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Molecular Biomarkers for the Evaluation of Colorectal Cancer

Guideline from the American Society for Clinical Pathology, College of American Pathologists, Association of Molecular Pathology, and American Society of Clinical Pathology

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METHODS USED TO PRODUCE THE GUIDELINE

Panel Composition

The College of American Pathologists (CAP), the American Society for Clinical Pathology (ASCP), the Association For Molecular Pathology (AMP), and the American Society of Clinical Oncology (ASCO) convened an Expert Panel (EP) consisting of pathologists, geneticists, oncologists, biostatisticians, laboratory technologists, and a methodologist to develop an evidence-based guideline to help establish standard molecular marker testing, guide targeted therapies, and advance personalized care for patients. All four organizations appointed a representative to serve as a co-chair, with one taking a leadership role (AS). All four organizations approved the appointment of panel members. The EP and the methodologist performed the systematic evidence review. An advisory panel (AP) of pathologists, oncologists, and patient advocates also helped in the development of the guideline. The role of the AP members was to provide guidance and feedback on the key questions for the literature search, vet the draft guideline statements prior to the public comment period, and to review and provide feedback for the manuscript and supplemental digital content.

Conflict of Interest (COI) Policy

Prior to acceptance on the expert or advisory panel, potential members completed a joint guideline conflict of interest (COI) disclosure process, whose policy and form (in effect July 2011) require disclosure of material financial interest in, or potential for benefit of significant value from, the guideline's development or its recommendations 12 months prior through the time of publication. The potential members completed the COI disclosure form, listing any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Examples of conflicts of interest with relevant commercial entities were provided to the participants using a Conflict of Interest (COI) Policy Supplemental Information Evaluation of *KRAS*, *BRAF* and MMR for Colorectal Cancer document.

The ASCP/CAP/AMP/ASCO joint guideline conflicts of interest policy uses the following criteria to define relationships that could be interpreted as constituting an actual, potential, or apparent conflict:
1. Stock options or bond holdings in a relevant commercial entity or self-directed pension plan
2. Research grants from a relevant commercial entity
3. Employment (full or part-time) by a relevant commercial entity
4. Ownership or partnership in relevant corporate entities, including equities and stock options
5. Consulting or advisory fees from relevant commercial entities
6. Other remuneration from relevant commercial entities, including free or discounted products or equipment, trips, accommodations, tickets to sports or entertainment events, etc.
7. Non-remunerative positions of influence in a relevant commercial entity such as officer, board member, trustee, spokesperson, advisor
8. Royalties from relevant commercial entities
9. Intellectual property rights, i.e., patents issued or pending
10. Lecture or speaker fees/honoraria from relevant commercial entities
11. Other relationships, e.g., research collaborations, to be identified with details, as needed

All project participants were required to disclose conflicts prior to beginning and continuously throughout the project's timeline. All disclosed conflicts were reviewed by a joint COI Review Committee composed of staff officials from each of the respective organizations. The joint COI Review Committee agreed, by majority vote, on any resolution of actual or perceived conflicts of interest.

Only one of the co-chairs could receive research support from a relevant commercial entity (no other relevant relationship was allowed). At least 51% of the Expert Panel had no existing or future relationships planned with relevant commercial entities during the development and publication of the practice guidelines. For the remaining 49%, such relationships did not preclude Expert Panel membership. At the discretion of the Co-Chairs, these individuals were asked to recuse themselves from discussing topics and abstained from voting on any decisions or approvals relevant to their relationships. Expert panel members' disclosed conflicts are listed in the appendix of the manuscript. Advisory panel

members had a disclosure requirement, but conflicts were not subject to management by the COI Review Committee.

ASCP, CAP, AMP, and ASCO provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist.

Literature Review and Analysis

The Expert Panel met 11 times through teleconference webinars from July 27, 2013 to September 24, 2015. Additional work was completed via electronic mail. The panel met in person on three occasions (July 26 and 27, 2013, Houston, Texas; Dec 7 and 8, 2013, San Francisco, California; Feb 14 and 15, 2015, Bethesda, Maryland) to review evidence to date and draft recommendations. Additionally, the panel co-chairs met monthly to monitor the project's progress.

Prior to the in-person meeting, the expert panel formed the following key questions on which to base the literature search:

I. What biomarkers are useful to select patients with colorectal cancer (CRC) for targeted and conventional therapies?
   1. Do *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *PTEN*, MMR/MSI, *MLH1* methylation, and gene expression profiling, provide independent prognostic information and/or therapeutically predictive response information for colorectal cancer?
   2. Does *KRAS* provide independent prognostic information and/or therapeutically predictive response information?
   3. Is extended *RAS* testing (such as *NRAS*, exons 1-4, including codons 12, 13, 61, 146) indicated for targeted or conventional therapies?
      a. Does the *KRAS* G13D mutation provide therapeutically predictive response information?
   4. Does *BRAF* provide independent prognostic information and/or therapeutically predictive response information?
   5. Does *PIK3CA* provide independent prognostic information and/or therapeutically predictive response information??
   6. Does *PTEN* provide independent prognostic information and/or therapeutically predictive response information??
   7. Does deficient MMR (dMMR) (detected by MSI or IHC) provide independent prognostic information?
      - Does dMMR provide independent prognostic information in metastatic and in Stage II, III, adjuvant therapy setting?
      - Does dMMR provide similar or different independent prognostic information in Lynch and sporadic MSI?
      - Does dMMR/MSI provide therapeutically predictive response information?
      - Does dMMR provide therapeutically predictive response information in metastatic and/or in Stage II, III, adjuvant therapy?
      - Does dMMR provide similar or different therapeutically predictive response information in Lynch and sporadic MSI?
   8. Does MLH1 methylation provide independent prognostic information and/or therapeutically predictive response information?
   9. Does gene expression profiling provide independent prognostic information and/or therapeutically predictive response information?

II. How should tissue specimens be processed for biomarker testing for CRC management?

   10. What is the optimal CRC specimen to be tested?
   11. How should CRC specimens be processed for molecular testing?
   12. What factors should be evaluated in the selection of tissue specimens to be tested?

III. How should biomarker testing for CRC management be performed?

13. What are the minimum analytic requirements for testing for each marker?
14. What is the appropriate algorithm for CRC molecular testing?
15. What additional considerations are there for biomarker testing?

IV. How should molecular testing of CRC be implemented and operationalized?

16. For what biomarkers in addition to MMR status should patients with hereditary nonpolyposis colorectal cancer (HNPCC or Lynch Syndrome) be tested?
17. Are there specific CRC biomarker testing algorithms that should be used?
18. What is the optimal time for CRC molecular biomarker testing results to be reported?

V. Are there emerging genes/biomarkers that should routinely tested in CRC?

19. What is the optimal time for CRC molecular biomarker testing results to be reported?
20. What research is needed to validate their use?

All expert panelists participated in the systematic evidence review (SER). Each level of the SER (title-abstract, full text review, and data extraction) was performed in duplicate by two members of the expert panel. All expert panelists and a methodologist performed adjudication of the conflicts. Articles meeting the inclusion criteria were assessed for strength of evidence, methodological rigor, and confirmation of validity by the methodologist. Supplemental Figure 1 displays the results of the literature review. All articles were available as discussion or background references. All members of the expert panel participated in developing draft recommendations, reviewing open comment feedback, finalizing and approving final recommendations and writing/editing of the manuscript.

Peer Review

A public open comment period was held from March 30 through April 22, 2015. Twenty one draft statements (8 recommendations, 10 expert consensus opinions, and 3 no recommendation) were posted online on the AMP Web site www.amp.org. The open comment period was publicized via joint society communications announcements and the following societies were deemed to have interest:

- American Society for Clinical Pathology (ASCP)
- College of American Pathologists (CAP)
- Association for Molecular Pathology (AMP)
- American Society for Clinical Oncology (ASCO)
- Association of Directors of Anatomic and Surgical Pathology (ADASP)
- Arthur Purdy Stout Society (APSS)
- Association of Pathology Chairs (APC)
- Canadian Association of Pathologists (CAP-APC)
- United States & Canadian Academy of Pathology (USCAP)
- Quality Initiative in Interpretive Pathology (QIIP) Canadian Partnership Against Cancer
- Society to Improve Diagnoses in Medicine (SIDM)
- Roger G Haggitt Gastrointestinal Pathology Society (GIPS)
- European Society for Medical Oncology (ESMO)
- American Association for Clinical Chemistry (AACC)
- American College of Medical Genetics and Genomics (ACMG)
- Association of Community Cancer Centers (ACCC)
- National Comprehensive Cancer Network (NCCN)
- American Cancer Society
- Partnership Against Cancer American Cancer Society
- Cancer Research and Prevention Foundation
- Cancer Leadership Council
- Union for International Cancer Control

- Fight Colorectal Cancer
- Colon Cancer Alliance
- US Food and Drug Administration (FDA)
- Centers for Medicare & Medicaid Services (CMS)
- Centers for Disease Control and Prevention (CDC)
- Veteran's Affairs (VA) and Department of Defense (DOD)

The website received 248 comments in total (Agree and Disagree responses were also captured). All eight recommendations achieved between 73% to 94% agreement. All ten expert consensus opinion statements achieved between 66% to 90% agreement. Teams of 3 to 4 of expert panel members were assigned 3 to 5 draft recommendations for which to review all comments received and provide an overall summary to the rest of the panel. Following panel discussion, and the final quality of evidence assessment, the panel members determined whether to maintain the original draft recommendation as is, revise it with minor language change, or consider it as a major recommendation change. Resolution of all changes was obtained by majority consensus of the panel using nominal group technique (rounds of email discussion and multiple edited recommendations) amongst the panel members. The final recommendations were approved by the expert panel with a formal vote. The panel considered the risks and benefits throughout the whole process in their considered judgment process. Formal cost analysis or cost effectiveness was not performed.

Organizational review was instituted to review and approve the guideline. ASCP assigned the review to a Special Review Panel. For the CAP, an independent review panel (IRP) representing the Council on Scientific Affairs was nominated to review and approve the guideline. The CAP IRP was masked to the expert panel and vetted through a COI process. The AMP approval process required the review of the Publications and Communications Committee Chair and Executive Committee in order to ensure AMP's protection from liability or other problems due to the publication's content. The Publications and Communications Committee Chair enlisted the assistance of any Subdivision Leadership or Board member in this review. Concurrent reviews by the PCC Chair and Executive Committee are permitted but not required. The ASCO approval process required the review and approval of the Clinical Practice Guidelines Committee.

Dissemination Plans

Final dissemination of the guideline will be a joint process between the four organizations. There are plans to host a resource page which will include a link to the manuscript and supplement, summary of the recommendations, social media as well as patient information guides. The guideline will be promoted and presented at various society meetings.

Systematic Evidence Review (SER)

The objective of the SER was to determine to develop an evidence-based guideline to help establish standard molecular marker testing, guide targeted therapies, and advance personalized care for patients If of sufficient quality, findings from this review could provide an evidence base to support the development of the guideline. The scope of the SER and the key questions (KQs) were established by the EP in consultation with the methodologist prior to beginning the literature search.

Search and Selection

A comprehensive search for literature was performed in MEDLINE using the OvidSP (8/1/2013) and PubMed (9/17/2013) interfaces. The initial MEDLINE search encompassed the publication dates of 1/1/2008 to 8/1/2013 (OvidSP) and 1/1/2008 to 9/17/2013 (PubMed). A supplemental literature search was performed utilizing Scopus (9/25/2013) to identify relevant articles published in journals not indexed in MEDLINE and published between 1/1/2008 and 9/25/2013. The literature search of the electronic databases involved two separate searches in each database, the first using MeSH terms and keywords for the concepts "Colorectal Cancer", "Biomarkers", "Treatment" and "Treatment Outcomes", and the second using terms for the concepts "Colorectal Cancer", "Biomarkers, and "Laboratory Methods". Limits were set for human studies published in English, and a publication filter was applied to exclude lower levels of evidence such as letters, commentaries, editorials, and case reports. The Ovid search was

rerun on 2/12/2015 to identify articles published since 8/1/2013. The Ovid, PubMed, and Scopus search strategies are included as Supplemental Figure 2.

In addition to the searches of electronic databases, an Internet search of international health organizations, the National Guidelines Clearinghouse, and Guidelines International Network was conducted for existing relevant guidelines or protocols. Guidelines were included if they were published since 2008 in English. The proceedings of the meetings of the American Society of Clinical Oncology (ASCO and ASCO-GI), European Society for Medical Oncology (ESMO), and the American Association for Cancer Research (AACR) from the years 2012 and 2013 were also searched for relevant abstracts.

A focused examination of all systematic reviews retrieved by the initial literature search and retained after full text review was performed to identify primary research studies not already included. In addition, recommendations from the expert panel were reviewed, and the reference lists of all articles deemed eligible for inclusion were scanned for relevant reports. The results of all searches were combined and deduplicated.

Selection at all levels was based on predetermined inclusion/exclusion criteria.
Included were:

1. Patients of all ages with colorectal or rectal cancer with a pathology diagnosis of adenocarcinoma or adenocarcinoma with neuroendocrine differentiation, either primary or metastatic
2. Patients of all ages
3. Patients with cancer of any invasive stage
4. Biomarker testing such as *KRAS*, MMR/MSI, *BRAF*, *NRAS*, *PIK3CA*, *PTEN*, *MLH1* methylation, or gene expression profiles.
5. Comparative studies
6. Human studies
7. Studies published in English

Not included were:

1. All other tumor primaries and types (i.e., non-colorectal or non-rectal cancers, tumor types other than adenocarcinoma or adenocarcinoma with neuroendocrine differentiation)
2. Patients with non-invasive tumors (i.e., intraepithelial, dysplasia, in situ, polyps without carcinoma)
3. Studies of colorectal cancers without biomarker testing, novel biomarkers (e.g., VEG-F, XRCC1, Insulin GroMut-h Factor, E, ERCC, micro-RNA, TS, GCC, LINE, CIMP, HER2, CIN Status (LOH), and germline (genetics only) testing)
4. Non-English language articles
5. Animal studies
6. Studies published prior to 2002
7. Non-comparative studies, letters, commentaries, editorials
8. Studies that did not address at least one of the defined inclusion criteria
9. Studies that did not present new evidence
10. Studies with less than 50 patients per comparison arm

Outcomes of Interest

The primary outcomes of interest included survival outcomes and performance characteristics of laboratory testing assays. Survival outcomes included: overall survival (OS), disease-free survival (DFS), progression free survival (PFS), recurrence-free survival, time to recurrence, response to therapy (e.g., complete and partial response). Laboratory data and test performing characteristics included: percent mutation, concordance of detected mutations between primary and metastatic mutations (number of cases (%) with mutations versus number of cases with no mutations in the gene of interest), concordance of mutations (synchronous primary versus metastatic, metachronous primary versus metastatic, between synchronous metastases, between metachronous metastases), sensitivity and specificity of testing methods.

Data Extraction & Management

Following the initial search, titles and abstracts of retrieved studies were reviewed by two expert panel members for relevancy. Conflicts were resolved by initial reviewers and further adjudicated by a project co-chair, if necessary. Those deemed relevant to the key questions that met inclusion criteria and none of the exclusion criteria were moved on to full text review. Full text articles were reviewed for relevancy by two expert panel members to determine eligibility, and conflicts were resolved by the initial reviewers and further adjudicated by a project co-chair, if necessary. In cases of duplication of reporting study results, the most inclusive were retained. Data extraction was performed by one expert panel member and audited by a methodologist. Any discrepancies in data extraction were resolved by discussion. A bibliographic database was established in EndNote (Thomson Reuters, Carlsbad, CA) to track all literature identified and reviewed during the study.

Quality Assessment Methods

An assessment of the quality of the evidence was performed for all retained studies following application of the inclusion and exclusion criteria. Using this method, studies deemed be of low quality would not be excluded from the systematic review, but would be retained and their methodological strengths and weaknesses discussed where relevant. Studies would be assessed by confirming the presence of items related to both internal and external validity, and which are all associated with methodological rigor and a decrease in the risk of bias. These items were assessed as being either yes, no, partial, not reported (NR), or not applicable (N/A) in the following way:

Clinical Practice Guidelines (CPGs) and Systematic Reviews (SRs) were assessed for quality by confirming the following attributes were considered and incorporated in its design as recommended by the Institute of Medicine (IOM).[1] (Summarized in Supplemental Table 1)

- Based on a systematic review (this was not assessed for SRs)
- Included a multidisciplinary panel
- Patient preferences were considered
- Important patient sub-types were considered
- Methods were well-described and reproducible
- Information on potential conflicts of interest were gathered and disclosed
- Quality of the evidence was assessed
- Strength of the evidence was rated
- CPG includes a plan for updating
- Sources of funding are disclosed

Meta-analyses (MAs) were assessed in a similar fashion to CPGs according to the following criteria:

- Based on a systematic review
- Methods were well-described and reproducible
- Quality of the evidence was assessed
- Any planned pooling was stated a priori
- Limitations of the analysis are discussed
- Sources of funding are disclosed

Randomized Control Trials (RCTs) and Quasi-RCTs were assessed for quality according to reporting and full description of:

- Randomization method fully-described
- Details on any blinding was provided
- Provided details of all planned analyses
- Stated the expected effect size and described the statistical power calculation
- Reported the length of follow-up
- Provided a description of the baseline characteristics for all patients by treatment/assessment arm
- Sources of funding are disclosed

Non-randomized clinical trials (NRCTs), prospective cohort studies (PCS), and retrospective cohort studies (RCS) were assessed according to:

- Balance between treatment/assessment groups
- Reporting of baseline characteristics
- Reporting if any adjustments were made where baseline differences were detected
- Sources of funding

Supplemental Table 1 summarizes the quality assessment criteria by study design.

Each study was assessed individually, and then each study type was summarized. Finally, a summary of the overall quality of the evidence was given considering the evidence in totality.

Quality Assessment Results

A total of 62[2-63] studies, comprising 39 systematic reviews, with or without meta-analyses,[2-4, 6, 7, 9, 11-17, 19-22, 24-26, 28-30, 32-34, 36-41, 51, 52, 58-62] two meta-analyses,[8, 27] one RCT,[56] 9 prospective cohort studies,[23, 31, 35, 42, 44-46, 53, 57] and 11 retrospective cohort studies[5, 10, 18, 43, 47-50, 54, 55, 63] were obtained that met the inclusion criteria. Each of the included studies was assessed for quality against specific risk of bias criteria as described in the Methods, and a summary of these assessments appears with each recommendation. The tabulated results of the assessment for each recommendation can be found in Supplemental Tables 2 through 11.

Assessing the Strength of Recommendations

The overarching goal of the panel i was to develop an evidence-based guideline to help establish standard molecular marker testing, guide targeted therapies, and advance personalized care for patients.

Development of recommendations required that the panel review the identified evidence and make a series of key judgments:

1) What are the significant findings related to each KQ or outcome? Determine any regulatory requirements and/or evidence that support a specific action.
2) What is the overall strength of evidence supporting each KQ or outcome? Strength of evidence is graded as Convincing, Adequate or Inadequate, based on four published criteria (Supplemental Table 12). Strength of evidence is a key element in determining the strength of a recommendation.
3) What is the strength of each recommendation? There are many methods for determining the strength of a recommendation based on the strength of evidence and the magnitude of net benefit or harm. However, such methods have rarely (if ever) been applied to the area of biomarker molecular testing practice for colorectal cancer. Therefore, the method for determining strength of recommendation has been modified for this application (Supplemental Table 11), and is based on the strength of evidence and the likelihood that further studies will change the conclusions. Recommendations not supported by evidence (i.e., evidence was missing or insufficient to permit a conclusion to be reached) were made based on consensus expert opinion. Another potential consideration is the likelihood that additional studies will be conducted that fill gaps in knowledge.
4) What is the net balance of benefits and harms? The consideration of net balance of benefits and harms will focus on the core recommendations to adopt specific biomarker molecular testing for colorectal cancer.

Supplemental Table 1 – Quality Assessment Criteria by Study Design

Criteria	Study Design			
	Clinical Practice Guideline (CPG)/Systematic Review (SR)	**Meta-analyses**	**Randomized Control Trial (RCT)/Quasi-randomized Controlled Trial (QRCT)**	**Non-randomized Controlled Trial (NRCT)/Prospective Cohort Study (PCS)/Retrospective Control Study (RCS)**
Based on a systematic review	✓ (CPG only)	✓		
Included a multidisciplinary panel	✓			
Patient preferences were considered	✓			
Important patient sub-types were considered	✓			
Methods were well-described and reproducible	✓	✓		
Information on potential conflicts of interest were gathered and disclosed	✓			
Quality of the evidence was assessed	✓	✓		
Strength of the evidence was rated	✓			
CPG includes a plan for updating	✓			
Sources of funding are disclosed	✓	✓	✓	✓
Any planned pooling was stated a priori		✓		
Limitations of the analysis are discussed		✓		
Randomization method fully-described			✓	
Details on any blinding was provided			✓	
Provided details of all planned			✓	

analyses				
Stated the expected effect size and described the statistical power calculation			✓	
Reported the length of follow-up			✓	
Provided a description of the baseline characteristics for all patients by treatment/assessment arm			✓	✓
Balance between treatment/assessment groups				✓
Reporting if any adjustments were made where baseline differences were detected				✓

Supplemental Table 2 – Quality Assessment Results for Statement 1

Author	Year	Multi-disciplinary panel	Patient preferences considered	Important patient sub-types considered	Well-described and reproducible methods	COIs are examined	Rated quality of the Evidence	Rated strength of the evidence	Includes a plan for updating	Funding source	Overall risk of bias assessment
Systematic review (N=29)											
Adelstein BA et al[2]	2011	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low-moderate
Allegra C et al[3]	2009	Y	NR	NR	Y	Y	NR	NR	NR	Non-industry	Low-moderate
Baas J et al[4]	2011	NR	NR	NR	Y	Y	NR	NR	NR	Non-industry	Moderate
Chen J et al[6]	2013	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low-moderate
Dahabreh I et al[7]	2011	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low-moderate
Health Quality Ontario[12]	2010	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low
Hoyle M et al[13]	2013	Y	NR	NR	Y	Y	Y	Y	NR	Non-industry	Low-moderate
Ibrahim EM et al[15]	2010	NR	NR	NR	Y	Y	NR	NR	NR	NR	Moderate
Jiang Z et al[16]	2013	NR	NR	Y	Y	Y	NR	NR	NR	Non-industry	Low-moderate
Ku G et al[17]	2012	NR	NR	NR	Y	Y	NR	NR	NR	Non-industry	Moderate
Lin A et al[19]	2011	NR	NR	NR	Y	Y	NR	NR	NR	Non-industry	Low-moderate
Linardou H et al[21]	2008	Y	NR	Y	Y	Y	NR	NR	NR	Non-industry	Low-moderate

Loupakis F et al[22]	2012	NR	NR	NR	Y	Y	NR	NR	NR	None	Moderate
Mao C et al[24]	2013	NR	NR	NR	Y	Y	Y	Y	NR	NR	Low-moderate
Mao C et al[26]	2012	NR	NR	Y	Y	Y	NR	NR	NR	Non-industry	Moderate
Petrelli F et al[28]	2012	NR	NR	NR	Y	NR	NR	NR	NR	NR	Moderate
Petrelli F et al[29]	2011	NR	NR	Y	Y	NR	NR	NR	NR	NR	Moderate
Petrelli F et al[30]	2013	NR	NR	NR	Y	Y	NR	NR	NR	NR	Low-moderate
Qui LX et al[32]	2010	NR	NR	NR	Y	Y	NR	NR	NR	NR	Moderate
Ren J et al[33]	2012	NR	Y	Y	Y	Y	Y	Y	NR	Non-industry	Low
Tsoukalas N et al[36]	2012	NR	NR	NR	Y	NR	NR	NR	NR	NR	Moderate
Vale C et al[37]	2012	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low-moderate
Yang ZY et al[39]	2012	NR	NR	Y	Y	Y	NR	NR	NR	Non-industry	Moderate
Zhang L et al[40]	2011	NR	NR	NR	Y	NR	NR	NR	NR	NR	Moderate
Zhou S et al[41]	2012	NR	NR	Y	Y	NR	Y	Y	NR	None	Low-moderate
Ross JS et al[52]	2012	NR	NR	NR	N	NR	NR	NR	NR	NR	High
Sorich MJ	2015	NR	NR	Y	Y	Y	NR	NR	NR	Non-	Low-

et al[59]										industry	moderate
Xu Q et al[60]	2013	NR	NR	Y	Y	NR	NR	NR	NR	NR	Low-Moderate
Ibrahim EM et al[14]	2011	NR	NR	NR	Y	NR	NR	NR	NR	NR	Moderate

Author	**Year**	**Based on systematic review**	**Reproducible methods**	**Quality assessment of included studies**	**Planned pooling stated a priori**	**Limitations of the study**	**Funding source**	**Overall risk of bias assessment**
Meta-analysis (N=2)								
De Roock W et al[8]	2010	NR	Y	NR	Y	Y	Non-industry	Low-moderate
Modest DP et al[27]	2012	NR	Y	NR	Y	Y	Industry	Moderate

Author	**Year**	**Provided details on randomization**	**Provided details on blinding**	**Provided details on any planned analysis**	**Expected effect size calculation and power calculation**	**Reported on length of follow-up**	**Reported on any differences in patient characteristics**	**Funding source**	**Overall risk of bias assessment**
Randomized controlled trials (N=1)									
Douillard JY et al[56]	2013	NR	NR	NR	NR	NR	Y	Partial industry	Low-moderate

Author	**Year**	**Was there balance between treatment/assessment groups?**	**Reporting of baseline characteristics (and any differences detected between groups)**	**Reporting of any adjustment when differences were present**	**Funding source**	**Overall risk of bias assessment**
Prospective cohort studies (N=1)						
Etienne-Grimali MC et al[57]	2014	Y	Y	Y	Non-industry	Low
Retrospective cohort studies (N=1)						
Bando H et al[55]	2013	Y	Y	NR	Non-industry	Low

Supplemental Table 3 – Quality Assessment Results for Statement 2

Author	Year	Multi-disciplinary panel	Patient preferences considered	Important patient sub-types considered	Well-described and reproducible methods	COIs are examined	Rated quality of the Evidence	Rated strength of the evidence	Includes a plan for updating	Funding source	Overall risk of bias assessment
Systematic reviews (N=7)											
Parsons MT et al[51]	2012	N	N	Y	Y	Y	N	N	N	Non-industry	Low-moderate
Mao C et al[25]	2011	N	N	Y	Y	N	N	N	N	Non-industry	Moderate
Lin J et al[20]	2011	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low
Baas J et al[4]	2011	N	N	N	Y	Y	N	N	N	Non-industry	Moderate
Xu Q et al[60]	2013	NA	NA	Y	Y	NR	NR	NR	NR	NR	Low-Moderate
Yuan ZX et al[62]	2013	NA	NA	Y	Y	Y	Y	NR	NR	Non-industry	Low
Cui D et al[58]	2014	NA	NA	Y	Y	NR	NR	NR	NR	Non-industry	Moderate

Supplemental Table 4 – Quality Assessment Results for Statement 3

Author	Year	Multi-disciplinary panel	Patient preferences considered	Important patient sub-types considered	Well-described and reproducible methods	COIs are examined	Rated quality of the Evidence	Rated strength of the evidence	Includes a plan for updating	Funding source	Overall risk of bias assessment
Systematic Reviews (N=2)											
DesGuetz G et al[9]	2009	NR	NR	NR	Y	Y	NR	NR	NR	NR	Moderate
Guastadisegni C et al[11]	2010	NR	NR	NR	Y	Y	NR	NR	NR	Non-industry	Low-moderate

Supplemental Table 5 – Quality Assessment Results for Statement 4

Author, RefID	Year	Multi-disciplinary panel	Patient preferences considered	Important patient sub-types considered	Well-described and reproducible methods	COIs are examined	Rated quality of the Evidence	Rated strength of the evidence	Includes a plan for updating	Funding source	Overall risk of bias assessment
Systematic reviews (N=5)											
Mao C et al[25]	2011	N	N	Y	Y	N	N	N	N	Non-industry	Moderate
Cui D et al[58]	2014	NA	NA	Y	Y	NR	NR	NR	NR	Non-industry	Moderate
Yang ZY et al[61]	2013	NA	NA	Y	Y	NR	Y	NR	NR	Non-industry	Low
Xu Q et al[60]	2013	NA	NA	Y	Y	NR	NR	NR	NR	NR	Low-Moderate
Yuan ZX et al[62]	2013	NA	NA	Y	Y	Y	Y	NR	NR	Non-industry	Low

Supplemental Table 6 – Quality Assessment Results for Statement 5

Author	Year	Multi-disciplinary panel	Patient preferences considered	Important patient sub-types considered	Well-described and reproducible methods	COIs are examined	Rated quality of the Evidence	Rated strength of the evidence	Includes a plan for updating	Funding source	Overall risk of bias assessment
Systematic reviews (N=2)											
Yang ZY et al[39]	2012	N	N	Y	Y	Y	N	N	N	Non-industry	Moderate
Mao C et al[26]	2012	NR	NR	Y	Y	Y	NR	NR	NR	Non-industry	Moderate

Supplemental Table 7 – Quality Assessment Results for Statement 6

Author	Year	Multi-disciplinary panel	Patient preferences considered	Important patient sub-types considered	Well-described and reproducible methods	COIs are examined	Rated quality of the Evidence	Rated strength of the evidence	Includes a plan for updating	Funding source	Overall risk of bias assessment
Systematic reviews (N=4)											
Wang ZH et al[38]	2012	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low-moderate
Lin J et al[20]	2011	NR	NR	Y	Y	Y	Y	Y	NR	Non-industry	Low
Baas J et al[4]	2011	NR	NR	NR	Y	Y	NR	NR	NR	Non-industry	Moderate

Shen Y et al[34]	2012	NR	NR	Y	Y	NR	NR	NR	NR	NR	Moderate

Supplemental Table 8 – Quality Assessment Results for Statement 7

Author	Year	Was there balance between treatment/assessment groups?	Reporting of baseline characteristics (and any differences detected between groups)	Reporting of any adjustment when differences were present	Funding source	Overall risk of bias assessment
Retrospective cohort studies (N=2)						
Cejas P et al[5]	2012	Y	N/A	N/A	Partial industry	Low-moderate
Vakiani E et al[54]	2012	Y	N/A	N/A	NR	Low

Supplemental Table 9 – Quality Assessment Results for Statement 14

Author	Year	Was there balance between treatment/assessment groups?	Reporting of baseline characteristics (and any differences detected between groups)	Reporting of any adjustment when differences were present	Funding source	Overall risk of bias assessment
Prospective cohort studies (N=8)						
Ma ESK et al[23]	2009	Y	N/A	N/A	Non-industry	Low
Pinto P et al[31]	2011	Y	N/A	N/A	NR	Low
Tol J et al[35]	2010	Y	Y	N/A	Partial industry	Low-moderate
Buxhofer-Ausch V et al[42]	2013	Y	N/A	N/A	Non-industry	Low
Chang YS et al[44]	2010	Y	N/A	N/A	Non-industry	Low

Chen Y et al[45]	2009	Y	N/A	N/A	Non-industry	Low
Chow L et al[46]	2012	Y	N/A	N/A	Non-industry	Low
Sundstrom M et al[53]	2010	Y	N/A	N/A	Industry	Low
Retrospective cohort studies (N=7)						
Franklin et al[10]	2010	Y	N/A	N/A	NR	Low
Laosinchai-Wolf et al[18]	2011	Y	N/A	N/A	Industry	Moderate
Carotenuto P et al[43]	2010	Y	N/A	N/A	Non-industry	Low
Cavallini A et al[63]	2010	Y	N/A	N/A	Non-industry	Low
Kristensen LS et al[47]	2010	Y	N/A	N/A	Non-industry	Low
Kristensen LS et al[48]	2012	Y	N/A	N/A	Non-industry	Low
Lang AH et al[49]	2011	Y	N/A	N/A	NR	Low

Supplemental Table 10 – Quality Assessment Results for Statement 18

Author	**Year**	**Was there balance between treatment/assessment groups?**	**Reporting of baseline characteristics (and any differences detected between groups)**	**Reporting of any adjustment when differences were present**	**Funding source**	**Overall risk of bias assessment**
Prospective Cohort Studies (N=4)						
Ma ESK et al[23]	2009	Y	N/A	N/A	Non-industry	Low
Chen Y et al[45]	2009	Y	N/A	N/A	Non-industry	Low
Chow L et al[46]	2012	Y	N/A	N/A	Non-industry	Low
Sundstrom M et al[53]	2010	Y	N/A	N/A	Industry	Low
Retrospective Cohort Studies (N=2)						
Nardon E et al[50]	2010	Y	N/A	N/A	Non-industry	Low

Vakiani E et al[54]	2012	Y	N/A	N/A	NR	Low

Supplemental Table 11 – Quality Assessment Results for Statement 19

Author	Year	Provided details on randomization	Provided details on blinding	Provided details on any planned analysis	Expected effect size calculation and power calculation	Reported on length of follow-up	Reported on any differences in patient characteristics	Funding source	Overall risk of bias assessment
Randomized controlled trials (N=1)									
Douillard JY et al[56]	2013	NR	NR	NR	NR	NR	Y	Partial industry	Low-moderate

Supplemental Table 12. Grades for Strength of Evidence

Convincing • Two or more Level 1[a] or 2 studies (study design and execution) that had an appropriate number and distribution of challenges[b] and reported consistent[c] and generalizable[d] results. • One Level 1 or 2 study that had an appropriate number and distribution of challenges and reported generalizable results.
Adequate • Two or more Level 1 or 2 studies that lacked the appropriate number and distribution of challenges OR were consistent but not generalizable.
Inadequate • Combinations of Level 1 or 2 studies that show unexplained inconsistencies OR combinations of one or more lower quality studies (Level 3 or 4) OR expert opinion.

Reprinted from Teutsch et al[64] with permission from Macmillan Publishers Ltd: The evaluation of genomic applications in practice and prevention (EGAPP) initiative: methods of the EGAPP working group. *Genet Med.* 11(1):3-14, copyright 2009.

[a] Level 1 studies include systematic reviews of Level 2 studies, Level 2 studies include randomized clinical trials (RCT) of good quality, Level 3 studies include RCTs of poor quality, comparative studies with concurrent controls, and comparative study without concurrent controls. Level 4 studies include case series with either post-test or pre-test/post-test outcomes.

[b] Based on number of possible response categories and required confidence in results.

[c] Consistency can be assessed formally by testing for homogeneity, or, when data are limited, less formally using central estimates and range of values.

[d] Generalizability is the extension of findings and conclusions from one study to other settings.

Supplemental Table 13: Grades for Strength of Recommendations

Designation	Recommendation	Rationale
Strong Recommendation	Recommend for or against a particular molecular testing practice for colorectal cancer (Can include must or should)	Supported by convincing or adequate strength of evidence, high or intermediate quality of evidence and clear benefit that outweighs any harms
Recommendation	Recommend for or against a particular molecular testing practice for colorectal cancer (Can include should or may)	Some limitations in strength of evidence (adequate or inadequate), and quality of evidence (intermediates or low), balance of benefits and harms, values, or costs but panel concludes that there is sufficient evidence and/or benefit to inform a recommendation
Expert Consensus Opinion	Recommend for or against a particular molecular testing practice for colorectal cancer (Can include should or may)	Serious limitations in strength of evidence (inadequate or insufficient), quality of evidence (inadequate or low), balance of benefits and harms, values or costs, but panel consensus is that a statement is necessary
No Recommendation	No recommendation for or against a particular molecular testing practice for colorectal cancer	Insufficient evidence or agreement of the balance of benefits and harms, values, or costs to provide a recommendation

Data derived from Guyatt, et al.[65]

Supplemental Table 14. Summary of Studies

Study type and evidence	Number of studies (Number of patients n(N)	Comparison	Tests used*	Overall Survival (OS)	Progression Free Survival (PFS)	Overall Response Rate (ORR)
KRAS [2-4, 6-8, 12-17, 19, 21, 22, 24, 26-30, 32, 33, 36, 37, 39-41, 52, 55-57, 59]						
33 papers: 28 SR 2 M-A 1 RCT 1 PCS 1 RCS	311(74,546)	*KRAS* Mut + versus *KRAS* Mut– -anti-EGFR inhibitor to wild-type patients -anti-EGFR inhibitor treatment independent of *KRAS* status G13D versus 12, 13 versus other, 13D versus other, high EGFR Gene Copy Number (GCN) versus low EGFR-GCN	PCR (including qPCR, PCR, AS-PCR, PCR-RFLP), direct sequencing, pyro-sequencing, FISH, CISH, Sanger, surveyor analysis, SISH, ARMS, Scorpion, hybridization, topographic, genotypic, AD, melting curve analysis, TTGE, HPLC, capillary sequencing, allelic discrimination, SSCP, ASO, MALDI-ToF analysis and WAVE-based SURVEYOR analysis, Luminex xMAP	21 pooled OS: 14 found significant differences *KRAS* wild-type > *KRAS* mutation (N=6) *KRAS* wild-type + anti-EGFR inhibitor > *KRAS* mutation given CT alone (N=4) G13D mutations > codon 12 (N=1) G13D > other mutations (N=1) high EGFR GCN with anti-EGFR inhibitors > low EGFR GCN (N=1) anti-EGFR	21 pooled PFS: 20 found significant differences anti-EGFR inhibitor to CT for *KRAS* wild-type patients > CT alone (N=15), although one of these found the difference in 3rd-line patients only (1413), and one found a disadvantage for patients with the *PIK3CA* exon 20 mutation (869) *KRAS* wild-type > mutation (N=2) G13D mutation > other mutation (N=1)	16 pooled ORR: 14 found significant differences adding an anti-EGFR inhibitor + CT in wild-types patients > CT alone (N=8) *KRAS* wild-type patients > mutation patients (N=4) G13D mutations > codon 12 mutations (N=1) codon 13 > other mutations (N=1) adding an anti-EGFR inhibitor > BSC alone in wild-type patients (N=1)

				inhibitor > Best Supportive Care (BSC) in a cohort of all wild-type patients (N=1) anti-EGFR inhibitor > CT alone (N=2)	G13D mutation > codon 12 (N=1) codon 13 > other (N=1) high EGFR GCN > low EGFR GCN (N=1)	
BRAF [4, 20, 25, 51, 55, 57, 58, 60-62]						
8 SRs 1 PCS 1 RCS	118(16,477**)	*BRAF* Mut + patients with *BRAF* Mut- patients (N=3) *BRAF* Mut + *BRAF* Mut – CT +/- anti-EGFR MoAbs (N=1) correlation study (N=1)	direct sequencing (N=2), pyro-sequencing (N=2), AS, AD, PCR amplification, qPCR, Sanger, rtPCR, genotyping+DS, PCR clamping, melting curve analysis, allele-specific PCR, DNA sequencing, Taqman SNP assay	*BRAF* wild-type patients > *BRAF* mutations (N=4)	*BRAF* wild-type patients > *BRAF* mutations (N=4)	*BRAF* wild-type patients > *BRAF* mutations (N=5)
PIK3CA [4, 20, 26, 55, 61, 66]						
PIK3CA 5 SRs 1 RCS	30(2613)	*PIK3CA* Mut+ versus Mut - (N=4) exon 20 mutations	Direct sequencing (N=3) pyro sequencing (N=3)	4 pooled OS: 3 found significant differences	4 pooled PFS: 3 found significant differences	2 pooled ORR: 1 found significant differences Exon 9 > exon 20

		versus no exon 20 mutations (N=1) All Mut - versus *BRAF* Mut+ and *P1K3CA* Mut +(N=1)	Sanger (N=2) allelic discrimination (N=1) PCR amplification, AS+PCR, genotyping, rtPCR, DNA sequencing, Luminex xMAP	*PIK3CA* Mut - > *PIK3CA* Mut + (N=3)	*PIK3CA* Mut - > *PIK3CA* Mut + (N=2) Exon 9 > exon 20 mutations (N=1)	mutations (N=1)
PTEN[4, 20, 34, 38]						
4 SRs	31(2545)	loss of *PTEN* expression compared with normal *PTEN* expression (N=4)	IHC (N=3) FISH (N=2)	3 pooled OS: 1 found significant differences normal *PTEN* expression > loss of *PTEN* expression (N=1)	3 pooled PFS: 2 found significant differences normal *PTEN* expression > loss of *PTEN* expression (N=2)	2 pooled ORR: 2 found significant differences normal *PTEN* expression > loss of *PTEN* expression (N=2)
MSI/MSS[9, 11, 51, 67]						
4 SRs	127(27,044)	MSI with MSS (N=3) positive with negative MLH1 promoter methylation (N=1)	IHC (N=1) PCR (N=1) flow cytometry (N=1) image analysis	2 pooled OS: 1 found significant differences MSS>MSI (N=1)	2 pooled PFS: 1 found significant differences MSI>MSS (N=1)	1 pooled ORR: No significant differences found

			(N=1)			
CTC[68, 69]						
2 SRs	23(2,487)	CTC vs no-CTC (N=2)	RT-PCT (N=2) IMP (N=1) ICS (N=1)	NR	1 pooled DFS: 1 found significant difference No-CTC > CTC (N=1)	NR

Abbreviations: AD, Allelic Discrimination PCR; ARMS, Amplification Refractory Mutation System; ASO, Allele-Specific Oligonucleotide; AS-PCR, Allele-Specific-Polymerase Chain Reaction; BRAF, proto-oncogene B-Raf/v-Raf murine sarcoma viral oncogene homolog B; BSC, Best Supportive Care; CISH, Chromogenic In Situ Hybridization ; CT, chemotherapy; CTC, Circulating Tumor Cells; EGFR, Epidermal Growth Factor Receptor; FISH, Fluorescence In Situ Hybridization; HPLC, High-Performance Liquid Chromatography; HTA, Health Technology Assessment; IHC, Immunohistochemistry; KRAS, Kirsten RAt Sarcoma viral oncogene homolog; M-A, meta-analysis; MALDI-TOF, Matrix-Assisted Laser Desorption/Ionization-Time of Flight; MLH1, MutL homolog 1; MoAbs, monoclonal antibodies; MSI, MicroSatellite Instability; MSS, Microsatellite Stable; Mut+, mutation positive; Mut-, mutation negative;n, number of studies, N, number of patients; NR, Not Reported; ORR, Objective Response Rate; OS, Overall Survival; PCR, Polymerase Chain Reaction; PCR-RFLP, Polymerase Chain Reaction-Restriction Fragment Length Polymorphism; PCS, prospective cohort study; PFS, Progression-Free Survival; PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase Catalytic subunit Alpha; PTEN, Phosphatase and TENsin homolog; qPCR, quantitative PCR; RCS, retrospective cohort study; RCT, randomized controlled trial; SISH, Silver In Situ Hybridization; SSCP, Single-Strand Conformation Polymorphism; SR, systematic review; TTGE, Tissue TransGlutaminase Enzyme; xMAP, Multiplex assay

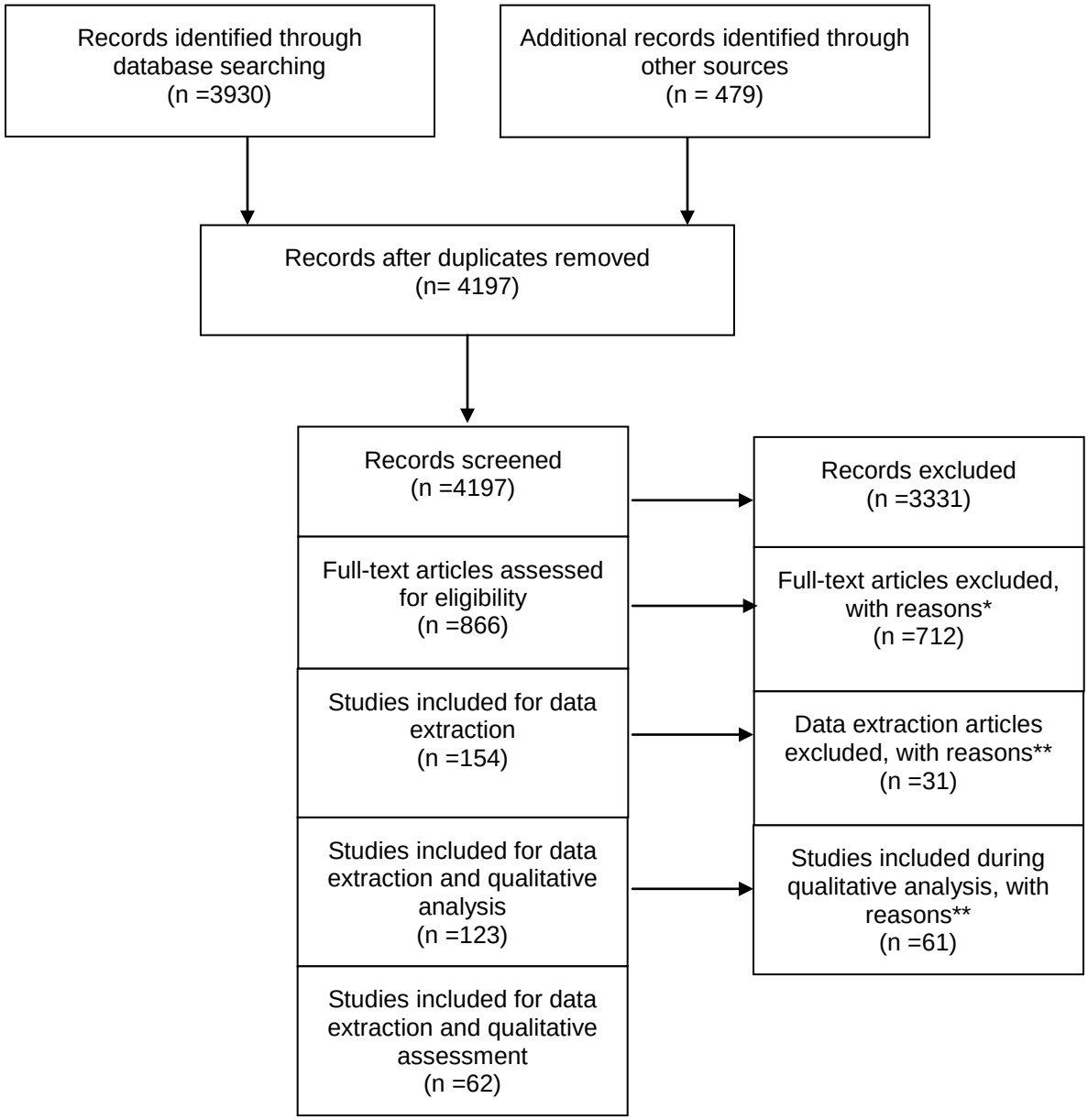
*Codons studied for *KRAS*: G13D, 13, 12, 59, 61, 117, 146; for *BRAF*: V600/V600E (N=10),D549C, 599, K601E, 466, 469, MLH1 (N=1) exon 15/codon 11 (N=1); for *PIK3CA*: Exon 9 (N=6), Exon 20 (N=5), Exon 7 (N=1), Exon 8 (N=1), Exon 18 (N=1), Exon 19 (N=1); for PTEN: not reported.

**Yang et al[61]: Total number of patients not reported

Supplemental Table 15. Emerging evidence on prognostic and predictive colorectal molecular markers

Author, year	Title	Markers studied/ Assays used	Prognostic or predictive
Akiyoshi T et al,[70] 2012	Predicting the response to preoperative radiation or chemoradiation by a microarray analysis of the gene expression profiles in rectal cancer.	Microarray data	Predictive of response to CRT
Bertagnolli MM et al,[71] 2009	p27Kip1 in stage III colon cancer: implications for outcome following adjuvant chemotherapy in cancer and leukemia group B protocol 89803.	p27Kip1 IHC	Prognostic
Dvorak J et al,[72] 2012	[Prognostic significance of changes of tumor epidermal growth factor receptor expression after neoadjuvant chemoradiation in patients with rectal adenocarcinoma].	EGFR expression	Prognostic
Guo GF et al,[73] 2011	Autophagy-related proteins Beclin-1 and LC3 predict cetuximab efficacy in advanced colorectal cancer.	Beclin-1 and LC3 IHC	Predictive: Low expression associated with better outcomes of cetuximab treated CRC-
Kim JC et al,[74] 2009	Chemoresponsiveness associated with canonical molecular changes in colorectal adenocarcinomas.	TGF-beta2 expression	Predictive: Preserved expression associated with response to fluoropyrimidine therapy
Li P et al,[75] 2013	ERCC1, defective mismatch repair status as predictive biomarkers of survival for stage III colon cancer patients receiving oxaliplatin-based adjuvant chemotherapy.	ERCC1 IHC	Predictive of survival
Licitra L et al,[76] 2013	Predictive value of epidermal growth factor receptor expression for first-line chemotherapy plus cetuximab in patients with head and neck and colorectal cancer: analysis of data from the EXTREME and CRYSTAL studies.	EGFR IHC	Not predictive of response to cetuximab
Negri FV et al,[77] 2008	Biological predictive factors in rectal cancer treated with preoperative radiotherapy or radiochemotherapy.	TS IHC	Predictive of response to RCT
Vogelaar FJ et al,[78] 2010	Clinical impact of different detection methods for disseminated tumor cells in bone marrow of patients undergoing surgical resection of colorectal liver metastases: a prospective follow-up study.	Bone marrow tumor cells IHC and RT-PCR	Prognostic
Walsh MD et al,[79] 2009	HLA-DR expression is associated with better prognosis in sporadic Australian clinicopathological Stage C colorectal cancers.	HLADR IHC	Prognostic
Zhang W et al,[80] 2011	A let-7 microRNA-binding site polymorphism in 3'-untranslated region of *KRAS* gene predicts response in wild-type *KRAS* patients with metastatic colorectal cancer treated with cetuximab monotherapy.	TG/GG *KRAS* genotype polymorphism	Predictive of response to cetuximab
Zlobec I et al,[81] 2010	TIA-1 cytotoxic granule-associated RNA binding protein improves the prognostic performance of CD8 in mismatch repair-proficient colorectal cancer.	IHC	Prognostic
Diehl F et al,[82] 2008	Circulating mutant DNA to assess tumor dynamics.	ctDNA	Prognostic

Supplemental Figure 1. Literature Review Flow Diagram



*Excluded based on expert opinion, did not fall within project scope or meet inclusion/exclusion criteria.
**Excluded based on expert opinion, did not meet minimum quality standards, presented incomplete data or data that were not in useable formats
Adapted from Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6:e1000097[83]

Supplemental Figure 2: Literature search strategies

Ovid Search Strings

Concept 1: Colorectal Cancer
1. Colorectal Neoplasms/
2. exp Colonic Neoplasms/
3. exp Colorectal Neoplasms, Hereditary Nonpolyposis/
4. Rectal neoplasms/
5. or/1-4
6. Adenocarcinoma/
7. exp Colon/
8. 6 and 7
9. ((colon or colorectal or rectal) adj3 (cancer or carcinoma or adenocarcinoma or neoplas$ or malignan$ or tumor$)).ti,ab.
10. ((lynch adj4 syndrome) and (colon or colorectal or rectal)).ab,ti.
11. (non?polyposis adj8 (familial or syndrome)).ab,ti.
12. 5 or 8 or 9 or 10 or 11

Concept 2: Biomarkers
1. exp Adaptor Proteins, Signal Transducing/
2. exp Antigens, Neoplasm/
3. Base Pair Mismatch/
4. exp Base Sequence/
5. exp Cell Adhesion Molecules/
6. DNA Mismatch Repair/
7. DNA Methylation/
8. DNA Binding Proteins/
9. DNA, Neoplasm/
10. Gene Amplification/
11. exp Gene Expression/
12. Gene Expression Profiling/
13. Gene Expression Regulation, Neoplastic/
14. Genetic Heterogeneity/
15. Genes, ras/ or Genes, erbB-1/ or Genes, erbB-2/
16. Genetic Markers/
17. Genetic Testing/
18. MicroRNAs/
19. Microsatellite instability/
20. exp Phenotype/
21. exp Phosphatidylinositol 3-Kinases/
22. exp Promoter Regions, Genetic/
23. exp Proto-Oncogene Proteins/
24. PTEN Phosphohydrolase/
25. Proto-Oncogene Proteins B-raf/
26. exp ras Proteins/
27. Receptor, Epidermal Growth Factor/
28. exp RNA, Messenger/
29. exp Tumor Markers, Biological/
30. B?raf.ab. /freq=2
31. K?ras.ab. /freq=2
32. MMR.ab. /freq=2
33. "CpG islands".ti,ab.

34. (mismatch adj3 repair).ab. /freq=2
35. PIK3CA.ab. /freq=2
36. (microRNA$ or miRNA$).ab. /freq=2
37. biomarker$.ab. /freq=2
38. (tumor adj3 marker$).ab. /freq=2
39. germ$ mutation.ab. /freq=2
40. (genom$ adj3 (analys#s or rearrangement? or sequenc$)).ab. /freq=2
41. ((cpg or dna) adj3 methy$).ab. /freq=2
42. cimp.ab. /freq=2
43. transcriptome.ab. /freq=2
44. interactome.ab. /freq=2
45. (GCC adj2 expression).ab. /freq=2
46. "guanylyl cyclase c".ab,ti.
47. ("long interspersed nuclear element?1" or LINE?1).ti,ab.
48. (microarray adj5 analysis).ab. /freq=2
49. ("microsatellite instability" or MSI$).ab. /freq=2
50. PTEN.ab. /freq=2
51. (VEGF$ or XRCC1 or EGFR or HER?2 or MIR?21 or IGF$ or "insulin growth factor$" or ERCC?1 or "long non?coding" or MLH?1 or "MutL homolog").ab. /freq=2
52. (gene adj3 (expression$ or signature$)).ab. /freq=2
53. (predictive adj2 marker$).ab. /freq=2
54. (somatic adj3 mutation$).ab. /freq=2
55. (germ$ adj2 polymorphism).ti,ab.
56. "copy number variation$".ti,ab.
57. (("CIN status" or "chromosomal instability") and (LOH or "loss of heterozygosity")).ti,ab.
58. or/1-57

Concept 3: Outcomes

1. Analysis of Variance/
2. Cluster Analysis/
3. Decision Support Techniques/
4. Diagnosis, Differential/
5. Disease Progression/
6. Disease-Free Survival/
7. Drug Resistance, Neoplasm/
8. exp Early Diagnosis/
9. Kaplan-Meier Estimate/
10. Multivariate Analysis/
11. "Predictive Value of Tests"/
12. Prognosis/
13. Risk Assessment/
14. "Sensitivity and Specificity"/
15. exp Survival Analysis/
16. Survival Rate/
17. exp Treatment Outcome/
18. ((improve$ or overall or disease$ or time) adj3 survival).ab,ti.
19. ((prognos$ or predict$ or therap$ or treatment) adj3 (marker$ or value or respons$)).ab,ti.
20. ((progression$ or recurrence$) adj3 (time or survival)).ab,ti.
21. "response rate".ab,ti.
22. non?respon$.ab,ti.
23. ('clinical usefulness' or (prediction adj3 ability)).ab,ti.
24. (statistical$ adj3 significan$).ab,ti.
25. prognos$.ab. /freq=3

26. RECIST.ab,ti.
27. or/1-26

Concept 4: Treatment
1. Antibodies, Monoclonal/
2. Antibodies, Monoclonal, Humanized/
3. Antimetabolites, Antineoplastic/
4. Antineoplastic Agents/
5. Antineoplastic Combined Chemotherapy Protocols/
6. exp Combined Modality Therapy/
7. Fluorouracil/
8. Leucovorin/
9. Camptothecin/
10. Chemotherapy, Adjuvant/
11. Combined Modality Therapy/
12. Drug Therapy, Combination/
13. Drug Combinations/
14. Phenylurea Compounds/
15. Molecular Targeted Therapy/
16. Neoadjuvant Therapy/
17. Organoplatinum Compounds/
18. Oxonic Acid/
19. Protein Kinase Inhibitors/
20. Tegafur/
21. Pyridines/
22. Individualized Medicine/
23. Anti-Inflammatory Agents, Non-Steroidal/
24. Aspirin/
25. (chemotherap$ or chemoradiotherap$ or chemoradiation or chemosensitivit$).ti,ab.
26. ((personal$ or individual$) adj (medicine or treatment or therapy)).ab,ti.
27. (cetuximab$ or ?folfox$ or folfiri$ or bevacizumab$ or benzimidazole$ or 5?fluorouracil$ or 5?FU or camptothecin$ or irinotecan$ or regorafenib$ or capecitabine$ or panitumumab$ or oxaliplatin$ or S?1 or tegafur$ or oteracil$ or gimeracil$ or avastin$ or fluorouracil$ or trastuzumab$).ab,ti.
28. ("MEK inhibitor$" or TKI$ or PKI$).ab,ti.
29. (anti?egfr or (EGFR adj3 antibody)).ab,ti.
30. (adjuvant or neoadjuvant or epigenetic).ti,ab.
31. ((EGFR or kinase) adj3 inhibitor$).ab,ti.
32. (drug adj5 respons$).ab,ti.
33. (aspirin or NSAID$ or COX2).ab. /freq=2
34. (target$ or therap$ or agent$ or treatment$).ab. /freq=3
35. or/1-34

Concept 5: Laboratory Testing Methods
1. *DNA mutational analysis/
2. *High-Throughput Nucleotide Sequencing/
3. *Oligonucleotide Array Sequence Analysis/
4. *Molecular Diagnostic Techniques/
5. exp *Molecular Typing/
6. *Neoplastic Cells, Circulating/
7. *Comparative Genomic Hybridization/
8. *nucleic acid denaturation/
9. exp *Polymerase Chain Reaction/
10. exp *Sequence Analysis, DNA/

11. *immunohistochemistry/
12. *fluorescent antibody technique/
13. *fluorescent antibody technique, direct/
14. *fluorescent antibody technique, indirect/
15. *Genome-Wide Association Study/
16. exp *Nucleic Acid Amplification Techniques/
17. fixatives/
18. formaldehyde/
19. paraffin embedding/
20. tissue fixation/
21. exp *Transfection/
22. *Radioimmunoassay/
23. exp *Enzyme-Linked Immunosorbent Assay/
24. *Chromatography, High Pressure Liquid/
25. exp *Molecular Probe Techniques/
26. exp *Molecular Probes/
27. *Polymorphism, Restriction Fragment Length/
28. *Polymorphism, Single-Stranded Conformational/
29. ((real?time or reverse or chain) adj3 polymerase).ab,ti.
30. (HPLC or HRMA or RFLP or smart?amplification or sequencing or pyrosequencing or PNA?enriched or RT?PCR or PCR?invader or TaqMan or multiplexing or "laser capture").ab. /freq=2
31. ((melting or sequence or chain) adj2 analysis).ab. /freq=2
32. (("gene expression" or mutation$) adj3 (analys#s or status or profiling)).ab. /freq=2
33. (formalin or paraffin or FFPE or PCR).ab. /freq=2
34. (test$ adj3 (implementation or validat$)).ti,ab.
35. (analytic$ adj3 (method$ or sensitivity or requirement$)).ab,ti.
36. ("life technologies" or quantstudio or agilent or raindance or qiagen or bio?rad or "ion torrent" or illumina or roche or fluidigm or snapshot or mi?seq or hi?seq or high?seq).ab. /freq=2
37. "tumor cell enrichment".ab,ti.
38. (ChIP?seq$ or ChIP?array$ or microarray or "Sanger seq$").ab. /freq=2
39. ((Parallel or next?gen$ or target$ or deep or multiplex) adj3 seq$).ab. /freq=2
40. (DNA adj3 extract$).ab. /freq=2
41. (Circulating adj (tumor cells or nucleic acid or DNA)).ab,ti.
42. (probe adj2 amplification).ab,ti.
43. (macro?dissection or micro?dissection or "laser capture" or fresh?frozen or immunohistochem$ or IHC or "in situ hybridi#ation" or FISH).ab. /freq=2
44. or/1-43

Publicaton Filter:
1. Meta-Analysis as Topic/
2. meta analysis.pt.
3. meta?analy$.tw.
4. (pooled analy$ or statistical pooling or mathematical pooling or statistical summar$ or mathematical summar$ or quantitative synthes?s or quantitative overview).tw.
5. (systematic adj (review$ or overview?)).tw.
6. (exp Review Literature as topic/ or review.pt. or exp review/) and systematic.tw.
7. or/1-6
8. (selection criteria or data extraction or quality assessment or jadad scale or methodological quality).ab.
9. (study adj selection).ab.
10. 8 or 9
11. review.pt.
12. 10 and 11

13. exp randomized controlled trials as topic/ or exp clinical trials, phase III as topic/ or exp clinical trials, phase IV as topic/
14. (randomized controlled trial or clinical trial, phase?III or clinical trial, phase?IV).pt.
15. random allocation/ or double blind method/ or single blind method/
16. (randomi$ control$ trial? or rct or phase?I or phase?II or phase?III or phase?IV or phase?1 or phase?2 or phase?3 or phase?4).tw.
17. or/13-16
18. exp clinical trial/ or exp clinical trial as topic/
19. (clinical trial or clinical trial, phase II or controlled clinical trial).pt.
20. 18 or 19
21. ((singl$ or doubl$ or treb$ or tripl$) adj3 (blind$ or mask$ or dummy)).tw.
22. (allocated adj3 random).tw.
23. (clinic$ adj3 trial$1).tw.
24. ((experimental or study or research) adj3 design).tw.
25. placebos/
26. or/21-25
27. practice guidelines/
28. (practice adj3 guideline?).tw.
29. practice guideline.pt.
30. or/27-29
31. comparative study.pt.
32. consensus development conference.pt.
33. consensus development conference, nih.pt.
34. evaluation studies.pt.
35. or/31-34
36. research support, nih, extramural.pt.
37. research support, nih, intramural.pt.
38. research support, non us gov't.pt.
39. research support, us gov't, non phs.pt.
40. research support, us gov't, phs.pt.
41. or/36-40
42. 7 or 12 or 17 or 20 or 26 or 30 or 35 or 41
43. (comment or interview or letter or editorial or note or erratum or short survey or news or newspaper article or patient education handout or case report or historical article).pt.
44. 42 not 43
45. (colon or colorectal or rectal).ab,ti.
46. 44 and 45

Search #1: Concepts 1, 2, 3, 4 and publication filter.
Search #2: Concepts 1, 2, 5 and publication filter.

Both searches were run and duplicates were removed. Limits were set for human-only studies [NOT (animal NOT human)], studies published in English only, and with the publication dates 1/1/2008 – 8/1/2013. Unique references from both searches were pooled for title/abstract review. The searches were rerun on 2/12/2015 to identify articles published since 8/1/2013.

PubMed – Search #1

(((("adaptor proteins, signal transducing"[mh] OR "antigens, neoplasm"[Mh] OR "base pair mismatch"[mh:noexp] OR "base sequence"[mh] OR "cell adhesion molecules"[Mh] OR "dna mismatch repair"[mh:noexp] OR "dna methylation"[mh:noexp] OR "dna-binding proteins"[mh:noexp] OR "dna, neoplasm"[mh:noexp] OR "gene amplification"[mh:noexp] OR "gene expression"[Mh] OR "gene expression profiling"[mh:noexp] OR "gene expression regulation, neoplastic"[mh:noexp] OR "genetic heterogeneity"[mh:noexp] OR "genes, ras"[mh:noexp] OR "genes, erbb-1"[mh:noexp] OR "genes, erbb-2"[mh:noexp] OR ("genetic markers"[MeSH Terms] OR ("genetic testing"[mh:noexp] OR ("micrornas"[mh:noexp] OR ("microsatellite instability"[mh:noexp] OR "phenotype"[MeSH Terms] OR "phosphatidylinositol 3-kinases"[MeSH Terms]) OR "promoter regions, genetic"[MeSH Terms]) OR "proto-oncogene proteins"[MeSH Terms]) OR ("pten phosphohydrolase"[mh:noexp] OR "proto-oncogene proteins b-raf"[mh:noexp] OR "ras proteins"[MeSH Terms]) OR "receptor, epidermal growth factor"[mh:noexp] OR "rna, messenger"[MeSH Terms]) OR "tumor markers, biological"[MeSH Terms]) AND (Clinical Trial[ptyp] OR Clinical Trial, Phase II[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp] OR Comparative Study[ptyp] OR Evaluation Studies[ptyp] OR Meta-Analysis[ptyp] OR Randomized Controlled Trial[ptyp] OR Research Support, N I H, Extramural[ptyp] OR Consensus Development Conference[ptyp] OR Consensus Development Conference, NIH[ptyp] OR Practice Guideline[ptyp] OR Research Support, N I H, Intramural[ptyp] OR Research Support, Non U S Gov't[ptyp] OR Research Support, U S Gov't, Non P H S[ptyp] OR Research Support, U S Gov't, P H S[ptyp] OR Review[ptyp] OR systematic[sb] OR Validation Studies[ptyp])) AND ((((((((((((((((((((((("dna mutational analysis"[Majr:noexp] OR "high-throughput nucleotide sequencing"[Majr:noexp]) OR "oligonucleotide array sequence analysis"[Majr:noexp]) OR "molecular diagnostic techniques"[Majr:noexp]) OR "molecular typing"[Majr]) OR "neoplastic cells, circulating"[Majr:noexp]) OR "comparative genomic hybridization"[Majr:noexp]) OR "nucleic acid denaturation"[Majr:noexp]) OR "fixatives"[Majr:noexp]) OR "polymerase chain reaction"[Majr]) OR "sequence analysis, dna"[Majr]) OR "immunohistochemistry"[Majr:noexp]) OR "fluorescent antibody technique"[Majr:noexp]) OR "fluorescent antibody technique, direct"[Majr:noexp]) OR "fluorescent antibody technique, indirect"[Majr:noexp]) OR "genome-wide association study"[Majr:noexp]) OR "nucleic acid amplification techniques"[Majr]) OR "fixatives"[Majr:noexp]) OR "formaldehyde"[Majr:noexp]) OR "paraffin embedding"[Majr:noexp]) OR "tissue fixation"[Majr:noexp]) OR "transfection"[Majr]) OR "radioimmunoassay"[Majr:noexp]) OR "enzyme-linked immunosorbent assay"[Majr]) OR "chromatography, high pressure liquid"[Majr:noexp] OR "molecular probe techniques"[majr] OR "Molecular probes"[majr OR "polymorphism, restriction fragment length"[majr:noexp] OR "polymorphism, single-stranded conformational"[majr:noexp])) AND ((((("colorectal neoplasms"[MeSH Terms:noexp] OR "colonic neoplasms"[MeSH Terms]) OR "colorectal neoplasms, hereditary nonpolyposis"[MeSH Terms]) OR "rectal neoplasms"[MeSH Terms:noexp]) OR (("adenocarcinoma, mucinous"[MeSH Terms:noexp] OR "adenocarcinoma"[MeSH Terms:noexp]) AND "colon"[MeSH Terms])) AND (("2008/01/01"[PDAT] : "2013/12/31"[PDAT]) AND English[lang])

PubMed Search #2

(((((Clinical Trial[ptyp] OR Clinical Trial, Phase II[ptyp] OR Clinical Trial, Phase I[ptyp] OR Clinical Trial, Phase III[ptyp] OR Clinical Trial, Phase IV[ptyp] OR Comparative Study[ptyp] OR Evaluation Studies[ptyp] OR Meta-Analysis[ptyp] OR Randomized Controlled Trial[ptyp] OR Research Support, N I H, Extramural[ptyp] OR Consensus Development Conference[ptyp] OR Consensus Development Conference, NIH[ptyp] OR Practice Guideline[ptyp] OR Research Support, N I H, Intramural[ptyp] OR Research Support, Non U S Gov't[ptyp] OR Research Support, U S Gov't, Non P H S[ptyp] OR Research Support, U S Gov't, P H S[ptyp] OR Review[ptyp] OR systematic[sb] OR Validation Studies[ptyp]) AND ((((("colorectal neoplasms"[MeSH Terms:noexp] OR "colonic neoplasms"[MeSH Terms]) OR "colorectal neoplasms, hereditary nonpolyposis"[MeSH Terms]) OR "rectal neoplasms"[MeSH Terms:noexp]) OR (("adenocarcinoma, mucinous"[MeSH Terms:noexp] OR "adenocarcinoma"[MeSH Terms:noexp]) AND "colon"[MeSH Terms]))) AND ((((((((((((((("analysis of variance"[MeSH Terms:noexp] OR "cluster analysis"[MeSH Terms:noexp]) OR "decision support techniques"[MeSH Terms:noexp]) OR "diagnosis, differential"[MeSH Terms:noexp]) OR "disease progression"[MeSH Terms:noexp]) OR "disease-free survival"[MeSH Terms:noexp]) OR "drug resistance, neoplasm"[MeSH Terms:noexp]) OR "early diagnosis"[MeSH Terms]) OR "kaplan-meier estimate"[MeSH Terms:noexp]) OR "multivariate analysis"[MeSH Terms:noexp]) OR "predictive value of tests"[mh:noexp]) OR "prognosis"[MeSH Terms:noexp]) OR "risk assessment"[MeSH Terms:noexp]) OR "sensitivity and specificity"[mh:noexp]) OR "survival analysis"[MeSH Terms]) OR "survival rate"[MeSH Terms:noexp]) OR "treatment outcome"[MeSH Terms])) AND ((((((((((((((((((("antibodies, monoclonal"[MeSH Terms:noexp] OR "antibodies, monoclonal, humanized"[MeSH Terms:noexp]) OR "antimetabolites, antineoplastic"[MeSH Terms:noexp]) OR "antineoplastic agents"[MeSH Terms:noexp]) OR "antineoplastic combined chemotherapy protocols"[MeSH Terms:noexp]) OR "combined modality therapy"[MeSH Terms]) OR "fluorouracil"[MeSH Terms:noexp]) OR "leucovorin"[MeSH Terms:noexp]) OR "camptothecin"[MeSH Terms:noexp]) OR "chemotherapy, adjuvant"[mh:noexp] OR "drug therapy, combination"[MeSH Terms:noexp]) OR "drug combinations"[MeSH Terms:noexp]) OR "phenylurea compounds"[MeSH Terms:noexp]) OR "molecular targeted therapy"[MeSH Terms:noexp]) OR "neoadjuvant therapy"[MeSH Terms:noexp]) OR "organoplatinum compounds"[MeSH Terms:noexp]) OR "oxonic acid"[MeSH Terms:noexp]) OR "protein kinase inhibitors"[MeSH Terms:noexp]) OR "tegafur"[MeSH Terms:noexp]) OR "pyridines"[MeSH Terms:noexp]) OR "individualized medicine"[MeSH Terms:noexp]) OR "anti-inflammatory agents, non-steroidal"[MeSH Terms:noexp] OR "aspirin"[MeSH Terms:noexp])) AND ("adaptor proteins, signal transducing"[Majr] OR "antigens, neoplasm"[Majr] OR "base pair mismatch"[majr:noexp] OR "base sequence"[Majr] OR "cell adhesion molecules"[Majr] OR "dna mismatch repair"[majr:noexp] OR "dna methylation"[majr:noexp] OR "dna-binding proteins"[majr:noexp] OR "dna, neoplasm"[majr:noexp] OR "gene amplification"[majr:noexp] OR "gene expression"[Majr] OR "gene expression profiling"[majr:noexp] OR "gene expression regulation, neoplastic"[majr:noexp] OR "genetic heterogeneity"[majr:noexp] OR "genes, ras"[majr:noexp] OR "genes, erbb-1"[majr:noexp] OR "genes, erbb-2"[majr:noexp] OR ("genetic markers"[Majr] OR ("genetic testing"[majr:noexp] OR ("micrornas"[majr:noexp] OR ("microsatellite instability"[majr:noexp] OR "phenotype"[Majr] OR "phosphatidylinositol 3-kinases"[Majr]) OR "promoter regions, genetic"[Majr]) OR "proto-oncogene proteins"[Majr]) OR ("pten phosphohydrolase"[majr:noexp] OR "proto-oncogene proteins b-raf"[majr:noexp] OR "ras proteins"[Majr]) OR "receptor, epidermal growth factor"[majr:noexp] OR "rna, messenger"[Majr]) OR "tumor markers, biological"[Majr])) AND (("2008/01/01"[PDAT] : "2013/12/31"[PDAT]) NOT "animals"[MeSH Terms:noexp]) AND English[lang]

Scopus Search Strategy

(((((TITLE-ABS-KEY(colorectal OR colon OR rectal) AND TITLE-ABS-KEY(cancer OR carcinoma OR neoplasm OR neoplasia))) AND ((TITLE-ABS-KEY(molecular OR biomarker OR KRAS OR BRAF OR MSI OR MMR OR NRAS OR PIK3CA OR PTEN OR MIR21 OR MLH1) AND TITLE-ABS-KEY("laboratory method" OR technique OR validation OR implementation))) AND (guideline OR metaanalysis OR systematic OR "randomized controlled" OR "clinical trial")) AND NOT (mouse OR mice OR animal OR murine OR "cell line"))) or ((((TITLE-ABS-KEY(colorectal OR colon OR rectal) AND TITLE-ABS-KEY(cancer OR carcinoma OR neoplasm OR neoplasia))) AND ((TITLE-ABS-KEY(molecular OR biomarker OR KRAS OR BRAF OR MSI OR MMR OR NRAS OR PIK3CA OR PTEN OR MIR21 OR MLH1) AND TITLE-ABS-KEY((treatment OR chemotherapy OR therapy) AND (outcome OR survival OR progression OR recurrence))) AND (guideline OR metaanalysis OR systematic OR "randomized controlled" OR "clinical trial")) AND NOT (mouse OR mice OR animal OR murine OR "cell line"))) AND (LIMIT-TO(SUBJAREA,"MEDI") OR LIMIT-TO(SUBJAREA,"BIOC") OR LIMIT-TO(SUBJAREA,"PHAR") OR LIMIT-TO(SUBJAREA,"HEAL")) AND (LIMIT-TO(PUBYEAR,2013) OR LIMIT-TO(PUBYEAR,2012) OR LIMIT-TO(PUBYEAR,2011) OR LIMIT-TO(PUBYEAR,2010) OR LIMIT-TO(PUBYEAR,2009) OR LIMIT-TO(PUBYEAR,2008) OR LIMIT-TO(PUBYEAR,2007))

Unique results published in journals not indexed in MEDLINE were added to the evidence pool.

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