum cancer/ (150551)
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$)).ti,ab,ot,hw. (243311)
3 CRC.ti,ab,ot. (13754)
4 ((cecum or cecal or caecum or caecal or il?eoc?ecal or il?eoc?ecum) adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$)).ti,ab,ot. (1631)
5 (large intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$)).ti,ab,ot. (1625)
6 (lower intestin$ adj3 (cancer$ or neoplas$ or oncolog$ or malignan$ or tumo?r$ or carcinoma$ or adenocarcinoma$ or metasta$ or meta-sta$)).ti,ab,ot. (17)
7 or/1-6 (246582)
8 k ras oncogene/ (4844)
9 (k ras or kras or V-Ki-ras$ 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).af. (15425)
10 (Kirsten adj3 (murine or rat) adj3 sarcoma$).ti,ab,ot. (391)
11 (thera?screen$ or therascreen$).af. (57)
12 (Cobas adj3 (k ras or kras or V-Ki-ras$ or V-K-ras or c-ki-ras or c-k-ras or ki-ras or ki ras)).af. (8)
13 (sanger sequencing adj3 (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)).af. (14)
14 (pyrosequencing adj3 (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)).af. (25)
15 ((HRM or HRMA) adj3 (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)).af. (13)
16 (high resolution adj3 melt$ adj3 (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)).af. (8)
17 (SNapShot adj3 (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)).af. (5)
18 (Next generation sequencing adj3 (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)).af. (1)
19 high resolution melting analysis/ (632)
20 19 and (8 or 9 or 10) (57)
21 or/8-18,20 (15677)
22 7 and 21 (5546)
23 limit 22 to yr="2000 -Current" (4874)
24 limit 23 to embase (4388)
25 remove duplicates from 24 (4385)

Appendix 2: Related NICE guidance

Cancer service guidance

- Improving outcomes in colorectal cancer. Cancer service guidance (2004). Available from: http://guidance.nice.org.uk/CSGCC

Clinical guideline

- Colorectal cancer: the diagnosis and management of colorectal cancer. NICE clinical guideline CG131 (2011). Available from http://guidance.nice.org.uk/CG131 Date of review: TBC. CG131 updates and replaces TA93 Irinotecan, oxaliplatin and raltitrexed for advanced colorectal cancer, and incorporates TA100 Capecitabine and oxaliplatin in the adjuvant treatment of stage III (Dukes' C) colon cancer and TA105 Laparoscopic surgery for the treatment of colorectal cancer and TA61 Capecitabine and tegafur uracil for metastatic colorectal cancer

Technology appraisals

- Colorectal cancer (metastatic) 2nd line: cetuximab, bevacizumab and panitumumab (review). NICE technology appraisal guidance TA242 (2012). Available from: http://guidance.nice.org.uk/TA242. Date for review: January 2015. Replaces TA150 Colorectal cancer (metastatic) - cetuximab (terminated appraisal) and partially updates TA118 Colorectal cancer (metastatic) - bevacizumab and cetuximab
- Bevacizumab in combination with oxaliplatin and either fluorouracil plus folinic acid or capecitabine for the treatment of metastatic colorectal cancer. NICE technology appraisal guidance TA212 (2010). Available from: http://guidance.nice.org.uk/TA212. Date for review: TBC.
- Cetuximab for the first line treatment of metastatic colorectal cancer. NICE technology appraisal guidance TA176 (2009). Available from: http://guidance.nice.org.uk/TA176. 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."

NICE pathways

- NICE Pathway (November 2011) Colorectal cancer. Available from: http://pathways.nice.org.uk/pathways/colorectal-cancer

Quality standards

- Colorectal cancer. NICE quality standard QS20 (August 2012). Available from: http://guidance.nice.org.uk/QS20

Under development

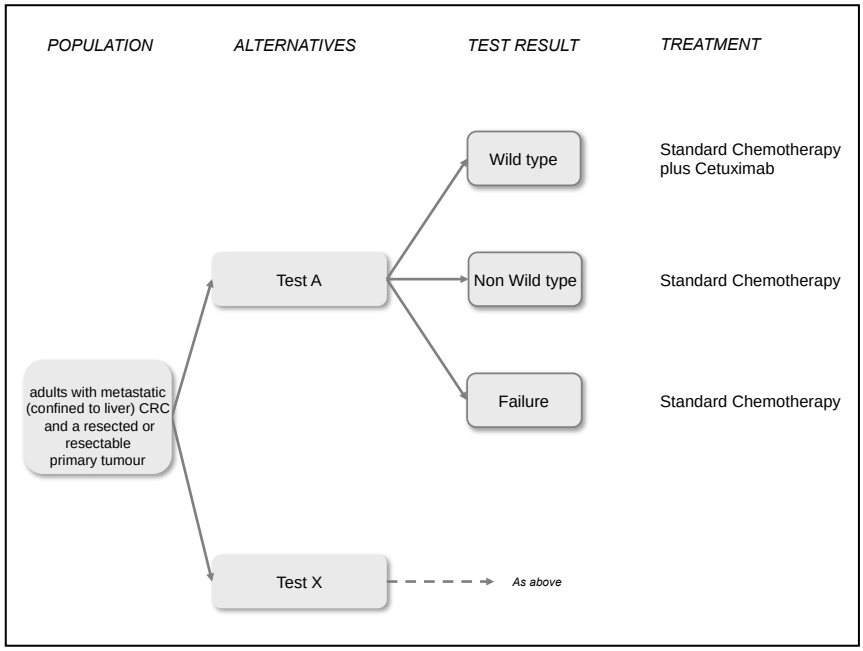
- Aflibercept for the treatment of metastatic colorectal cancer which has progressed following prior oxaliplatin-based chemotherapy. NICE technology appraisal (publication expected October 2013). http://guidance.nice.org.uk/TA/Wave0/617

Terminated

- Panitumumab in combination with chemotherapy for the treatment of metastatic colorectal cancer (terminated NICE technology appraisal TA240). "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." (December 2011). http://guidance.nice.org.uk/TA240

Appendix 3: Draft model structure

Decision tree modelling test phase



Model structure as used in TA 176: [170]