Technology Assessment

Technology Assessment Program

Systematic Reviews on Selected Pharmacogenetic Tests for Cancer Treatment: *CYP2D6* for Tamoxifen in Breast Cancer, *KRAS* for anti-EGFR antibodies in Colorectal Cancer, and *BCR-ABL1* for Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia

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Systematic Reviews on Selected Pharmacogenetic Tests for Cancer Treatment: *CYP2D6* for Tamoxifen in Breast Cancer, *KRAS* for anti-EGFR antibodies in Colorectal Cancer, and *BCR-ABL1* for Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia

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Tufts EPC

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Peer Reviewers

We wish to acknowledge individuals listed below for their review of this report. This report has been reviewed in draft form by individuals chosen for their expertise and diverse perspectives. The purpose of the review was to provide candid, objective, and critical comments for consideration by the EPC in preparation of the final report. Synthesis of the scientific literature presented here does not necessarily represent the views of individual reviewers.

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Summary

Background

The challenges in the integration of cancer pharmacogenetics and targeted therapies in clinical practice should require evidence of benefit to the patients (a favorable balance of harms and benefits of testing), cost-effectiveness for the healthcare system, incorporating patient preferences, improving provider education, and anticipating potential ethical and social implications. It is possible that pharmacogenetic testing and the subsequent use of targeted therapies will add cost without producing clinically meaningful improvements in patient outcomes. In the absence of data that can address its clinical utility and value, integration of pharmacogenetic testing in the healthcare system is not straightforward.

This Technology Assessment assesses the evidence on the benefits and harms of three pharmacogenetic tests employed for three different diseases pertinent to the Medicare beneficiary population: variations in *CYP2D6* and response to tamoxifen in breast cancer; variations in *KRAS* and response to cetuximab and panitumumab in colorectal cancer and variations in *BCR-ABL1* and response to imatinib, dasatinib and nilotinib in chronic myeloid leukemia. The Coverage and Analysis Group at the Centers for Medicare and Medicaid Services (CMS) requested this report from The Technology Assessment Program (TAP) at the Agency for Healthcare Research and Quality (AHRQ). AHRQ assigned this report to the following Evidence-based Practice Center: Tufts EPC (HHSA 290 2007 100551).

Methods

We performed three systematic reviews of the published literature to address the following Key Questions for each of the aforementioned topics:

1) Does the genetic test result predict response to therapy?
2) What patient- and disease-related factors affect the test results, their interpretation or their predictive response to therapy?
3) How does the gene testing impact the therapeutic choice?
4) What are the benefits and harms or adverse effects for patients when managed with gene testing?

We searched MEDLINE from inception until August 2009. For the *CYP2D6* and *KRAS* systematic reviews the search was updated through the end of March 2010. Details on which studies are considered eligible to address these key questions are described in the Methods Section of the full Technology Assessment. Briefly, eligible are studies that report primary data to address the aforementioned questions. These include studies on patients with the diseases of interest and presented information on patient-relevant outcomes (mortality; disease progression; and treatment failure, as defined in each study) stratified by the genetic factor. Especially for Key Question 2, eligible are studies that performed interaction analyses between test results and the factor of interest. For Key Question 3 we consider only studies that explicitly describe changes in treatment

plans before and after testing. For Key Question 4, we refer to evidence on benefits and harms beyond what is covered in Key Question 1 (prediction of response to therapy).

Results

Our literature searches did not identify any eligible studies for Key Questions 2 (no study reported statistical interactions), 3 (no study explicitly reported treatment plans before and after testing) or 4 (no study explicitly reported evidence on benefits or harms beyond what is covered in Key Question 1).

Thus, we summarize here the relevant evidence identified for Key Question 1 for each topic. **Table S1** shows the number of studies that were included in the three systematic reviews.

Section 1: Variations in *CYP2D6* and response to tamoxifen in breast cancer

There were no consistent associations between *CYP2D6* polymorphisms and outcomes in tamoxifen treated women with breast cancer across 16 studies included in the systematic review. The included studies were generally small in size, followed poor analytic practices, and differed both in the direction and in the formal statistical significance of their results. It is unclear whether pharmacogenetic testing of germline (heritable) mutations in *CYP2D6* can predict differential response to adjuvant tamoxifen in women with non-metastatic breast cancer. Further, evidence is severely limited for tamoxifen-treated women with metastatic disease. Our conclusions are analogous to the 2009 American Society of Clinical Oncology (ASCO) practice guideline update.

We documented extensive heterogeneity in the definitions of *CYP2D6*-derived metabolizer categories across eligible studies. Determining the clinically meaningful genetic comparisons in a multi-allelic system is challenging, and may be subjected to offers authors opportunities for data dredging. Most studies were relatively small and thus underpowered to detect what would be a plausible effect size for the modification of response to tamoxifen by a single polymorphism.

We found no evidence on whether patient or disease relevant factors affect the association between *CYP2D6*-derived metabolizer status and outcomes in tamoxifen treated women. Such evidence would be obtained by examining interaction effects between the factors of interest and metabolizer status. However, no study performed such analyses. Several studies performed simple adjustments for patient level factors. This is not only noninformative, but also questionable from an analytic standpoint.

Section 2: Variations in *KRAS* and response to cetuximab and panitumumab in colorectal cancer

We identified 47 eligible studies. Of those, 37 were conducted in the second-line metastatic setting, 8 were conducted in the first line metastatic setting and 2 were conducted in the neo-adjuvant setting. When treated with anti-EGFR antibodies, patients with *KRAS* mutations were less likely to experience treatment benefit, compared to patients whose tumors were wild-type for *KRAS* mutations, for all outcomes assessed.

These results were confirmed in several RCT-based analyses of progression-free survival that demonstrated a significant treatment-by-*KRAS* mutation interaction in three out of the four cases where such analyses were reported.

The direction of effect was consistent among studies, and formal significance was achieved in the majority of individual studies that reported information on the clinically relevant outcomes of overall and disease-free survival. Most studies pertained to patients who had received previous cytotoxic chemotherapy. These observations are analogous to guidance provided recently by ASCO, the Food and Drug Administration (FDA), and the European Medicines Agency (EMeA).

Although few studies were conducted in the first line setting, for all outcomes and particularly for treatment failure, the predictive ability of *KRAS* mutations was lower compared to that observed in pre-treated patients. This observation argues for the need for further studies in the first line setting.

Regarding the two different agents, cetuximab and panitumumab, the predictive ability of *KRAS* mutations appeared to be similar. However, the bulk of available evidence for this comparison was related to studies assessing panitumumab as monotherapy, and in all cases in patients pre-treated with cytotoxic chemotherapy.

Section 3: Variations in *BCR-ABL1* and response to imatinib, dasatinib and nilotinib in chronic myeloid leukemia

We identified 31 eligible studies. The presence of *any* BCR-ABL1 mutation (that is when considering all mutations together) does not appear to predict differential response to tyrosine kinase inhibitor (TKI) treatments (defined as imatinib-, dasatinib-, and nilotinib-based regimens). There is consistent evidence that presence of the relatively rare T315I mutation can predict TKI treatment failure, mainly in terms of hematologic and cytogenetic response.

The fact that presence of *any BCR-ABL1* mutation does not appear to differentiate response to TKI therapies is emblematic of the complexity of this topic: different mutations may confer different resistance to each of the three drugs. Exploring such relationships with systematic reviews of published aggregate data is extremely challenging. Other approaches, including collaborative registries of CML patients are much better suited to address such questions.

Further, the majority of evidence pertains to the short term surrogate outcomes of hematologic, cytogenetic or molecular response. Data on overall or progression-free survival are sparse. Finally, most evidence is on second line TKI treatments, especially dasatinib and nilotinib, and originates from a small number of referral cancer centers where those agents were first-tested before becoming more widely available.

Table S1: Evidence map of studies included in this report.

	Breast Cancer *CYP2D6*		Colorectal Cancer *KRAS*[a]						CML *BCR-ABL1**					
	Tamoxifen		Cetuximab		Panitumumab		Both		Imatinib			Dasatinib		Nilotinib
	Adjuvant	Metastatic	1st line	2nd line	1st line	2nd line	1st line	2nd line	1st line	2nd line	2nd line	3rd line	2nd line	
Mortality	7 (1)	0	4 (2)	20 (1)	1 (1)	2 (1)	0	3 (0)	1 (0)	0	0	0	0	
Progression	14 (2)	1 (0)	6 (3)	23 (1)	1 (1)	3 (1)	0	5 (0)	1 (0)	1 (0)	0	0	0	
Treatment Failure	0	1 (0)	7 (4)	28 (1)	1 (1)	3 (1)	0	6 (0)	HR	1 (0)	4 (0)	9 (0)	1 (0)	3 (0)
									CyR	1 (0)	4 (0)	12 (0)	1 (0)	3 (0)
									MolR	0	0	1 (0)	0	0
									Comp	1 (0)	0	0	0	0

Summary table of studies included in this report, organized by topic and outcomes assessed. Numbers in parentheses are RCT-based analyses. HR, Hematologic response. CyR=cytogenetic response. MolR= Molecular response. Comp=composite definition (i.e. hematologic + molecular). Here we only count as "RCTs" randomized trials that compare using vs not using tamoxifen, anti-EGFR antibodies or tyrosine kinase inhibitors, respectively, for the three topics.

[a] Two additional prospective single arm studies assessed pathologic response in patients receiving cetuximab in combination with chemoradiotherapy for rectal cancer.

Crosscutting issues

Here we summarize a range of methodological issues that we identified across the three topics. These issues are applicable to all three tests, which have quite different characteristics. In the first topic (*CYP2D6* polymorphisms and tamoxifen response modification) we evaluate germline polymorphisms, i.e., heritable common variations. In the other two cases we examine somatic mutations, i.e., genetic variations that are not heritable, and may evolve during the course of the disease. In the *KRAS* case mutations were relatively frequent, but the T315I mutation in *BCR-ABL1* is relatively rare. We comment only on methodological issues that we came across in the three topics; broader consideration of this literature is outside the scope of this work.

Study design issues

- Treatment-by-gene (polymorphism or mutation) interactions were often not formally assessed.
- It may not be necessary to design new studies to address pharmacogenetic associations. It is possible to "repurpose" already completed RCTs in which the drugs of interest are tested against a suitable comparator, by genotyping tissue samples from enrollees.
- Studies often had small sample sizes and thus would not be able to reliably identify effects as small as those anticipated for most pharmacogenetic tests (unlike the relatively large effects observed for *KRAS*) or effects determined by rare genetic variations (such as *BCR-ABL1* mutations).

Outcomes

- Most studies assessed surrogate short term outcomes of treatment failure, as defined by imaging or laboratory measurements. Data on the clinical outcomes of overall or progression-free survival are sparse.
- In all three examples, we found no evidence on whether testing impacts on therapeutic decisions, or on harms associated with testing and its downstream effects.

Heterogeneity in the classification of genetic factors

- We documented extensive heterogeneity in way genetic factors were grouped and analyzed across the included studies.
- These and other challenges limit the usefulness of meta-analysis of aggregate level data, and are better addressed by meta-analyses of individual patient data.

Statistical analyses

- Adjustments for potential confounding factors were often not based on sound epidemiological principles.
- Adjustments for multiple comparisons were not documented in the included studies. This is a major issue in genetic epidemiology, because of the large number of possible hypotheses that can be examined.

Other issues

- Multiple studies on each topic frequently originated from a limited number of specialized centers, posing problems in identifying nonoverlapping populations, and potentially threaten the generalizability of the findings.

Introduction

The next decade may hold great promise for using genetic information to prevent diseases and to manage patients afflicted with these conditions. In the era of evidence-based medicine, the clinical usefulness of alternative prevention and management strategies should be demonstrated, rather than assumed. The subtyping of diseases by cancer genetics and genomics has opened the door to targeted therapies. The ability to target specific therapies to those individuals who can benefit from them will become increasingly urgent among patients with advanced or life-threatening diseases where fewer alternative treatments may be available. The challenges in the integration of cancer pharmacogenetics and targeted therapies in clinical practice require evidence of benefit to the healthcare system, incorporating patient preferences, improving provider education, and anticipating potential ethical and social implications. It is possible that pharmacogenetic testing and the subsequent use of targeted therapies will add cost without producing clinically meaningful improvements in patient outcomes. In the absence of data that can address its clinical utility and value, integration of pharmacogenetic testing in the healthcare system can be a challenge.

Definition of genetic and pharmacogenetic tests

There are several definitions of genetic tests that are currently available, including the Secretary's Advisory Committee on Genetic Testing[a]. For the purpose of this report we consider tests of human DNA, RNA or proteins for diagnosis, prognosis, risk prediction, treatment guidance or patient monitoring or other clinical purposes, through detection of heritable or nonheritable genetic variations.

A pharmacogenetic test is a specific type of genetic test. It aims to identify patients' differential response to specific pharmacotherapies. Pharmacogenetic tests are meant to guide treatment strategies, patient evaluations and decisions based on their ability to predict response to treatment in particular clinical contexts.

Pharmacogenetic tests evaluated in this technology assessment

The Coverage and Analysis Group at the Centers for Medicare and Medicaid Services (CMS) requested this report on several pharmacogenetic tests relevant to the Medicare beneficiary population from The Technology Assessment Program (TAP) at the Agency for Healthcare Research and Quality (AHRQ). AHRQ assigned this report to the following Evidence-based Practice Center: Tufts EPC (HHSA 290 2007 100551). The aforementioned tests were selected after discussions between AHRQ, CMS and Tufts EPC as examples of tests that are relevant to the Medicare population, evaluate common disease conditions, and meet the definitions of a (pharmaco)genetic test.

These tests are: *CYP2D6* testing and response to tamoxifen in patients with breast cancer; *KRAS* testing and response to cetuximab or panitumumab in patients with colorectal cancer; and *BCR-ABL1* mutation testing and response to imatinib, dasatinib

[a] Department of Health and Human Services Secretary's Advisory Committee on Genetic Testing. Request for public comment on a proposed classification methodology for determining level of review for genetic tests. 65(236), 76643-76645. 2000. Federal Register. 2000

and nilotinib in patients with chronic myeloid leukemia. Below is a short description of these tests. More detailed information per test is discussed later in this report.

CYP2D6 and response to tamoxifen in patients with breast cancer

Tamoxifen is a pro-drug of more biologically potent antagonists of the estrogen receptor in breast tissue such as 4-hydroxytamoxifen and endoxifen, the latter of which is now considered the most active form of tamoxifen metabolites. They are biotransformed through a complicated metabolic pathway, in which CYP2D6 is a key enzyme. Several variant alleles of the *CYP2D6* gene have different enzymatic activity and can potentially affect the pathway and metabolite transformation, potentially impacting on patients' response to tamoxifen treatment.(1) These variants are found in up to 40 percent of the general population.

KRAS and response to cetuximab or panitumumab in patients with colorectal cancer

Cetuximab targets epidermal growth factor receptor (*EGFR*), a member of the subclass I of the receptor tyrosine kinase superfamily, which is overexpressed in up to 80 percent of colorectal cancers. Panitumumab is a similarly acting drug. Both drugs block the activation of an EGFR-derived cascade of biochemical events that can ultimately stimulate cellular proliferation, invasion and metastasis. *KRAS* is an oncogene implicated in the *EGFR* pathway. Mutations in *KRAS* can initiate continuous activation of the downstream part of the *EGFR*-derived cascade regardless of *EGFR*-dependent activation. Thus, examining the mutation status of *KRAS* has the potential to predict therapeutic outcomes of treatment strategies involving cetuximab(2;3) or panitumumab.

BCR-ABL1 and response to imatinib, dasatinib and nilotinib in patients with chronic myeloid leukemia

BCR-ABL1 is a chimeric oncogene (a cancer-causing gene generated by the fusion of parts of genetic material that are normally not adjacent) arising from the translocation between chromosomes 9 and 22. The gene encodes an enzyme (protein) called tyrosine kinase, which is central to the pathogenesis of chronic myelogeneous leukemia. Imatinib, dasatinib and nilotinib are tyrosine kinase inhibitors, i.e., drugs that bind to the tyrosine kinase enzyme and inhibit its activity in a competitive fashion. Specific mutations in *BCR-ABL1* have been shown to confer resistance to imatinib both *in vitro* and *in vivo*, by affecting the binding of the drug to the tyrosine kinase enzyme. Based of these observations, the detection of mutations of the *BCR-ABL1* gene has been proposed as a pharmacogenetic test with potential impact on management decisions.(4)

Organization of this technology assessment report

We first describe common methods used throughout this Technology Assessment. We then present the systematic review of each topic in detail, in separate sections. Each of these sections includes a background, additional methods (e.g., topic-specific search strategies, eligibility criteria, or outcomes), results, and discussion:

- Section 1 is variations in *CYP2D6* and response to tamoxifen in breast cancer.
- Section 2 is variations in *KRAS* and response to cetuximab and panitumumab in colorectal cancer.

- Section 3 is variations in *BCR-ABL1* and response to imatinib, dasatinib and nilotinib in chronic myeloid leukemia.

The final part of the Technology Assessment discusses crosscutting methodological issues across the three topics.

Generic methods common to the three topics

This Technology Assessment is based on systematic reviews of the literature. CMS and AHRQ defined the following generic Key Questions that are common to all three topics: The topic-specific Key Questions are obtained from the generic ones by substituting the pharmacogenetic test, the drug intervention, and the cancer of interest. The topic-specific Key Questions are listed in the Methods section of each topic.

Generic Key Questions

Key Question 1: Does a gene test result predict response to therapy?

Outcomes of interest ("response") are clinical outcomes such as overall survival, recurrence, and disease progression, and no response to treatment by imaging or laboratory criteria.

Key Question 2: What patient- and disease-related factors affect the test results, their interpretation or their predictive response to therapy?

Examples of patient-level factors are age, sex (when applicable), or racial/ethnic descent. Examples of disease-related factors are tumor type or cancer stage.

Key Question 3: How does the gene testing impact the therapeutic choice?

Only studies that explicitly describe changes in treatment plans before and after testing are considered here. For example, in that case one could record the number (percentage) of times therapy planned before genetic testing changed after the test.

Key Question 4: What are the benefits and harms or adverse effects for patients when managed with gene testing?

Any cognitive, behavioral or other health effects of testing with the three tests of interest. These can include direct effects of the process of testing (e.g., increased anxiety). We would include here downstream effects of testing that are beyond those described in Key Question 1.

Systematic review process

A systematic review is a structured analysis of the literature that follows a series of predefined steps to answer a collection of well defined key questions: Searching of the literature, screening of citations for inclusion according to predefined criteria, critically reviewing publications in full text to assess their methodological and reporting quality and extract data as per protocol, and synthesizing individual study results qualitatively or quantitatively (meta-analysis).

Details on each systematic review are given in the pertinent sections and Appendices. Here we describe general methods that are common to all three sections.

Literature searches and screening of citations

For each of the three topics we performed electronic searches in OVID MEDLINE® using specific search strategies from inception through the 4th week of August 2009 with no language restriction. (The actual search strategies are described in the individual Sections.) We limited searches to humans. The first few hundred (300-500) citations in each topic were screened jointly by four authors to ensure that screening criteria were well understood and applied uniformly. Thereafter, three authors screened nonoverlapping sets of the remaining citations.

We complemented the electronic literature searches by perusing the reference lists of included papers and of several relevant narrative or systematic reviews.

Generic eligibility criteria

For each of the three topics the following generic eligibility criteria were set for all Key Questions. Additional topic specific criteria are described in the Methods section of the specific topics.

Briefly, eligible are studies that report primary data to address the aforementioned questions. These include studies on patients with the diseases of interest and presented information on patient-relevant outcomes (mortality; disease progression; and treatment failure, as defined in each study) stratified by the genetic factor. For Key Question 2, we demanded data from interaction tests.

1. The study associated predefined genetic variations with clinical outcomes in patients treated with the drug of interest
    a. Clinical outcomes of interest were overall or cause specific mortality, recurrence, relapse, or disease progression as defined by each study, and the corresponding time to event outcomes (e.g., time-to-death, time-to-progression).
    b. Treatment failure was defined failure by radiologic criteria for colorectal solid cancers or laboratory criteria (lack of hematologic, cytogenetic or molecular response) for CML.
    c. Somatic genetic variations must be assessed in malignant tissue obtained before administering the drug of interest (for the 2nd and 3rd topic, *KRAS* and *BCR-ABL1*). Germline genetic variations may have been ascertained at any time (1st topic, *CYP2D6*).
2. Eligible designs are
    a. Randomized or nonrandomized studies comparing patient management with versus without pharmacogenetic testing.
    b. Association studies of pharmacogenetic testing and clinical outcomes in patients treated with the drug. These studies resemble a "case-only" design, and assume that the test result *does not predict* the clinical outcome in untreated patients.
    c. Association studies of pharmacogenetic testing and clinical outcomes examining both patients who received and patients who did not receive the drug of interest. These studies have the opportunity to examine interactions between the test results and treatment type. This analysis can inform on

whether the test predicts clinical outcomes in treated rather than untreated patients.[b]

3. At least 10 patients analyzed (total).

Especially for Key Question 2 eligible are studies that performed interaction analyses between test results and the factor of interest.[c] For Key Question 3 we consider only studies that explicitly describe changes in treatment plans before and after testing. For Key Question 4, we refer to evidence on benefits and harms beyond what is covered in Key Question 1 (prediction of response to therapy).

Data abstraction

We extracted bibliographic information (first author name, journal and year of publication), as well as information on study design including inclusion criteria, patient characteristics (demographic and staging information), treatment characteristics (dosing schedule and compliance), details on genetic testing and frequency of specific genotypes or mutation positions, definitions of outcomes, and numerical data, as described in the individual sections. If a paper did not explicitly report these data, but cited other publications instead, we consulted the cited publications.

Identification of studies with overlapping populations

We took particular care to identify studies with at least partially overlapping populations. We cross-checked author names, institutions, patient enrollment periods, citations to previous studies, patient numbers, descriptions of treatment protocols, and supplementary materials of all eligible papers to identify sets of potentially overlapping publications. We defined "at least partially overlapping" studies that had any patients in common, because it is often impossible to determine the degree of overlap. In the two systematic reviews (*KRAS* and *BRC-ABL1*) where we found such studies, we created undirected graphs to depict which publications were at least partially overlapping. We included all publications in qualitative analyses, but only nonoverlapping publications in meta-analyses. This avoids overcounting, but may introduce undercounting.

Quality assessment

As of this writing, there are no validated quality items that are specific for assessing pharmacogenetic tests. Therefore, we did not assign quality grades (A, B or C) to studies included in this Technology Assessment. However, we abstracted information on aspects of the design and conduct of the individual studies that we believe is important for interpreting these data. These items include description of patient sampling (representative of parent population or convenience sampling), design of the parent study, (e.g., original data from a well-conducted randomized controlled trial), availability of samples on a large majority of study participants, whether clinically representative patients were assessed, assay methodology (i.e., whether it is predefined and

[b] A study in both treated and untreated patients that does not perform such interaction analyses between test results and treatment type is effectively as informative as a prediction study in treated-only patients.

[c] Studies that simply perform adjustments for the factors of interest do not inform on e.g., how much the strength of the pharmacogenetic association changes across the levels of the factor of interest.

standardized), and analysis techniques (blinding of assessors of test to clinical outcomes and *vice versa*, and whether appropriate statistical analyses including multivariable adjustments are performed).(5)

Outcomes

Outcomes of interest were organized into three categories.

- Mortality: Includes all-cause or disease-specific mortality. Apart from analyses of cumulated deaths by a specific time point, pertinent to this category are also time-to-death (overall survival or cause-specific survival) analyses.
- Progression: Includes disease progression, as defined by each study, or a composite of progression or death. Examples are recurrence free survival for resectable breast cancer, which is typically defined as time from study inclusion to local or distant recurrence or death from any causes, and progression free survival for metastatic colorectal cancer, which is typically defined as time from study entry to disease progression assessed by radiologic tests or death from any causes. Apart from analyses of cumulated progression events by a specific time point, pertinent to this category are also time-to-progression (progression-free survival) analyses.
- Tumor responses: These are typically short-term index of treatment efficacy, which may or may not be reliable surrogate outcomes of progression or mortality. For example tumor response is defined based on radiologic imaging criteria in colorectal cancer, whereas several different response assessments such as hematologic (by conventional blood counts and bone marrow examination), cytogenetic (by conventional karyotype analysis or fluorescent in situ hybridization), molecular response (by quantitative real time polymerase chain reaction of *BCR-ABL1* transcripts) are performed in chronic myeloid leukemia.

Synthesis

As will be described in the Results Sections, most eligible studies evaluated associations of genetic test results and outcomes of interest in patients who received the treatment of interest. For mortality and disease progression we evaluate *strength of the association* between test results and outcomes; for the endpoint of treatment failure we also evaluate test performance by means of sensitivity, specificity and likelihood ratios.

Analysis of the strength of the association between test results and outcome of interest

We abstracted data to calculate odds ratios or extracted odds ratio estimates to quantify the strength of the association between test results and outcomes (for analyses of cumulated events) or time-to-clinical-event analyses. We captured both unadjusted (crude) and adjusted effects.

When deemed appropriate, we performed meta-analyses of odds or hazard ratios using the DerSimonian and Laird random effects model. We tested for between-study heterogeneity with Cochran's *Q* (considered statistically significant at $p<0.10$) and assessed its extent with I^2. I^2 ranges between 0 and 100 percent and expressed the proportion of between study variability that is attributed to heterogeneity rather than chance.(6;7) We did not set criteria for interpreting I^2 values.

Analysis of the ability of the pharmacogenetic test to predict treatment failure

For each genetic test, we calculated its sensitivity and specificity to predict the occurrence of an event of interest (e.g., progression of disease, relapse or death) using standard methods. Sensitivity is the ability of the test to maximize true positives. Specificity is the ability of the test to minimize false positives.[d]

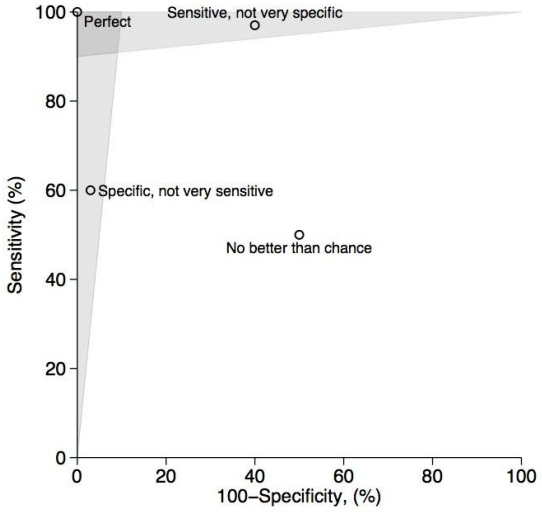
A particularly informative graph plots sensitivity against 100% minus their specificity in a plot (commonly known as the ROC space plot). The closer a study point is to the upper left corner of the plot, the better its performance (**Figure 1**).

The *positive* and *negative likelihood ratios* (LR+ and LR-, respectively[e]) quantify the change in the certainty of the "diagnosis" (prediction) conferred by the results of the test. More specifically, the likelihood ratios transform the *pretest odds* to the *posttest odds* of a given prediction:

$$posttest\ \ odds = pretest\ \ odds \times LR$$

For a positive test result, the LR+ would be used in the above relationship; for a negative result, the LR- would be used. Typically, a LR+ of 10 or more and a LR- of 0.1 or less are considered to represent informative tests.(8) Other, more lenient boundaries for LR+ and LR- can be used. The choice of the boundaries is dependent on the decisional context of the test. Studies with high LR+ and low LR- can be readily identified in the square sensitivity/100%-specificity plot, as shown in **Figure 1**.

Figure 1. Square plot of sensitivity versus 100%-specificity



[d] Sensitivity is defined as the proportion of people who will experience the outcome that are correctly identified by genetic test. Specificity is the proportion of people who will not experience the outcome that are correctly identified as such by the genetic test. Sensitivity and specificity range between 0 and 100% and higher values imply better diagnostic ability.

[e] The likelihood ratios can be conveniently calculated as follows:

$$LR+ = \frac{sensitivity}{1-specificity}, \quad LR- = \frac{1-sensitivity}{specificity}$$

If a given pharmacogenetic test has very good ability to predict clinical outcomes of interest, its LR+ will be high (will greatly increase the odds of a positive diagnosis) and its LR- will be low (will diminish substantially the likelihood of the positive diagnosis). A completely non-informative pharmacogenetic test would have likelihood ratios equal to 1 (does not transform the pre-test odds substantially in the equation listed in the text).

Four hypothetical studies are depicted in the square sensitivity/100%-specificity plot. The closer a study is to the upper-left corner of the plot, the better its diagnostic ability. Studies lying on the major diagonal of the plot have no diagnostic ability (no better than chance). Studies lying on the left shaded area have LR+ of 10 or more. Studies lying on the top shaded are have LR- of 0.1 or less. Studies lying on the intersection of the grey areas (darker grey polygon) have both LR+>10 and LR-<0.1.(8)

When deemed appropriate, we performed meta-analyses of sensitivity and specificity using a bivariate model with exact binomial likelihood,(9;10) as described in the topic-specific Methods sections.

Section 1: Variations in *CYP2D6* and response to tamoxifen in breast cancer

1.1 Background

Endocrine therapies targeting estrogen action are the mainstay of treatment for breast cancers expressing estrogen or progesterone receptors (ER/PR), which constitute more than 80 percent of all breast cancers. In fact, endocrine therapies for ER/PR expressing breast cancer are considered as some of the most successful forms of "targeted therapy", since women whose tumors do not express the ER/PR receptors are not expected to derive benefit from endocrine treatments.(11) Currently, the two main forms of endocrine treatments for breast cancer are selective estrogen receptor modulators (SERMs) and aromatase inhibitors.(12)

SERMs act by selectively behaving as antagonists (inhibitors) or agonists of the ER in different tissues. Tamoxifen and its metabolites exert their activity by binding to the ER and blocking the binding of estrogen (competitive inhibition) in breast tissue, while acting as ER agonists in bone and endometrial tissue. This prevents the activation of ER-mediated signaling and suppresses ER-dependent tumor cell proliferation. Aromatase inhibitors block the conversion of adrenal androgens to estrogen by aromatase, an enzyme expressed in peripheral tissues. Peripheral aromatization is the main source of estrogen in women for whom the ovaries are no longer a source of estrogen due to ovarian ablation or, most commonly menopause. The focus of this review is tamoxifen resistance, and aromatase inhibitors will not be considered further.

Based on extensive randomized clinical trial evidence, tamoxifen is considered the standard of care for premenopausal women with ER/PR positive breast cancer, as well as a valid option for the treatment of post-menopausal women. Although an individual-patient data meta-analysis of 194 randomized controlled trials (145,000 patients) has demonstrated that tamoxifen reduces the risk of breast cancer relapse by about 50 percent and the risk of breast-cancer specific mortality by about 30 percent, a substantial number of women with ER-positive breast cancer develop disease relapse following surgical resection despite tamoxifen use.(11) In addition, response to tamoxifen in the metastatic setting is usually short lived and disease progression is inevitable. Tamoxifen resistance has been extensively investigated and a variety of biological mechanisms are considered as potentially mediating treatment resistance, including cross talk of the ER/PR-activated pathway and growth-factor signaling pathways, activation of alternative (non-ER-dependent) signaling pathways, loss of ER expression and ER mutations (a rare cause of resistance).(13-15)

Inherited polymorphisms that influence tamoxifen metabolism are increasingly recognized as a source of between-individual variation in treatment response.(16) Cytochrome P450 (CYP) enzymes play a major role in the metabolism of tamoxifen and its conversion to active metabolites. Tamoxifen is converted into N-desmethylatamoxifen and 4-hydroxytamoxifen, by the cytochrome 450 (CYP) enzymes CYP3A4/5 and CYP2D6, respectively.(17) These metabolites in turn undergo oxidation and are converted into endoxifen (4-hydroxy-N-desmethyltamoxifen), which is the

pharmacologically active metabolite of tamoxifen. Based on the higher blood concentration, binding affinities and in vitro potency of tamoxifen metabolites compared to tamoxifen, it appears that these metabolites are the primary effectors of tamoxifen activity.(18;19)

To date, more than 75 *CYP2D6* variants have been recognized.(20)[a] The majority of those variants appear to be functionally silent, do not influence enzymatic activity, and are commonly referred to as "extensive metabolizer" (EM) alleles. Other variants of the *CYP2D6* gene result in the absence of enzymatic activity ("null" variants) and are referred to as "slow metabolizer" (SM) alleles. A third group of variants have intermediate enzymatic activity and are called "intermediate metabolizer" (IM) alleles.(16;20) Duplication or multiplication of normal and null activity alleles has been reported, resulting in "ultra rapid metabolizer" (UM) and slow metabolizer phenotypes, respectively.(21;22)

Because intermediate and slow metabolizer genotypes are associated with reduced enzymatic activity, patients who carry these genotypes may have impaired conversion of tamoxifen to its active metabolites, endoxifen and N-desmethylatamoxifen.(23) Indeed, both in vitro and in vivo studies have demonstrated that SM and IM genotypes are associated with lower levels of tamoxifen metabolite concentrations compared to EM genotypes, although a substantial portion of the variability in metabolite concentrations is not explained by *CYP2D6* genotype. In addition, drugs that inhibit CYP2D6 (such as selective-serotonin reuptake inhibitors (SSRIs)[b], haloperidol and amiodarone) have also been shown to reduce tamoxifen metabolite concentrations, both in animal models and in humans. The potential association of SSRI use and reduced tamoxifen levels (and activity) is of particular relevance in breast cancer therapeutics, because SSRIs are often coprescribed with tamoxifen to alleviate hot flashes (a common tamoxifen side effect).

Based on these observations, it has been hypothesized that breast cancer patients with slow (and intermediate) metabolizer genotypes who are treated with tamoxifen may have worse clinical outcomes compared to patients with extensive metabolizer genotypes.(23)

[a] A comprehensive list is available at: http://www.cypalleles.ki.se/cyp2d6.htm (last accessed April, 22nd, 20100).

[b] Fluoxetine (Prozac), paroxetine (Paxil), bupropion (Wellbutrin), and duloxetine (Cymbalta) can substantially inhibit CYP2D6 activity. Citalopram (Celexa), escitalopram (Lexapro), desvenlafaxine (Pristiq), and sertraline (Zoloft) are weaker inhibitors. Venlafaxine (Effexor) appears to have no effect on CYP2D6 activity.

1.2 Methods

The reader is referred to the Generic Methods Section for a description of methods common to all three topics examined in this review. Herein we describe the topic-specific Key Questions, as well as additional topic-specific methods.

Key Questions

1) Does *CYP2D6* testing predict response to tamoxifen therapy?
2) What patient- and disease-relevant factors affect the test results, their interpretation or their predictive response to therapy?
3) How does the gene testing impact the therapeutic choice?
4) What are the benefits and harms or adverse effects for patients when managed with gene testing?

The reader is referred to the Generic Methods section in the beginning of this Technology Assessment for a description of the Key Questions.

Eligibility criteria

Eligible were studies that fulfilled the generic eligibility criteria listed in the Generic Methods section. Briefly, eligible studies reported on women with breast cancer and presented information on clinical outcomes (mortality and/or overall survival; disease recurrence or progression free survival or time-to-progression or response rates) stratified by *CYP2D6* polymorphism status. We considered all study designs (prospective and retrospective), tamoxifen doses, and dosing schedules.

Extracted data

See the Generic Methods Section for commonly extracted items across the three cancer topics. Data specifically collected for studies pertinent to the current topic include the percentage of postmenopausal women, details of treatment setting (metastatic versus nonmetastatic disease, stage distribution, proportion with lymph node involvement, proportion with positive estrogen and progesterone receptor status, proportion positive for *HER2*, tamoxifen doses, potential cotreatments, information on comedications that inhibit *CYP2D6* enzymatic activity, and adherence to tamoxifen schedule.

Deducing metabolizer status from *CYP2D6* genotypes

To demonstrate the heterogeneity in the definition of *CYP2D6*-derived genotypes in the included studies we used a simple, if arbitrary, algorithm to group genotypes in the slow, intermediate, and extensive metabolizer categories. We classified genotypes with two, one, or none of the "slow" (impaired) *CYP2D6* alleles as slow, intermediate and extensive metabolizers, respectively. Allele classification was based on a curated CYP allele classification database.[a]

[a] http://www.cypalleles.ki.se/cyp2d6.htm; last accessed December 7th, 2009.

Outcomes

For studies evaluating tamoxifen in the adjuvant setting, clinical outcomes of interest were mortality (survival), recurrence (as defined in each study), and the composite outcome of death or recurrence.

For studies in the metastatic setting, clinical outcomes of interest were mortality (survival), disease progression (as defined in each study) or the composite outcome of death or progression.

Synthesis

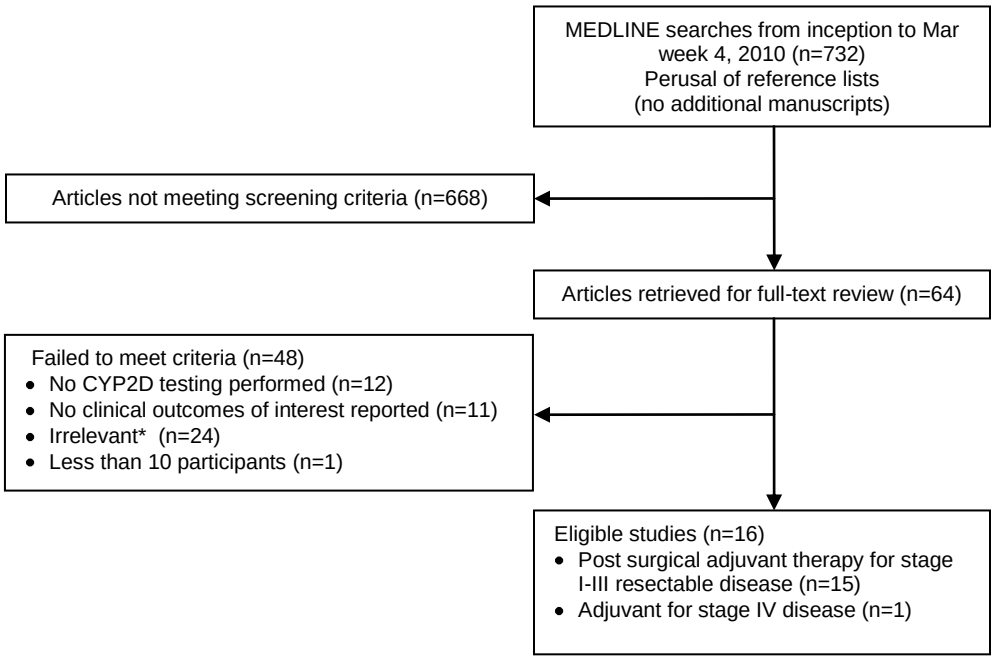
We did not perform quantitative synthesis (meta-analysis) because of extensive heterogeneity in the definition of slow, intermediate and extreme metabolizers across eligible studies (see 1.3 Results). Instead, we present results from individual studies on the strength of the associations between *CYP2D6* testing results and clinical outcomes. We list odds ratios and 95 percent confidence intervals (for analyses of cumulated events) or hazard ratios and 95 percent confidence intervals (for time-to-event analyses).

1.3 Results

Literature flow

Our searches returned 590 citations. After screening of titles and abstracts 51 were retrieved and reviewed in full text. Sixteen publications were finally accepted (**Figure 2**).(15;24-38) **Appendices B** and **C** show the list of included and excluded citations, respectively.

Figure 2. *CYP2D6* and tamoxifen for breast cancer: literature flow



* "Irrelevant" includes publications with no primary data, studies on healthy population, and studies on medications that inhibit CYP2D6.

Characteristics of included studies

Table 1 summarizes the characteristics of the 16 eligible studies.(15;24-38)

Patients

Briefly, all but one(27) studies pertained to women who received adjuvant tamoxifen for nonmetastatic breast cancer. Mean or median participant age was 65 or older in 7 out of 13 studies that reported this information. As shown in **Table 1**, the distribution of breast cancer stages varied across studies. Similarly, the proportion of women with positive lymph node status, positive estrogen or progesterone receptor status was quite heterogeneous across studies. In terms of racial composition, 9 studies pertained to White populations, 6 studies pertained to East Asian populations, and one study enrolled predominantly African-American women.

Treatments

Tamoxifen dosing was not reported in the majority of studies.

Design

Ten studies were retrospective.(26;28-32;34;35) In the remaining five(15;24;25;27;33), outcome assessment was described as prospective and one study combined a prospective and a retrospective cohort.(38) The majority of studies (n=9) assessed the ability of *CYP2D6* testing to predict outcomes only in women who received tamoxifen.(15;24;26-28;30;31;34;35) This design essentially assumes that *CYP2D6* testing has no predictive ability for breast cancer outcomes in patients who did not receive tamoxifen.[a] Two studies were "repurposed" RCTs, i.e., RCTs that were reanalyzed to examine the ability of *CYP2D6* status to predict outcomes.(25;33) The "repurposed" RCTs analyzed women who received tamoxifen (one arm), as well as women who received other treatments (comparator arm), and have the opportunity to examine pharmacogenetic effects of *CYP2D6* genotyping by testing the interaction of genotypes and treatments.[b] However, as will be discussed later, none of the repurposed RCTs performed interaction tests. Effectively they were analyzed in the same way as the aforementioned 12 studies.

Sample sizes in the 16 studies ranged between 21 and 1361. Median followup duration ranged from 20 to 150 months, and was longer than 56 months in 13 studies. There was extensive overlap in the patient populations included in the following studies: Goetz 2005(25), Goetz 2008(15), Scroth 2007(32) and Scroth 2009(38). In addition, there was complete overlap of the patient populations in Kiyotani 2008(26) and Kiyotani 2010(37).

[a] If *CYP2D6* status predicted, e.g. breast cancer outcome irrespective of treatment, studies that evaluate only tamoxifen treated women would still find an association between *CYP2D6* status and patient outcomes; however the association would not be specific to tamoxifen treated people, and therefore would not be evidence for a pharmacogenetic interaction.

[b] Testing for interaction answers whether an observed association between *CYP2D6* status and patient outcomes is specific to those who received tamoxifen, or is independent of treatment. This analysis would not invoke the assumption outlined in the previous footnote.

Table 1. *CYP2D6* and tamoxifen for breast cancer: characteristics of included studies

Author, year Ethnicity (Country)	Sample size, *n* Study design Sampling	Selection criteria	Median age, *y* [range] (% post-menopause)	-Treatment setting -Stage distribution, *n (%)* -LN involvement, *n (%)*	ER/PR+, *%* [method] HER2+, *%* [method]	Follow-up in months Median [range]	-CYP2D6 inhibitors -TAM adherence
Prospective – adjuvant setting							
Goetz, 2005(25) White 92% (USA)	Total 256 Post-hoc analysis of a multicenter RCT[a] Convenience sampling	Postmenopausal with node-negative disease (T1c or T2N0M0) ≥65 years with node-positive disease (T1NxM0 or T2N1M0)	68 [42-83] (100)	-Adjuvant -Tumor >30 mm, 54 (21) -96 (38)	-ER+: 100 -HER2+: NR	137 [68-169][b]	NR
Wegman, 2005(33) NR (Sweden)	TAM 112; No TAM 114[c] Post-hoc analysis of a multicenter 2x2 RCT Convenience sampling	Postmenopausal women age <70 y Unilateral operable breast cancer Histologically verified lymph node metastasis or a tumor >30 mm Fresh-frozen tissue available	<70 y [NR] (100)	-Adjuvant -Tumor >30 mm, 25 (11); Node+, tumor ≤20 mm, 89 (39); Node+, tumor >20 mm, 112 (50) -201 (89)	-ER+: 69 [NR] -HER2+: NR	128 [2-223]	NR
Goetz, 2008(15) NR (USA)	Total 110 Post-hoc analysis of RCT Convenience sampling	Postmenopausal women with resected, node-negative, ER+ breast cancer	65 [42-84] (100)	-Adjuvant -Tumor size ≥3 cm, 22 (20) -0	-ER +: 100 [IHC or quantitative] PR +: NR -HER2 +: 19 [3+ IHC]	150 [68-186][a]	-CYP2D6 inhibitors groups with slow metabolizers. -NR
Bijl, 2009(24) NR (Netherlands)	TAM 85 Post-hoc analysis of TAM-treated women from a prospective population-based cohort study[d]	Women started on TAM between 1991 and 2005, with follow-up >=180 days and available genotype.	76 [NR] NR	NR	NR	NR	-Analyses adjusted for CYP2D6 inhibitors -NR
Retrospective – adjuvant setting							

[a] NCCTG 89-30-52.
[b] Alive patients only.
[c] 226 out of 679 postmenopausal women from 960 trial participants (i.e., 24% of total trial participants)
[d] The Rotterdam Study a prospective cohort study that included 7983 women from the general population aged >55 years

Wegman, 2007(34) Whites (Sweden)	TAM 677 Retrospective cohort study[e] Convenience sampling	Postmenopausal patients with stage II/III breast cancer from the South East Health Care Region of Sweden (1986-1997)	69 [50-96][f] (100)	-Adjuvant -II, 581 (86); III, 96 (14) -171(28)	-ER+: 100 [NR] -HER2+: NR	85 [0.5-215]	NR
Schroth, 2007(32) Whites (Germany)	TAM 206; 280 chemotherapy or no therapy[g] Retrospective non-RCS Convenience sampling	Primary invasive breast cancer, diagnosed between 1986 and 2000 Follow-up ≥ 8 months and tissue for genotyping available	TAM-treated: 68 [40-92] No-TAM: 56 [29-88] (NR)	-Adjuvant -NR -TAM: 57 (31) No TAM: 118 (44)	All TAM-treated where -ER+ [NR] -HER2+: NR	71 [4-227]	-Not accounted for in the analysis due to incomplete data -NR
Nowell, 2005(29) Whites 81%; African-American 19% (USA)	TAM 165; No TAM 172 Single center Retrospective non-RCS Convenience sampling	Primary invasive breast cancer "Registered" on hospital tumor registry Received any type of adjuvant therapy as part of first-line therapy No prior history of cancer	41% <50 y, 59% >50 y (NR)	-Adjuvant -I, 106 (31); II node-, 71 (21); II node+, 95 (28); III, 49 (15); IV, 16, (5) -(see above)	NR	~62[h] [NR]	NR
Xu, 2008(35) East Asian (China)	TAM 152; chemotherapy 141 Single-center retrospective non-RCS Convenience sampling	Primary breast cancer with adjuvant TAM, no concurrent chemo DNA from samples available	NR[i]	-Adjuvant -TAM: 0, 8 (5); I, 104 (68); II, 40 (26); No TAM: I, 24 (17); II, 91 (65); III, 22 (16) -TAM: 10 (7) No TAM 59 (42)	ER+: TAM-treated: 125 (82); No TAM: 24 (16) [IHC or dextran coated charcoal method] PR+: TAM-treated: 95 (67); No TAM: 51 (36) [IHC or dextran coated charcoal method] HER2+: TAM-treated: 21 (14); No TAM: NR [IHC]	TAM 63 [4-122]; No TAM 120 [4-193]	-None of the eligible subjects in the TAM group were on CYP2D6 inhibitors
Kiyotani, 2008(26) East Asian (Japan)	TAM 67 Retrospective cohort "Consecutive" patients	Inflammatory breast cancer, adjuvant TAM, ER + tumor.	50 [34-82] (48)	-Adjuvant -NR	ER+: (96) [IHC] PR+: (90) [IHC] HER2+: (5) [IHC]	96 [19-259]	-None of patients received SSRIs. -NR

[e] 235 patients had participated in an RCT of 2 vs. 5 years TAM.
[f] Mean [range].
[g] An additional 135 patients who received chemotherapy + TAM or had unclear estrogen receptor status were excluded.
[h] Approximated from reported person-years.
[i] Age reported as >50 or <50 years only.

Newman, 2008(28) Whites (United Kingdom)	TAM 115 (47 BRCA1, 68 BRCA2) Retrospective cohort study Convenience sampling	Probands from breast cancer families from a single cancer genetics center, BRCA1 or BRCA2 positive, received TAM following surgery	BRCA1: 41 [26-68] BRCA2: 44 [27-68] (NR)	-Adjuvant -NR -BRCA1: 22 (47); BRCA2: 25 (37)	BRCA1: ER+, 58 [NR] BRCA2: ER+, 91 [NR] HER2 +: NR	120 [NR][j]	-4 patients concomitantly taking SSRIs were included.
Okishiro, 2009(30) East Asian (Japan)	TAM 173 (+/- chemotherapy) Retrospective cohort study Convenience sampling[k]	Primary, ER+ breast cancer, received adjuvant TAM (with or without chemotherapy)	47 [22-73] (22)	-Adjuvant -NR -50 (29)	ER+: 91 [IHC] PR+: 86 [IHC] HER2 +: 8 [FISH or IHC]	56 [8-109]	-Patients receiving paroxetine concomitantly with TAM excluded -NR
Ramón y Cajal, 2009(31) Whites (Spain)	TAM 91 (+/- chemotherapy) Retrospective single-center cohort study Convenience sampling	Patients evaluated at the study center in 2007 Receiving TAM (monotherapy or concomitantly with chemotherapy) Radiotherapy and/or chemotherapy-treated patients were included	51 [28-79] (40)	-Adjuvant -I, 30 (33); II, 39 (43); III, 22 (24) -45 (50)	-ER+: 100 [NR] -HER2+: NR	108 [91-133][l]	-Not accounted for in the analysis due to incomplete data -NR
Schroth, 2009(38) NR (Germany, USA)	TAM 1361 (1325 included in analyses) Combined analysis of a retrospective cohort study and a post-hoc analysis of a multicenter RCT Convenience sampling ("consecutive" patients from the German cohort)	Histologically confirmed breast cancer patients recommended to receive 5 years of adjuvant TAM therapy ER/PR + tumors	66 [37-93] (96)	-Adjuvant -I-III (distribution NR) -449 (34)	ER+: (97) [IHC] PR+: (74) [IHC] HER2+: NR	76 [2-244]	-Not accounted for in the analysis due to incomplete data -Not accounted for in the analysis due to "insufficient control"
Toyama, 2009(36) East Asian (Japan)	TAM 156 Retrospective single-center	Primary node-negative invasive breast cancer Patients planned to	59.1 [33-89] (NR)	-Adjuvant -NR -0	ER+: (96) [IHC] PR+: (80) [IHC] HER2+: (6) [IHC]	95 [25-249]	-NR

[j] Patients were followed-up for a median 10 years or until death.
[k] Study reports "serial" sampling of patients.
[l] Mean age [range].

	cohort study Convenience sampling ("consecutive" patients)	receive 2-5 years of adjuvant tamoxifen					
Kiyotani, 2010(37) East Asian (Japan)	TAM 282 Retrospective multicenter cohort study Convenience sampling	Primary breast cancer patients with localized hormone-receptor positive disease Scheduled to receive 5 years of TAM	51 [31-83] (53%)	-Adjuvant -NR -48 (17%)	ER+: (74) [IHC] PR+: (69) [IHC] HER2+: (2) [IHC]	85 (10-282)	-NR
Prospective – metastatic setting							
Lim HS, 2007(27) East Asian (Korea)	TAM 21 Single center prospective cohort study	Histologically/ cytologically confirmed ER+ or PR+ breast cancer	46.5 [31-70] (NR)	-Metastatic -Stage IV, 11 (52), Recurrent disease, 10 (48) -NR	ER+: 100 [IHC] HER2: 10 [IHC or FISH]	20 [7-54]	-Patients receiving CYP2D6 inhibitors or inducers within 28 days of study enrollment were excluded -NR

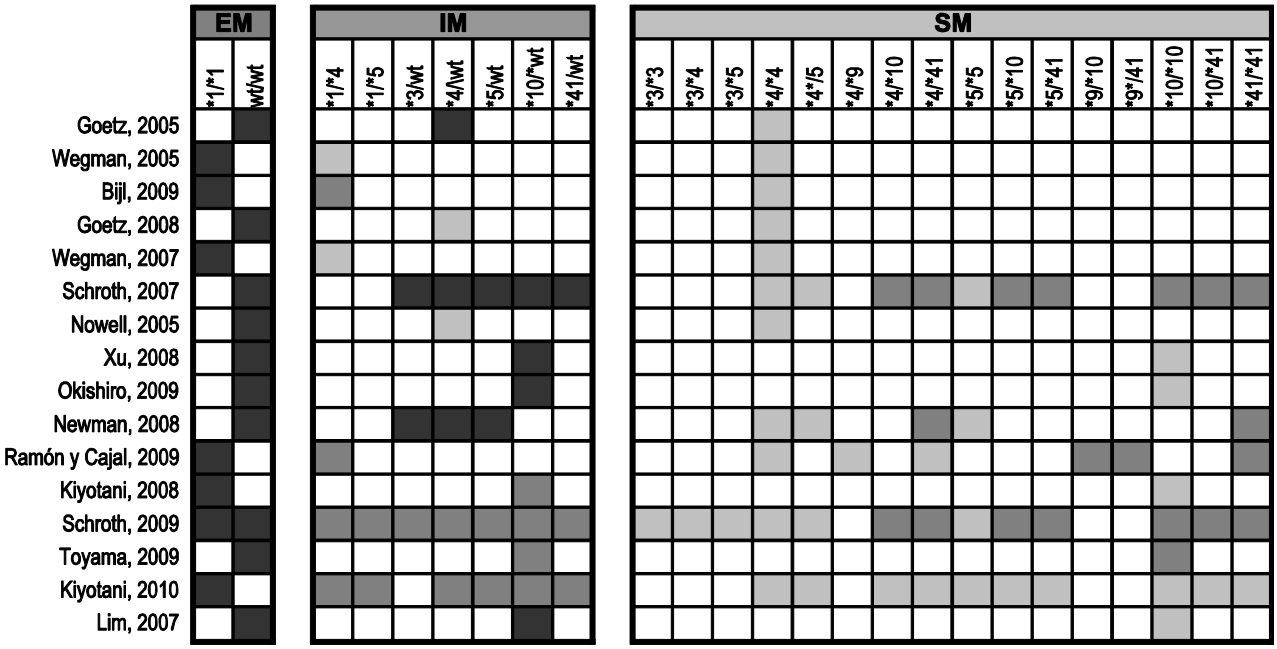
Studies are grouped according to whether outcomes were ascertained prospectively or retrospectively in the adjuvant or metastatic setting. Within each group studies are ordered by decreasing sample size.
ER, estrogen receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; LN, lymph node; Non-RCS, non-randomized comparative study; NR, not reported; PR, progesterone receptor; RCT, randomized clinical trial; TAM, tamoxifen.

Heterogeneity in the classification of genotypes to categories of enzymatic activity

Patients can be classified as slow, intermediate or extensive metabolizers of tamoxifen according to the activity of the CYP2D6 enzyme. Genotypic testing of *CYP2D6* is a surrogate of the actual enzymatic activity of the CYP2D6 protein in a given patient. However, different studies classified the same genotypes into different categories of (predicted) enzymatic activity.

Table 2 provides details on which genotypes were considered as slow, intermediate or extensive metabolizers by each study. **Figure 3** summarizes the information in **Table 2**. It is evident that there is considerable heterogeneity in the definition of metabolizer status across studies. This heterogeneity should be kept in mind when comparing the results of individual studies between them, and is the main reason why a meta-analysis was not performed in this topic.

Figure 3. *CYP2D6* and tamoxifen for breast cancer: heterogeneity in the definition of slow, intermediate and extensive metabolizers across studies



We grouped genotypes into extensive, intermediate and slow metabolizers (EM, IM and SM, respectively) following the conventions outlined in Section 1.2. The big boxes correspond to the three metabolizer groups. Filled cells imply that a genotype has been assessed in a given study. The color of the cells corresponds to each study's definitions of metabolizer status (EM = dark gray; IM = medium gray; SM = light gray).
Genotypes *1/*1 and wt/wt (which we considered as EM) were consistently considered as EM by all studies. On the contrary, the genotypes that we defined as IM were variably treated as EM, IM or SM by different studies (all three colors appear in the "IM" box). Similarly, genotypes that we defined as SM were treated both as SM and IM by the studies. Genotypes *4/*4, *4/*5 and *5/*5 were consistently treated as SM by all studies that investigated them. Studies are presented in the same order as in **Table 1**. Not all genotypes reported in the study by Ramon y Cajal 2009 are depicted in the table (see **Table 2** for the exact classification in that study).

Several observations can be made in **Figure 3**. Allowing for the caveats mentioned in the "1.2 Methods" section, ideally all green cells would fall into the EM box, all blue cells would fall into the IM box, and all red cells in the SM box. By definition, some empty cells in this figure (corresponding to alleles that were *not assessed*) fall into the "wild type" category. For example, Goetz 2005 did not assess for the *5 allele. Therefore, e.g. the *5/*5 genotype is implicitly in the "wild type" wt/wt category (i.e., would be green in the figure). Further, some studies, (e.g., Ramon y Cajal 2009), classified genotypes in a complex and not adequately supported way (**Table 2**).

Table 2. *CYP2D6* and tamoxifen for breast cancer: genotype detection methods, metabolic phenotype definitions and frequency of genotypes in eligible studies

Study (first author, year)	Genotyping method	Source of DNA for genotyping	Available sample, *n* Genotyping success, n (*%*)	Genotypes		Frequency, *n* (%)
Prospective outcome assessment – adjuvant setting						
Goetz, 2005	TaqMan® Allelic Discriminatory Assay	Tissue with high tumor in PET (n=213) or fresh buccal mucosal tissue (n=10)	190 (85)[a]	**SM**	*4/*4	13 (7)
				IM	-	-
				EM	*4/wt wt/wt	40 (21) 137 (72)
Wegman, 2005	PCR-RFLP[b]	Fresh-frozen tumor tissue	226 (100)	**SM**	*4/*4 *4/*1	9 (4) 55 (24)
				IM	-	-
				EM	*1/*1	162 (72)
Goetz, 2008	TaqMan® Allelic Discriminatory Assay	Tissue with high tumor in PET or fresh buccal mucosal tissue	110 (100)[c]	**SM**	*4/*4, *4/wt, or on CYP2D6 inhibitor	32 (29)
				IM	-	-
				EM	wt/wt	78 (71)
Bijl, 2009	TaqMan® Allelic Discriminatory Assay	Whole blood[d]	85 (79)	**SM**	*4/*4	4 (5)
				IM	*4/*1	29 (34)
				EM	*1/*1	52 (61)
Retrospective outcome assessment –adjuvant setting						
Nowell, 2005[e]	TaqMan® Allelic Discriminatory Assay	Normal LN (or other normal tissue) in PET	337 (100)	**SM**	*4/*4 *4/wt	8 (2) 97 (28)
				IM	-	-
				EM	wt/wt	240 (71)
Schroth, 2007	MALDI-TOF MS and TaqMan® real-time quantification assays[f]	Normal breast tissue from PET	TAM-treated: 206 (197 genotyped, 95.6%) No-TAM: NR	**SM**	Two null alleles (*4/*5)	14 (7)
				IM	Two *10/*41 or one null allele (*4/*5) together with one *10/*41 allele	16 (8)
				EM	Homozygous EM (no *4/*5 alleles and not more than one *10/*41 allele) Heterozygous EM (one null allele (*4/*5) and no *10/*41 allele)	18 (60)
Wegman, 2007	PCR RFLP	Microdissected frozen breast tumor tissue	677 (100)	**SM**	*4/*4	35 (5)
				IM	*4/*1	186 (27)
				EM	*1/*1	475 (70)
Xu, 2008	PCR RFLP	Blood or tumor (fresh-frozen), or tumor-free lymph nodes (PET)	152 (100)[g]	**SM**	*10/*10	72 (48)
				IM	-	-
				EM	*10/wt or wt/wt	80 (52)
Kiyotani, 2008	Multiplex PCR-	Blood	67 (100)[h]	**SM**	*10/*10	15 (22)

a b c

b c

c

[d] Based on Bijl MJ, et al. Br J Clin Pharmacol, 2008. 65 (4):558.

[e] Although *CYP2D6**3 and *6 were also examined, individuals with these allele(s) were not analyzed separately from "wild type" due to low frequency.

[f] *CYP2D6**10 was not in Hardy Weinberg equilibrium, but the meaning of this departure is unclear.

[g]

[h] Other genotypes [*CYP2D6*

	based Invader assay and Taqman assay			**IM**	*1/*10	23 (34)
				EM	*1/*1	20 (30)
Newman, 2008	TaqMan® Allelic Discriminatory Assay	Peripheral blood lymphocytes	115 (100)	**SM**	*3,*4, or *5/*3, *4, or *5[i]	8 (7)
				IM	-	-
				EM	wt/*3,*4, or *5 wt/wt	40 (35) 67 (58)
Okishiro, 2009	TaqMan® Allelic Discriminatory Assay	Peripheral blood mononuclear cells	173 (100)	**SM**	*10/*10	40 (23)
				IM	-	-
				EM	wt/wt, *10/wt	132 (77)
Ramón y Cajal, 2009	Microarray hybridization method (AmpliChip CYP450 GeneChip®)	Whole blood	91 (100)	**SM**	*4/*4 *3/*4 *4/*41 *4/*9	5 (6) 1 (1) 2 (2 2 (2)
				IM	*9/*10 *9/*41 *41/*41 *1/*4 *1/*5 *1/*6 *2/*4 *2/*5 *2/*20 *35/*4 *1/*10 *1/*9 *1/*41 *35/*10 *2/*9 *2/*41 *35/*41 *35/*9	4 (4) 1 (1) 1 (1) 9 (10) 7(8) 1 (1) 2 (2) 2 (2) 1 (1) 1 (1) 3 (3) 3 (3) 7 (7) 1 (1) 2 (2) 3 (3) 1 (1) 1 (1)
				EM	*1/*1 *1/*2 *1/*35 *2/*2 *2/*35 *1xN/*2 *3xN/*41	11 (12) 10 (11) 3 (3) 4 (4) 1 (1) 1 (1) 1 (1)
Schroth, 2009	German cohort: MALDI-TOF MS and TaqMan® real-time quantification assays[j] USA cohort: TaqMan® Allelic Discriminatory Assay	Blood (601 samples) Tumor (fresh-frozen, 101 samples; PET, 659 samples)	1325 (97)	**SM**	*3/*3 *3/*4 *3/*5 *4/*4 *4/*5 *5/*5	79 (46)
				IM	wt/*3 wt/*4 wt/*5 wt/*10 wt/*41 *1/*3 *1/*4 *1/*5 *1/*10 *1/*41 2x*2/*3	637 (48)

[i] For analyses of recurrence and recurrence free survival, patients on CYP2D6 inhibitors were also included.
[j] *CYP2D6**10 was not in Hardy Weinberg equilibrium.

					2x*2/*4 2x*2/*5 2x*2/*10 2x*2/*41	
				EM	wt/wt wt/*1 *1/*1 wt/2x*2 *1/2x*2 2x*2/2x*2	609 (6)
Toyama, 2009	TaqMan® Allelic Discriminator y Assay	Tumor (fresh-frozen)[k]	154 (99)	**SM**	*10/*10	28 (18)
				IM	wt/*10	62 (40)
				EM	wt/wt	64 (42)
Kiyotani, 2010	Taqman assay and multiplex PCR-based Invader assay			**SM**	*5/*5 *5/*10 *5/*21 *5/*41 *10/*10 *10/*10-*10 *10/*21 *10/*36-*36 *10/*41	63 (22)
				IM	*1/*4 *1/*5 *1/*10 *1/*10-*10 *1/*14 *1/*21 *1/*36-*36 *1/*41 *1-*1/*10	136 (48)
				EM	*1/*1	83 (29)
Prospective outcome assessment –metastatic setting						
Lim HS, 2007	Long PCR and PCR-RFLP	Peripheral blood leucocytes	21 (NR)	**SM**	*10/*10	12 (57)
				IM	-	-
				EM	wt/wt, wt/*10	9 (43)

EM, extensive metabolizer; HWE, Hardy-Weinberg equilibrium; IM, intermediate metabolizer; MALDI-TOF MS, matrix-assisted, laser desorption/ioanization, time-of-flight mass spectrometry; PCR, polymerase chain reaction; PET, Paraffin embedded tissue; RFLP, restriction fragment length polymorphism; SM, slow metabolizers.

[k] Results were compared with DNA extracted from blood for 50 patients (100% concordance).

Key Question 1. Does *CYP2D6* testing predict response to tamoxifen therapy?

Fifteen studies evaluated the association of *CYP2D6* testing results with clinical outcomes in the adjuvant setting.(15;24-26;28-35) Their results are summarized below.

Studies in the adjuvant setting – mortality

Strength of the association between CYP2D6 testing results and mortality

Table 3 summarizes odds ratios (from analyses of cumulated deaths) and hazard ratios (from time-to-death analyses) for slow or intermediate metabolizers compared to extensive metabolizers.

Only seven studies reported analyses on overall survival (mortality) outcomes.(24;28;29;32) None demonstrated a statistically significant relationship between *CYP2D6*-defined metabolizer status and overall survival or mortality in either adjusted or unadjusted analyses. The point estimates in five of seven studies suggested a trend towards increased mortality with slow compared to extensive metabolizers among tamoxifen-treated women, but the corresponding confidence intervals were wide.

It is interesting to note that two of the seven studies were nonrandomized studies informing on clinical outcomes in tamoxifen-treated versus non tamoxifen-treated women.[a](29;32) However, neither of the two performed interaction tests to evaluate whether the strength of the association between *CYP2D6*-defined metabolizer status and mortality differs by treatment type. If anything, in the Nowell 2005 study the hazard ratios for mortality in the two tamoxifen treated strata are very similar, suggesting that a formal interaction test would be statistically nonsignificant. In the other study (Schroth 2007) such a comparison cannot be made (no effect sizes are reported among women who were not treated with tamoxifen).

Many of the studies presented regression-adjusted estimates of the effect of CYP2D6 genotype on mortality risk, frequently for factors that could not confound the genotype-response association.

[a] The study by Scroth 2009 only reported on the tamoxifen-treated arm of patients participating in the US North Central Cancer Treatment Group (NCCTG) 89-30-52 trial and thus is not counted among the "repurposed RCT" group of studies.

Table 3. *CYP2D6* and tamoxifen for breast cancer: mortality (adjuvant setting)

Author, year	Study arm (patient number)	Mortality Event/patient, *n (%)*			OR (95% CI)	Overall survival times (median survival time in years)			Crude HR (95% CI) Adjusted HR (95% CI), [covariates]
		SM	**IM**	**EM**		**SM**	**IM**	**EM**	
Goetz, 2005	TAM (190)	NR	-	NR	NR	NR	-	NR	*SM vs EM* 1.73 (0.79-3.76) **1.12 (0.50-2.50), [S]**
Nowell, 2005	TAM (162)	7/48 (15)	-	27/114 (24)	*SM vs EM* 0.51 (0.19-1.43)	0.028[a]	-	0.046[n]	*SM vs EM* **0.77 (0.32-1.81), [A, S, ER, PR]**
	No TAM (175)	18/49 (37)	-	48/126 (38)	*SM vs EM* 0.94 (0.45-1.96)	0.063[n]	-	0.080[n]	*SM vs EM* **0.79 (0.42-1.26), [A, S, ER, PR]**
Schroth, 2007	TAM (197)	NR	NR	NR	NR	NR	NR	NR	*SM+IM vs EM* 1.73 (0.88-3.41)
	No-TAM (NR)	NR	NR	NR	NR	NR	NR	NR	NR
Newman, 2008	TAM (115)	NR	NR	NR	NR	NR	NR	NR	*SM vs EM+IM* 3.5 (0.8-15.4)[b]
Bijl, 2009[c]	TAM (85)	4/4 (100)	19/29 (66)	30/52 (58)	1.69 (0.67-4.23)	NR	NR	NR	*IM vs EM* 1.5 (0.8-2.8) *SM vs EM* 1.9 (0.6-5.6)
Schroth, 2009	TAM (1325)	18/79 (23)	114/637 (18)	102/609 (17)	NR	NR	NR	NR	*SM+IM vs EM* **1.15 (0.88-1.51), [S][d]**
Toyama, 2009	TAM (154)	NR	NR	NR	NR	NR	NR	NR	*"**p=NS**" by log-rank test*

Adjusted effects shown in bold font. Covariate key: A, Age; S, Severity; ER, Estrogen receptor status; PR, Progesterone receptor status.

[a] Deaths/person-year.

[b] This study also defined a group of patients with "overall reduced CYP2D6 activity", defined as SM genotype or use of a CYP2D6 inhibitor. Comparing this group with all other patients, the HR for OS=2.5 (0.8-8.2), adjusted for LN status

[c] This study reports mortality from a population-based cohort of breast cancer patients. No information is provided for the stage at diagnosis or line of treatment of eligible patients. We abstracted mortality data for "incident TAM users".

[d] Analyses were stratified by menopausal status, and subcohort assignment (prospective versus retrospective).

Studies in the adjuvant setting – recurrence

Strength of the association between CYP2D6 testing results and recurrence

Table 4 summarizes odds ratios (from analyses of cumulated events) and hazard ratios (from time-to-event analyses) for slow or intermediate metabolizers compared to extensive metabolizers.

Fourteen studies reported time-to-tumor recurrence outcomes. Most studies did not report statistically significant relationships between *CYP2D6*-derived metabolizer status and recurrence or time-to-recurrence. Six studies reported significant associations between slower (slow or intermediate) versus extensive metabolizer status and increased odds for recurrence or shorter time to recurrence for at least one analysis. Again, studies that had information for women treated with tamoxifen and women not treated with tamoxifen did not analyze metabolizer status by treatment interactions.

Many of the studies presented regression-adjusted estimates of the effect of CYP2D6 genotype on disease recurrence risk, frequently for factors that did not satisfy the causal structure of a confounder of the genotype-response association.

Table 4. *CYP2D6* and tamoxifen for breast cancer: recurrence (adjuvant setting)

Study (first author, year)	Study arm	Recurrence or mortality Events/patient, *n (%)*			OR (95% CI)	Recurrence free survival times (median survival time in years)			Crude HR (95% CI) Adjusted HR (95% CI), [covariates]
		SM	IM	EM		SM	IM	EM	
Goetz, 2005	TAM (190)	NR	-	NR	NR	NR	-	NR	*SM vs EM*[a] 2.44 (1.22-4.90) **1.86 (0.91-3.82), [S]**
Nowell, 2005	TAM (162)	10/48 (21)	-	38/112 (34)	*SM vs EM* 0.51 (0.21-1.19)	0.019[b]	-	0.073[q]	*SM vs EM* **0.67 (0.33-1.35), [A, S, ER, PR]**
	No TAM (175)	18/46 (39)	-	53/120 (44)	*SM vs EM* 0.81 (0.38-1.71)	0.075[q]	-	0.110[q]	*SM vs EM* **0.69 (0.40-1.18), [A, S, ER, PR]**
Wegman, 2005	TAM (79)	6/24 (25)	-	25/52 (48)	*SM vs EM*[c] 0.36 (0.10-1.16)	NR	-	NR	NR
	No TAM (78)	15/23 (65)	-	27/55 (49)	*SM vs EM*[d] 1.94 (0.64-6.17)	NR	-	NR	NR
Wegman, 2007	TAM (677)	2/35 (6)	42/186 (23)	103/475 (22)	*SM+IM vs EM* 0.90 (0.60-1.33)	NR	NR	NR	NR
Schroth, 2007	TAM (197)	5/14 (36)	5/16 (31)	Homozygous EM 17/118 (14) Heterozygous EM 14/49 (29)	*SM+IM vs (homozygous) EM* 2.97 (1.18-7.43)	NR	NR	NR	*SM+IM vs (homozygous) EM* 1.89 (1.10-3.25)
	No TAM (NR)	NR	NR	NR	NR	NR	NR	NR	"Differences were not observed in the control group"
Goetz, 2008[e]	TAM (110)	67/78	NR	24/32	*SM vs EM* 2.03 (0.62-6.30)[f]	NR	NR	NR	NR
Kiyotani, 2008	TAM-treated (67)	7/15	4/23	1/20	*SM vs EM* 16.6 (1.8-158.1) *IM vs EM* 4.0 (0.4-39.2) *SM vs IM+EM* 6.7 (1.7-26.4)[g]	NR	-	NR	NR
Newman, 2008	TAM (115)	NR	NR	NR	NR	NR	NR	NR	*SM vs EM* 2.9 (0.9-9.4)[hi]

[a] Time to recurrence (i.e., "relapse-free time" – death events were excluded from analysis) was reported as HR = 2.71 (1.15-6.41) in univariate analysis and 1.85 (0.76-4.52), [S] in multivariate analysis.
[b] Deaths/person-year.
[c] "Distant" recurrence-free survival. No definition is reported.
[d] "Distant" recurrence-free survival. No definition is reported.
[e] Most analyses performed for groups defined by combination of CYP2D6 genotype and HOXB13/IL17BR gene expression ratio.
[f] Estimated at 5 years.
[g] Estimated at 10 years.

Xu, 2008	TAM (152)	NR	NR	NR	NR	NR	-	NR	SM vs EM **4.7 (1.1-20.0), [A, S, ER, PR, T]**
	No TAM (141)	NR	NR	NR	NR	NR	-	NR	NR
Okishiro, 2009	TAM (173)	NR	-	NR	NR	63	-	54	*SM vs IM+EM* 0.94 (0.34-2.60) **0.60 (0.18-1.92), [S, PR, T]**
Ramón y Cajal, 2009	TAM (91)	NR	NR	NR	NR	8.2	9.5	9.8	NR[j]
Schroth, 2009	TAM (1325)	26/79 (33)	176/637 (28)	135/609 (22)	NR	NR	NR	NR	*SM+IM vs EM* **1.33 (1.06-1.68), [S][k]**
Toyama, 2009	TAM (154)	NR	NR	NR	NR	NR	NR	NR	*"**p=NS**" by log-rank test*
Kiyotani, 2010	TAM (282)	18/63 (29)	20/135 (15)	3/84 (3)	NR	NR	NR	NR	*IM vs EM* **4.44 (1.31-15), [S][l]** *SM vs EM* **9.52 (2.79-32.45), [S][m]** Test-for-trend p-value<0.001

[h] Study reported HR for "time to recurrence".

[i] Study also defined a group of patients with "overall reduced CYP2D6 activity", defined as SM genotype or use of a CYP2D6 inhibitor. Comparing this group with all other patients the HR for recurrence free survival was 1.9 (0.8-4.8), adjusted for LN status.

[j] This study reported a non-significant p-value=0.413 for "disease free survival" in its main analysis that compared specific genotype groups, set a priori. However, in a post hoc-analysis comparing a poor-metabolizer group (*4/*4, *****4/*****41, *1/*5, and *2/*5) versus all other genotypes, the reported p-value was 0.016.

[k] Analyses were stratified by menopausal status, and subcohort assignment (prospective versus retrospective).

[l] Analyses were stratified by menopausal status, and subcohort assignment (prospective versus retrospective).

[m] Analyses were stratified by menopausal status, and subcohort assignment (prospective versus retrospective).

Studies in the metastatic setting

A single small study from Korea (**Table 5**, n=16 analyzed samples) reported a statistically significant association between slow (versus extensive) metabolizer status and increased odds for progression and shorter time-to-progression.(27) In addition, the study also demonstrated a higher rate of "clinical benefit", defined as complete or partial response or stable disease for 24 weeks, among extensive metabolizers (p=0.02).

Table 5. *CYP2D6* and tamoxifen for breast cancer: progression (metastatic setting)

Author, year	Progression events, n (%)				Median TTP (months)			
	SM	IM	EM	OR, 95% CI	SM	IM	EM	HR, 95% CI
Lim HS, 2007	12 (100)	-	4 (44)	53 (3-1105) [a]	5.0	-	21.8	*SM vs IM+EM* 3.7 (1.3-10.7) **3.7 (1.2-11.0) [S, ER, PR, T]**

Covariate key: A =Age; S= Severity; ER = Estrogen receptor status; PR = Progesterone receptor status; T = treatments.

Key Question 2: What patient- and disease-related factors affect the test results, their interpretation or their predictive response to therapy?

None of the included studies performed analyses for interaction between the aforementioned factors and *CYP2D6*-derived metabolizer status to predict response to therapy.

Key Question 3: How does the gene testing impact the therapeutic choice?

No study explicitly reported details on changes in treatment plans before and after testing.

Key Question 4: What are the benefits and harms or adverse effects for patients when managed with gene testing?

No study explicitly reported evidence on benefits or harms beyond what is covered in Key Question 1.

[a] Continuity correction k=0.5 was used to calculate the OR.

1.4 Discussion

There were no consistent associations between *CYP2D6* polymorphism status and outcomes in tamoxifen treated women with breast cancer across 16 studies included in our systematic review. The included studies were generally small in size, followed poor analytic practices, and differed both in the direction and in the formal statistical significance of their results. It is questionable whether pharmacogenetic testing of germline (heritable) variations in *CYP2D6* can predict differential response to adjuvant tamoxifen in women with non-metastatic breast cancer. Further, evidence is severely limited for tamoxifen-treated women with metastatic disease. Our conclusions are analogous to the 2009 ASCO practice guideline update, which states "Given the limited evidence, *CYP2D6* testing is currently not recommended in the preventive setting".(39)

We documented extensive heterogeneity in the definitions of *CYP2D6*-derived metabolizer categories across the 16 studies (**Figure 3**). To demonstrate between-study differences in genotype categorizations, we used an arbitrary algorithm that assigned patients with two, one, or none "slow" alleles to slow, intermediate, or extensive metabolizer categories, respectively. We show that studies are inconsistent in their classification of genotypes in metabolizer groups. Further, these inconsistencies are irreconcilable and not dependent of the algorithm we used to standardize metabolizer groups. **Figure 3** shows that genotypes in our intermediate metabolizer group were "shifted" towards the extensive or slow metabolizer groups in different studies. Determining the clinically meaningful genetic comparisons in a multi-allelic system is challenging, and offers opportunities for data dredging. Biological rationale can be invoked retroactively and with relative ease(40) in support of even non-intuitive genotype comparisons. For example, the main analysis in the Ramon y Cajal 2009 study was statistically non-significant. In additional analyses the authors grouped 29 different genotypes in a non-intuitive way, and report a p-value of 0.016. Efforts to standardize the definitions of metabolizer groups based on genotype information would allow uniform reporting and facilitate patient-level synthesis of results across studies.

We found no evidence on whether patient or disease relevant factors affect the association between *CYP2D6*-derived metabolizer status and outcomes in tamoxifen treated women. Such evidence would be obtained by examining interaction effects between the factors of interest and metabolizer status. However, no study performed interaction analyses. Several studies performed simple adjustments for patient level factors. This is not only not informative, but also questionable from a methodological standpoint, as will be discussed below. Arguably, this lack of evidence on patient-level factors may be a moot point, given the equivocal epidemiological evidence on the overall association between testing and outcomes in tamoxifen-treated patients.

Similarly, we found no evidence on whether testing impacts on therapeutic decisions, or on harms associated with testing and its downstream effects. Like all tests, genetic testing exerts most of its effects in an indirect way: test results affect subsequent patient management decisions, which in turn impact on patient-relevant outcomes.(41) Harms are often reported inadequately in RCTs(42) and nonrandomized studies of interventions,(43) and reporting may be even worse for studies of medical tests.

As of this writing there are no validated quality items for evaluating the methodological quality of studies on pharmacogenetic tests. However, methodological observations can be made from basic epidemiological principles.(44) In fact, several contributions to the literature have drawn attention to problems with studies of modification of tamoxifen response.(44-47)

First, most studies are relatively small and thus probably underpowered to detect what would be a plausible effect size for modification of response to tamoxifen and susceptible to type I error (false positive findings). Lash 2009 argues that based on pharmacologic data, and the knowledge that the effect of tamoxifen cannot be bigger than that of aromatase inhibitors, a plausible effect size for the pharmacogenetic effect of *CYP2D6* on survival would be at best modest (relative effect no greater than 1.55).(44)

By definition, a pharmacogenetic interaction implies that the genetic factor has differential effects on outcomes in treated versus untreated patients. Almost universally, the studies we identified made the assumption that effects in untreated patients are expected to be zero. Studies based on RCT data provide an opportunity to test for interactions between treatment and *CYP2D6*-derived metabolizer status, by analyzing both treated and untreated women. No study performed such tests. Instead, all studies implicitly assume that there is no association between *CYP2D6*-derived metabolizer status and outcomes in women not treated with tamoxifen. Testing for gene by treatment interactions (when possible) is more than a formality; it presents the opportunity to triangulate results on the main effects, i.e., to perform a "reality check" on whether all analyses point to the same direction.

Finally, many studies followed poor analytic practices, by performing statistical adjustments for factors that cannot confound the relationship between *CYP2D6* and survival (or other outcomes). Mendelian randomization (the natural randomization of genotypes during mitosis) protects the relationship between *germline* (heritable) variations in *CYP2D6* and outcomes from confounding.[ref] For example, *CYP2D6* genotypes are distributed randomly (equally) across women who take SSRIs or other *CYP2D6* inhibiting medications and women who do not take such drugs. Because comedication cannot affect anyone's *CYP2D6* genotype, it *cannot confound* the relationship of *CYP2D6* and survival. **Table 6** discusses some examples of factors or design characteristics that can confound or bias relationship in classical epidemiology, but not in genetic epidemiology. As discussed elsewhere,(48) genetic associations are not immune to bias; rather, a different set of considerations is applicable to such studies. Multiplicity of comparisons, data dredging, population stratification, and misclassifications of outcomes and genotypes and various biases, such as reporting and publication bias and local-literature bias, are the most common threats to the validity of associations between genetic factors and treatment effect modification.

Table 6. *CYP2D6* and tamoxifen for breast cancer: examples of adjustments that are not epidemiologically sound in the effect modification case (*See* also Lash 2009(47))

Factor/characteristic	Why adjustment for factor is inappropriate
Comedication (or other factors)	• Mendelian randomization: *CYP2D6* genotypes are randomly distributed across medication use patterns. • Even if *CYP2D6* predisposed to specific medications, adjustments for medication use are unwarranted: The medication is in the causal path between the genotype and the outcome, and therefore should not be adjusted for (this is called "endogeneity").
Breast cancer stage	• Mendelian randomization
Adherence to tamoxifen	• Mendelian randomization • If *CYP2D6* affects adherence to tamoxifen, it is an intermediary in the causal path and should not be adjusted for
Sampling bias (convenience sampling of tissues)	• If genotyping is performed after sampling mendelian randomization protects from confounding

Section 2: Variations in *KRAS* and response to cetuximab and panitumumab in colorectal cancer

2.1 Background

The epidermal growth factor receptor (EGFR, also known as HER1 or c-erbB-1) is a transmembrane growth factor receptor with tyrosine kinase activity. EGFR is a member of the human epidermal growth factor receptor (HER) family which includes 3 more members: HER2 (HER-2/c-erbB-2/NEU), HER3 (HER-3/c-erbB-3), and HER4 (HER-4/c-erbB-4). EGFR is activated by the binding of ligands such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), or amphiregulin. Ligand binding induces a series of biochemical events[a] that activate intrinsic tyrosine kinase activity of the intracellular domains of the receptor complex. Subsequently, the now activated kinase domain of the receptor phosphorylates intracellular substrates, resulting in the activation of several downstream signaling pathways.(49) The most important are the RAS-RAF-mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol 3-kinase (PI3K) -Akt pathway and the phospholipase Cγ pathway.(50) EGFR-mediated signaling has been implicated in several cellular processes including cell proliferation, resistance to apoptosis, enhanced cell motility and neoangiogenesis. These processes are central to the pathogenesis of a variety of epithelial cancers, including lung, breast and colorectal cancer.

The central role of EGFR in cancer pathogenenesis, along with evidence indicating that EGFR expression in tumor tissues may predict adverse outcomes, has motivated an extensive translational effort to develop therapies specifically targeting this receptor. Colorectal cancer, were overexpression of EGFR occurs frequently, has long been considered a good candidate disease for implementing EGFR-targeted therapies such as antibodies targeting the EGFR protein.

Monoclonal anti-EGFR antibodies were first produced in the 1990s.(51-53) These antibodies target the extracellular domain of EGFR and are believed to exert their effects through a variety of mechanisms, including blocking EGFR activation, causing increased EGFR internalization and inducing cell-mediated immune cell cytotoxicity.(49) Currently, two monoclonal anti-EGFR antibodies are licensed in the USA for the treatment of colorectal cancer: cetuximab[b] and panitumumab.[c] Cetuximab is a chimeric mouse-human monoclonal antibody, whereas panitumumab is a fully human monoclonal antibody.(54) Both agents have been evaluated for the treatment of metastatic colorectal cancer, both as monotherapy and in combination with cytotoxic chemotherapy. Although initial studies used EGFR expression as a requirement for enrollment, it appears that both cetuximab and panitumumab are active, irrespective of EGFR expression.(55-57) Furthermore, their anti-tumor activity appears to be similar (although direct randomized comparisons have not been reported) but panitumumab has lower immunogenicity and

[a] These include receptor autophosphorylation and receptor dimerization (either homodimerization or heterodimerization involving other members of the *ERBB* family).
[b] ER-K0034, Erbitux, Merck-Serono KgaA, Darmstadt, Germany; ImClone Systems Inc, New York, NY.
[c] ABX-EGF, Vectibix; Amgen Inc, Thousand Oaks, CA.

causes fewer infusion reactions (because it is fully human).(54)

Although both anti-EGFR antibodies have demonstrated anti-tumor activity in colorectal cancer patients in a variety of treatment settings, response rates have been relatively low (around 10% for chemotherapy pre-treated patients) and the majority of patients experience disease progression while under treatment.(58-61) Low response rates and high rates of progression have motivated a number of translational investigations attempting to identify the molecular basis of resistance to anti-EGFR antibodies. One hypothesis that has been explored in a large number of studies is that genetic aberrations of the genes encoding downstream effectors of EGFR-mediated signaling could be related to resistance to anti-EGFR antibody therapy.(54)

Kirsten-RAS (*KRAS*), a member of the rat sarcoma virus (*ras*) gene family of oncogenes, is a central signaling "node" that integrates several signaling cascades controlling gene transcription, including many EGFR-mediated pathways.(62;63) *KRAS* is frequently mutated in epithelial cancers including colorectal cancer, where the mutational frequency is estimated to be approximately 35-45 percent. Mutations occur in certain "mutational hotspots", corresponding to codons 12 and 13 of the KRAS protein. Although mutations affecting other protein residues (including codon 61) have been reported, they represent a minority of reported mutations.(64) *KRAS* mutations result in continuous activation of the downstream RAS-RAF-MAPK or PI3K pathways, regardless of whether the EGFR is activated or pharmacologically blocked (constitutive activation).(3;54) Such activation increases the transcription of oncogenes implicated in cancer progression and metastasis.

Given the central role of *KRAS* in integrating EGFR-mediated signaling, and the ability of *KRAS* mutations to induce EGFR-independent cancer growth, it was hypothesized that the presence of *KRAS* mutations could abrogate the effects of anti-EGFR antibodies and precludes any beneficial effects of antibody therapy. In this framework, *KRAS* mutations may predict resistance (lack of response) to anti-EGFR antibodies. Here we report a systematic review of the available evidence on the ability if *KRAS* testing to predict response to treatment with cetuximab or panitumumab in colorectal cancer patients.

2.2 Methods

The reader is referred to the Generic Methods section for a description of methods common to all three topics examined in this review. Herein we describe the topic-specific Key Questions, as well as additional topic-specific methods.

Key questions

1) Does *KRAS* testing predict response to cetuximab or panitumumab therapy?
2) What patient- and disease-related factors affect the test results, their interpretation or their predictive response to therapy?
3) How does the gene testing impact the therapeutic choice?
4) What are the benefits and harms or adverse effects for patients when managed with gene testing?

The interpretation of the Key Questions is provided in the Generic Methods section.

Literature search strategy

The actual search strategy is listed in **Appendix A**. Briefly, we searched OVID MEDLINE, from inception to March week 3, 2010, using combinations of the terms "cetuximab", "panitumumab", "colorectal cancer" and their synonyms. We did not use terms for *KRAS* to increase the sensitivity of the searches.

Eligibility criteria

Eligible were studies that fulfilled the generic eligibility criteria listed in the Generic Methods section. Eligible studies reported on human subjects with colon or rectal cancer and presented information on clinical outcomes (mortality and/or overall survival; disease recurrence and/or progression free survival or time-to-progression or response rates) stratified by *KRAS* mutational status.

We considered all study designs (prospective and retrospective), treatment settings (first line versus second line or higher), treatment strategies (anti-EGFR antibody monotherapy versus combination with cytotoxic chemotherapy).

Extracted data

See the Generic Methods Section for commonly extracted items across the three cancer topics. Data specifically collected for studies pertinent to the current topic include tumor characteristics (including tumor anatomic location and stage), information regarding the number and types of prior chemotherapy regimens received, number of patients participating in the study and included in genotyping analyses, *KRAS* mutation testing methodology and results of *KRAS* genotyping, outcomes assessed and outcomes stratified by *KRAS* mutation status.

Outcomes

As described in the Generic Methods section three categories of outcomes were analyzed: mortality (all-cause or from colorectal cancer); progression, or the composite outcome of death or progression; and treatment failure, defined as absence of tumor response by imaging. We accepted each study's definition of colorectal cancer-specific mortality, cancer progression or treatment failure.

Synthesis

Mortality and disease progression

For time-to-event outcomes (overall and progression-free survival or time to progression) we did not perform any quantitative synthesis, because most studies did not report sufficient statistics necessary for meta-analysis. Instead, we summarize studies qualitatively. We list odds ratios and 95% confidence intervals (for analyses of cumulated events) or hazard ratios and 95% confidence intervals (for time-to-event analyses).

Treatment failure by imaging

For this outcome we performed two different sets of analyses (see Generic Methods). The first set evaluated the *test performance* of *KRAS* mutation testing for predicting treatment failure at a specific time point. The second set of analyses evaluated the *strength of association* between the presence of *KRAS* mutations and treatment failure.

To assess test performance we calculated the sensitivity, specificity, LR+ and LR- of *KRAS* testing to predict adverse outcomes as described in the Generic Methods

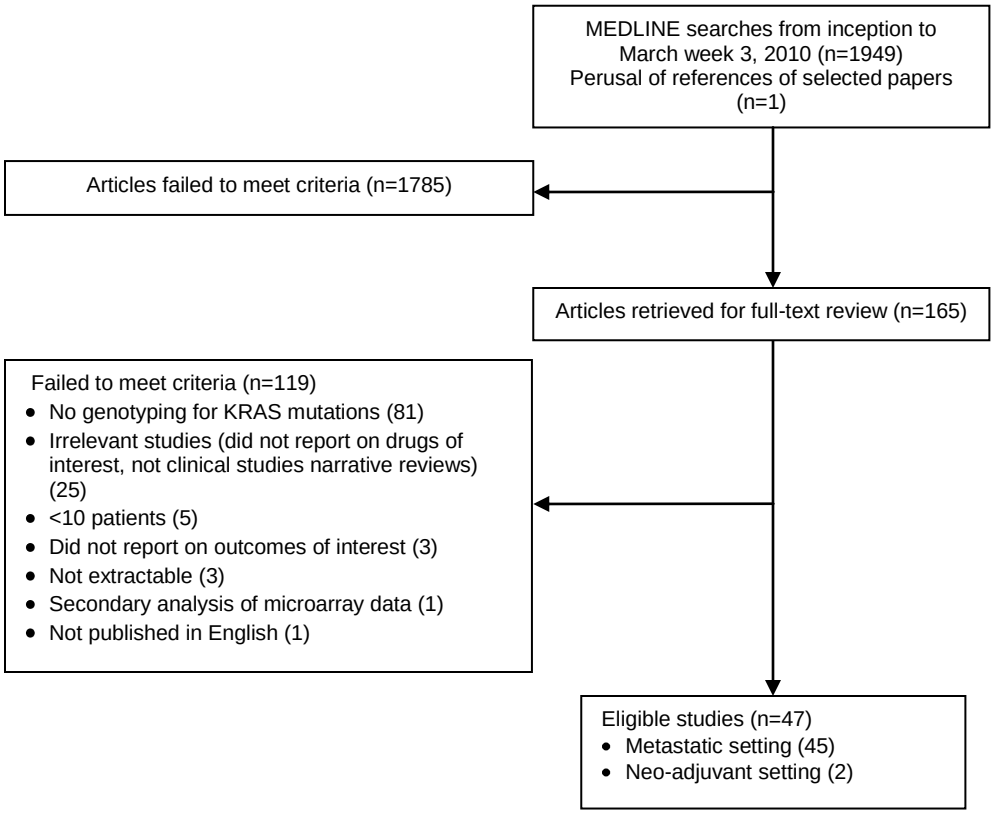
Because treatment failure was reported by the majority of studies, and reporting was relatively uniform, we performed meta-analysis to calculate summary estimates of the association of KRAS mutations with "lack of response" as well as their predictive accuracy for the same outcome. We took care not to include in the meta-analysis studies with overlapping populations, to avoid duplication of information. When several overlapping publications were identified, we selected the one with the largest number of patients. The methods for quantitative synthesis are presented in the Generic Methods section.

2.3 Results

Literature flow

Our searches returned 1949 citations. After screening of titles and abstracts 164 were retrieved and reviewed in full text. Forty-six publications were finally eligible.(58;59;61;65-92) **Appendices B** and **C** show the list of included and excluded citations, respectively. **Figure 4** summarizes the literature flow.

Figure 4: *KRAS* and anti-EGFR antibodies for colorectal cancer: literature flow



Characteristics of included studies

Tables 8 and **9** summarize the characteristics of the 47 eligible studies.(58;59;61;65-108)

Patients

Briefly, two studies were conducted in the neo-adjuvant setting,(65;70) eight studies pertained to patients who received anti-EGFR antibodies as part of the first regimen received for metastatic disease,(58;61;91-96) and all other studies included patients with metastatic disease who had previously been treated with cytotoxic chemotherapy. In studies conducted in the metastatic setting, the majority of patients had received prior treatment with at least one chemotherapy regimen; both the number and types of treatment regimens administered varied across studies. Mean or median participant age was 65 or older in 6 of the 41 studies that reported relevant information, and 60 or older in 32 of the 40 studies that reported relevant information. As shown in **Table 8** and **Table 9**, the distribution of anatomic tumor location (colon versus rectum) varied across studies and was often not reported. In terms of racial composition, the information was not reported in most studies; no study was exclusively conducted in the USA. Thirty four studies were conducted in Europe, 4 studies pertained to East Asian populations and 8 studies were conducted in a multinational setting (all 8 were prospective multicenter studies) and 1 study reported on a combined USA and European group of patients.

Treatments

All but two studies pertained to patients who received anti-EGFR antibodies for metastatic colorectal cancer. Two studies pertained to the use of cetuximab in combination with cytotoxic chemotherapy and radiotherapy for the treatment of rectal cancer before surgery (neo-adjuvant study) and their results will be discussed separately.(65;70) Thirty five studies reported exclusively on patients receiving cetuximab (as monotherapy in 4 studies; in combination with cytotoxic chemotherapy in 23 studies; both as monotherapy and in combination in 8 studies), 4 on patients receiving panitumumab (in 3 studies as monotherapy and in 1 study as part of combination therapy) and 6 on mixed patient populations receiving both drugs (panitumumab as monotherapy and cetuximab both as monotherapy and in combination in all cases). Anti-EGFR antibody dosing was commonly not mentioned, particularly in reports of retrospective studies. Given that many of the patients in these studies were participants in larger, multicenter clinical trials, drug dosing in the studies included in this report can be expected to be similar to that employed in the prospective trials. Treatment strategies are summarized in **Table *7***.

Table 7. *KRAS* and anti-EGFR antibodies for colorectal cancer: treatment strategies employed in the eligible studies

Treatment strategy	Monotherapy	Combination therapy	Both	TOTAL
Cetuximab	4	23	8	*35*
Panitumumab	3	1	0	*4*
Both	0	0	6[a]	*6*
TOTAL	*7*	*24*	*14*	***45***

This table presents the treatment strategies employed in the 45 studies reporting on patients with metastatic colorectal cancer. The studies by Debucquoy 2009 and Bengala 2009 are not included in this table because of the different treatment strategies usually employed in the neoadjuvant setting.

[a] In all 6 studies cetuximab was administered both as monotherapy and in combination with cytotoxic chemotherapy, whereas panitumumab was administered as monotherapy.

Design

Only 3 studies (including both neoadjuvant studies) explicitly stated that sample collection and subgroup analysis by *KRAS* testing was a pre-specified aim of the study.(59;65;70) In the remaining 44, outcome assessment was frequently described as prospective, while *KRAS* testing was performed on archival samples, indicating that the major driver of inclusion into each study was sample availability. The majority of studies (41 studies) assessed the ability of *KRAS* testing to predict outcomes only in patients who received anti-EGFR antibodies. This design essentially assumes that *KRAS* testing has no predictive ability for colorectal cancer outcomes in patients who did not receive anti-EGFR antibodies.[b] Seven studies presented analyses based on RCTs.(58;59;61;77;91;93;95) These RCT-based analyses included patients who received cetuximab or panitumumab (alone or in combination with chemotherapy) ("antibody" arm), as well as patients who received other treatments (chemotherapy alone or best supportive care, "comparator" arm), and provide an opportunity to examine pharmacogenetic effects of *KRAS* genotyping by testing the interaction of genotypes and treatments.[c]

Both cetuximab and panitumumab have been investigated by an extensive network of phase II and phase III clinical trials. Many of the centers participating in these trials have analyzed tissue samples from their patient cohorts for *KRAS* mutations and have presented a number of post hoc analyses, often including patients treated outside the clinical trials. When the clinical trial results are published, analyses stratified by *KRAS* status are often presented. In addition, several "pooled" analyses of such analyses have been published, resulting in substantial overlap in the published reports. Using the conventions described in the Generic Methods Section we identified studies reporting on (partially or completely) overlapping patient populations (Figure **5**).

Sample sizes in the 45 studies conducted in the metastatic setting ranged between 12 and 440 patients[d]. The seven analyses based on RCTs included 1588 patients treated with anti-EGFR antibodies and 1481 patients treated in the "comparator arms", for a total of 3069 patients assessed for *KRAS* mutations[e]. In the metastatic setting, median follow-up duration ranged from 9 to 26 months, and was commonly not reported.[f]

[b] As discussed regarding the predictive effect of *CYP2D6* polymorphisms for response to tamoxifen, if *KRAS* status predicted colorectal cancer outcome irrespective of treatment, studies that evaluate only anti-EGFR treated patients would still find an association between *KRAS* status and patient outcomes; however the association would not be specific to tamoxifen treated people, and therefore would not be evidence for a pharmacogenetic interaction.

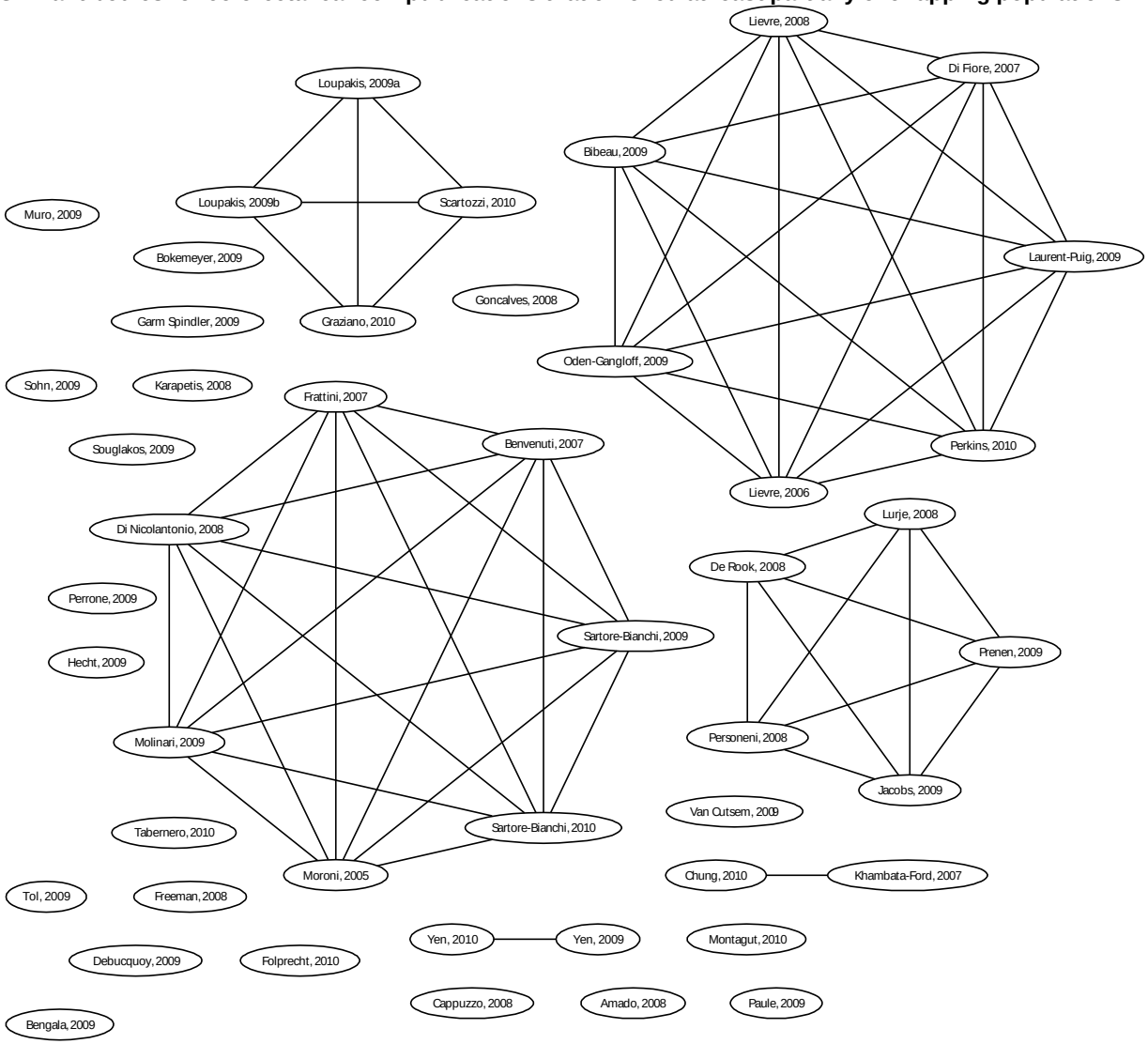
[c] Testing for interaction answers whether an observed association between *KRAS* status and patient outcomes is specific to those who received anti-EGFR antibodies, or is independent of treatment. This analysis would not invoke the assumption outlined in the previous footnote.

[d] Numbers refer to individuals assessed for *KRAS* mutations and treated with anti-EGFR antibodies.

[e] Numbers refer to patients assessed for *KRAS* mutations. In the study by Folprecht 2010 both arms received cetuximab.

[f] Numbers do not include studies where duration of follow-up since the original diagnosis of metastatic disease was reported.

Figure 5. *KRAS* and anti-EGFR antibodies for colorectal cancer: publications that enrolled at least partially overlapping populations.



Overlap between included studies. Each publication is represented by an ellipse. Studies reporting on partially or overlapping cohorts of patients are presented as a group of ellipses linked amongst themselves with lines. Refer to the Generic Methods Section for a description of how overlap was assessed.
*Studies selected for meta-analysis to avoid duplication of information (see later in this section).

Table 8. *KRAS* and anti-EGFR antibodies for colorectal cancer: characteristics of included studies (first line therapy)

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	Patients in first line therapy (%)	Prior chemo-therapy	-Treatment regimen -Adherence	Median followup, *mo* [range]
Randomized controlled trials								
Bokemeyer, 2009(58)[b] (Multinational)	-233 -Retrospective analysis based on phase II RCT -Convenience sampling	Adult patients with Histologically-confirmed EGFR-expressing CRC with at least one radiologically measurable lesion and PS≤2.	Cetuximab arm: 59 [24-82] (50) Not receiving cetuximab: 60 [30-82] (55)	-Colon 127 (55); rectum 105 (45); colon + rectum 1 (0.4) -Metastatic 233 (100)	86[c]	NA	-2 arm study: arm A: FOLFOX-4, arm B: FOLFOX-4+cetuximab until progressive [d]disease or toxicity -In the cetuximab group 84% of patents had RDI≥80%	NR
Hecht, 2009(93) (Multinational)	-865[e] (from 1053) -Prospective analysis based on a phase III RCT[f] -Patients were assigned to two cohorts (oxaliplatin-based and irinotecan-based chemotherapy) based on physician preference. Both cohorts received bevacizumab and patients were randomized into panitumumab *vs*. control arms -Convenience sampling	Pathologically diagnosed metastatic CRC patients with no prior chemotherapy of biologic therapy for metastatic disease. Measurable disease and ECOG PS ≤0.	Oxaliplatin, bevacizumab, panitumumab: 61 [28-88] (56) Oxaliplatin, bevacizumab: 62 (22-89) [58] Irinotecan, bevacizumab, panitumumab: 60 [35-84] (49) Irinotecan, bevacizumab: 59 [23-80] (62)	-Colorectal 1053 (100) -Metastatic 1053 (100)	100 (for metastatic disease)	231 patients (37%) had received prior adjuvant chemotherapy	-97% received panitumumab 6 mg/kg as their first dose; 95% received bevacizumab 5 mg/kg as their first dose - the proportion of patients receiving a RDI ≥85% of bevacizumab and each chemotherapy agent in both chemotherapy cohorts was lower in the panitumumab arms (33% for panitumumab *vs.* 42% for control for Ox-CT; 34% for panitumumab *v* 44% for control for Iri-CT)	Oxaliplatin, bevacizumab: 12 [0-26] Irinotecan, bevacizumab: 9 [0.3-24]

[a] Number of included patients typically assessed for baseline clinical characteristics, not necessarily the same as the number assessed for KRAS mutations.
[b] Data abstracted for the subgroup of patients assessed for *KRAS* mutations.
[c] 33 patients (of whom 14 were in the cetuximab+FOLFOX arm) had received adjuvant chemotherapy.
[d] Initial dose 400 mg/m^2 over 2 hours followed by a weekly dose of 250 mg/m^2 over one hour.
[e] Data abstracted for the subgroup of patients assessed for *KRAS* mutations.
[f] Availability for tissue was an inclusion criterion in the study.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	Patients in first line therapy (%)	Prior chemo-therapy	-Treatment regimen -Adherence	Median followup, *mo* [range]
Tol, 2009 (Netherlands)(91)	-736[g] -Prospective analysis[h] of RCT between CB and CBC -Convenience sampling	>18 y, WHO PS 0-1 Measurable colon or rectal cancer with metastatic disease No indication of curative surgery No previous systemic chemotherapy or neoadjuvant chemotherapy <6 mo[i]	62 [27-83] (60)	-Colon 336 (46); Rectum 202 (27); Rectosigmoid 198 (27) -Metastatic 736 (100)	100	None	-Capecitabine 2000 mg/m^2 d1-14 + oxaliplatin 130 mg/m^2 d1 + bevacizumab 7.5 mg/kg d1; q 3 wk. (CB) vs. the same regimen plus cetuximab 250 mg/m^2 d1; q 3 wk[j] (CBC) -NR	23
Van Cutsem, 2009(61)[k] (Multinational)	-540 -Retrospective analysis based on a phase III RCT -Convenience sampling	Adult patients with colorectal adenocarcinoma and unresectable metastatic disease	Cetuximab arm: 61 [22-79] (62) Not receiving cetuximab: 62 [22-79] (53)	-Colon, 317 (59); rectum, 217 (40); other, 6 (1) -Metastatic 540 (100)	84[l]	NR	-FOLFIRI arm: irinotecan 180 mg/m^2 BSA; leucovorin or L-leucovorin 400 or 200 mg, respectively/m^2; 5-FU 400 mg/m^2 followed by continuous infusion for 46 hours of 2400 mg/m^2 Cetuximab+FOLFIRI arm: cetuximab 400 mg/m^2, followed by weekly 250 mg/m^2 -NR	NR
Folprecht, 2010(95) (Germany + Austria)	-111 -Retrospective analysis based on a phase II RCT	-Patients with unresectable, Histologically confirmed metastatic colorectal cancer. Only patients with	FOLFOX arm: 65 [57-71][m] (64)	-Colorectal cancer with liver metastases 111 (100)	100	-18 (16%) of patients had received	-Cetuximab (400 mg/m2 on day 1, followed by 250 mg/m2 weekly thereafter every 2	NR

[g] The number of patients randomized and assessed for baseline characteristics. 368 were treated with cetuximab. Baseline characteristics are for the 736 subjects.
[h] It is unclear whether the assessment of KRAS mutations was also prospectively planned.
[i] Those with sensory neuropathy, symptomatic CNS metastasis, bleeding diathesis, coagulation disorders, significant cardiovascular disease, previous other cancers <5 y excluding squamous or basal carcinoma of the skin or carcinoma in-situ of the cervix were excluded.
[j] 400 mg/m^2 for the first course.
[k] Data abstracted for the subgroup of patients that underwent KRAS mutational testing.
[l] 16% of the patients had received adjuvant chemotherapy. Patients were eligible if no prior chemotherapy for metastatic disease had been administered.
[m] Age [interquartile range].

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	Patients in first line therapy (%)	Prior chemo-therapy	-Treatment regimen -Adherence	Median followup, *mo* [range]
	-Patients were randomly assigned to two alternative chemotherapy regimens (FOLFOX or FOLFIRI), both in combination with cetuximab -Convenience sampling (based on tissue availability)	liver metastases and no other evidence of metastatic disease were enrolled. -Patients having received previous chemotherapy (except adjuvant chemotherapy ≥6 months before enrollment) were excluded	FOLFIRI arm: 62 [56-68] (64)	-Metastatic disease 111 (100)		prior adjuvant chemothe rapy. No patient had received chemothe rapy in the metastatic setting.	weeks, followed by either FOLFOX6 (day 1, oxaliplatin 100 mg/m2, folinic acid 400 mg/m2, and fluorouracil 400 mg/m2 intravenous bolus, then 2400 mg/m2 over 46 h continuous infusion, or FOLFIRI (day 1, irinotecan 180 mg/m2, fluorouracil and folinic acid as described for the FOLFOX group)	
Single arm studies								
Bengala, 2009(65) (Italy)	-40 -"Correlative study" based on a phase II prospective single arm clinical trial of cetuximab + chemoradiotherapy for rectal cancer -From all 40 patients (representative)	-Histologically confirmed locally advanced rectal cancer, stage T3-T4; N0-N1 rectal carcinoma with no evidence of distant metastasis	61 [28-77] (85)	-Rectum (100) -Locally advanced rectal cancer 40 (100)	100	NA	-Cetuximab 400 mg/m^2 loading dose for three, then 250 mg/m^2 weekly, followed by cetuximab 250 mg/m^2 weekly plus 5-FU (225 mg/m^2/day IV 7 days a week for 5 weeks) + radiotherapy [15–18 MV photon beams, at 1.8–2 Gy/fraction up to 50–50, 4 Gy in 25–28 daily fractions for 5 days a week] -Median number of cetuximab: 8; 70% of the patients completed the planned doses; 38 patients underwent radical surgery (one refused surgery and the other had disease progression under	NR

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	Patients in first line therapy (%)	Prior chemo-therapy	-Treatment regimen -Adherence	Median followup, *mo* [range]
							therapy and was operated on[n])	
Debucquoy, 2009(70) (Belgium)	-41 Prospective phase I/II clinical trial -From all 41 patients (representative)	-Histologically proven rectal adenocarcinoma, stage T3-T4 and/or N1-N2, ECOC performance status ≤2 and acceptable liver, renal and hematological parameters[o]	NR	-Rectum (100) -Advanced rectal cancer 41 (100)	100	NA	-Capecitabine [650 mg/m2 twice daily (4 patients), or 850mg/m2 twice daily (37 patients)] + cetuximab (loading dose 400 mg/m2, followed by 250mg/m2 weekly) + radiotherapy (1.8 Gy/day for 25 days) -NR	32 [5-46]
Yen, 2009(92) (Taiwan)	-76 -Retrospective analysis of a cohort -Convenience sampling	-Metastatic colorectal cancer treated with FOLOX or FOLFIRI+cetuximab where tumor and peripheral blood samples available, measurable lesions by CT	64 [39-83] (58)	-Colon 55 (72) Rectum 21 (28) -Metastatic disease 76 (100)	NR	NR	-Cetuximab + FOLFOX, 54 (71) ; cetuximab + FOLFIRI, 22 (29)	20 [4-34]
Tabernero, 2010(94) (Spain)[p]	-62 -Secondary analysis based on a prospective multicenter cohort study of cetuximab monotherapy followed by sequential assignment to different cetuximab-FOLFIRI combinations -Convenience	-Adults with histologically confirmed, EGFR-expression positive metastatic colorectal cancer.	65 [39-80] (63)	-Colon 36 (58); rectum 23 (37); colon/rectum 3 (5) -Metastatic 62 (100)	100	-11 patients (18%) had received prior adjuvant or neoadjuvant chemotherapy	-Cetuximab monotherapy 6 wk, followed by cetuximab plus FOLFIRI until disease progression. -Patients in the control arm received cetuximab as a 400 mg/m^2 initial dose then 250 mg/m^2 per wk; patients in the dose-escalation arms received 400 to 700 mg/m^2 every second	NR

[n] This patient was considered a treatment failure in the original report.
[o] Eligibility criteria were abstracted from Machiels et al., Ann Oncol, 2007.
[p] Some patient characteristics were extracted from Tabernero et al., Ann Oncol, 2009.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	Patients in first line therapy (%)	Prior chemo-therapy	-Treatment regimen -Adherence	Median followup, *mo* [range]
	sampling (based on tissue availability)						wk. -In the monotherapy phase, 97% of patients achieved RDI≥90% for cetuximab. In the combination phase, 70% achieved an RDI≥90%	
Yen, 2010(96) (Taiwan)	-95 -Retrospective cohort study -Convenience sampling	-Adults with histologically confirmed metastatic colorectal cancer -Tumor DNA availability -Life expectancy >3 months, prior cytotoxic or radiation therapy completed at least 4 weeks prior to enrollment	66 [39-86] (58)	-Colon 71 (75); rectum 24 (25)	NR	NR	-Cetuximab + FOLFOX 74 (78); cetuximab + FOLFIRI 21 (22)	NR

BSA, body surface area; BSC, best-supportive care; CAPOX, capecitabine + oxaliplatin; CB, capecitabine, oxaliplatin, bevacizumab; CBC, capecitabine, oxaliplatin, bevacizumab, cetuximab; CNS, central nervous system; CPT-11, irinotecan, EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; FOLFIRI, irinotecan, fluorouracil, and folinic acid; FOLFOX, oxaliplatin, fluorouracil, and folinic acid; IAH, intra-artery hepatic infusion; IHC, immunohistochemistry; LV5FU2, leucovorin, 5-fluorouracil; NA, not applicable; NR, not reported; PS, performance status; RCT, randomized controlled trial; RDI, relative dose intensity; WHO, World Health Organization.

Table 9. *KRAS* and anti-EGFR antibodies for colorectal cancer: characteristics of included studies (salvage therapy)

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
Randomized controlled trials								
Amado, 2008(59) (Belgium, Italy)	-427 -Pre-specified subgroup analysis based on a randomized phase III RCT (panitumumab + BSC versus BSC alone) -Prospective selection of patients	-Patients with EGFR expressing metastatic colorectal cancer progressing after fluoropyrimidine therapy, having previously received oxaliplatin and irinotecan	Panitumumab arm: 62 [27-79] (56) among KRAS positive and 63 [29-82] (67) among KRAS negative BSC arm: 62 [27-83] (64) among KRAS positive and 63 [32-81] (64) among KRAS negative	-Colon 286 (67) Rectal 141 (33) -Metastatic 427 (100)	0	-Fluoropyrimidine-based, oxaliplatin, irinotecan -133 patients in the panitumumab arm and 137 in the BSC arm had received 2 prior lines of chemotherapy; 64 patients in the panitumumab arm and 73 in the BSC arm had received 3 prior lines of chemotherapy	-2 arm study: Arm A: panitumumab 6 mg/kg every 2 weeks + BSC versus; Arm B: BSC alone -Mean number of panitumumab infusions was 10 (median=8) in KRAS negative patients and 4.9 (median=4) in KRAS positive patients	NR
Karapetis, 2008(77) (Multinational)	-394 -Prospective analysis[b] of RCT -Convenience sampling	-Advanced CRC, failed all prior chemo, no prior treatment with EGFR inhibitor.	63 [29-88] (64)	-Colon 332 (58); rectum 133 (23); both sites 107 (19) -NR (all subjects had "advanced CRC")	0	-Thymidylate synthase inhibitor 394 (100); irinotecan 380 (96); oxaliplatin 385 (98); radiotherapy 127 (32)	-Cetuximab loading dose 400mg/m^2, followed by 250mg/m^2 weekly	NR
Single arm studies								
Moroni, 2005(85) (Italy)	-31 -Retrospective cohort study of patient enrolled in cetuximab or panitumumab clinical trials -Convenience sampling	-EGFR expressing metastatic colorectal cancer	65 [41-85] (71)	-NR -Metastatic disease 31 (100)	35[c]	-Patients had received a variety of regimens for metastatic disease. -FOLFOX 18 (58); FOLFIRI 16 (52); any irinotecan-based regimen 20 (65); other 9 (29)	-Panitumumab monotherapy, 10 (32); Cetuximab monotherapy, 12 (39); Cetuximab + irinotecan - based	NR

[a] Number of included patients typically assessed for baseline clinical characteristics, not necessarily the same as the number assessed for KRAS mutations.
[b] It is unclear whether the assessment of KRAS mutations was also prospectively planned.
[c] 11 patients had not received any chemotherapy regimen for metastatic disease. Adjuvant chemotherapy data were not reported.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
							chemotherapy, 9 (29) -NR	
Lievre, 2006(80) (France)	-30 -Retrospective multi-center cohort -Convenience sampling	-Metastatic colorectal adenocarcinoma -Underwent a surgical resection of primary tumor -Received cetuximab	62 [41-78] (63)	NR	10	-FOLFIRI 22 (73); FOLFOX 20 (67); LV5FU2 11 (37); IAH 6 (20)	-Cetuximab alone 1 (3); cetuximab + irinotecan 25 (83); cetuximab + FOLFIRI 4 (13) -NR	NR
Benvenuti, 2007(66) (Italy)	-48 -Retrospective single-center cohort (patients were enrolled in clinical trials evaluating panitumumab treatment) -Convenience sampling	-EGFR-expressing metastatic colorectal cancer	61 [39-84] (63)	-NR -Metastatic disease 48 (100)	23[d]	-Number of prior regimens[e]: 2-3, 58 (45); 4-5, 60 (46); 6-8, 12 (9) -Patients received cetuximab+irinotecan therapy when disease progression had been observed during or within 3 months of receiving an irinotecan-based regimen	-Cetuximab alone 12 (25); Panitumumab alone 25 (52); Cetuximab + irinotecan-based chemotherapy 11 (23) -NR	NR
Di Fiore, 2007(72) (France)	-59 -Multi-center retrospective cohort -Convenience sampling	-Metastatic colorectal cancer -Treated with cetuximab (2004-2005) -Tumor DNA available	NR	NR	0	≥1 regimen	-Cetuximab + irinotecan or oxaliplatin -NR	NR
Frattini, 2007(73) (Switzerland)	-27 -Retrospective single center cohort (18 of the patients participated in clinical trials of cetuximab) -Unclear	-Histologically confirmed metastatic colorectal cancer -EGFR expression by the primary tumor [IHC]	67 [29-84] (67)	-Colon (19) + rectal (8) -Metastatic disease 27 (100)	15	-FOLFOX (11), CPT-11 (9); FOLFIRI (8), CAPOX (4), other (7)	-Cetuximab + irinotecan-based, 23 (85); cetuximab + CAPOX-based 4 (15)	NR

[d] Percentage of patients receiving first line treatment for metastatic disease. The percentage of patients who had received adjuvant chemotherapy was not reported.

[e] Line of treatment for metastatic disease.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
							-NR	
Khambata-Ford, 2007(78) (Multinational)	-110 -Prospective multi-center cohort[f] -Unclear	-Metastatic CRC, at least one prior chemo regimen or refused treatment, pretreatment core tumor biopsy of metastatic lesion available	61 [25-89] (50)	NR	0	NR	-Cetuximab loading dose 400mg/m^2, followed by 250mg/m^2 weekly, eligible for dose escalation q 3 wk up to max of 400mg/m^2 -NR	NR
Cappuzzo, 2008(68) (Italy)	-85 -Retrospective cohort -Convenience sampling	-Chemo-refractory metastatic colorectal cancer -Received cetuximab-based therapy -Previously evaluated for EFGR by FISH	63 [NR] (64)	-Colon 65 (77); Rectum 20 (24) -NR	0	-Irinotecan 71 (84) and/or oxaliplatin 72 (85)	-Irinotecan 180 mg/m^2 q2wk + cetuximab 250 mg/m^2 qwk[g] (NR for other concurrently used agents) -NR	NR
De Rook, 2008(69) (Belgium)	-113 -Retrospective multi-center cohort -Convenience sampling	-Metastatic EGFR expressing colorectal adenocarcinoma receiving cetuximab treatment (monotherapy or in combination with irinotecan)	60 [11][h] (70)	-NR -Metastatic disease 113 (100)	0	-Irinotecan-based therapy	-Cetuximab monotherapy, 30 (27); cetuximab +irinotecan, 83 (73) -NR	94 (25-473)[i]

[f] It is unclear the assessment of KRAS mutations were also prospectively planned.
[g] 400 mg/m^2 for the first week.
[h] Mean [SD].
[i] Time since diagnosis of metastatic disease.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
Di Nicolantonio, 2008(71) (Italy, Switzerland)	-113 -Retrospective 2-center cohort study, 63 of the patients participated in clinical trials -Convenience sampling	-Histologically confirmed, EGFR expressing metastatic colorectal cancer -Evidence that the treatment outcome could be attributable only to panitumumab or cetuximab	63 [NR] (64)	-Colon, 70 (62); rectum, 43 (38) -Metastatic disease (100)	12	-100 patients (88) had received prior adjuvant chemotherapy; 53 (47) had received 2 prior lines of chemotherapy; 35 (31) had received 3 or more	-Cetuximab monotherapy, 36 (32); panitumumab monotherapy, 26 (23); cetuximab + chemotherapy, 51 (45) -NR	NR
Freeman, 2008(73) (Multinational)	-62 -Retrospective analysis of multi-center cohort[j] -Convenience sampling	-Metastatic colorectal cancer, disease progression on chemo, tumor samples available for analysis.	62 [29-85] (60)	NR	0	-Fluoropyrimidine, irinotecan, and/or oxaliplatin	-Panitumumab 6 mg/kg q 2 wk or 2.5 mg/kg q 1 wk -NR	NR
Goncalves, 2008(76) (France)	-32 -Retrospective cohort study -Convenience sampling	-EGFR positive metastatic colorectal cancer treated with cetuximab at a single center	58 (36-78) (50)	-Colon, 21 (65); rectum, 11 (35) -NR	6	-Irinotecan 29 (91); none 2 (6); fluoropyrimidine and oxaliplatin 1 (3)	-Cetuximab (loading dose 400 mg/m2 followed by 250 mg/kg weekly) + irinotecan (180 mg/m2 every other week), 26 (81); cetuximab dose escalation in irinotecan refractory patients, 3 (9); cetuximab+FOLFIRI, 3 (9) NR	19.1 [NR]
Lievre, 2008(79) (France)	-89[k] -Retrospective multi-center cohort -Convenience sampling	-Histologically confirmed metastatic adenocarcinoma -Treated with cetuximab as US FDA administration	NR	NR	0	-Irinotecan-based chemotherapy	-Cetuximab alone 2 (2); cetuximab + irinotecan 79 (88); cetuximab + FOLFIRI 9 (10)	10

[j] Pooled retrospective analysis of 3 Phase II trials.
[k] 114 patients are assessed in a pooled analysis.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
		guidelines -Treatment response assessable					-NR	
Lurje, 2008(83) (Multinational)	-130 -Retrospective analysis of phase II study -Convenience sampling	-Metastatic CRC, failed two prior regimens or adjuvant therapy plus one regimen	≤54 y, 36; 54-64 y, 45; ≥65 y, 49 [NR] (49)	-Colon 99 (76); rectum 31 (24) -Metastatic disease 130 (100)	0	-Number of prior regimens: 2-3, 58 (45); 4-5, 60 (46); 6-8, 12 (9) -Patients were refractory to irinotecan, oxaliplatin and fluoropyrimidines	-Cetuximab 400 mg/m^2 loading dose followed by 250 mg/m^2 weekly[l] -NR	12
Personeni, 2008 (108) (Belgium)	-96 (of whom 87 were tested for *KRAS* mutations) -Retrospective cohort study (4 centers) -Convenience sampling	-Patients with metastatic colorectal cancer treated at 4 Belgin centers, patients were participants in prospective studies of cetuximab treatment	59 [26-80] (56)	-NR -Metastatic disease 96 (100)	0	-Cetuximab was administered as third- or subsequent-line of treatment.	-Cetuximab monotherapy, 18 (21); cetuximab + irinotecan, 69 (79). Cetuximab loading dose 400 mg/m^2 followed by 250 mg/m^2 weekly, 75 (86); cetuximab dose escalation (up to 500 mg/m^2), 12 (14).	NR
Bibeau, 2009(67) (France)	-69 -Retrospective cohort study (2 centers) -Convenience sampling	-Irinotecan-based chemotherapy-refractory patients with metastatic colorectal cancer	60 [23-81] (67)	-Colon, 24 (35); sigmoid, 22 (32%); rectum, 21 (30); other, 2 (3) -Metastatic disease 69 (100)	0	-All patients had failed irinotecan-based chemotherapy and 78% had received at least two lines of treatment for metastatic disease	-Cetuximab "standard dosage"+irinotecan , 68 (99) ; FOLFIRI+cetuximab, 1 (1) -NR	19.3 [0.3 to 34.4][m]
Garm Spindler, 2009(75) (Denmark)	-71 -Retrospective single-center cohort -Convenience sampling	-Metastatic colorectal adenocarcinoma refractory to prior fluouropyrimidine,	61 [38-77] (52)	-Colon 24 (34); rectosigmoideum 22 (31); rectum 25 (35)	0	-Fluoropyrimidine, oxaliplatin, and irinotecan regimes	-Irinotecan 350mg/m^2 + cetuximab 400mg/m^2 loading	8 [2-30]

[l] Data extracted from Lenz et al., J Clin Oncol, 2006.
[m] Median follow-up [95% confidence interval].

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
		oxaliplatin, and irinotecan regimens , minimum of 3 cycles of chemo, measurable disease by RECIST		-Metastatic Sites 1-2, 33 (46); 3-5, 38 (54)			with weekly 250mg/m^2 -NR	
Jacobs, 2009(97) (Belgium)[n]	-220 -Retrospective multicenter cohort (patients were enrolled in clinical trials evaluating cetuximab) -Convenience sampling	-Irinotecan refractory colorectal cancer patients with EGFR-expressing tumors	NR [NR] (NR)	-NR -Metastatic disease 220 (100)	0	NR	-Irinotecan-based chemotherapy 220 (100) -NR	NR
Laurent-Puig, 2009(98) (France)	-173 -Retrospective multicenter cohort -Convenience sampling	-Irinotecan-refractory metastatic colorectal cancer	NR [NR] (NR)	-NR -Metastatic disease 173 (100)	<1	-Number of previous lines of treatment: none 1 (<1); 1, 13 (8); 2, 78 (45); 3, 49 (28); 4, 20 (12); 5 or more, 12 (7)	-Cetuximab + irinotecan 141 (82); cetuximab + FOLFIRI 28 (16); cetuximab alone 3 (2)[o] -NR	NR
Loupakis, 2009(82) (Italy)	-102 -Multi-center Retrospective cohort -Convenience sampling	-Measurable and evaluable EGFR+ adenocarcinoma -Progressed ≤3 mo after start of irinotecan-based regimens -Received salvage cetuximab-irinotecan regimen -Paraffin-embedded samples from primary and/or metastatic lesions available[i]	62 [38-78] (59)	-NR -84 (82) had multiple metastatic sites; 12 (12) had only hepatic metastasis	0	-Irinotecan-based regimens	-Irinotecan 130-180 mg/m^2 q2wk + cetuximab 250 mg/m^2 qwk[p] (only 2 received cetuximab monotherapy) -NR	21

[n] This study also included a small (n=67) cohort from France that received standard treatment for colorectal cancer. All patients in this cohort were WT for KRAS mutations. We did not extract data for these patients.

[o] Treatment was not reported for one patient.

[p] 400 mg/m^2 for the first week, and 500 mg/m^2 if administered every 2 weeks.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
Loupakis, 2009(81) (Italy)	-138[q] -Multi-center Retrospective cohort -Convenience sampling	-Measurable and evaluable EGFR+ adenocarcinoma -Progressed ≤3 mo after start of irinotecan-based regimens -Received salvage cetuximab-irinotecan regimen -Paraffin-embedded samples from primary lesions available	61 [42-77] (55)	-NR -105 (76) had multiple metastatic sites	0	-Irinotecan-based regimens	-Irinotecan 180 mg/m^2 q2wk + cetuximab 250 mg/m^2 qwk[r] -NR	NR
Molinari, 2009(84) (Switzerland)	-12[s] -Single-center retrospective cohort -Convenience sampling	-Metastatic colorectal cancer (adenocarcinoma) -Both synchronous and metachronous metastasis -Registered in a local cancer database	67 [48-94] (63)[t]	-Colon (76); Rectum (24) -Synchronous metastases (66); metachronous metastases (34)	8	-Irinotecan-based chemotherapy	-Cetuximab alone 11 (92); Cetuximab + irinotecan 1 (8) -NR	NR
Muro, 2009(86) (Japan)	-52[u] -Retrospective analysis of multi-center Phase II study -Convenience sampling	-CRC with progressive disease after Fluoropyrimidine, irinotecan, and/or oxaliplatin -At least one lesion >20mm in diameter, EGFR expression on >1% of tumor cells	59 [23-77] (65)	-Colon 30 (58); rectal 22 (42) -Metastatic disease 52 (100)	0	-Fluoropyrimidine, irinotecan, and/or oxaliplatin	-Panitumumab at 6mg/kg q 2 wks -Panitumumab dosing: median 6 infusions (range, 2-20)	26 [5-42]

[q] KRAS 12, 13, 61, and 146 mutations were assessed. 76 patients assessed for KRAS 61 and 146 mutations only.
[r] 400 mg/m^2 for the first week, and 500 mg/m^2 if administered every 2 weeks.
[s] Those who received cetuximab only. Other 26 patients did not receive cetuximab.
[t] Data extracted from the entire cohort including those who did not receive cetuximab.
[u] Only 16 samples available for KRAS genotyping. This was augmented with 8 additional samples from another Phase 1 panitumumab study in patients with metastatic disease for a total of 24 KRAS samples.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
Oden-Gangloff, 2009(87) (France)	-64 -Retrospective cohort study -Convenience sampling	-Chemorefractory metastatic colorectal cancer patients treated with cetuximab based chemotherapy for whom tissue was available for genotyping	60 [20-82][v] (70)	-NR -Metastatic disease 64 (100)	0	-Patients had received a mean of 2 previous chemotherapy regimens (in metastatic setting) and90% were refractory to irinotecan-based treatment	-Cetuximab + irinotecan or oxaliplatin based chemotherapy 63 (98); cetuximab monotherapy 1 (2) -NR	NR
Paule, 2009(99) (France)	-23 -Retrospective cohort study -Convenience sampling	-Primary EGFR-expression positive colorectal cancer with liver metastases -Resistant to irinotecan	60 [29-80] (65)	-Colon 23 (100) -Metastatic disease	0	-Irinotecan-based chemotherapy 23 (100)	-Cetuximab (loading dose 400 mg/m2 followed by 250m/m2 weekly) + irinotecan (350 mg/m2every for 6 cycles), 23 (100) -NR	NR
Perrone, 2009(88) (Italy)	-32 -Single-center retrospective cohort -Convenience sampling	Metastatic colorectal cancer Refractory to irinotecan[w]	58 [36-78] (63)	-NR -Stage IV at diagnosis23 (72) and metachronous metastasis 9 (28) (NR)	0	5FU or fluoropyrimidines, oxaliplatin, irinotecan	-Irinotecan 300 mg/m^2 q3wk + cetuximab 250 mg/m2 qwkx -NR	NR
Prenen, 2009(89) (Belgium)	-200 -Retrospective single center cohort 197 of the patients participated in clinical trials -Convenience sampling	-Metastatic colorectal adenocarcinoma receiving cetuximab treatment (monotherapy or in combination with irinotecan)	61 [26-89] (60)	-NR -Metastatic 200 (100)	0	-Irinotecan-based therapy	-Cetuximab monotherapy, 16 (8); cetuximab +irinotecan, 184 (92)[y] -NR	NR

[v] Mean age [range].
[w] Refractory to irinotecan was defined as disease progression during treatment or <3 months of the end of the last received cycle.
[x] 400 mg/m^2 for the first week
[y] 197 (98.5%) of patients were participants in clinical trials evaluating cetuximab monotherapy or cetuximab+irinotecan-based combinations.

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
Sartore-Bianchi, 2009(100) (Italy)	-132 -Retrospective cohort study -Convenience sampling	-Metastatic EGFR-expression positive colorectal cancer -"Evidence that treatment outcome could be attributable to administration of panitumumab or cetuximab"	63.5 [26-85] (65)	-Colon 78 (59); Rectum 31 (23); Recto-sigmoid junction 19 (14); other 4 (3) -Metastatic disease 132 (100)	10	-Number of previous lines of treatment: none 13 (10); 1, 19 (14); 2, 65 (49); 3, 29 (22); 4 or more, 6 (5) -Irinotecan-based chemotherapy 117 (89); fluoropyrimidine/capecitabine-based chemotherapy 115 (87); oxaliplatin-based 105 (80)	-Cetuximab + irinotecan-based chemotherapy 94 (71); panitumumab monotherapy 23 (17); cetuximab monotherapy 15 (11) -NR	NR
Sohn, 2009(101) (Korea)	-66 -Retrospective cohort study -"Prospective patients" -Convenience sampling (2 patients were excluded based on tissue availability)	-Histologically confirmed metastatic colorectal cancer that had received at least one course of irinotecan-based chemotherapy -Had experienced disease progression during prior irinotecan-based chemotherapy or within 3 months	58 [28-77] (61)	-NR -Metastatic disease 66 (100)	0	-Number of previous lines of treatment: 1, 2 (3); 2, 22 (33); 3, 42 (64)	-Cetuximab + FOLFIRI 50 (76); cetuximab + irinotecan 16 (24) -NR	NR
Souglakos, 2009(102) (USA + Greece)	-92[z] -Retrospective cohort study; patients were treated in the USA and Greece -Convenience sampling	-Histologically confirmed metastatic colorectal cancer -Colectomy samples available for molecular analysis	59 [23-86] (52)[aa]	-Colon 135 (80); rectum 33 (20) [bb] -Metastatic disease 92 (100) [cc]	0	-Number of previous lines of treatment: 1, 37 (40); ≥2, 55 (60)	-Cetuximab + chemotherapy -NR	NR

[z] The study enrolled a total of 168 patients of whom 100 received cetuximab in combination with chemotherapy. Of those 100, 92 received this treatment in the second-line setting and the study reports clinical outcomes only for them.
[aa] Data refer to the overall cohort (168 individuals).
[bb] Data refer to the overall cohort (168 individuals).
[cc] Data refer to the overall cohort (168 individuals).

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
Sartore-Bianchi, 2009(90) (Italy, Switzerland)	-110 -Retrospective single center cohort study -Convenience sampling	-EGFR expressing metastatic colorectal cancer	64 [26-85] (65)	-Colon, 69 (63); sigmoid, 11 (10); rectum, 28 (25); other, 2 (2) -Metastatic disease (100)	12[dd]	-Irinotecan-based, 95 (86); fluoropyrimidine/ capecitabine-based, 93 (85); oxaliplatin-based, 84 (76)	-Panitumumab monotherapy, 22; Cetuximab monotherapy, 14; Cetuximab + irinotecan-based chemotherapy, 74 -NR	NR
Chung, 2010(103) (USA)	-88 -Retrospective cohort study of patients enrolled in a clinical trial of cetuximab monotherapy -Convenience sampling	-Metastatic colorectal cancer -Patients had to have material for KRAS mutation analysis and a mass-spectrometry derived proteomic profile (64/88 patients)	57 [NR] (57)[ee]	-Colorectal cancer 88 (100) -Metastatic disease	0	NR	-Cetuximab loading dose 400mg/m^2, followed by 250mg/m^2 weekly, eligible for dose escalation q 3 wk up to max of 400mg/m^2 -NR	NR
Graziano, 2010(104) (Italy)	-138 (4 patients had non-assessable samples and 13 were found to carry BRAF mutations and were excluded) -Retrospective cohort study -Convenience sampling	-Metastatic EGFR-expression positive colorectal cancer resistant to irinotecan-based chemotherapy -Available tumor from the primary cancer	65 [41-77] (54)	-NR -Metastatic disease 138 (100)	0	-All patients had failed prior irinotecan-based chemotherapy -Treatment in the study was "third line"	-Cetuximab (loading dose 400 mg/m2 followed by 250 mg/kg weekly) + irinotecan (180 mg/m2 every other week), 138 (100) -NR	NR
Montagut, 2010(106) (Spain)	-48 -Retrospective single center cohort study -"consecutive patients" -Convenience sampling (based on tissue availability)	-Histologically confirmed metastatic colorectal cancer -Available tissue for molecular analysis	<65 y, 26; ≥65 y, 22 [NR] (65)	-Colon 36 (75); rectum 12 (25) -Metastatic disease 48 (100)	2	-47 (98) of patients had failed prior chemotherapy - Number of previous lines of treatment: 1, 7 (15); ≥2, 40 (83)	- Cetuximab (loading dose 400 mg/m2 followed by 250 mg/kg weekly) + chemotherapy [irinotecan-based, 44 (92); oxaliplatin-	NR

[dd] Individuals that received no previous cancer treatments for advancd disease prior to anti-EGFR antibody treatment.
[ee] Data refer to the overall cohort (88 individuals).

Author, year (Country)	-Sample size[a], *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Cancer site -Staging [criteria] distribution, *n (%)*	% Patient in First-line Therapy	Prior chemotherapy	-Treatment -Regimen Adherence	Median followup, *mo* [range]
							based 4 (8)] -NR	
Perkins, 2010(105) (France)	-42 -Retrospective cohort study -Convenience sampling	-Histologically proven, EGFR-expression positive metastatic colorectal adenocarcinoma	61 [44-78] (57)	-NR -Metastatic disease 42 (100)	2	-Number of previous lines of treatment: none, 1 (2); 1, 16 (38); 2, 17 (40); 3, 5 (12); 4 or more 3 (7)	-Cetuximab + irinotecan 37 (88); cetuximab + FOLFIRI 2 (5); cetuximab alone 2 (5); panitumumab alone 1 (2) -NR	10 [NR]
Scartozzi, 2010(107) (Italy)	-112 -Retrospective cohort study -Convenience sampling	-Histologically proven EGFR-expression positive metastatic colorectal cancer -Previous treatment with irinotecan -Progressive disease under irinotecan-based chemotherapy	64 [36-81] (63)	-NR -Metastatic disease 112 (100)	0	-Number of previous lines of treatment: 1, 13 (12); 2-3, 99 (88) -All patients had received prior oxaliplatin-based chemotherapy	-Cetuximab + FOLFIRI 36 (32); cetuximab + irinotecan 76 (68) -NR	NR

BSA, body surface area; BSC, best-supportive care; CAPOX, capecitabine, oxaliplatin; CB, capecitabine, oxaliplatin, bevacizumab; CBC, capecitabine, oxaliplatin, bevacizumab, cetuximab; CNS, central nervous system; CPT-11, irinotecan; EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; FOLFIRI, irinotecan, fluorouracil, and folinic acid; FOLFOX, oxaliplatin, fluorouracil, and folinic acid; IAH, intra-artery hepatic infusion; IHC, immunohistochemistry; LV5FU2, leucovorin, 5-fluorouracil; NA, not applicable; NR, not reported; PS, performance status; WHO, World Health Organization.

Mutation testing characteristics

The methods and results of *KRAS* mutation analyses are presented in **Tables 10** and **11**. The majority of studies only assessed codons 12 and 13 of the KRAS gene, using direct sequencing or allele-specific methods. The commonly employed technologies are commercially available.

Table 10. *KRAS* and anti-EGFR antibodies for colorectal cancer: detection and reporting of mutations (first line therapy)

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability *(%)*	Mutation Frequency, n (%)								Comment
					Exon 1						Exon 2	Wild-type (%)	
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Randomized controlled trials													
Bokemeyer, 2009	233 [344]	NR [PET, NR]	PCR clamping and melting curve	NR	NR	NR	NR	NR	NR	NR	Not assessed	134 (58) of whom 61 received cetuximab	Any mutation+ 99 (42%) of whom 52 were in the cetuximab arm
Hecht, 2009	865 [1053]	NR [PET, NR]	ARMS-PCR (DxS)	NR	346 (40)						Not assessed[b]	519 (60) of whom 258 received panitumumab	
Tol, 2009	520 [755]	P 520 (100) [PET, NR]	ARMS-PCR (DxS)	1%	NR	NR	NR	NR	NR	NR	Not assessed	314 (60)	7 most prevalent KRAS mutations in codon 12 (n=6) and 13 (n=1) were assessed 206 (40%) had KRAS mutation.
Van Cutsem, 2009	540 [1217]	NR [PET, NR]	PCR clamping and melting curve	NR	NR	NR	NR	NR	NR	NR	Not assessed	348 (64) of whom 172 received cetuximab	Any Mutation+ 192 (36%) of whom 105 were in the cetuximab arm
Folprecht, 2010[c]	99 [111][d]	P 44 (44) & M 55 (56) [PET]	ARMS-PCR (DxS) & DS	NR	29 (29)						Not assessed	70 (71)	
Single arm studies													

[a] The number of patients who were enrolled into a trial and randomized (randomized clinical trials only).
[b] The mutations assessed by the employed Method were extracted from Amado, J Clin Oncol, 2008.
[c] All patients received cetuximab.
[d] Of whom 94 were assessable for response.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)								Comment
					Exon 1						Exon 2	Wild-type (%)	
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Bengala, 2009	39[e]	P [Microdissected PET, NR]	Bi-DS	NR					9 (23)		Not assessed	30 (77)	
Debucquoy, 2009	40	P [PET, NR]	AS-Taqman allelic discrimination assay	NR	NR	NR	NR	NR	NR	NR	Not assessed	27 (69)	Any mutation+ 12 (31%)
Yen, 2009[f]	76 [76]	P 76 (100) [FFT, NR][g]	DS on tumor samples, membrane array on peripheral blood	NR	NR	NR	NR	NR	NR	NR	NR	Tumor: 43 (57) Blood: 46 (61)	Any mutation+ 33 (43%) Tumor: Codon 12, 11 (33%); Codon 13, 9 (27%); Codon 15, 7 (21%); Codon 18, 1 (3%); Codon 20, 2 (6%); Codon 30, 2 (6%); Codon 31, 1 (3%) Blood: mutations in codons 12,13,15,18 no reported individually Membrane array compared to primary tumor sequence data: sensitivity = 84%, specificity=95%.
Tabernero, 2010	48 [62]	NR [PET]	PCR clamping and melting curve technique	NR	0	0	4 (8)	1 (2)	9 (19)	5 (10)	Not assessed	29 (60)	

[e] The patient who did not undergo surgery (and was excluded from all analyses of response) did not have a *KRAS* mutation.
[f] Although not reported in the paper, the authors confirmed that this was a "first-line study".
[g] Patients' RNA was also isolated from peripheral blood samples.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)							Comment
					Exon 1						Exon 2	Wild-type (%)	
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Yen, 2010[h]	95 [95]	P 95 (100) [PET, FFT]	DS[i]	NR	23 (24)						Not assessed	72 (76)	The study also assessed mutations in codons 15, 18, 20, 30 and 31. Overall, 41 mutations (43%) were identified. Mutations in codons 12, 13, 15 and 18 (33 mutations, 35%) were considered as "activating".

ARMS, amplification refractory mutation system; AS-PCR, allele-specific PCR; b-DS, bi-directional direct sequencing; DS, direct sequencing; LN, lymph node metastasis; LCR, Ligase chain reaction; M, metastatic tumor; NR, not reported; P, primary tumor; PCR, polymerase chain reaction; PET, paraffin-embedded tissue; PS, pyrosequencing.

[h] Although not reported in the paper, the authors confirmed that this was a "first-line study".
[i] Data extracted from Yen, Clin Cancer Res, 2009.

Table 11. *KRAS* and anti-EGFR antibodies for colorectal cancer: detection and reporting of mutations (salvage therapy).

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)							Wild-type (%)	Comment
					Exon 1						Exon 2		
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Randomized controlled trials													
Amado, 2008[b]	427 [463]	"Mostly" from P 410 (96) [PET, NR]	AS-PCR	NR	14 (3)	14 (3)	70 (16)	15 (4)	40 (22)	29 (16)	Not assessed	243 (60)	Any mutation+ 184 (43%)
Karapetis, 2008	394 [572]	NR	DS 2	NR	17 (10)	9 (5)	61 (36)	11 (6)	48 (28)	20 (12)	Not Assessed	164 (42)	"Exon 2" sequenced. Any mutation+ 230 (58%) Other mutations noted: G12R: 2 (1); G13A: 1 (1); G13C: 1 (1); G13V: 1 (1)
Single arm studies													
Moroni, 2005	31	NR [PET, NR]	DS	NR	1 (3)	0	3 (10)	0	2 (6)	4 (13)	Not assessed	21 (68)	Any mutation+ 10 (32%)
Lievre, 2006	30	P 30 (100) [FFT, NR]	DS	NR	1 (3)	2 (7)	5 (17)	1 (3)	1 (3)	3 (10)	Not assessed	17 (57)	Exon 1 was assessed
Benvenuti, 2007	48	NR [PET]	DS	NR			10 (21)			6 (14)	Not assessed	32 (67)	
Di Fiore, 2007	59	P 53 (90); M 6 (10) [PET, NR]	DS only, DS + SNaPsho	NR	NR	NR	NR	NR	NR	NR	Not assessed	43 (73); 37 (63)[d]	"Exon2" was examined 16 (27%) or 22 (37%)[e] had KRAS mutations.

[a] The number of patients who were enrolled into a trial and randomized (randomized clinical trials only).
[b] Three patients had the G12R *KRAS* mutation. One patient carried two mutations (G12D and G12R).

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%)*	Mutation Frequency, n (%)							Wild-type (%)	Comment
					Exon 1						Exon 2		
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
			t assay + PCR-LCR[c]										
Frattini, 2007	27	P (100) [PET]	Bi-DS	NR	0	1 (4)	4 (15)	2 (7)	0	3 (11)	Not assessed	17 (63)	
Khambata-Ford, 2007	80	M 80 (100) [FFT, NR]	DS 2	NR	NR	NR	NR	NR	NR	NR	Not assessed	50 (63)	"Exon 2" sequenced, but no specific mention of identified variants Any mutation+ 30 (38%)
Cappuzzo, 2008	80	P 65 (76); M 20 (24) [NR]	Surveyor ® DNA endonuclease	NR	NR	NR	NR	NR	NR	8 (10)	NR	38 (48)	Any mutations 42 (53%) "Exons 1 and 2" were assessed
De Rook, 2008[f]	113	NR (100) [PET, NR]	Allelic discrimination assay and bi-DS	NR	4 (4)	3 (3)	17 (15)	3 (3)	10 (9)	9 (8)	Not assessed	67 (59)	Any mutation+ 46 (41%)
Di Nicolantonio, 2008	113	NR [PET, NR]	DS	NR	NR	NR	NR	NR	NR	NR	Not assessed	79 (70)	Any mutation+ 34 (30%)

[d] The former is for DS only, and the latter for DS partially (95% of patients) complimented by SNaPshot assay and PCR-LCR.
[e] The former is for DS only, and the latter for DS partially (95% of patients) complimented by SNaPshot assay and PCR-LCR.
[c] SNaPshot assay and PCR-LCR as complement to DS was performed in 41 (95%) out of 43 patients with no mutation detected by DS.
[f] One patient carried two mutations (G12S and G12V).

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)							Wild-type (%)	Comment
					Exon 1						Exon 2		
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Freeman, 2008	62	NR	DS	NR	NR	NR	NR	NR	NR	NR	0	38 (61)	Exon 2 was also sequenced Any mutation+:24 (39%) in either codon 12 or 13
Goncalves, 2008	32	P and/or M [12, PET; 20 FT, NT]	DS	NR	0	1 (3)	6	0	3 (9)	4 (13)	Not assessed	18 (56)	Any mutation+ 14 (44%)
Lievre, 2008	114[g]	P 83 (73); M 31 (27) [FFT or PET, NR]	TaqMan ® allelic discrimination assay[h]	20	NR	NR	NR	NR	NR	Not assessed	Not assessed	65 (73)	24 patients (27%) had mutation in KRAS gene.
Lurje, 2008	130	NR	DS 12, 13	NR	NR	NR	NR	NR	NR	NR	Not assessed	88 (68)	Any mutation+ 42 (32%)
Personeni, 2008	87 [96]	NR [PET, NR]	Allelic discrimination assay	NR				29 (33)			Not assessed	58 (67)	
Bibeau, 2009	64	P (77), M (22), Local (1) [PET, NR]	Multiplex primer extension method sequencing	NR	NR	NR	NR	NR	NR	NR	Not assessed	37 (58)	Any mutation+ 27 (42%)

[g] "Pooled" cohort.
[h] All identified mutations were verified with DS.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)							Wild-type (%)	Comment
					Exon 1						Exon 2		
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Garm Spindler, 2009	64	P & M 31 (48) P 33 (52) [PET, NR]	DxS qPCR kit (7 mutations in codons 12 and 13)	NR	NR	NR	NR	NR	NR	NR	Not assessed	42 (66)	Samples from 59 patients were also assessed with DS. Any mutation+ 22 (34%)
Jacobs, 2009	212 [220]	NR [NR]	Allelic discrimination assay and bi-DS	NR				91 (43)			Not assessed	169 (80)	
Laurent-Puig, 2009	169 [173]	NR [FFT or PET, NR][i]	TaqMan® allelic discrimination assay[j]	20				53 (31)			Not assessed	116 (69)	
Loupakis, 2009	93	P&M 43 (46); P 45 (48); M 5 (5) [PET, NR]	DS 12/13	NR	NR	NR	NR	NR	NR	NR	Not assessed	53 (60); 27 (56)[k]	2 out of 43 (5%) patients with samples form both primary and metastatic lesions available and evaluable had discrepant test results between primary and metastatic tumor. 35 (40%) and 21 (48%) had KRAS mutation in primary (total n=88) and metastatic (total n=48) tumors, respectively. PTEN (by immunohistochemistry)

[i] Mutation detection was described in Lievre, J Clin Oncol, 2008.
[j] All identified mutations were verified with DS.
[k] The former is primary tumor (total n=88) and the later.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)							Wild-type (%)	Comment
					Exon 1						Exon 2		
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
													was also assessed and outcome results were also presented with any KRAS mutations and PTEN positive vs. negative.
Loupakis, 2009	138	P 138 (100) [PET, NR]	PS	NR	NR	NR	NR	NR	NR	NR	7 (5)	79 (57)	51 (37%) had KRAS mutations in codon 12 or 13. 1 (0.7%) had KRAS mutation in codon 146. Only codons 12, 13, 61, and 146 were examined BRAF mutations were also assessed and outcome results were also presented by any KRAS 61 or 146 or BRAF mutations positive vs. negative.
Molinari, 2009	12	P&M&LN 5 (42); P&M 7 (58) [PET, NR]	DS	NR	1 (8)	0	1 (8)	3 (25)	0	0	Not assessed	7 (58)	All 5 patients with mutation in the KRAS gene had concordant results between primary and metastatic tumor, and lymph node metastasis.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)							Wild-type (%)	Comment
					Exon 1						Exon 2		
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Muro, 2009	24	NR	DxS	NR	NR	NR	NR	NR	NR	NR	Not assessed	14 (58)	Any mutation+: 10 (42)
Oden-Gangloff, 2009[l]	64	NR [PET 55 (86); FFT 9 (14), NR]	Multiplex primer extension method sequencing	NR	1 (2)	2 (3)	3 (5)	1 (2)	5 (8)	5 (8)	Not assessed	46 (72)	Any mutation+ 18 (28%)
Paule, 2009	19 [23]	NR [PET, NR]	TaqMan® allelic discrimination assay	NR				3 (16)			Not assessed	16 (84)	
Perrone, 2009	29	P&M 12 (38); P 12 (38); M 8 (25) [PET, NR]	DS	NR	1 (3)	0	3 (10)	0	1 (3)	2 (7)	Not assessed	22 (76)	2 out of 7 patients with mutation in the KRAS gene had discrepant test results between primary and metastatic tumor. Any mutation+ 7 (24%)
Prenen, 2009	199	P 199 (100) [PET, NR]	AS-PCR	NR	NR	NR	NR	NR	NR	NR	Not assessed	122 (61)	Any mutation+ 77 (39%)

[l] One patient carried the G13C (c37G>T) mutation.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)								Comment
					Exon 1						Exon 2	Wild-type (%)	
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Sartore-Bianchi, 2009[m]	109	P or liver M [PET, NR]	DS	NR	5 (5)	1 (1)	8 (7)	5 (5)	5 (5)	8 (7)	Not assessed	77 (71)	Any mutation+ 32 (29%)
Sartore-Bianchi, 2009	131 [132]	P 130 (98); M 2 (2) [PET, NR]	DS	NR				35 (27)			Not assessed	96 (73)	
Sohn, 2009	66 [68]	P 62 (94); M 4 (6) [PET, NR]	DS	NR			21 (32)			6 (9)	Not assessed	39 (59)	
Souglakos, 2009	92 [92]	P 92 (100) [PET, NR]	Sequenom mass-spectrometric genotyping (USA) and Sanger DS (Greece); results were cross-confirmed	NR				32 (35)			Not assessed	60 (65)	

[m] One patient carried two mutations (G12R and G13D). In addition one patient carried the G13V mutation.

Study (first author, year)	Number tested [Number enrolled][a], *n*	Tumor site, *n (%)* [Source, time between collection and DNA extraction, *h*]	Mutation detection method	Detection ability (*%*)	Mutation Frequency, n (%)								Comment
					Exon 1						Exon 2	Wild-type (%)	
					Codon 12					Codon 13	Codon 61		
					C34 G>A (G12S)	C34 G>T (G12C)	C35 G>A (G12D)	C35 G>C (G12A)	C35 G>T (G12V)	C38G>A (G13D)			
Chung, 2010[n]	64 [88]	M 66 (100) [FFT, NR]	DS	NR			26 (41)				Not assessed	38 (59)	
Graziano, 2010	134 [138]	P 134 (100) [PET, NR]	Pyrosequencing	NR			58/121 (48)[o]					63 (52)	No data is presented regarding the 13 BRAF mutation carriers.
Montagut, 2010	48 [48]	NR [PET, NR]	DS	NR	0	0	3 (6)	1 (2)	3 (6)	5 (10)	Not assessed	36 (75)	
Perkins, 2010	42 [42]	P & M [FFT or PET, NR]	TaqMan® allelic discrimination assay[p]	20			19 (45)				Not assessed	23 (55)	
Scartozzi, 2010	112 [112]	P 112 (100) & M NR (NR) [PET, NR]	DS	NR	6 (5)	0	13 (12)	5 (4)	5 (4)	10 (9)	Not assessed	69 (62)	Also detected 4 patients (4%) with codon 12 G12R mutations

[n] Data extracted from Khambata-Ford, J Clin Oncol, 2007.
[o] Four patients had non-assessable samples. In addition, 13 patients carrying BRAF mutations were excluded from all outcomes reported in the study pertain to 121 patients.
[p] All identified mutations were verified with DS.

ARMS, amplification refractory mutation system; AS-PCR, allele-specific PCR; b-DS, bi-directional direct sequencing; DS, direct sequencing; FFT= fresh frozen tissue; LN, lymph node metastasis; LCR, Ligase chain reaction; M, metastatic tumor; NR, not reported; P, primary tumor; PCR, polymerase chain reaction; PET, paraffin-embedded tissue; PS, pyrosequencing.

Key Question 1. Does *KRAS* testing predict response to cetuximab or panitumumab therapy?

All but two studies evaluated the association of *KRAS* testing results with clinical outcomes in patients with colorectal cancer in the metastatic setting and their results are summarized bellow. Two studies evaluated the association of *KRAS* with clinical outcomes in the neoadjuvant setting, exclusively for patients with advanced rectal cancer, and are discussed separately at the end of this topic.(65;70)

Mortality

Strength of the association between KRAS testing results and mortality

Tables 12 and **13** summarize KRAS associations with mortality in the first- and second-line settings, respectively. Only 30 studies reported analyses on overall survival and/or mortality outcomes. In the anti-EGFR antibody treated arms time-to-event analyses were presented in 28 studies. In 15 of those, a statistically significant detrimental effect of *KRAS* mutations was observed. In all 22 studies that reported relevant information, median overall survival in *KRAS* positive patients was shorter compared to the median overall survival of wild-type patients. Mortality analyses were presented in 5 studies, all of which demonstrated higher mortality risk in *KRAS* mutated patients although data were sparse and confidence intervals were wide.

As previously mentioned, 7 of the studies were based on RCTs. Of those, 2 studies assessed the treatment-by-*KRAS* mutation interaction. The test was statistically significant in the study by Karapetis 2008.(59) ($p=0.01$) indicating that difference between the anti-EGFR antibody treatment arm and the comparator arm was present in the wild-type group of patients but not the group of patients harboring *KRAS* mutations. The interaction test was non-significant in the study by van Cutsem 2009 ($p=0.44$) but the direction of effects was consistent.(61)

Table 12. *KRAS* and cetuximab for colorectal cancer: mortality (first line therapy)

Author, year	Study arm (patient number)	Event/patient, n (%)		OR (95% CI)	P value	Median Survival Time, mo		HR (95% CI)	P value
		Mutation+	Mutation-			Mutation+	Mutation-		
Randomized controlled trials									
Bokemeyer, 2009[a]	FOLFOX-4 + cetuximab	NR	NR	NR	NR	NR	NR	NR	NR
	FOLFOX-4	NR	NR	NR	NR	NR	NR	NR	NR
Hecht, 2009 (Multinational)	ChBP cohort								
	Oxaliplatin-based	NR	NR	NR	NR	19.3	20.7	NR	NR
	Irinotecan-based	NR	NR	NR	NR	17.8	"Not estimable"[b]	NR	NR
	ChB cohort								
	Oxaliplatin-based	NR	NR	NR	NR	19.3	24.5	NR	NR
	Irinotecan-based	NR	NR	NR	NR	20.5	20	NR	NR
Tol 2009	CBC (1')	NR	NR	NR	NR	17.2	21.8	NR	0.06
	CB (1')	NR	NR	NR	NR	24.9	22.4	NR	0.82
Van Cutsem, 2009	FOLFIRI+cetuximab	83/105 (79)	104/172 (60)	2.47 (1.41-4.30)	0.002	17.5	24.9	NR	NR
	FOLFIRI	65/87 (75)	115/176 (65)	1.57 (0.88-2.78)	0.16	17.4	21	NR	NR
Folprecht, 2010	FOLFOX + cetuximab	NR	NR	NR	NR	NR	NR	NR	NR
	FOLFIRI + cetuximab	NR	NR	NR	NR	NR	NR	NR	NR
Single arm studies									
Yen, 2009	Cetuximab + chemotherapy	NR	NR	NR	NR	NR	NR	6.67 (2.70-12.50)	<0.0001
Tabernero, 2010	Cetuximab monotherapy phase	NR	NR	NR	NR	NR	NR	NR	NR

[a] Nine patients were not assessable for response in this study.
[b] Per study report.

	Cetuximab + FOLFIRI phase	NR	NR	NR	NR	NR	NR	NR	NR
	Overall analysis (full follow-up period)	NR	NR	NR	NR	NR	NR	NR	NR
Yen, 2010[c]	Cetuximab+ chemotherapy	NR	NR	NR	NR	NR	NR	6.76 (3.16-14.48)	<0.0001

CI, confidence interval; FOLFIRI, irinotecan, fluorouracil, and folinic acid; FOLFOX, oxaliplatin, fluorouracil, and folinic acid; HR, hazard ratio; NR, not reported; OR, odds ratio.

[c] If the analysis is limited to "activating KRAS mutations" (per study definition), the OS HR=9.91 (4.53-21.65).

Table 13. *KRAS* and anti-EGFR antibodies for colorectal cancer: mortality (Salvage therapy)

Author, year	Study arm (patient number)	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
		Mutation+	Mutation-			Mutation+	Mutation-		
Randomized controlled trials									
Amado, 2008	Panitumumab + BSC	79/84 (94)	107/124 (86)	2.51 (0.89-7.09)	0.12	4.9	8.1	NR	
	BSC	95/100 (95)	110/119 (92)	1.55 (0.30-4.80)	0.62	4.4	7.6	NR	
Karapetis, 2008	Cetuximab	NR	NR	NR	NR	4.5	9.5	NR	NR
	BSC	NR	NR	NR	NR	4.6	4.8	NR	NR
Single arm studies									
Moroni. 2005	Cetuximab +/- chemotherapy; panitumumab monotherapy	NR	NR	NR	NR	NR	NR	NR	NR
Lievre, 2006	Cetuximab-based (Salvage only)	10/13 (77)	7/14 (50)	3.3 (0.50-26.1)	0.15	6.9	16.3	NR	0.02
Benvenuti, 2007	Panitumumab or Cetuximab +/- irinotecan	NR	NR	NR	NR	NR	NR	NR	NR
Di Fiore, 2007	Cetuximab + irinotecan or oxaliplatin	NR	NR	NR	NR	NR	NR	NR	NR
Frattini, 2007	Cetuximab + chemotherapy	NR	NR	NR	NR	NR	NR	NR	NR
Khambata-Ford, 2007	Cetuximab	NR	NR	NR	NR	NR	NR	NR	NR
Cappuzzo, 2008	Cetuximab-based	NR	NR	NR	NR	9.5	10.8	NR	0.3[a]
De Rook, 2008	Cetuximab +/- irinotecan	NR	NR	NR	NR	6.8	10.8	1.61 (1.09-2.44) [adjusted][b]	0.020
Di Nicolantonio, 2008	Cetuximab +/- chemotherapy; panitumumab monotherapy	NR	NR	NR	NR	NR	NR	NR	0.0869
Freeman, 2008	Panitumumab	22/24 (92)	29/38 (76)	3.41 (0.67-17.4)	0.23	5.6	10.7	2.00 (1.11-3.33)	<0.015

[a] Subgroup analysis by EFGR by FISH reported.
[b] Age, gender, skin toxicity, previous chemotherapies, and treatment regimen were used as covariates.

Author, year	Study arm (patient number)	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
		Mutation+	Mutation-			Mutation+	Mutation-		
Goncalves, 2008	Cetuximab + chemotherapy; cetuximab	NR	NR	NR	NR	13.8	20.8	NR	0.472
Lievre, 2008	Cetuximab-based (primary data n=88)	NR	NR	NR	NR	10.1	14.3	NR	0.026
	Cetuximab-based (Pooled analysis n=113)	NR	NR	NR	NR	10.1	14.3	NR 2.4 (1.4-4.1), [skin toxicity]	0.0017 0.001
Lurje, 2008	Cetuximab	NR	NR	NR	NR	4.9	6.6	1.59 (1.05-2.40)	0.02
Personeni, 2008	Cetuximba +/- irinotecan	NR	NR	NR	NR	NR	NR	NR	NR
Bibeau, 2009	Cetuximab + irinotecan-based chemotherapy	NR	NR	NR	NR	8.7	10.8	1.6 (0.8-2.9) [adjusted][c]	0.147 0.151 [adjusted] [d]
Garm Spindler, 2009	Cetuximab + irinotecan	NR	NR	NR	NR	8.7	11.1	NR	0.46
Jacobs, 2009	Cetuximab +/- chemotherapy[e]	NR	NR	NR	NR	7	11	NR	NR
Laurent-Puig, 2009	Cetuximab +/- irinotecan-based chemotherapy	NR	NR	NR	NR	8.4	14.4	NR	<0.001
Loupakis, 2009	Cetuximab +/- irinotecan	NR	NR	NR	NR	6.1	13.5	2.2 (1.5-4.5)	0.0004
Loupakis, 2009[f]	Cetuximab + irinotecan	NR	NR	NR	NR	9.7	14.7	1.4 (0.57-4.2)	0.39
Molinari, 2009	Cetuximab +/- irinotecan	NR	NR	NR	NR	NR	NR	NR	NR
Muro, 2009	Panitumumab	NR	NR	NR	NR	NR	NR	NR	NR
Oden-Gangloff, 2009	Cetuximab + irinotecan-based chemotherapy	NR	NR	NR	NR	NR	NR	NR	NR
Perrone, 2009	Cetuximab + irinotecan	NR	NR	NR	NR	NR	NR	NR	NR
Prenen, 2009	Cetuximab +/- irinotecan	NR	NR	NR	NR	6.5	11.3	2.00 (1.45-2.70)	<0.0001

[c] Covariates used in the multivariate analyses not reported.
[d] Covariates used in the multivariate analyses not reported.
[e] Patients were recruited from clinical trials investigating diverse treatment strategies.
[f] Only codon 61 and 146 were assessed for mutations if patients had no mutations in codon 12 or 13 (n=76).

Author, year	Study arm (patient number)	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
		Mutation+	Mutation-			Mutation+	Mutation-		
Paule, 2009[g]	Cetuximab + irinotecan	3/3 (100)	13/16 (81)	1.81 (0.08 – 43.99)[h]	0.71	NR	NR	NR	NR
Sartore-Bianchi, 2009	Cetuximab +/- chemotherapy; panitumumab monotherapy (109)	NR	NR	NR	NR	NR	NR	NR	0.1127
Sartore-Bianchi, 2009	Cetuximab +/- irinotecan-based chemotherapy or panitumumab monotherapy	NR	NR	NR	NR	NR	NR	1.72 (1.017-2.903)	0.043
Sohn, 2009	Cetuximab + irinotecan-based chemotherapy	NR	NR	NR	NR	7	18	2.42 (1.2-4.7) [adjusted for skin toxicity and number of prior chemotherapy regimens]	0.001 [log-rank] 0.009 [adjusted for skin toxicity and number of prior chemotherapy regimens]
Souglakos, 2009	Cetuximab + chemotherapy	NR	NR	NR	NR	NR	NR	NR	NS
Chung, 2010	Cetuximab	NR	NR	NR	NR	NR	NR	NR	NR
Graziano, 2010	Cetuximab + irinotecan	NR	NR	NR	NR	NR	NR	2.3 (1.46-3.7) [adjusted for sex, age, ECOG performance status, number of metastatic sites, CEA levels]	0.004 [adjusted for sex, age, ECOG performance status, number of metastatic sites, CEA levels]

[g] Mortality data at 4 years.
[h] Continuity correction k=0.5 was used to calculate the odds ratio.

Author, year	Study arm (patient number)	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
		Mutation+	Mutation-			Mutation+	Mutation-		
Montagut, 2010	Cetuximab + chemotherapy	NR	NR	NR	NR	NR	NR	NR	0.1
Perkins, 2010	Cetuximab +/- irinotecan-based chemotherapy or panitumumab monotherapy	NR	NR	NR	NR	6	14	NR	0.02
Scartozzi, 2010	Cetuximab +/- irinotecan-based chemotherapy	NR	NR	NR	NR	9.5	16	NR	0.0003

BSC, best supportive care; CEA, carcinoembryonic antigen; CI, confidence interval; ECOG, Eastern Cooperative Oncology Group; FISH, fluorescent in situ hybridization; FOLFIRI, irinotecan, fluorouracil, and folinic acid; FOLFOX, oxaliplatin, fluorouracil, and folinic acid; HR, hazard ratio; NR, not reported; NS, non-significant; OR, odds ratio.

Progression

Strength of the association between KRAS testing results and recurrence

Tables 14 and **15** summarize KRAS associations with disease progression in the first- and second-line settings, respectively. Thirty eight studies reported progression-free-survival or time-to-progression analyses. The outcomes were referred to as "PFS" in 33 of the studies and as "TTP" in 5 of the studies. In all studies reporting disease progression information, median PFS or TTP was shorter among patients with *KRAS* positive tumors, compared to wild-type patients and the difference was statistically significant in 25 studies.

As previously mentioned, 7 of the studies were based on RCTs, six of which reported on PFS. In the antibody-treated arms of these studies, PFS was shorter among *KRAS* mutated patients compared to wild-type patients in all cases (and the difference was statistically significant in two of the studies that reported a relevant p-value). The studies by Amado 2008, Karapetis 2008 and van Cutsem 2009 assessed the treatment-by-*KRAS* mutation interaction test p-value was <0.0001, 0.0001 and 0.07 respectively. Bokemeyer 2009 did not report an interaction test. However, we observed that in cetuximab-treated patients presence of *KRAS* mutations had a hazard ratio of 2.2, while the corresponding HR in the comparator arm was 0.7. The confidence intervals of these estimates were not overlapping, suggesting a significant interaction between *KRAS* and treatment. In all three studies, the anti-EGFR antibody treatment appeared to be beneficial for the group of patients with wild type tumors but not the patients whose tumors harbored *KRAS* mutations.

Table 14. *KRAS* and cetuximab for colorectal cancer: progression (first line therapy)

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
Randomized controlled trials												
Bokemeyer, 2009[a]	FOLFOX-4 + cetuximab	Modified WHO	PFS NR	q 8 wk	39/52 (75)	30/61 (49)	3.1 (1.39-6.92)	0.009	5.5	7.7	2.23 (1.38-3.62)	0.0009
	FOLFOX-4	Modified WHO	PFS NR	q 8 wk	26/47 (55)	48/73 (66)	0.64 (0.30-1.37)	0.34	8.6	7.2	0.71 (0.44-1.15)	0.1655
Hecht, 2009	ChBP cohort	RECIST	PFS Radiologic progression or death	q 12 wk								
	Oxaliplatin-based				NR	NR	NR	NR	10	10	NR	NR
	Irinotecan-based				NR	NR	NR	NR	8	13	NR	NR
	ChB cohort	RECIST	PFS Radiologic progression or death									
	Oxaliplatin-based				NR	NR	NR	NR	11	12	NR	NR
	Irinotecan-based				NR	NR	NR	NR	12	13	NR	NR
Tol, 2009	CBC (1')	RECIST	PFS Progression or death	q 9 wk	NR	NR	NR	NR	8.1	10.5	NR	0.04
	CB (1')	RECIST	PFS Progression or death	q 9 wk	NR	NR	NR	NR	12.5	10.6	NR	0.80
Van Cutsem, 2009	FOLFIRI+cetuximab	Modified WHO	PFS NR	q 8 wk	58/105 (55)	76/172 (44)	2.79 (1.75-4.47)	<0.001	7.6	9.9	NR	NR
	FOLFIRI	Modified WHO	PFS NR	q 8 wk	43/87 (49)	95/176 (54)	0.83 (0.50-1.39)	0.57	8.1	8.7	NR	NR

[a] Nine patients were not assessable for response in this study.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
Folprecht, 2010 (Germany + Austria)	FOLFOX + cetuximab	RECIST	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
	FOLFIRI + cetuximab	RECIST	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Single arm studies												
Yen, 2009	Cetuximab	RECIST	PFS Progression or death	NR	NR	NR	NR	NR	NR	NR	3.95 (2.09-6.71)	<0.0001
Tabernero, 2010	Cetuximab monotherapy phase	WHO	PFS Radiologically confirmed disease progression	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Cetuximab + FOLFIRI phase	WHO	PFS Radiologically confirmed disease progression	NR	NR	NR	NR	NR	NR	NR	NR	NR
	Overall analysis (full follow-up period)	WHO	PFS Radiologically confirmed disease progression	NR	NR	NR	NR	NR	6	9	2.13 (NR)	0.0475
Yen, 2010[b]	Cetuximab + chemotherapy	RECIST	PFS Tumor progression of death from any cause	NR	NR	NR	NR	NR	NR	NR	4.15 (2.66-6.48)	<0.0001

CI, confidence interval; FOLFIRI, irinotecan, fluorouracil, and folinic acid; FOLFOX, oxaliplatin, fluorouracil, and folinic acid; F/U, follow-up; HR, hazard ratio; NR, not reported; OR, odds ratio; PFS, progression-free survival; PS, performance status; RECIST, Response Criteria In Solid Tumors; TTP, time-to-progression; WHO, World Health Organization.

[b] If the analysis is limited to "activating KRAS mutations" (per study definition), the PFS HR=5.81 (3.50-9.66).

Table 15. *KRAS* and anti-EGFR antibodies for colorectal cancer: progression (salvage treatment)

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
Randomized controlled trials												
Amado, 2008	Panitumumab	RECIST	PFS Progression or death	q 4-8 wk	76/84 (90)	115/124 (93)	0.74 (0.27-2.01)	0.74	7.4	12.3	NR	NR
	BSC	RECIST	PFS Progression or death	q 4-8 wk	95/100 (95)	114/119 (96)	0.83 (0.23-2.96)	0.97	7.3	7.3	NR	NR
Karapetis, 2008	Cetuximab	RECIST	PFS Progression or death	q 8 wk	NR	NR	NR	NR	1.8	3.7	NR	NR
	BSC	RECIST	PFS Progression or death	q 8 wk	NR	NR	NR	NR	1.8	1.9	NR	NR
Single arm studies												
Moroni. 2005	Cetuximab +/- chemotherapy; panitumumab monotherapy	RECIST	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Lievre, 2006	Cetuximab-based (Any lines of therapy)	RECIST	TTP Progression	NR	13/13 (100)	17/17 (100)	NE (NE)	NR	0	4.8	4.0 (0.99-16.1)	0.06[a]
	Cetuximab-based (Salvage	RECIST	TTP Progression	NR	13/13 (100)	14/14 (100)	NE (NE)	NR	0	4.3	2.9 (0.72-11.7)	0.14[b]

[a] Estimated from reported individual patient data with an assumption that all patients progressed.
[b] Estimated from reported individual patient data with an assumption that all patients progressed.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
	only)											
Benvenuti, 2007	Panitumumab or Cetuximab +/- irinotecan	RECIST	PFS[c] First documented tumor progression or death	NR	NR	NR	NR	NR	NR	NR	NR	0.0443 [d]
Di Fiore, 2007	Cetuximab + irinotecan or oxaliplatin	RECIST	TTP Progression or death	NR	NR	NR	NR	NR	3	5.5	NR	0.015
Frattini, 2007	Cetuximab + chemotherapy	RECIST	NR	q 6 wk	NR	NR	NR	NR	NR	NR	NR	NR
Khambata-Ford, 2007	Cetuximab	WHO	PFS Progression or death	q 9 wk	NR	NR	NR	NR	2.1	2.2	1.4 (0.87-2.6)	0.14
Cappuzzo, 2008	Cetuximab-based	RECIST	TTP Progression	q 2 mo	NR	NR	NR	NR	4.4	5.4	NR	0.2
De Rook, 2008	Cetuximab +/- irinotecan (113)	RECIST	PFS Progression, death from any cause or censoring	q 6 wk[e]	NR	NR	NR	NR	3	6	NR	0.074
Di Nicolantonio, 2008	Cetuximab +/- chemotherapy; panitumumab monotherapy	RECIST	PFS Progression or death	q 6-8 wk	NR	NR	NR	NR	NR	NR	NR	0.0275
Freeman,	Panitumum	RECIST or	PFS	Q8 or 9 wk	24/24 (100)	37/38 (97)	1.96 (0.08-	>0.9	1.9	4.1	2.50 (1.43-5.00)	<0.002

[c] The study referred to this end-point both as "PFS" and "TTP".
[d] Patients with KRAS mutations had reduced PFS compared to wild-type patients.
[e] Until week 24, 30 or 36; q 12 weeks thereafter.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
2008	ab	WHO	Progression of death				50.09)					
Goncalves, 2008	Cetuximab + chemotherapy; cetuximab	WHO	Progression or death	q 2 mo[f]	NR	NR	NR	NR	4.7	3.9	NR	0.968
Lievre, 2008	Cetuximab-based (primary data n=88)	RECIST	PFS Progression or death from any causes	NR	NR	NR	NR	NR	2.3	7.2	NR	0.0001
Lurje, 2008	Cetuximab	WHO	PFS Progression or death	q 6 wk	NR	NR	NR	NR	1.3	1.4	1.49 (1.01-2.20) wt referent	0.02
Personeni, 2008	Cetuxmab +/- irinotecan	RECIST	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Bibeau, 2009	Cetuximab + irinotecan-based chemotherapy	RECIST	PFS Progression or death from any cause	CT q 2-3 m	NR	NR	NR	NR	3	5.3	1.8 (1.1-3.1) [adjusted][g]	0.024 0.021 [adjusted][h]
Garm Spindler, 2009	Cetuximab + irinotecan	RECIST	PFS Progression or death	NR	NR	NR	NR	NR	2.3	8.0	NR	<0.008 8
Jacobs, 2009	Cetuximab +/- chemotherapy[i]	RECIST	PFS Progression of disease or death from any cause	NR	NR	NR	NR	NR	NR	NR	NR	NR
Laurent-Puig, 2009	Cetuximab +/- irinotecan-	RECIST	PFS NR	NR	NR	NR	NR	NR	3	8	NR	<0.001

[f] For the first 6 mo and every 3 mo thereafter until disease progression.
[g] Covariates in the multivariate analyses not reported.
[h] Covariates in the multivariate analyses not reported.
[i] Patients were recruited from clinical trials investigating diverse treatment strategies.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
	based chemotherapy											
Loupakis, 2009	Cetuximab +/- irinotecan	RECIST	PFS Progression or death from any causes	q 8 wk	NR	NR	NR	NR	3.1	4.2	2.2 (1.4-3.7)	0.003
	Cetuximab-based (Pooled analysis n=113)	RECIST	PFS Progression or death from any causes	NR	NR	NR	NR	NR	2.1	7.4	NR 3.3 (2.0-5.4), [skin toxicity]	0.0000 0014 0.0001
Loupakis, 2009[j]	Cetuximab + irinotecan	RECIST	PFS Progression or death from any causes	q 8 wk	NR	NR	NR	NR	3.8	5.1	2.2 (1.1-9.1)	0.028
Molinari, 2009	Cetuximab +/- irinotecan	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Muro, 2009	Panitumumab	RECIST	PFS Progression or death	q 8 wk	NR	NR	NR	NR	7.3	13.2	NR	NR
Oden-Gangloff, 2009	Cetuximab + irinotecan-based chemotherapy	RECIST	TTP Progression or death	"on clinical basis"	NR	NR	NR	NR	3	5	2.08 (1.06-4.00) [adjusted][k]	0.034 0.032 [adjusted][l]
Paule, 2009[m]	Cetuximab + irinotecan	RECIST	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Perrone, 2009	Cetuximab + irinotecan	RECIST	PFS Progression or death from any causes	q 9 wk	10/10 (100)	22/22 (100)	NE (NE)	NR	4.5	8.5	3.0 (1.1-7.9)	0.03[n]

[j] Only codon 61 and 146 were assessed for mutations if patients had no mutations in codon 12 or 13 (n=76).
[k] Gender, age, previous number of treatment, and TP53 status was used as covariates.
[l] Gender, age, previous number of treatment, and TP53 status was used as covariates.
[m] Mortality data at 4 years.
[n] Estimated from reported individual patient data with an assumption that all patients progressed.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
Prenen, 2009	Cetuximab +/- irinotecan	RECIST	PFS NR	NR[o]	NR	NR	NR	NR	3	6	1.79 (1.53-2.09)[p]	Log-rank p-value< 0.0001
Sartore-Bianchi, 2009	Cetuximab +/- chemotherapy; panitumumab monotherapy (109)	RECIST	PFS NR	q 6-8 wk	NR	NR	NR	NR	NR	NR	NR	0.0815
Sartore-Bianchi, 2009	Cetuximab +/- irinotecan-based chemotherapy or panitumumab monotherapy	RECIST	PFS NR	q 6-8 wk	NR	NR	NR	NR	NR	NR	1.65 (1.041 – 2.601)	0.033
Sohn, 2009	Cetuximab + irinotecan-based chemotherapy	RECIST	PFS Disease progression or death from any cause	q 6-8 wk	NR	NR	NR	NR	2	6	1.7 (0.1-3.1) [adjusted for skin toxicity and number of prior chemotherapy regimens]	0.005 [logrank] 0.068 [adjusted for skin toxicity and number of prior chemotherapy regimens]

[o] Patients were participating in clinical trials of cetuximab. Follow-up was "as planned in these trials".
[p] Reported hazard ratio was inverted for consistency.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
Souglakos, 2009	Cetuximab + chemotherapy	RECIST	PFS Documented progression or death	NR	NR	NR	NR	NR	3	5	1.5 (0.9 – 2.3)	0.09
Chung, 2010	Cetuximab	RECIST	"PFS" Measured progression of disease[q]	NR	NR	NR	NR	NR	NR	NR	1.45 (0.90-1.99)	0.18
Graziano, 2010	Cetuximab + irinotecan	RECIST	PFS Progression or death from any cause	NR	NR	NR	NR	NR	NR	NR	2.3 (1.46-3.7) [adjusted for sex, age, ECOG performance status, number of metastatic sites, CEA levels]	0.004 [adjusted for sex, age, ECOG performance status, number of metastatic sites, CEA levels]
Montagut, 2010	Cetuximab + chemotherapy	RECIST	TTP[r] Documented tumor progression or death	NR	NR	NR	NR	NR	8	25	NR	0.01
Perkins, 2010	Cetuximab +/- irinotecan-based chemotherapy or panitumumab monotherap	RECIST	PFS Tumor progression or death from any cause	NR	NR	NR	NR	NR	4	8	NR	<0.001

[q] The definition provided for this outcome is typically described as time-to-progression (TTP).
[r] Although the study reports "TTP" the definition used is that of PFS.

Author, year	Study arm (patient number)	Response criteria	PFS vs. TTP Definition	Frequency of F/U	Event/patient, *n (%)*		OR (95% CI)	*P* value	Median Survival Time, *mo*		HR (95% CI)	*P* value
					Mutation+	Mutation-			Mutation+	Mutation-		
	y											
Scartozzi, 2010	Cetuximab +/- irinotecan-based chemotherapy	RECIST	PFS Clinical progression or death from any cause	q 8 wk	NR	NR	NR	NR	2	4	NR	0.0001

CEA, carcinoembryonic antigen; CI, confidence interval; FOLFIRI, irinotecan, fluorouracil, and folinic acid; FOLFOX, oxaliplatin, fluorouracil, and folinic acid; F/U, follow-up; HR, hazard ratio; NR, not reported; OR, odds ratio; PFS, progression-free survival; PS, performance status; RECIST, Response Criteria In Solid Tumors; TTP, time-to-progression; WHO, World Health Organization.

Response

Strength of the association between KRAS testing results and response

Tables 14 and **17** summarize KRAS associations with treatment failure (by radiologic criteria) in the first- and second-line settings, respectively. Overall, failure rates were higher in patients with *KRAS* mutations rather than wild-type patients. Particularly in studies of patients who had received prior chemotherapy, the response rates in the presence of *KRAS* mutations were typically very low (often 0, see **Table 17**).

All of the RCT-based analyses reported response results in antibody-treated and comparator arms, stratified by *KRAS* mutation status. The 5 RCTs enrolling first-line patients presented lower sensitivity and specificity compared to the 2 RCTs enrolling patients pre-treated with cytotoxic chemotherapy. Four of the 5 RCTs included a non-anti-EGFR antibody arm (in Folprecht 2010 all patients received cetuximab). Among these studies, predictive accuracy was higher in the antibody-treated arms compared to the comparator arms. Only the study by van Cutsem 2009 presented the results of a treatment-by-KRAS mutation interaction test. The test was statistically significant (p=0.03) indicating that the relative response rate of anti- EGFR antibody treated patients compared to those in the comparator arms was different between *KRAS* mutated and wild-type patients.

Table 16. *KRAS* and anti-EGFR antibodies for colorectal cancer: treatment failure by radiologic criteria (first line therapy)

Author, year	Study arm (patient number)	Response criteria [RECIST or WHO]	Definition of response [cutoff point and timing]	Patient, *n*				Sensitivity (95% CI)	Specificity (95% CI)	LR + (95% CI)	LR – (95% CI)
				Mutation +		Mutation –					
				Resp – (TP)	Resp + (FP)	Resp – (FN)	Resp + (TN)				
Randomized controlled trials											
Bokemeyer, 2009[a]	FOLFOX-4 + cetuximab	Modified WHO	CR+PR	34	17	22	37	0.61 (0.47, 0.74)	0.69 (0.54, 0.80)	1.9	0.6
	FOLFOX-4	Modified WHO	CR+PR	22	23	42	27	0.34 (0.23, 0.47)	0.54 (0.39, 0.68)	0.8	1.2
Hecht, 2009	ChBP cohort Oxaliplatin-based	RECIST	CR+PR	72	63	100	101	0.42 (0.34, 0.50)	0.62 (0.54, 0.69)	1.1	0.9
	Irinotecan-based	RECIST	CR+PR	33	14	26	31	0.56 (0.42, 0.69)	0.69 (0.53, 0.82)	1.8	0.6
	ChB cohort Oxaliplatin-based	RECIST	CR+PR	70	55	89	114	0.44 (0.36, 0.52)	0.67 (0.60, 0.74)	1.4	0.8
	Irinotecan-based	RECIST	CR+PR	24	15	30	28	0.44 (0.31, 0.59)	0.65 (0.49, 0.79)	1.3	0.9
Tol, 2009	CBC (1')	RECIST	CR+PR	53	45	61	97	0.46 (0.37, 0.56)	0.68 (0.60, 0.76)	1.47	0.78
	CB (1')	RECIST	CR+PR	44	64	78	78	0.36 (0.28, 0.45)	0.55 (0.46, 0.63)	0.8	1.16
Van Cutsem, 2009	FOLFIRI+cetuximab	Modified WHO	CR+PR	67	38	70	102	0.49 (0.40, 0.58)	0.73 (0.65, 0.80)	1.8	0.7
	FOLFIRI	Modified WHO	CR+PR	52	35	100	76	0.34 (0.27, 0.42)	0.68 (0.59, 0.77)	1.1	1.0
Folprecht, 2010	FOLFOX + cetuximab	RECIST	CR+PR	16	11	20	47	0.44 (0.28, 0.62)	0.81 (0.69, 0.90)	2.3	0.7
	FOLFIRI + cetuximab	RECIST	CR+PR								
Single arm studies											
Yen, 2009	Cetuximab + chemotherapy	RECIST	CR+PR (numbers represent mutation status as defined by primary tumor sample)	24	4	7	41	0.77 (0.59, 0.90)	0.91 (0.79, 0.98)	8.71	0.25
Tabernero, 2010	Cetuximab monotherapy phase	WHO	CR+PR	9	0	21	8	0.30 (0.15, 0.49)	1.00 (0.63, 1.00)	>100	0.7

[a] Nine patients were not assessable for response in this study.

	Cetuximab + FOLFIRI phase	WHO	CR+PR	13	6	13	16	0.50 (0.30, 0.70)	0.73 (0.50, 0.89)	1.83	0.69
	Overall analysis (full follow-up period)	WHO	CR+PR	13	6	13	16	0.50 (0.30, 0.70)	0.73 (0.50, 0.89)	1.83	0.69
Yen, 2010[b]	Cetuximab+ chemotherapy	RECIST	CR+PR	32	9	8	46	0.80 (0.64, 0.91)	0.84 (0.71, 0.92)	4.89	0.24

CB, capecitabine, oxaplatin, bevacizumab; CBC, capecitabine, oxaplatin, bevacizumab, cetuximab; ChB, chemotherapy, bevacizumab; ChBP, chemotherapy, bevacizumab, panitumumab;

[b] If the analysis is limited to "activating KRAS mutations" (per study definition), then TP = 29, FP = 4, FN = 8, TN = 46.

Table 17. *KRAS* and anti-EGFR antibodies for colorectal cancer: treatment failure by radiologic criteria (salvage therapy)

Author, year	Study arm (patient number)	Response criteria [RECIST or WHO]	Definition of response [cutoff point and timing]	Patient, *n*				Sensitivity (95% CI)	Specificity (95% CI)	LR + (95% CI)	LR – (95% CI)
				Mutation +		Mutation –					
				Resp – (TP)	Resp + (FP)	Resp – (FN)	Resp + (TN)				
Randomized controlled trials											
Amado, 2008	Panitumumab	RECIST	CR+PR	69	0	21	87	0.77 (0.67, 0.85)	1.00 (0.96, 1.00)	>100	0.23
	BSC	RECIST	CR+PR	68	0	103	0	0.40 (0.32, 0.48)	NA	NA	NA
Karapetis, 2008	Cetuximab	RECIST	"Objective response"	80	1	102	15	0.44 (0.37, 0.51)	0.94 (0.70, 1.00)	7.03	0.6
	BSC	RECIST	"Objective response"	83	0	113	0	0.42 (0.35, 0.50)	NA	NA	NA
Single arm studies											
Moroni, 2005	Cetuximab +/- chemotherapy; panitumumab monotherapy	RECIST	CR+PR	8	2	13	8	0.38 (0.18, 0.62)	0.80 (0.44, 0.97)	1.9	0.77
Lievre, 2006	Cetuximab-based (Any lines of therapy)	RECIST	CR+PR	13	0	6	11	0.68 (0.43, 0.87)	1.00 (0.72, 1.00)	>100	0.32
	Cetuximab-based (Salvage only)	RECIST	CR+PR	13	0	6	8	0.68 (0.43, 0.87)	1.00 (0.63, 1.00)	>100	0.32
Benvenuti, 2007	Cetuximab +/- irinotecan Panitumumab	RECIST	CR+PR	15	1	22	10	0.41 (0.25, 0.58)	0.91 (0.59, 1.00)	4.46	0.65
Di Fiore, 2007	Cetuximab + irinotecan or oxaliplatin	RECIST	CR+PR (DS)	16	0	31	12	0.34 (0.21, 0.49)	1.00 (0.74, 1.00)	>100	0.66
Frattini, 2007	Cetuximab + chemotherapy	RECIST	CR+PR	9	1	8	9	0.53 (0.28, 0.77)	0.90 (0.55, 1.00)	5.29	0.52
Khambata-Ford, 2007	Cetuximab	WHO	CR+PR+SD	27	3	26	24	0.51 (0.37, 0.65)	0.89 (0.71, 0.98)	4.58	0.55
Cappuzzo, 2008	Cetuximab-based	RECIST	NR ("based-on RECIST")	38	4	28	10	0.58 (0.45, 0.70)	0.71 (0.42, 0.92)	2.02	0.59
De Rook, 2008	Cetuximab +/- irinotecan	RECIST	CR+PR	32	0	26	22	0.55 (0.42, 0.68)	1.00 (0.85, 1.00)	>100	0.45
Di Nicolantonio, 2008	Cetuximab +/- chemotherapy; panitumumab monotherapy	RECIST	CR+PR	32	2	57	22	0.36 (0.26, 0.47)	0.92 (0.73, 0.99)	4.31	0.7

Author, year	Study arm (patient number)	Response criteria [RECIST or WHO]	Definition of response [cutoff point and timing]	Patient, *n*				Sensitivity (95% CI)	Specificity (95% CI)	LR + (95% CI)	LR – (95% CI)
				Mutation +		Mutation –					
				Resp – (TP)	Resp + (FP)	Resp – (FN)	Resp + (TN)				
Freeman, 2008	Panitumumab	RECIST or WHO (pooled analysis)	CR + PR	24	0	34	4	0.41 (0.29, 0.55)	1.00 (0.40, 1.00)	>100	0.59
			CR+PR (DS+ SnaPshot assay + PCR-LCR)	22	0	25	12	0.47 (0.32, 0.62)	1.00 (0.74, 1.00)	>100	0.53
			CR+PR+SD	13	3	15	28	0.46 (0.28, 0.66)	0.90 (0.74, 0.98)	4.8	0.59
			CR+PR+SD (DS+ SnaPshot assay + PCR-LCR)	17	5	11	26	0.61 (0.41, 0.78)	0.84 (0.66, 0.95)	3.76	0.47
Goncalves, 2008	Cetuximab + chemotherapy; cetuximab	WHO	CR+PR	12	2	7	11	0.63 (0.38, 0.84)	0.85 (0.55, 0.98)	4.11	0.44
Lievre, 2008	Cetuximab-based (primary data n=89)	RECIST	CR+PR	24	0	39	26	0.38 (0.26, 0.51)	1.00 (0.87, 1.00)	>100	0.62
	Cetuximab-based (Pooled analysis n=114)	RECIST	CR+PR	36	0	44	34	0.45 (0.34, 0.57)	1.00 (0.90, 1.00)	>100	0.55
Lurje, 2008	Cetuximab	WHO	CR+PR	37	0	65	12	0.36 (0.27, 0.46)	1.00 (0.74, 1.00)	>100	0.64
Personeni, 2008	Cetuximab + /- irinotecan	RECIST	CR + PR	29	0	33	28	0.47 (0.34, 0.60)	1.00 (0.86, 1.00)	>100	0.53
Bibeau, 2009	Cetuximab + irinotecan-based chemotherapy	RECIST	CR+PR	26	1	27	10	0.49 (0.35, 0.63)	0.91 (0.59, 1.00)	5.4	0.56
Garm Spindler, 2009	Cetuximab + irinotecan	RECIST	CR+PR	22	0	25	17	0.47 (0.32, 0.62)	1.00 (0.80, 1.00)	>100	0.53
Jacobs, 2009	Cetuximab +/- chemotherapy[a]	RECIST	CR+PR	86	1	67	52	0.32 (0.14, 0.55)	1.00 (0.69, 1.00)	>100	0.68
Laurent-Puig, 2009	Cetuximab +/- irinotecan-based chemotherapy	RECIST	CR+PR	52	1	64	52	0.63 (0.38, 0.84)	0.85 (0.55, 0.98)	4.11	0.44
Loupakis, 2009	Cetuximab +/- irinotecan	RECIST	CR+PR+SD6 (Primary tumors	33	2	40	13	0.45 (0.34, 0.57)	0.87 (0.60, 0.98)	3.39	0.63

[a] Patients were recruited from clinical trials investigating diverse treatment strategies.

Author, year	Study arm (patient number)	Response criteria [RECIST or WHO]	Definition of response [cutoff point and timing]	Patient, *n*				Sensitivity (95% CI)	Specificity (95% CI)	LR + (95% CI)	LR – (95% CI)
				Mutation +		Mutation –					
				Resp – (TP)	Resp + (FP)	Resp – (FN)	Resp + (TN)				
			only [n=88])								
			CR+PR+SD6 (Metastatic tumors only [n=48])	19	2	16	11	0.54 (0.37, 0.71)	0.85 (0.55, 0.98)	3.53	0.54
Loupakis, 2009	Cetuximab + irinotecan	RECIST	CR+PR (codon 12, 13, 61, 146 in all patients [n=138])	56	3	57	24	0.50 (0.40, 0.59)	0.89 (0.71, 0.98)	4.46	0.57
			CR+PR (codon 12, 13 only in all patients [n=138])	48	3	63	24	0.43 (0.34, 0.53)	0.89 (0.71, 0.98)	3.89	0.64
			CR+PR (codon 61, 146 only in patients free from mutations in 12 or 13 [n=76])	8	0	46	22	0.15 (0.07, 0.27)	1.00 (0.85, 1.00)	>100	0.85
Molinari, 2009	Cetuximab +/- irinotecan	RECIST	CR+PR	5	0	5	2	0.50 (0.19, 0.81)	1.00 (0.16, 1.00)	>100	0.5
Muro, 2009	Panitumumab	RECIST	CR+PR	10	0	10	4	0.50 (0.27, 0.73)	1.00 (0.40, 1.00)	>100	0.5
Oden-Gangloff, 2009	Cetuximab + irinotecan-based chemotherapy	RECIST	CR+PR	18	0	30	16	0.38 (0.24, 0.53)	1.00 (0.79, 1.00)	>100	0.62
Paule, 2009[b]	Cetuximab + irinotecan	RECIST	CR+PR	2	0	10	7	0.38 (0.18, 0.62)	0.80 (0.44, 0.97)	1.9	0.77
Perrone, 2009	Cetuximab + irinotecan	RECIST	CR+PR	7	0	15	10	0.32 (0.14, 0.55)	1.00 (0.69, 1.00)	>100	0.68
Prenen, 2009	Cetuximab +/- irinotecan	RECIST	CR+PR	76	1	85	37	0.47 (0.39, 0.55)	0.97 (0.86, 1.00)	17.94	0.54
Sartore-Bianchi, 2009	Cetuximab +/- irinotecan-based chemotherapy or panitumumab monotherapy	RECIST	CR+PR	33	2	72	24	0.68 (0.43, 0.87)	1.00 (0.72, 1.00)	>100	0.32

[b] Mortality data at 4 years.

Author, year	Study arm (patient number)	Response criteria [RECIST or WHO]	Definition of response [cutoff point and timing]	Patient, *n*				Sensitivity (95% CI)	Specificity (95% CI)	LR + (95% CI)	LR – (95% CI)
				Mutation +		Mutation –					
				Resp – (TP)	Resp + (FP)	Resp – (FN)	Resp + (TN)				
Sartore-Bianchi, 2009	Cetuximab +/- chemotherapy; panitumumab monotherapy	RECIST	CR+PR	30	2	57	20	0.34 (0.25, 0.45)	0.91 (0.71, 0.99)	3.79	0.72
Sohn, 2009	Cetuximab + irinotecan-based chemotherapy	RECIST	CR+PR	26	1	26	13	0.68 (0.43, 0.87)	1.00 (0.63, 1.00)	>100	0.32
Souglakos, 2009	Cetuximab + chemotherapy	RECIST	CR+PR	32	0	46	14	0.53 (0.28, 0.77)	0.90 (0.55, 1.00)	5.29	0.52
Chung, 2010	Cetuximab	RECIST	NR	NR	NR	NR	NR	NR	NR	NR	NR
Graziano, 2010	Cetuximab + irinotecan	RECIST	CR+PR	55	3	40	23	0.58 (0.47, 0.68)	0.88 (0.70, 0.98)	5.02	0.48
Perkins, 2010	Cetuximab +/- irinotecan-based chemotherapy or panitumumab monotherapy	RECIST	CR+PR	19	0	11	12	0.63 (0.44, 0.80)	1.00 (0.74, 1.00)	>100	0.37
Montagut, 2010	Cetuximab + chemotherapy	RECIST	CR+PR	12	0	25	11	0.32 (0.18, 0.50)	1.00 (0.72, 1.00)	>100	0.68
Scartozzi, 2010	Cetuximab +/- irinotecan-based chemotherapy	RECIST	CR+PR	43	0	45	24	0.49 (0.38, 0.60)	1.00 (0.86, 1.00)	>100	0.51

BSC, best supportive care; CB, capecitabine, oxaplatin, bevacizumab; CBC, capecitabine, oxaplatin, bevacizumab, cetuximab; CR, complete response; FN, false negative; FP, false positive; PR, partial response; Resp, response (defined as complete and partial remission); SD, stable disease; SD6, stable disease lasting >6 mo; TN, true negative; TP, true positive.

Meta-analysis: KRAS for predicting anti-EGFR antibody failure by radiological imaging

For the meta-analysis, we focused on 24 studies that appeared to have nonoverlapping populations (**Figure 5**) and were conducted in the metastatic setting. These studies reported on a total of 2938 patients of whom 1121 had KRAS mutations in codons 12 or 13. In the studies by Tol 2009 and Hecht 2009, anti-EGFR antibodies were administered with bevacizumab, an anti-VEGF antibody, and cytotoxic chemotherapy. Because these were the only studies that combined two targeted agents we only included them in sensitivity analysis. Finally, 22 studies[a] were included in the main meta-analysis.(58;59;61;68;74-78;81;86;88;94-102;106)

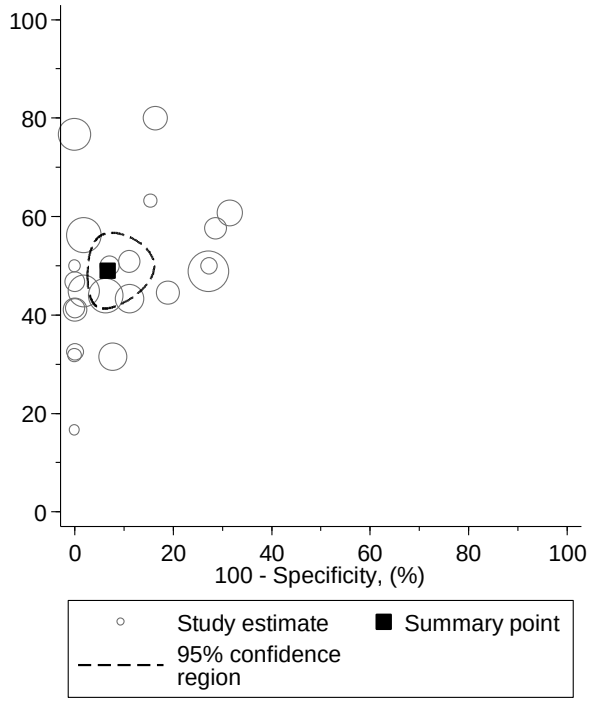
To assess whether specific factors may influence the ability of *KRAS* mutations to predict response to anti-EGFR antibodies, we performed the following subgroup analyses: percentage of patients who had not been exposed to prior chemotherapy (>80 percent unexposed versus >80 percent in second-line or higher), use of the anti-EGFR antibodies in combination with chemotherapy or as monotherapy (>80 percent monotherapy versus >80 percent in combination therapy), specific antibody used (>80 percent cetuximab versus >80 percent panitumumab). For each subgroup, bivariate meta-analysis of sensitivity and specificity was performed when more than 4 studies were available.

In the main analysis, the presence of *KRAS* mutations had a summary sensitivity of 0.49 (95% CI: 0.43, 0.55) and a summary specificity of 0.93 (95% CI: 0.87-0.97), corresponding to positive and negative likelihood ratios of 7.3 and 0.5, respectively. **Figure *6*** presents the results of the meta-analysis in the receiver operating characteristic (ROC) space.

In subgroup analysis, *KRAS* testing had high summary specificity (higher than 0.90 in all subgroups except in the first-line setting) whereas the summary specificity was low (ranging from 0.47 to 0.57 in the evaluated subgroups). The results of the bivariate meta-analysis of sensitivity and specificity are presented in **Table 18.**

[a] From 42 papers.

Figure *6*: *KRAS* and anti-EGFR antibodies for colorectal cancer: meta-analysis of treatment failure by imaging (sensitivity and specificity)



Meta-analysis for the ability of *KRAS* mutations to predict response to anti-EGFR antibodies. Each study is represented by a gray circle with size proportional to the study size. The summary point is represented by a black square and the dashed line represents the 95% confidence region of the summary estimate.

Table 18. *KRAS* and anti-EGFR antibodies for colorectal cancer: meta-analysis results for treatment failure by imaging (sensitivity, specificity and likelihood ratios)

	Number of studies (*KRAS* positive/ total)	Sensitivity (95% CI)	Specificity (95% CI)	LR+	LR-
Main analysis	22 (841, 2242)	0.49 (0.43, 0.55)	0.93 (0.87, 0.97)	7.3	0.5
Including combined antibody therapy (Tol 2009 and Hecht 2009)	24 (1121, 2938)	0.49 (0.44, 0.54)	0.92 (0.85, 0.96)	5.9	0.6
Combination with chemotherapy					
>80% monotherapy	6 (256, 621)	0.54 (0.43, 0.64)	0.95 (0.76, 0.99)	11	0.5
>80% combination therapy	16 (585, 1621)	0.47 (0.41, 0.53)	0.92 (0.85, 0.96)	6.2	0.6
Prior chemotherapy					
>80% second-line or higher	17 (598, 1618)	0.47 (0.41, 0.53)	0.96 (0.91, 0.98)	12.3	0.6
>80% first-line	5 (243, 624)	0.57 (0.46, 0.68)	0.76 (0.70, 0.81)	2.4	0.6
EGFR antibody					
Cetuximab	19 (738, 1979)	0.48 (0.42, 0.53)	0.91 (0.85, 0.95)	5.5	0.6
Panitumumab	3 (103, 263)	NA	NA	NA	NA

Results of subgroup analysis for the ability of *KRAS* mutations to predict response to anti-EGFR antibodies. Sensitivities and specificities are presented with their corresponding 95% CI. Positive and negative LRs were calculated form the summary sensitivity and specificity. LR = likelihood ratio.

We also assessed the strength of association between *KRAS* mutations and response to treatment with anti-EGFR antibodies by performing meta-analysis of predictive odds ratios. Overall, there was a significant association between the presence of *KRAS* mutations and failure anti-EGFR antibody treatment according to imaging criteria. Based on the main analysis of 22 independent studies (i.e., excluding studies with potential overlap, the 2 neoadjuvant studies and the studies by Tol 2009 and Hecht 2009 which evaluated a combination of two targeted agents), the summary odds ratio was

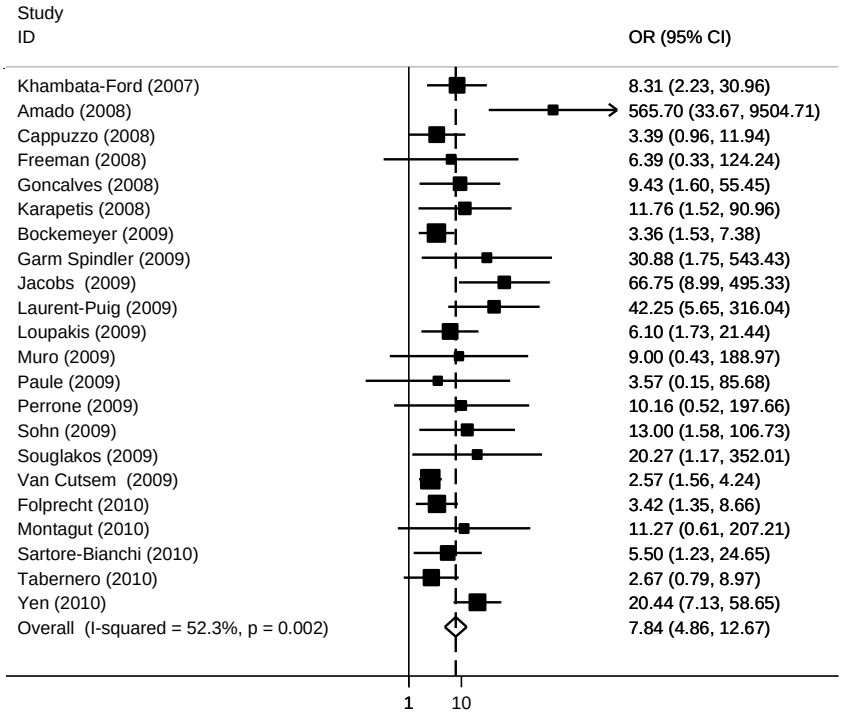
7.84 (95% CI: 4.86-12.67); p<0.001. **Figure 7** presents the forest plot for the results of the main analysis. The association between *KRAS* mutations and response to EGFR was robust in subgroup analyses. **Table 19** presents results from subgroup analyses although the predictive effect was much higher in the second-line or higher treatment setting.

Table 19. *KRAS* and anti-EGFR antibodies for colorectal cancer: meta-analysis results for treatment failure by imaging (odds ratio)

	Number of studies (*KRAS* positive/total)	Heterogeneity p-value; I^2	Summary odds ratio (95% CI); p-value
Main analysis	22 (841, 2242)	0.002; 52%	7.84 (4.86-12.67); <0.001
Including combined antibody therapy (Tol 2009 and Hecht 2009)	24 (1121, 2938)	<0.001; 70%	6.34 (3.96-10.15); <0.001
Combination with chemotherapy			
>80% monotherapy	6 (256, 621)	0.06; 53%	11.42 (3.42-38.12); <0.001
>80% combination	16 (585, 1621)	0.08; 52%	7.09 (4.20-11.98); <0.001
Prior chemotherapy			
>80% second-line or higher	17 (598, 1618)	0.28; 15%	10.99 (6.48-18.64); <0.001
>80% first line	5 (243, 624)	0.01; 68%	4.16 (2.13-8.12); <0.001
EGFR antibody			
Cetuximab	19 (738, 1979)	0.02; 46%	6.74 (4.30-10.56); <0.001
Panitumumab	3 (103, 263)	0.06; 65%	33.25 (1.86-594.88); 0.02

Results of subgroup analysis for the ability of *KRAS* mutations to predict response to anti-EGFR antibodies. Odds ratios are presented with their corresponding 95% CI. Heterogeneity was quantified with the Q and I^2 statistics.

Figure 7: *KRAS* and anti-EGFR antibodies for colorectal cancer: meta-analysis of treatment failure by imaging (odds ratio)



Forest plot of studies evaluating the association between *KRAS* mutations and anti-EGFR antibody failure by imaging. The point estimate of the odds ratio of each study is represented by a gray square. The size of the square is proportional to the weight of each study in the meta-analysis. Horizontal lines represent the accompanying 95% confidence interval. A diamond stands for the summary estimate of the OR. The width of the diamond represents the confidence interval around the summary estimate. Studies are ordered by year of publication and then by author name.

Studies in the neoadjuvant setting

Two small (40 and 41 enrolled patients) studies evaluated *KRAS* mutations as predictive factors for response to neoadjuvant therapy for patients with rectal cancer.(65;70) Both studies evaluated combinations of cytotoxic agents with cetuximab and radiotherapy.

The first study was a prospective phase II study from Italy (n=39 samples from patients included in the response analysis from a total of 40 enrolled - one patient refused surgery and was not assessable for response).(65) The study treatment consisted of neoadjuvant cetuximab and 5-fluorouracil, concurrently with radiotherapy. *KRAS* mutations were assessed before study treatment and response was assessed using the Dworak(109) assessment scale, in samples obtained by surgery. *KRAS* mutations were identified in 9 patients (23 percent) and were not associated with pathologic response to chemoradiotherapy (tumor grade regression 3-4 was observed in 1/9 patients with *KRAS* mutations versus 11/30 patients with wild-type tumors, p=0.119). Disease-free survival or overall survival analyses were not reported.

The second study was a prospective, phase I/II study from France (n=39 samples from a total of 41 patients enrolled) reported on the ability of *KRAS* mutations to predict pathologic response and disease-free survival in the neoadjuvant setting.(70) The study treatment consisted of neoadjuvant capecitabine in combination with cetuximab and radiotherapy and at the time of reporting had a median follow-up of 32 months (range: 4.8-46.2). *KRAS* mutations were assessed before study treatment and response was assessed using the Dworak(109) and Wheeler(110) assessment scales, independently by two pathologists, in samples obtained by surgery. *KRAS* mutations were identified in 12 patients (31 percent) and were not associated with pathologic response to chemoradiotherapy (details not reported). Per study report, *KRAS* positive tumors "tended to show regression" using the Wheeler scale (p=0.09) but not using the Dworak scale (p=0.36). In addition, *KRAS* mutations showed no correlation with tumor downstaging (p=0.69). Disease-free survival results were not presented by *KRAS* status.

Key Question 2: What patient- and disease-related factors affect the test results, their interpretation or their predictive response to therapy?

None of the included studies performed analyses for interaction between the aforementioned factors and *KRAS* mutations to predict response to therapy.

Key Question 3: How does the gene testing impact the therapeutic choice?

No study explicitly reported details on changes in treatment plans before and after testing. However, *KRAS* testing is already used to guide treatment in several clinical settings, and thus by definition affects treatment choice in these settings. The absence of the information requested by Key Question 3 is essentially an issue of reporting.

Key Question 4: What are the benefits and harms or adverse effects for patients when managed with gene testing?

No study explicitly reported evidence on benefits or harms beyond what is covered in Key Question 1

2.4 Discussion

Our systematic review of studies on the ability of *KRAS* mutation testing to predict response to treatment with the anti-EGFR antibodies cetuximab and panitumumab identified consistent evidence that *KRAS* testing can predict response to treatment in colorectal cancer patients. For all outcomes assessed, patients with *KRAS* mutations were less likely to experience benefit with anti-EGFR antibody treatment, compared to patients whose tumors were wild-type for *KRAS* mutations. The direction of the association is consistent for overall mortality, disease progression and treatment failure by radiologic imaging. In three out of four RCT-based analyses of progression free survival, the treatment-by-*KRAS* mutation interaction was significant and in the anticipated direction. In brief, a substantial body of evidence suggests that testing somatic *KRAS* mutations predicts differential response to anti-EGRF therapy in colorectal cancer patients.

Most individual studies reporting overall and disease-free survival reached formal statistical significance. Most studies pertained to patients who had received previous cytotoxic chemotherapy. Our conclusions are analogous to a recently published provisional clinical opinion by the American Society of Clinical Oncology,(111) the recent changes implemented by the Food and Drug Administration (FDA) on the product labels of both cetuximab and panitumumab[a], and similar decisions by the European Medicines Agency (EMeA).[b] Further, our results are in agreement with a previous systematic review that included a smaller number of studies of patients who were pre-treated with cytotoxic chemotherapy and only assessed treatment failure as an outcome.(112)

In seven studies in the first line setting the ability of *KRAS* mutations to predict adverse outcomes seemed to be lower compared to studies in pre-treated patients.(58;61;91) This was true for all outcomes and particularly for treatment failure by radiologic imaging. For example, the positive likelihood ratio for "ruling in" treatment failure under anti-EGFR antibody treatment based on the summary sensitivity and specificity was 12.3 for studies in the second-line or higher setting compared to 2.4 for studies in the first-line setting. A postulated explanation is that, in the salvage setting, many patients are resistant to cytotoxic chemotherapy, and most of their benefit comes from the anti-EGFR treatment. Therefore, in pretreated patients (salvage setting) the modifying effect of *KRAS* mutations on the anti-EGFR therapy *is not diluted* by the effect of cytotoxic chemotherapy, and is more readily observable. For these reasons, we argue that there is need for further research especially in the first line setting.(113) Ideally this can be studied in future RCTs of anti-EGFR agents by prespecifying analyses by *KRAS* status, or by "repurposing" already completed RCTs in which anti-EGFR antibodies were used as first line treatments. In the latter case, one would perform genetic analyses in archival tissue from RCT enrollees, and associate them with the prospectively recorded clinical outcomes.

The benefits of implementing *KRAS* mutation testing to guide treatment for

[a] http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm172905.htm; last accessed on December 7th, 2009.

[b] http://www.emea.europa.eu/humandocs/PDFs/EPAR/erbitux/089404en8.pdf; last accessed on December 7th, 2009.

colorectal cancer have been evaluated in a prospective cost-effectiveness analysis based on the National Cancer Institute of Canada Clinical Trials Group CO.17 (which was included in the present systematic review(77)[c]). The cost-effectiveness analysis concluded that implementing pharmacogenetic testing for *KRAS* mutations improves the incremental cost-effectiveness ratio of cetuximab over best supportive care (in the particular study setting), and that "from a health-care system perspective, it would not be efficient to fund cetuximab treatment for all patients with advanced colorectal cancer".(114)

Regarding the two different agents, cetuximab and panitumumab, the predictive ability of *KRAS* mutations appears to be similar, although the bulk of available evidence for this subgroup comparison was related to studies assessing panitumumab as monotherapy, and in all cases in patients pre-treated with cytotoxic chemotherapy. It should be noted that panitumumab is currently approved by the FDA only for use as a single agent for patients "with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens".[d]

In conclusion, this review identified a substantial body of evidence consisting of small retrospective analyses and analyses based on RCTs, assessing *KRAS* mutations as a pharmacogenetic test for predicting response to anti-EGFR antibodies.

[c] see Karapetis, 2008(77), a retrospective analysis of a phase III RCT comparing cetuximab plus best supportive care versus best supportive care for patients with chemotherapy pre-treated colorectal cancer.
[d] http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125147s080lbl.pdf; last accessed on December 7th, 2009.

Section 3: Variations in *BCR-ABL* and response to imatinib, dasatinib and nilotinib in chronic myeloid leukemia

3.1 Background

Chronic myeloid or myelogenous leukemia (CML) is a relatively uncommon hematological malignancy with approximately 5,000 new cases diagnosed annually.(115) CML was the first malignant disorder where a single chromosomal aberration was demonstrated to be fundamental to the etiology of the disease.(116) The Philadelphia chromosome, designated t(9;22)(q34;q11), a reciprocal translocation between chromosome 9 and 22, produces the BCR-ABL1 fusion protein,[a] a constitutively active tyrosine kinase that activates several signal transduction pathways. This ultimately causes abnormal bone marrow proliferation and the clinical manifestations of CML.

The identification of the *BCR-ABL1* fusion gene has also advanced the development of several laboratory tests, especially for molecular monitoring of residual disease after treatment. Tests for monitoring response include conventional G-banded karyotypic examination, fluorescence in situ hybridization (FISH), and quantitative reverse transcriptase polymerase chain reaction (RT-PCR).(117) For example, qualitative RT-PCR is routinely used for molecular monitoring of *BCR-ABL1* transcript levels to assess patient response to therapy. Another routinely used test for patient monitoring patient response is cytogenetic monitoring with conventional karyotypic examination.

The advent of imatinib a tyrosine kinase inhibitor (TKI) drug that specifically blocks the activity of the *BCR-ABL1* tyrosine kinase, has dramatically modified the clinical management of CML in this decade.(118) Imatinib as first-line therapy is now considered the standard of choice for most untreated patients with CML in chronic phase.(118) According to the 6-year followup results of a randomized trial[b] comparing interferon with imatinib in previously untreated chronic phase CML patients, about 80 percent of imatinib-treated patients enjoy long-term survival with no evidence of overt disease progression.(119) Some patients do not benefit from imatinib treatment. These include patients with suboptimal or no response to imatinib, patients who eventually develop resistance to first line therapy with imatinib, or those with CML in advanced disease (i.e., accelerated or blastic phase). Several therapeutic options are available for these patients, including high-dose imatinib,[c] the newer generation TKIs dasatinib and nilotinib,[d] and allogeneic stem-cell transplantation.(118)

The emergence of point mutations in the kinase domain of the *BCR-ABL1* gene is generally considered one of the most common and critical mechanisms of clinical

[a] Formally designated Bcr-Abl

[b] This is the IRIS trials, the International Randomized Study of Interferon versus STI571 (imatinib). This study included CML patients in the chronic phase who were previously untreated.

[c] 600 to 800 mg/d – versus 400 mg/d of the standard dose.

[d] Dasatinib and nilotinib are FDA approved second line treatments.

resistance to imatinib treatment.[e](120) Kinase domain mutations in *BCR-ABL1* was first identified by direct sequencing in 2001, in 11 patients who failed imatinib therapy.(121) Since then, several studies have examined these mutations in patients with clinical resistance to imatinib using several technologies.(122) To date, over 70 different mutation positions involving 57 different amino acids have been reported.(122) A particular mutation, T315I, is now considered to be a marker of poor response to all FDA-approved TKIs. The updated clinical recommendations by the European Leukemia Net (ELN) advocates performing mutation analysis in patients with suboptimal response or failure to any TKIs. ELN advocates that patients with the T315I mutation should consider allogeneic stem cell transplantation.(118)

In this Technology Assessment we perform a systematic review of the published evidence on the ability of mutation testing in the *BCR-ABL1* gene to predict patient response to therapy with TKI inhibitors. We consider separately first, second and third line treatments of CML.

[e] Other proposed mechanisms of clinical resistance to imatinib include *BCR-ABL1* gene-independent mechanisms such as pharmacokinetic factors, aberrant intracellular uptake of imatinib or clonal evolution, or *BCR-ABL*-related mechanisms like overexpression.(120)

3.2 Methods

The reader is referred to the Generic Methods Section for a description of methods common to all three topics examined in this review. Herein we describe the topic-specific Key Questions, as well as additional topic-specific methods.

Key Questions

5) Does *BCR-ABL1* mutation testing predict response to TKI therapy?
6) What patient- and disease-relevant factors affect the test results, their interpretation or their predictive response to therapy?
7) How does the gene testing impact the therapeutic choice?
8) What are the benefits and harms or adverse effects for patients when managed with gene testing?

The reader is referred to the Generic Methods section in the beginning of this Technology Assessment for a description of the Key Questions.

Literature search

We searched Ovid MEDLINE from inception to August week 4, 2009 using combinations of the terms "chronic myeloid leukemia", "imatinib", "dasatinib", "nilotinib" and their synonyms. The exact search strategy is listed in **Appendix A**. We also perused the reference list of eligible studies and of relevant review articles.

Eligibility criteria

In order to be eligible for this review, studies needed to meet the generic eligibility criteria. In addition, eligible studies included patients with *BCR-ABL1* positive CML, regardless of disease stage (chronic, accelerated, or blastic phase) or treatment context (first line, second line, or subsequent lines), who received imatinib, dasatinib, or nilotinib with or without concurrent other chemotherapy. Outcomes of interest were overall or CML specific mortality, CML progression (as defined by each study), or TKI treatment failure (lack of hematologic, cytogenetic, or molecular response, as defined by each study).

The main focus is on studies that performed mutation testing in samples obtained before the initiation of treatment (**Figure 8**). After discussions with AHRQ and TOO we decided to also abstract studies that performed mutation testing during treatment, to monitor patients for treatment failure (monitoring studies **Figure 9**). These are summarized separately at the end of this section.

Data extraction

The reader is referred to the Generic Methods section in the beginning of this Technology Assessment for a description of the Data extraction for common items across the three topics.

Technical specifications of tests to detect BCR-ABL1 mutations, and mutation frequency

We extracted information on tissue source (e.g., peripheral blood or tissue source), mutation detection method, tested gene region, assay detection sensitivity, identified mutations, and *BCR-ABL1* transcripts level (or trend) collected most recent to mutation testing.(122)

Laboratory guidelines have proposed an operational classification of mutations in the *BCR-ABL1* gene.(122;123) We recorded the frequency of each reported mutation in each study, and present a graphical summary of these frequencies

Outcomes of interest

As described in the Generic Methods section outcomes of interest were overall survival and cancer specific survival; progression-free or event-free survival (as defined by each study); and treatment failure. Typically, treatment failure is defined as absence of hematologic, cytogenetic, or molecular response to treatment, according to various criteria (European Leukemia Net, ELN,(118;124) criteria or other).

Definition of treatment categories

We grouped therapies into the following operational categories, irrespective of dose or dosing schedule unless noted otherwise:

1. Standard dose imatinib monotherapy (<=600 mg per day)
2. High dose imatinib monotherapy (>600 mg per day)
3. Imatinib-based combinations, which including non-TKI therapies added to imatinib
4. Dasatinib monotherapy
5. Nilotinib monotherapy
6. Miscellaneous. This category refers to (study) results of multiple TKI agents that do not provide separate data for each TKI[a]

Lines of TKI therapy

For the purpose of this report we used the following operational classification of studies:

1. First line TKI therapy studies: Studies in patients who have not received prior TKI treatment. Typically, these are studies of standard dose imatinib monotherapy.
2. Second line TKI therapy studies: Studies in patients in whom prior treatment with standard dose imatinib was not successful. Typically, these are studies of high dose imatinib monotherapy, imatinib-based combinations, dasatinib or nilotinib.
3. Third line TKI therapy studies: Studies in patients in whom first and second line therapies were not successful. These would generally be studies classified in the "miscellaneous" category in our operational classification of treatments.

Differential timing of mutation testing by line of TKI therapy

The following schematics help clarify the temporal relationship of *BCR-ABL1* mutation testing and treatment for first, second, and third line TKI therapy studies.

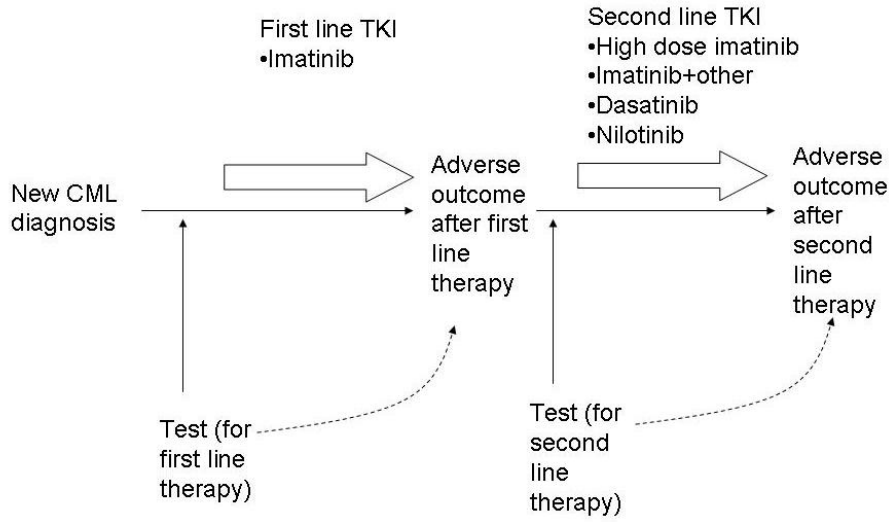
[a] Not about a concurrent administration of two (or more) different TKIs.

In first line TKI therapy studies mutation testing is performed in samples obtained before the initiation of first line therapy, as shown in **Figure 8**. The corresponding timing between testing and treatment is required for second and thirds line TKI therapy studies.

We also abstracted studies that used *BCR-ABL1* mutation testing during therapy, presumably to monitor patients for treatment failure (**Figure 9**). These studies are summarized separately.

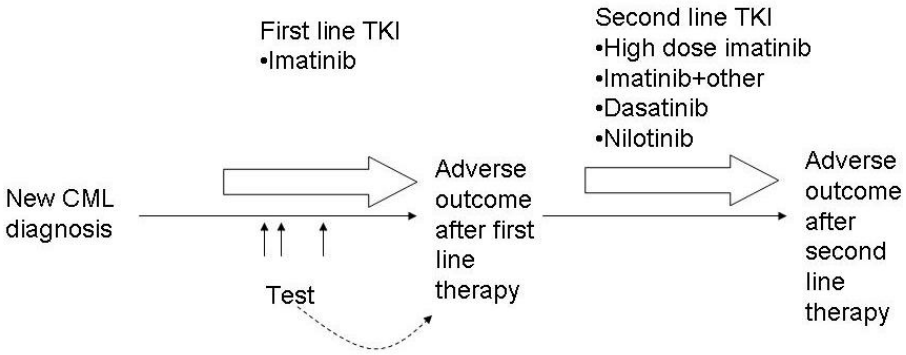
As described in the eligibility criteria, studies that perform mutation testing only among patients with treatment failure and do not associate this information with response to subsequent therapy are excluded (**Figure 10**).

Figure 8. *BCR-ABL1* and tyrosine kinase inhibitors for CML: timing of mutation testing and TKI therapy in studies predicting treatment failure



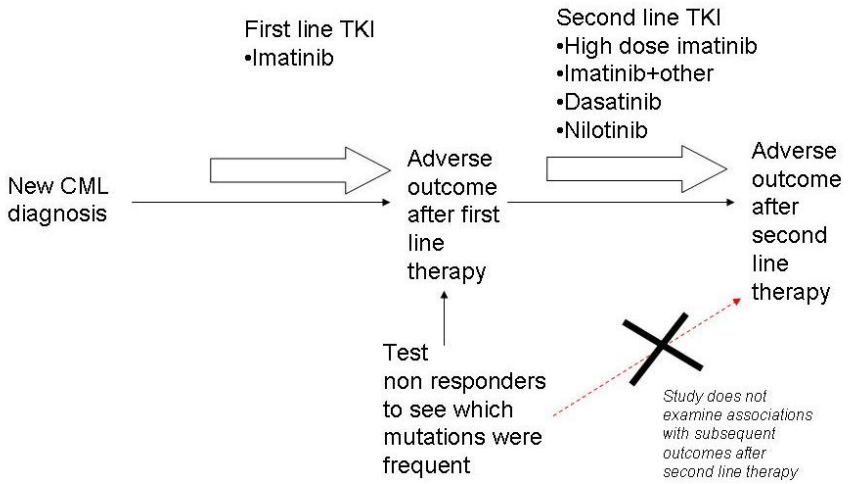
In studies predicting response to treatment (the main focus of this Section), mutation testing must be performed prior to initiation of first, second or third line TKI therapy (not shown for third line therapy studies).

Figure 9. *BCR-ABL1* and tyrosine kinase inhibitors for CML: timing of mutation testing in studies that monitor patients for treatment failure



In studies that monitor patient response, mutation testing is performed during therapy. Possible reasons are to identify treatment must be performed prior to initiation of first, second or third line TKI therapy (not shown for third line therapy studies).

Figure 10. *BCR-ABL1* and tyrosine kinase inhibitors for CML: timing of mutation testing relative to treatment initiation and outcome assessment in excluded studies



Studies that perform mutation testing only in non responders and do not associate this information with response to subsequent therapy are excluded from this report.

Synthesis

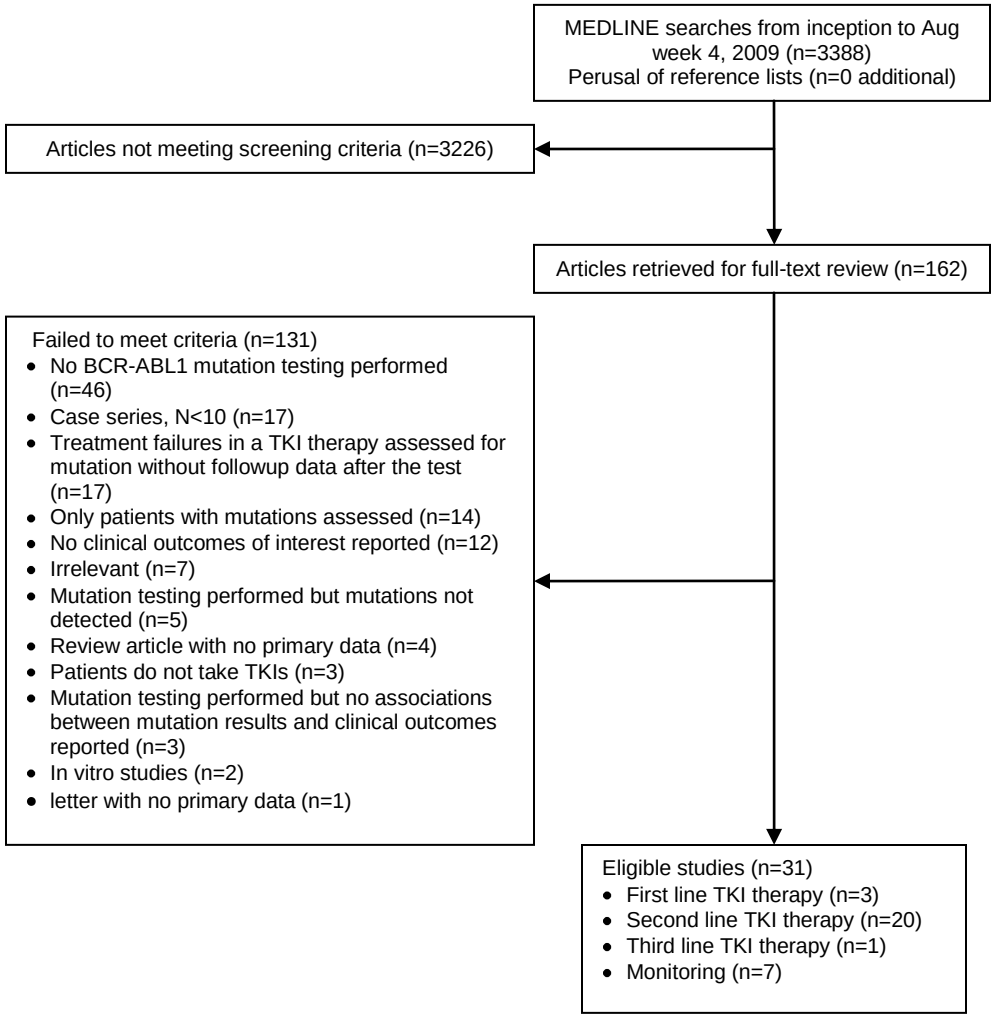
Please refer to the Generic Methods section for a description of study synthesis methodologies used in this Technology Assessment. We present results on the outcomes of overall or cancer specific survival, progression-free survival, and treatment failure separately for first, second, and third line TKI therapy studies, stratified by treatment category (as defined above).

We did not perform quantitative synthesis (meta-analysis). Data were very limited for survival or progression free survival. For the outcome of treatment failure there was extensive heterogeneity in the disease stage of included patients and in the definition of treatment failure across studies (section 3.3 Results). Instead, we perform qualitative syntheses through graphs and tables.

3.3 Results

Our electronic searches yielded 3388 studies, 162 of which were retrieved in full text (**Figure 11**). Finally, 31 publications were included. **Appendices A** and **B** list included and excluded papers, respectively.

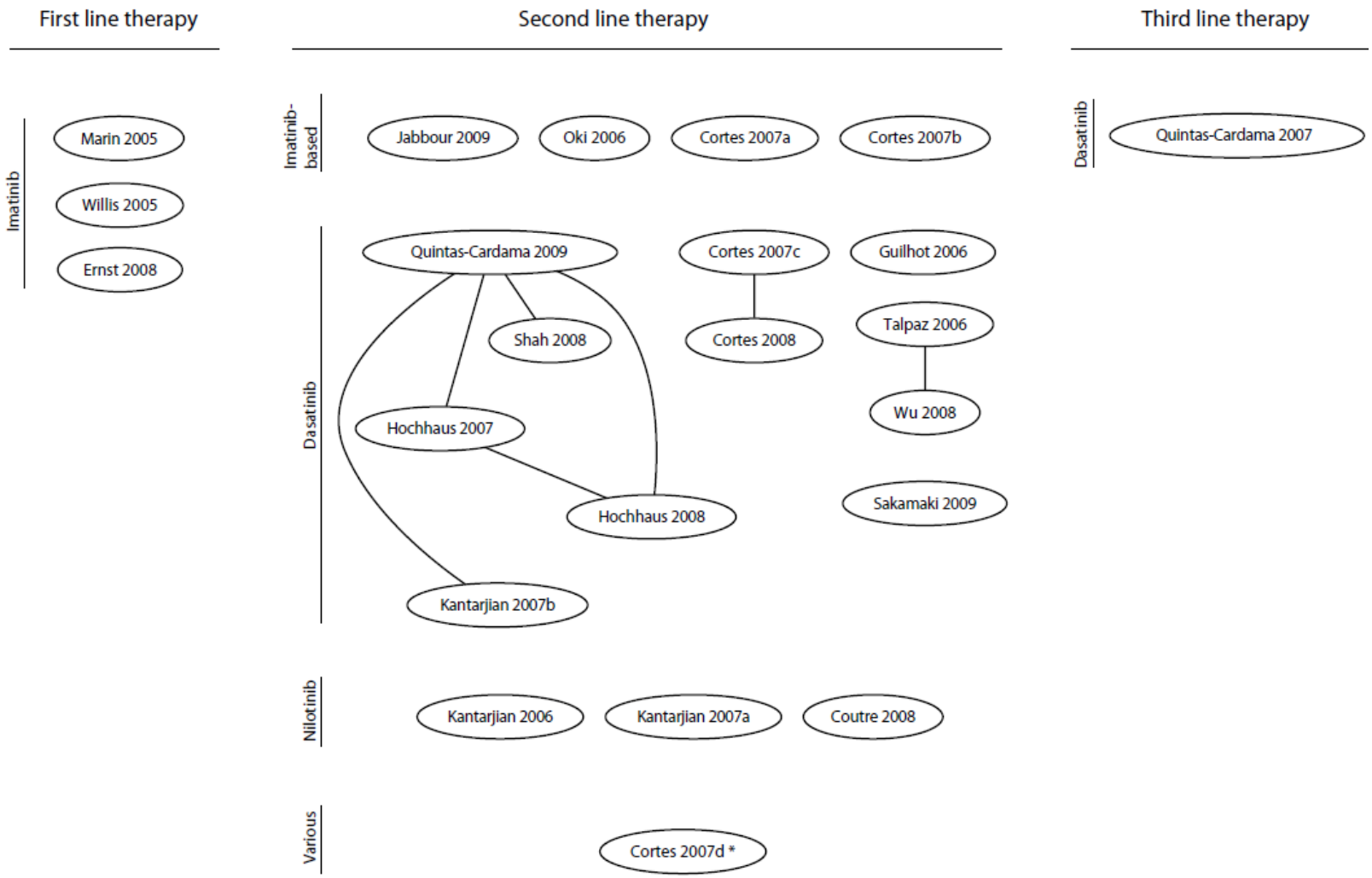
Figure 11. *BCR-ABL1* and tyrosine kinase inhibitors for CML: literature flow



Perusal of reference lists did not identify additional citations that were not found in the electronic searches.

Figure 12 summarizes which publications appear to have common sets of patients. Most publications originated from the MD Anderson Comprehensive Cancer Center.

Figure 12. *BCR-ABL1* and tyrosine kinase inhibitors for CML: potential overlaps in patient populations in first, second and third line therapy studies



Each ellipse is a citation included in the systematic review. Citations with at least partially overlapping patient populations are connected with a line. Refer to **Tables 18**, **21** and **28** to identify the citations of the depicted studies. Some second line therapy studies likely include some patients reported in the first line therapy studies. Similarly, Quintas-Cardama 2007 (third line therapy) likely includes patients who are also described in the first and second line therapy studies. *Cortes 2007d likely includes patients from several studies of dasatinib (not depicted). Note that Kantarjian 2007b belongs to the "miscellaneous" (various) treatment category.

Key Question 1: Does *BCR-ABL1* testing predict response to TKI therapy?

First line TKI therapy studies

Study characteristics

Three studies (from Germany, the UK, and the US) reported associations between *BCR-ABL1* mutation testing and clinical outcomes in patients with CML (**Table 20**).(125-127) All three were single arm cohorts of patients receiving imatinib. Two are retrospective cohorts(125;127) and the third is a prospective single center phase I/II trial of imatinib treatment. All three have small sample sizes (10, 66, and 120).

As depicted in **Figure 12** the three studies have nonoverlapping patient populations. Median ages ranged from 48 to 60 years, and approximately half of the participants were male. The two larger retrospective studies included patients in all disease stages (i.e., chronic, accelerated, or blastic phase), whereas the smaller phase I/II trial included mostly acute phase patients. Typically, baseline tumor load was not reported.

Mutation testing characteristics

Table 21 reports details on *BRC-ABL* mutation testing in the three studies. Studies considered various mutations as predisposing to adverse clinical outcomes. All studies tested for the T315I mutation. **Figure 13** presents a graphical summary of the frequencies of detected mutations in all studies.

Table 20. *BCR-ABL1* and tyrosine kinase inhibitors for CML: characteristics of included studies (first line therapy)

Author, year (Country)	-Sample size, *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	Staging distribution, *n (%)*	Disease status	Median recent tumor load (range)	Prior therapy	-Treatment -Adherence	Median followup, *mo* [range]
Ernst, 2008 (Germany) (125)	-120 -Retrospective single center cohort study[a] -Unclear	Patients with relapsed CML while on imatinib treatment (n=95) and patients with CML-CP with continuous CR for more than 2 years after imatinib monotherapy (n=25) were compared	Relapsed group[b]: 60 [20-80] (54) Responsive group: NR	Relapsed group[c]: CP 47 (50); AP 27 (28); BP 21 (22); Responsive group: CP 25 (100)	Median time since CML diagnosis 32 mo	NR	NR	-Imatinib 400-600 mg/d -NR	13 [1-44][d]
Willis, 2005 (USA)(127)	-66 -Retrospective analysis of a multi-center trial -Convenience sampling	NR	57 [31-80] (42)	CP 20 (30); AP 27 (40); BC 19 (29)	NR	NR	No prior imatinib HU 51 (77); IFN 37 (56); Cytarabine 18 (27); TopoII 11 (17); 6TG 6 (9); BU 5 (8); L-PAM 3 (5); others 4 (6)	-Imatinib 400 mg/d (CP); Imatinib 600 mg/d (AP or BC) -NR	NR
Marin, 2005 (UK)(126)[e]	-10 -Prospective single-center phase I/II trial -Unclear	Ph+ CML aged ≥18 y on imatinib 400 mg/d for ≥2 y Partially sensitive to imatinib but "suboptimal" response (≥minor CyR with a plateau in BCR-ABL1 transcripts)	48 [41-70] (60)	AP 1 (10); NR 9 (90)	Complete CyR 8 (80); partial CyR 1 (10); minor CyR 1 (10)	4.4% (0.03%-25%)	Imatinib 400 mg/d 10 (100); IFN 7 (70)	-Imatinib 400-600 mg/d + HHT 2x1.25 mg/m^2/d for 1-3 d for every 4wk (adjusted according to toxicity) -Median 6.5 cycles (range, 4-8)	6.5 [4-8][f]

AP, accelerated phase; BP, blastic phase; BU, busulfan; CP, chronic phase; CR, complete remission; CYR, cytogenetic response; HHT, homoharringtonine; HU, hydroxyurea; IFN, interferon; L-PAM, melphalan; NR, not reported. Ph+, Philadelphia chromosome positive; TopoII, topoisomerase II inhibitor; 6TG, 6-thioguanine

[a] Patients were participants in prospective multicenter trials.
[b] Median age [range] when starting imatinib.
[c] At imatinib resistance.
[d] Median time-to-hematologic relapse for patients who developed imatinib resistance. Responsive patients were in continuous response for at least 2 years.
[e] Addition of another agent to imatinib
[f] Duration of therapy

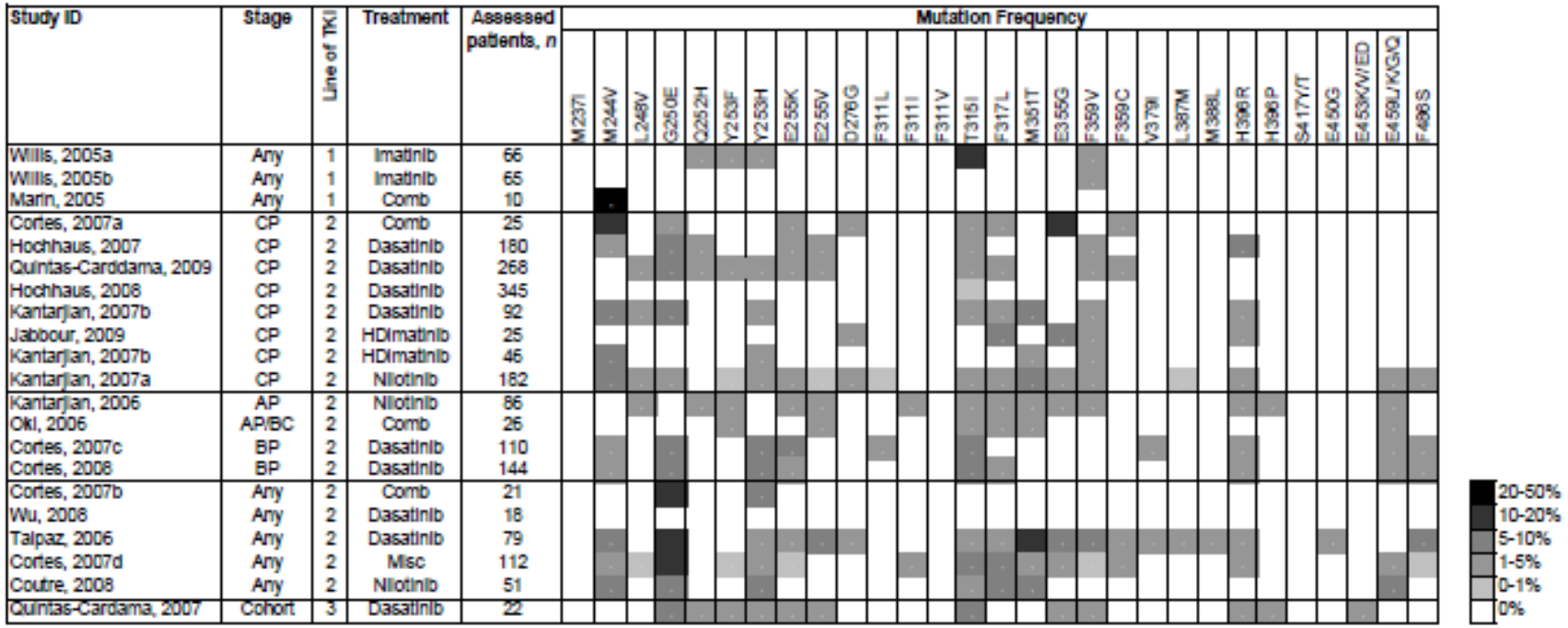
Table 21. *BCR-ABL1* and tyrosine kinase inhibitors for CML: detection and reporting of mutations conferring drug resistance

Study (first author, year)	Assessed patients, *n (% of included patients)*	Test Objective	Mutation detection method	Source (Tube) [process and time between collection and RNA extraction, *h*]	Detection sensitivity *(%)*	Mutation Frequency, n (%)	
						Mutation +	Mutation -
Ernst, 2008[a]	120 (100)	Response prediction	Nested PCR and DHPLC, DS	-Peripheral blood (commercial kits) -NR	-DHPLC: 0.1 for T315I/ M351T; 0.5 for Y253F; 1 for E255K -Sequencing: 10	Relapsed group: Any, 9 (9); T315I, 4 (4.2) Responsive group: Silent mutation, 1 (4); Normal polymorphism, 2 (2)	Relapsed group: 86 (91)
Willis, 2005	66 (100) 65 (98) [DS]	Predictive	ASO-PCR[b] DS	-Bone marrow -NR	-ASO-PCR: 0.1 -DS: NR	Any, 14 (21) [ASO-PCR] Any, 2 (3) [DS] T315I, 8 (12) [ASO-PCR] T315I, 0 [DS][c]	52 (79) [ASO-PCR] 63 (97) [DS]
Marin, 2005	10 (10)	Predictive	DS	-Peripheral blood or bone marrow -[NR]	-NR	Any, 2 (20) T315I, 0 (0)	8 (80)

ASO-PCR = allele-specific oligonucleotide polymerase chain reaction; DHPLC = denaturing high-performance liquid chromatography; DS = direct sequencing; PCR = polymerase chain reaction; PS = pyrosequencing; SS = subcloning and sequencing

[a] Data were extracted only regarding mutations detectable prior to imatinib treatment.
[b] Only 8 mutants
[c] DS detected 1 case with F359V, which was not included in ASO-PCR

Figure 13. *BCR-ABL1* and tyrosine kinase inhibitors for CML: frequencies of mutations



Mutation frequencies in the *BCR-ABL1* gene are depicted using a colorcode. When a study reported multiple different mutated codons in patients, each different codon was considered independently. One study reported results by ASO-PCR and direct sequencing separately (Willis 2005a and Willis 2005b, respectively).(127). The mutation testing by ASO-PCR assessed only 8 “common” mutations (Q252H, Y253F, Y253H, E255K, E255V, T315I, M351T, F359V). In this case we considered the frequency of the mutations in unassessed codons to be 0. Because one study reported several variants in three codons as single group (*F359C/I/V, Y253F/H, and E255K/V), the estimated frequency was divided and assigned evenly to each variant.(128) One study selectively reported only 8 codons. In one study(129) some patients with specific mutations (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R) were excluded. Ernst 2008(125) was excluded because of case-control design (i.e., patients with treatment failure and those in continuous complete cytogenetic remission were separately identified).

Abbreviations: ASO-PCR, allele-specific oligonucleotide polymerase chain reaction; AP, accelerated phase; BP, blastic phase; Comb, imatinib-based combination; CP, chronic phase; HD, high-dose.

Association of BCR-ABL1 mutation test results and mortality

Only Willis 2005 evaluated overall survival. The study found no statistically significant differences in the time-to-death among patients with versus without mutations (for all assessed mutations, hazard ratio not reported; log rank p=0.56).

Ability of BCR-ABL1 mutation testing to predict imatinib failure

Ernst 2008(125) and Willis 2005(127) reported relevant data. Ernst 2008 evaluated a composite of hematologic or cytogenetic response (no details on outcome definition). Willis 2005 evaluated complete or partial hematologic response (according to criteria described in reference(130)) or major cytogenetic response (as per the European Leukemia Net criteria, described in reference(118;124)). **Table 22** summarizes calculated predictive accuracy results.

Association of BCR-ABL1 testing results and disease progression outcomes

Only Willis 2005 evaluated disease progression, defined as loss of partial or complete hematologic response, major cytogenetic response, initiation of alternate therapy or death. The study found no statistically significant differences in the time-to-event among patients with versus without mutations (for all assessed mutations, hazard ratio not reported; log rank p=0.90).

Table 22. *BCR-ABL1* and tyrosine kinase inhibitors for CML: imatinib treatment failure(first line therapy)

Author, year	Outcome (followup)	*BCR-ABL1* mutations	Mutation, no response	Mutation, response	No mutation, no response	No mutation, response	Sensitivity (95 %CI)	Specificity (95% CI)	LR positive	LR negative
Willis, 2005	Hematologic response (ND)	All identified mutations	5	9	8	41	0.38 (0.14, 0.68)	0.82 (0.69, 0.91)	2.14	0.75
		T315I	1	7	12	43	0.08 (0.00, 0.36)	0.86 (0.73, 0.94)	0.55	1.07
	Major cytogenetic response (ND)	All identified mutations	11	3	31	17	0.26 (0.14, 0.42)	0.85 (0.62, 0.97)	1.75	0.87
		T315I	6	2	36	18	0.14 (0.05, 0.29)	0.90 (0.68, 0.99)	1.43	0.95
Ernst, 2008	Hematologic or cytogenetic response (1.1 y)	All identified mutations	9	0	86	25	0.09 (0.09, 0.17)	1.00 (0.86, 1.00)	5.15[a]	0.92
		T315I	4	0	91	20	0.04 (0.01, 0.11)	1.00 (0.83, 1.00)	1.97[b]	0.98

[a] A continuity correction of 0.5 was used in this calculation
[b] A continuity correction of 0.5 was used in this calculation

Second line TKI therapy studies

Study characteristics

In total 18 studies in 20 publications reported associations between *BCR-ABL1* mutations and clinical outcomes in patients with CML (**Table 23**). (128;129;131-148) As evident from **Figure 12**, at least several studies have at least partially overlapping patient populations.

Patients

Overall, studies included patients who had already failed imatinib therapy. In most studies, the vast majority of patients had also received other therapies such as interferon (**Table 23**). Patients at different stages of CML (chronic, blastic or accelerated phase) were included across studies. Typically, prospective phase I (dose-finding) studies included any disease stages, whereas other prospective studies generally focused on only one particular disease stage (see "Design", below). The median age of included patients ranged from 50 to 62 years. Typically, baseline *BCR-ABL1* transcript levels of patients were not reported. The median follow-up ranged 2 to 61 months. Most publications particularly in 2nd-line TKI treatments originated from MD Anderson Comprehensive Cancer Center."

Treatments

Overall, second line TKI therapy studies treated patients with high dose imatinib monotherapy (n=2),(129;131) imatinib-based combinations (n=3),(132-134) dasatinib (n=10),(128;129;135-144) nilotinib (n=3),(145-147) or a combination of TKI drugs ("miscellaneous" category, n=1).(148) Doses and dosing schedules differed (**Table 23**). Typically, compliance or blood concentrations of TKIs were not reported.

Design

Across all treatment types, in 5 studies outcome assessment was retrospective.(128;131;144;146;148) Outcome assessment was prospective or unclear in the remaining 13 studies.(129;132-143;145;147) Three studies were based on RCTs that performed mutation testing either as part of their original protocol, or in the context of post hoc analyses.(129;135;138) The remaining studies evaluated patients who received specific protocols. None of the RCT-based studies assessed interactions of mutation status with treatment type for predicting outcomes. Instead, they performed statistical analyses only within patients who received specific treatments, much like the single arm studies.

All studies but one(148) assessed the predictive ability of the mutation testing as part of subgroup analyses. The sample size of the studies ranged from 18 to 670 (median, 114), and mutations were assessed in 30 to 100 percent (median, 92 percent) of total study participants. One RCT-based study compared high-dose imatinib with dasatinib,(129), and another two RCT-based studies performed comparisons different dosing schedules of dasatinib.(135;138) Eleven prospective single arm studies published in 13 publications evaluated the effects of one particular second-line TKI.(132-134;136;137;139;140;142;143;145-148) Three retrospective studies evaluated the effects of one particular TKI.(128;131;144) Of these, one(128) assessed a portion of the patients

in three previous studies(129;135-137) and another(144) in the other previous study(142) (see **Figure 12**). Another retrospective study, in which at least several patients were likely to be included in some previous second line TKI therapy studies, assessed for chronological changes in mutation status as the main outcome.(148)

Table 23. *BCR-ABL1* and tyrosine kinase inhibitors for CML: characteristics of included studies (second line therapy)

Author, year (Country)	-Sample size, *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	Stage, *n (%)*	Disease status	Median Recent Tumor Load (range)	Prior therapy	-Treatment [n(%)] -Adherence	Median followup, *mo* [range]
High-dose Imatinib									
Jabbour, 2009 (USA)	-84 -Prospective single-center cohort -Convenience sampling	CML in CP Failure to standard-dose imatinib therapy	54 [18-79] (NR)	CP 84 (100)	Hematologic relapse 17 (20); Hematologic resistance 4 (5); cytogenetic relapse 33 (39); cytogenetic resistance 30 (36)	NR	Imatinib 400 mg/d 72 (86); Imatinib 300 mg/d 12 (14)	-Imatinib 800 mg/d 72 (86); Imatinib 600 mg/d 12 (14) -NR	61 [7-89]
Imatinib-based Combination Chemotherapy									
Cortes, 2007a (USA)	-26 -Prospective single-center phase I trial -Unclear	CML CP Imatinib failure (no or loss of CHR at 3 mo; no or loss of ≥ minimal CyR at 6 mo; no or loss of MCyR 12 mo) PS ≤2 (Zubrod) Cr and TBil <2 mg/dl WBC <30×10^9/L Effective contraception for women	62 [29-82] (46)	CP 26 (100)	NR	23.84% (2.4%->100%)	Imatinib ≥400 mg/d 26 (100); IFN + Ara-C 10 (38); IFN + HHT 2 (8); IFN + imatinib 1 (4); IFN + GMCSF 1 (4); decitabine 3 (12); allogeneic SCT 3 (12)	-Imatinib 300 mg d1-21 + tipifarnib 300 mg d1-14 (adjusted according to toxicity) ; q21d -Median 8 cycles (range, 1-49)	26[a]
Oki, 2006 (USA)	-28[b] -Prospective phase II single-center clinical trial -Unclear	Histologically confirmed CML-AP or myeloid BP of chemotherapy for at least 2 weeks before study entry	50 [26-75] (64)	AP 18 (68); BP 10 (32)	Imatinib resistance 25 (89)	NR	Imatinib only 10 (36); imatinib + other treatments 15 (54); no prior treatment 3 (11) IFN 10 (36); chemotherapy other than HU 4 (14); farnesyltrnsferase inhibitor 6 (21) homoharringtonine 4 (14) ; allogeneic SCT 3 (11)	-Imatinib 600 mg qd + Decitabine 15 mg/m^2 qd IV over 1 hour for 10 days (Days 1–5 and 8–12), approximately every 6 weeks as indicated by follow-up counts and marrow studies -91 cycles were administered in total; 2.5 cycles per patient (range 1-12)	NR

[a] Duration of therapy
[b] 25 of the patients had previously received imatinib (3 were newly diagnosed).

Cortes, 2007b (USA)	-23 -Prospective single-center phase I trial -Unclear	Patients >=16 years with imatinib resistant CML (CP, AP, BP)	55 [26-79] (NR)	CP 9 (39); AP 11 (48); BP 3 (13)	Imatinib resistance 23 (100)	NR	Imatinib 23 (100); IFN 16 (70); HU 7 (30); other [including homoharringtonine , decitabine, clofarabine] 7 (30)	-Imatinib 400 mg qd (600mg qd for advanced stages) + lonafarnib 100 mg bid -Dose escalation of lonafarnib was performed by 25 mg bid increments up to a maximum dose of 250 mg bid	CP: 8 [2-18] AP: 2 [1-4]
Dasatinib									
Shah, 2008 (Multinational)	-670 -Multi-center open-label, phase III, 2 x 2 factorial study of different dasatinib administration schedules -Unclear	Adults, CP-CML, acquired hematologic resistance or intolerance to imatinib therapy,	55 [18-84] (47)	CP 670 (100)	Imatinib resistance 491 (73); imatinib intolerance 171 (26)	NR	Imatinib 670 (100); IFN 349 (52); chemotherapy 175 (26); SCT 35 (5)	-4 groups: dasatinib 100 mg qd; 50 mg bid; 140mg qd; 70 mg bid -NR	NR [6-NR] [c]
Hochhaus, 2008 (Multinational)	-387 -Prospective multi-center phase II, open-label, single arm study -Unclear	Adults with CML-CP, imatinib resistant or intolerant	58 [21-85] (49)	CP 387 (100)	Imatinib resistance 288 (74); imatinib intolerance 99 (26)	NR	Imatinib 387 (100); IFN 252 (65); chemotherapy 135 (35); allo-SCT 38 (10); radiotherapy 9 (2)	-Dasatinib70 mg bid -84% of the patients received treatment for >6mo - Median actual daily dose of dasatinib, 101 mg	15 [NR]
Hochhaus, 2007 (Multinational)	-186[d] -Prospective multi-center phase II, open-label, single arm study -Unclear	Adults with CML-CP, imatinib resistant or intolerant	59 [24-79] (46)	CP, 186 (100)	Imatinib resistance 127 (68); imatinib intolerance 59 (32)	NR	Imatinib 186 (100); IFN 130 (70); SCT 17 (9)	-Dasatinib 70mg bid -Median actual daily dose of dasatinib, 101 mg	8 [0-11][e]

[c] Median treatment duration was 8 months (<1 month – 15 moths).
[d] The full cohort results are presented in Hochhaus, 2008.
[e] Median duration of dasatinib treatment.

Quintas-Cardama, 2009[f] (USA)	-293 -Retrospective analysis of 3 trials -Convenience sampling	CML-CP Participants of the previous dasatinib trials Imatinib failure (loss of MCyR or MCyR and CHR after achieving MCyR and CHR; or loss of CHR after achieving CHR only)	57 [18-85] (54)	CP 293 (100) NR	Loss of MCyR 151 (52); loss of MCyR and CHR 33 (11); loss of CHR in no MCyR 109 (37)	NR	Imatinib 293 (100); IFN 197 (67); allogeneic SCT 25 (9)	-Dasatinib 100-180 mg/d -NR	14 [0-31][g]
Kantarjian, 2009 (USA)	-317 -Subgroup analysis of RCT -Unclear	CML AP Imatinib resistance or tolerance	56 [17-84] (57)	AP 317 (100)	Imatinib resistance 233 (74); imatinib tolerance 84 (26)	NR	Imatinib ≤600 mg/d 176 (56); Imatinib >600 mg/d 141 (44); IFN 172 (54); others 140 (44); SCT 28 (9)	-Dasatinib 140 mg (once or in two divided dose)/d -NR	15 [0-35]
Guilhot, 2006 (Multinational)	-107 -Prospective phase II, open-label, single arm study -Unclear	CML-AP with primary or acquired hematologic resistance or intolerance to imatinib	57 [23-86] (51)	AP 107 (100)	Imatinib resistance 99 (93); imatinib intolerance 8 (8)	NR	Imatinib 107 (100); IFN 80 (75); chemotherapy 72 (67); SCT 19 (18); anagrelide 103 (96); radiotherapy 4 (4) typically	-Dasatinib 70 mg bid -NR	8 [0-13][h]
Cortes, 2008 (Multinational)	-157 -Combined analysis of 2 prospective, multi-center phase II, open label clinical trials (START-B and START-L) -Unclear	Imatinib resistant or intolerant myeloid (START-B) or lymphoid (START-L) BP-CML	START-B, 55 [21-81] (58) START-L, 50 [17-73] (52)	BP, 157 (100)	Imatinib resistance 141 (90)	NR	Imatinib 157 (100); IFN 76 (48); chemotherapy 103 (66); SCT 30 (19); radiotherapy 19 (12)	-Dasatinib 70 mg bid -Median duration of therapy, START-B, 3.5 mo; START-L, 2.9 mo Median average daily dose, START-B, 135 mg; START-L, 140 mg	3 [0-21]
Cortes, 2007[i]	-116 -Combined	Imatinib resistant or intolerant myeloid	START-B, 55 [21-71]	BP, 116 (100)	Imatinib resistance 105	NR	Imatinib 116 (100); IFN 61 (53);	-Dasatinib 70 mg bid -Median average daily	START-B, 4 [0-12];

[f] The original cohort results are presented in Shah, 2008; Hochhaus, 2008; and Katarjian, 2007b.
[g] Duration of therapy
[h] Median duration of study therapy [range].
[i] The full cohort results are presented in Cortes, 2008.

(Multinational)	analysis of 2 prospective, multi-center phase II, open label clinical trials -Unclear	(START-B) or lymphoid (START-L) BP-CML	(55) START-L, 47 [19-72] (52)		(91); imatinib intolerance 11 (9)		chemotherapy 82 (71); SCT 23 (20)	dose, START-B, 137mg; START-L, 140mg At 8 months 1 patient discontinued due to non-compliance	START-L, 3 (0-9)[j]
Talpaz, 2006 (USA)	-79[k] -Prospective multi-center phase I trial -Unclear	≥18 y Ph+ CML (or ALL)	56 [15-79] (56)	CP 40 (51); AP 11 (14); BC 28 (35)	Primary imatinib resistance 16 (19); acquired imatinib resistance 54 (64); intolerance to imatinib 12 (14)	ND	Imatinib 72 (86)	-Dasatinib 15-240 mg/d -NR	NR
Sakamaki, 2009 (Japan)	-41[l] -Prospective multi-center phase I/II trial -Unclear	Imatinib resistant or intolerant Ph+ CML	52 [27-73] (65)	CP 30 (73); AP/BC 11 (27)	Imatinib resistance 26 (63); imatinib intolerance 15 (37)	NR	Imatinib 41 (100); IFN 18 (44); others 40 (98); SCT 4 (10) NR	-Dasatinib 100-180 mg/d -Median dose ~98 mg/d	21[m]
Wu, 2008[n] (USA)	-18 -Retrospective analysis of phase I clinical trial -Convenience sampling	Patients with CML resistant or intolerant to imatinib treatment selected based on the availability of mononuclear cells and the lack of BCR-ABL kinase domain mutations[o]	55 [34-70] (39)	CP 11 (61); AP 1 (5); BP 6 (33)	Imatinib resistance 12 (67); imatinib intolerance 6 (33)	NR	Imatinib 18 (100); IFN 14 (78); HU 10 (56); chemotherapy other than HU 8 (44); homoharringtonine 5 (28); nilotinib 2 (11); farnesyltransferase inhibitor 2 (11); all-trans retinoic acid 2 (11)	-Dasatinib -NR	NR
Nilotinib									

[j] Median duration of therapy.
[k] Excluding 5 Ph+ ALL patients. Data on age, gender, and prior treatments were based on the entire population including 5 ALL patients.
[l] Excluding 13 Ph+ALL patients. Data on gender are based on the entire population including 13 ALL patients.
[m] Duration of therapy
[n] The full cohort results are presented in Talpaz, 2006.
[o] Other BCR-ABL mutations were allowed.

Kantarjian, 2007a (Multinational)	-280[p] -Prospective multi-center phase II study -Convenience sampling	Adults with CML-CP, imatinib-resistant or intolerant	58 [21-85] (51)	CP 280 (100)	Imatinib resistance 194 (69); imatinib intolerance 86 (31)	NR	Imatinib 280 (100); IFN 184 (66); HU 233 (83); cytarabine 71 (25); allogeneic or SCT 22 (8)	-Nilotinib 400mg bid -Median cumulative duration of nilotinib dose interruptions: 261 days [1-502] Median dose intensity: 797 mg/ day [151-1112]	NR[q]
Coutre, 2008 (Multiple countries)	-119 -Retrospective analysis of multicenter phase II trial -Convenience sampling	Imatinib-resistantr or -intolerant accelerated phase CML ≥18 y PS ≤2 (WHO)	57 [22-79] (56)	AP 119 (100)	Resistance 96 (81); intolerance 23 (19)	NR	Imatinib 119 (100); HU 109 (92); IFN 69 (58); Cytarabine 31 (26)	-Nilotinib 800-1200 mg/d -Median dose intensity 790 mg/d (range, 180-1149)	7 [0-20][s]
Kantarjian, 2006[t] (Multinational)	-106 -Prospective multi-center phase I study -Unclear	Adults with CML-AP, -BP or with CP in imatinib-resistant	NR	CP 17 (16); AP 56 (53); BP 33 (31)	Imatinib resistance 89 (84); imatinib intolerance 17 (16)	NR	NR	-Patients were assigned to one of nine cohorts receiving different nilotinib doses (from 50-1200mg qd and from 400-600 mg bid) -NR	NR
Miscellaneous									
Kantarjian 2007b[u] (Multinational)	-150 (101 in the dasatinib arm and 49 in the high-dose imatinib arm) -Prospective, multi-center phase II, open label, randomized (2:1) clinical -Unclear	CML-CP with primary or acquired resistance to conventional-dose (400-600mg) imatinib	Dasatinib arm, 51 [24-85] (52); Imatinib arm 51 [24-80] (45)	CP, 150 (100)	Imatinib resistance 150 (100)	NR	Dasatinib arm: hydroxyurea/anagrelide 97 (96); IFN 74 (73); chemotherapy 39 (39), SCT 7 (7). Imatinib arm: hydroxyurea/anagrelide 46 (94); IFN 33 (67); chemotherapy 18 (37), SCT 2 (4)	-Arm A: dasatinib, 70mg bid; Arm B: imatinib, 400 mg bid -NR	15 [1-21]

[p] Study enrolled 318 patients of whom 280 had at least 6 months of follow-up or had discontinued treatment and were included in this report.
[q] Median duration of exposure to nilotinib for the overall cohort (318 patients) was 245 days.
[r] Disease progression from chronic phase to accelerated phase (imatinib ≥600 mg/d); ≥50% increase in peripheral white blood cells, blasts, basophils, or platelets(imatinib ≥600 mg/d); lack of hematologic response after ≥4 wk (imatinib ≥600 mg/d); or presence of BCR-ABL mutation including L248, G250, Q252, Y253, E255, T315, F317, and H396 (imatinib <600 mg/d).
[s] Duration of therapy
[t] This report included 106 cases of CML and 13 cases of BCR-ABL positive ALL. We have only extracted data for CML patients.
[u] Patients with specific mutations (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R) known to be associated with imatinib resistance were excluded from the study.

Cortes, 2007 (USA)	-112 -Retrospective single-center cohort -Convenience sampling	Patients who failed or where intolerant to imatinib or second generation TKIS	51 [17-96] (NR)	CP 38 (34); AP 54 (54); BP 20 (20)	Imatinib resistance 107 (96); imatinib intolerance 5 (4)	NR	Imatinib 112 (100); IFN 69 (62)	-Dasatinib 56 (50); nilotinib 54 (48); bosutinib 2 (2) -NR	17 [4-31][v]

AP, accelerated phase; BP, blastic phase; BU, busulfan; CML, chronic myeloid leukemia; CHR, complete hematologic response; CP= chronic phase; CR, complete remission; CyR, cytogenetic response; Cr, creatinine; GMCSF, granulocyte-macrophage-colony-stimulating factor; HHT, (semisynthetic) homoharringtonine; HU, hydroxyurea; IFN, interferon-α; L-PAM, melphalan; MCyR, major cytogenetic response; NR, not reported; PS, performance status; SCT, stem cell transplantation; TBil, total bilirubin; 6TG, 6-thioguanine; TopoII, topoisomerase II inhibitor; WHO, World Health Organization.

[v] Time since second line TKI treatment initiation.

Characteristics of mutation testing and mutation frequencies

BRC-ABL1 mutations are somatic mutations. The mutational profile of CML can therefore evolve in the various stages of CML. We describe characteristics of mutation testing in the included studies according to stage, namely chronic (**Table 24)**, accelerated or blastic (**Table 25)** or any phase (**Table 26**). Typically, details on how frequently test samples were obtainable or how often mutation tests were successfully performed were not reported. Seventeen of 18 studies performed direct sequencing of the kinase domain of the BCR-ABL1 gene.(128;129;131-135;137;138;141-148) One study did not report technical specification of mutation tests in specific detail.(139) Three of 18 studies also performed denaturing high-performance liquid chromatography in addition to direct sequencing.(135;137;146) Rarely did studies report on tissue sources, how test samples were treated, durations between sampling and mutation tests, or detection sensitivity of the tests. The number of patients assessed for mutations ranged from18 to 563 (median, 112).

Studies reported various frequencies of mutations (**Figure 13**). Any mutations were detected at 24 to 59 percent for patients in the chronic phase, and 27 to 60 percent for accelerated or blastic phase. The T315I mutation was identified at up to 9 and 7 percent of patients in chronic phase and accelerated or blastic phase studies, respectively.

Table 24. *BCR-ABL1* and tyrosine kinase inhibitors for CML: technical specification of mutation testing and frequency of identified mutations (chronic phase)

Study (first author, year)	Assessed patients, *n (% of included patients)*	Staging	Mutation detection method	Source (Tube) [process and time between collection and RNA extraction, *h*]	Detection sensitivity *(%)*	Mutation Frequency, n (%)	
						Mutation +	Mutation -
High-dose Imatinib							
Kantarjian, 2007b[a]	138 (92)	CP	DS	NR	NR	Any 11 (24) T315I NR	35 (76)
Jabbour, 2009	25 (30)	CP	DS	NR	NR	Any 8 (32) T315I 0	17 (68)
Imatinib-based Combination Chemotherapy							
Cortes, 2007a	25 (96)	CP	DS	NR	NR	Any 13 (52) T315I 1 (4)	12 (48)
Dasatinib							
Shah, 2008	563 (84)[b]	CP	DHPLC; DS	PB (NR) [NR]	NR	Any 205(36)	358 (64)
Hochhaus, 2008	345 (89)	CP	NR	NR	NR	Any 139 (40) T315I 3 (9)	206 (60)
Hochhaus, 2007[c]	180 (97)	CP	DHPLC; DS	PB [NR]	NR	Any 73 (41) T315I 3 (2)	107 (59)
Quintas-Carddama, 2009	268 (91)	CP	DS	NR	NR	Any 157 (59) T315I 4 (1)	111 (41)
Kantarjian, 2007b[d]	138 (92)	CP	DS	NR	NR	Any 41 (45) T315I NR	51 (55)
Nilotinib							
Kantarjian, 2007a	182 (65)	CP	DS	PB (NR) [NR]	20	Any 77 (42) T315I 4 (2)	105 (58)

BM, bone marrow; DHPLC, denaturing high-performance liquid chromatography; DS, direct sequencing; PB, peripheral blood.

[a] Patients with specific mutations (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R) known to be associated with imatinib resistance were excluded from the study.
[b] 7 samples were not assessed successfully.
[c] Six individuals had mutations identified "at the site prior to study entry" and were excluded from the report due to lack of confirmation by the study central laboratory.
[d] Patients with specific mutations (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R) known to be associated with imatinib resistance were excluded from the study.

Table 25. *BCR-ABL1* and tyrosine kinase inhibitors for CML: technical specification mutation testing and frequency of detected mutations (accelerated or blastic phase)

Study (first author, year)	Assessed patients, *n (% of included patients)*	Staging	Mutation detection method	Source (Tube) [process and time between collection and RNA extraction, *h*]	Detection sensitivity *(%)*	Mutation Frequency, n (%)	
						Mutation +	Mutation -
Imatinib-based Combination Chemotherapy							
Oki, 2006	26 (89)	AP/BP	DS	PB/BM (NR) [NR]	NR	Any 7 (27) T315I 1 (4)	19 (73)
Dasatinib							
Kantarjian, 2009	292 (92)	AP	DS	NR	NR	Any 136 (47) T315I 20 (7)	156 (53)
Guilhot, 2006	100 (93)	AP	NR[a]	NR	NR	Any 60 (60) T315I 5 (5)	40 (40)
Cortes, 2008	144 (92)	BP	NR	NR	NR	Any 71 (49) T315I 10 (7)	73 (51)
Cortes, 2007c	110 (95)[b]	BP	DS	PB [NR]	NR	Any 54 (49) T315I 8 (7)	56 (51)
Nilotinib							
Kantarjian, 2006	86 (81)	AP	DS	PB (NR) [NR]	NR	Any 37 (43) T315I 2 (2)	49 (57)

BM, bone marrow; DHPLC, denaturing high-performance liquid chromatography; DS, direct sequencing; PB, peripheral blood

[a] "Exploratory assays".

[b] Mutation assessment was not performed at base line for 2/42 patients with available samples.

Table 26. *BCR-ABL1* and tyrosine kinase inhibitors for CML: technical specification of mutation testing and frequency of detected mutations (miscellaneous phases)

Study (first author, year)	Assessed patients, *n (% of included patients)*	Staging	Mutation detection method	Source (Tube) [process and time between collection and RNA extraction, *h*]	Detection sensitivity *(%)*	Mutation Frequency, n (%)	
						Mutation +	Mutation -
Imatinib-based Combination Chemotherapy							
Cortes, 2007b	21 (91)	Any	DS	NR	NR	Any 11 (52) T315I NR	10 (48)
Dasatinib							
Talpaz, 2006	79 (100)	Any	DS	NR	NR	Any 56[a] (71) T315I 7 (9)	23 (29)
Sakamaki, 2009	41 (100)	Any	NR	NR	NR	Any 7 (17) T315I 1 (2)[b]	34 (83)
Wu, 2008	18 (100)	Any	DS	PB/BM (NR) [NR]	NR	Any 3 (17) T315I 0	15 (83)
Nilotinib							
Coutre, 2008	51 (43)	Any	DHPLC + DS	PB (NR) [NR]	20	Any 29 (57) T315I 2 (7)	22 (43)
Miscellaneous							
Cortes, 2007d	112 (100)	Any	DS	PB/BM (NR) [NR]	10-20	Any 61 (54) T315I 10 (<1)	51 (46)

BM, bone marrow; DHPLC, denaturing high-performance liquid chromatography; DS, direct sequencing; PB, peripheral blood.

[a] 29 patients had multiple mutations

[b] This number is based on the entire population including 13 acute lymphoblastic leukemia patients.

Association of BCR-ABL1 mutation test results and mortality

No study evaluated overall survival.

Association of BCR-ABL1 testing results and disease progression outcomes

A single study (Jabbour 2009, median followup 61 months) evaluated event-free survival (defined as loss of partial or complete hematologic response, major cytogenetic response, progression to accelerated or blastic phase, or death from any causes) and transformation-free survival (defined as progression to accelerated or blastic phase, or death from any causes) in 25 patients treated with high-dose imatinib as second line TKI therapy.(131) The study found no statistically significant differences in the time-to-event among patients with versus without mutations (for all assessed mutations, hazard ratio not reported; log rank p=0.96 and 0.51 for event-free and transformation-free survival, respectively). The study found no patients with the T315I mutation but assessed only 30% of the entire patient cohort for the presence of mutations.

Ability of BCR-ABL1 mutation testing to predict TKI treatment failure in hematologic response

In total 16 studies published in 17 publications reported relevant data.(129;129;132-139;142-148) Studies defined treatment failure in non-uniform ways. Two most commonly employed definitions were absence of complete hematologic response or absence of major hematologic response. The latter was defined as absence of complete hematologic response or evidence of leukemia. Only three studies(129;139;141) give details on the timing of patient failure assessments. **Table 27, Figure 14**, and **Figure 15** summarize sensitivities, specificities and likelihood ratios of any mutation or the T315I mutation to predict absence of hematologic response.

When any *BCR-ABL1* mutation was considered (**Figure 14**), almost all studies reported sensitivity and specificity values that are not suggestive of strong predictive ability (i.e., they fall near or below the diagonal line in the plots). Most studies do not fall on areas suggestive of high positive or low negative likelihood ratios and the few that do have small sample sizes and sparse numbers (**Table 27**). The following observations can be made for studies of different treatment types when it comes to the presence of any mutation, regardless of disease stage:

- The 4 high-dose imatinib and imatinib-based combination studies were all above the diagonal, with sensitivity and specificity ranging from 0.35 to 0.83 and from 0.58 to 1.00, respectively.(129;132-134) However, these studies are small, the calculated sensitivity and specificity values have wide confidence intervals, and a range of different mutations was identified in each of them. Therefore, no robust conclusions can be made.
- Eight studies (9 publications(129;135-139;142-144)) pertained to dasatinib. As shown in **Figure 12** some have overlapping populations. Sensitivities and specificities ranged widely from 0.27 to 0.90 and from 0.14 to 0.87, respectively. However, studies were very near or on the diagonal, suggesting lack of predictive ability.
- For nilotinib, three studies had relevant data.(145-147) Sensitivity ranged from 0.56 to 0.71 and specificity ranged from 0.42 to 0.56 for all identified mutations.

When we considered the T315I mutation (**Figure 15**) the general pattern is different. Again, the majority of data pertain to dasatinib treated patients. Across all treatment types, the majority of studies are located in an area of the plot suggestive of high positive likelihood ratio, with high specificity values, albeit low sensitivity values. This implies that presence of the relatively rare T315I mutation is strongly predictive of hematologic failure. Because the T315I mutation is relatively rare, the confidence intervals of the calculated sensitivity and specificity values are wide (**Table 27**).

Table 27. *BCR-ABL1* and tyrosine kinase inhibitors for CML: treatment failure (hematologic response)

Author, year	Stage	Definition of Response (Criteria)	*BCR-ABL1* mutations	Patient, *n*				Sensitivity (95%CI)	Specificity (95%CI)	LR+	LR-
				Mutation +		Mutation –					
				TP	FP	FN	TN				
High-dose Imatinib											
Kantarjian, 2007b[a]	CP	CHR (Talpaz NEJM 2006)	All identified mutations	5	6	3	32	0.63 (0.24, 0.91)	0.84 (0.69, 0.94)	3.96	0.45
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Imatinib-based Combination											
Cortes, 2007a	CP	HR (Kantarjian Ann Intern Med 1995)	All identified mutations	5	8	1	11	0.83 (0.36, 1.00)	0.58 (0.33, 0.80)	1.98	0.29
			T315I	0	1	6	18	0.00 (0.00, 0.46)	0.95 (0.74, 1.00)	<0.01	1.06
Oki, 2006	AP/BP	CHR (ELN)	All identified mutations	6	1	11	8	0.35 (0.14, 0.62)	0.89 (0.52, 1.00)	3.18	0.73
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Cortes, 2007b	Any	CHR (Kantarjian Ann Intern Med 1995)	All identified mutations	11	0	9	1	0.55 (0.32, 0.77)	1.00 (0.03, 1.00)	>100	0.45
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Dasatinib											
Shah, 2008	CP	CHR (ELN)	All identified mutations	27	178	29	29	0.48 (0.35, 0.62)	0.14 (0.10, 0.19)	0.56	3.7
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Hochhaus, 2008	CP	CHR (ELN)	All identified mutations	11	128	19	187	0.37 (0.20, 0.56)	0.59 (0.54, 0.65)	0.9	1.07
			T315I	3	0	27	315	0.10 (0.02, 0.27)	1.00 (0.99, 1.00)	>100	0.9
Hochhaus, 2007	CP	CHR (ELN)	All identified mutations	8	65	9	98	0.47 (0.23, 0.72)	0.60 (0.52, 0.68)	1.18	0.88

[a] Patients with specific mutations (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R) known to be associated with imatinib resistance were excluded from the study.

			T315I	3	0	14	163	0.18 (0.04, 0.43)	1.00 (0.98, 1.00)	>100	0.82
Kantarjian, 2007b[b]	CP	CHR (Talpaz NEJM 2006)	All identified mutations	5	36	1	50	0.83 (0.36, 1.00)	0.58 (0.47, 0.69)	1.99	0.29
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Kantarjian, 2009	AP	Major HR (Guilhot Blood 2007)	All identified mutations	47	89	49	107	0.49 (0.39, 0.59)	0.55 (0.47, 0.62)	1.08	0.93
			T315I	9	2	87	194	0.09 (0.04, 0.17)	0.99 (0.96, 1.00)	9.19	0.92
Guilhot, 2006	AP	Major HR (Guilhot Blood 2006)	All identified mutations	16	44	18	22	0.47 (0.30, 0.65)	0.33 (0.22, 0.46)	0.71	1.59
			T315I	5	0	29	66	0.15 (0.05, 0.31)	1.00 (0.95, 1.00)	>100	0.85
Talpaz, 2006	Any	HR (Talpaz NEJM 2006)	All identified mutations	9	47	1	22	0.90 (0.55, 1.00)	0.32 (0.21, 0.44)	1.32	0.31
			T315I	3	0	7	69	0.30 (0.07, 0.65)	1.00 (0.95, 1.00)	>100	0.7
		Major HR (Talpaz NEJM 2006)	All identified mutations	11	45	3	20	0.79 (0.49, 0.95)	0.31 (0.20, 0.43)	1.13	0.7
			T315I	3	0	11	65	0.21 (0.05, 0.51)	1.00 (0.94, 1.00)	>100	0.79
		CHR (Talpaz NEJM 2006)	All identified mutations	16	40	8	15	0.67 (0.45, 0.84)	0.27 (0.16, 0.41)	0.92	1.22
			T315I	3	0	21	55	0.13 (0.03, 0.32)	1.00 (0.94, 1.00)	>100	0.88
Sakamaki, 2009	Any	Major HR (Talpaz NEJM 2006)	All identified mutations	3	4	8	26	0.27 (0.06, 0.61)	0.87 (0.69, 0.96)	2.05	0.84
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Wu, 2008	Any	CHR (Talpaz NEJM 2006)	All identified mutations	0	3	0	15	NE	0.83 (0.59, 0.96)	NE	NE
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Nilotinib											
Kantarjian, 2007a	CP	CHR[c] (Kantarjian NEJM 2002)	All identified mutations	24	38	10	48	0.71 (0.53, 0.85)	0.56 (0.45, 0.67)	1.6	0.53

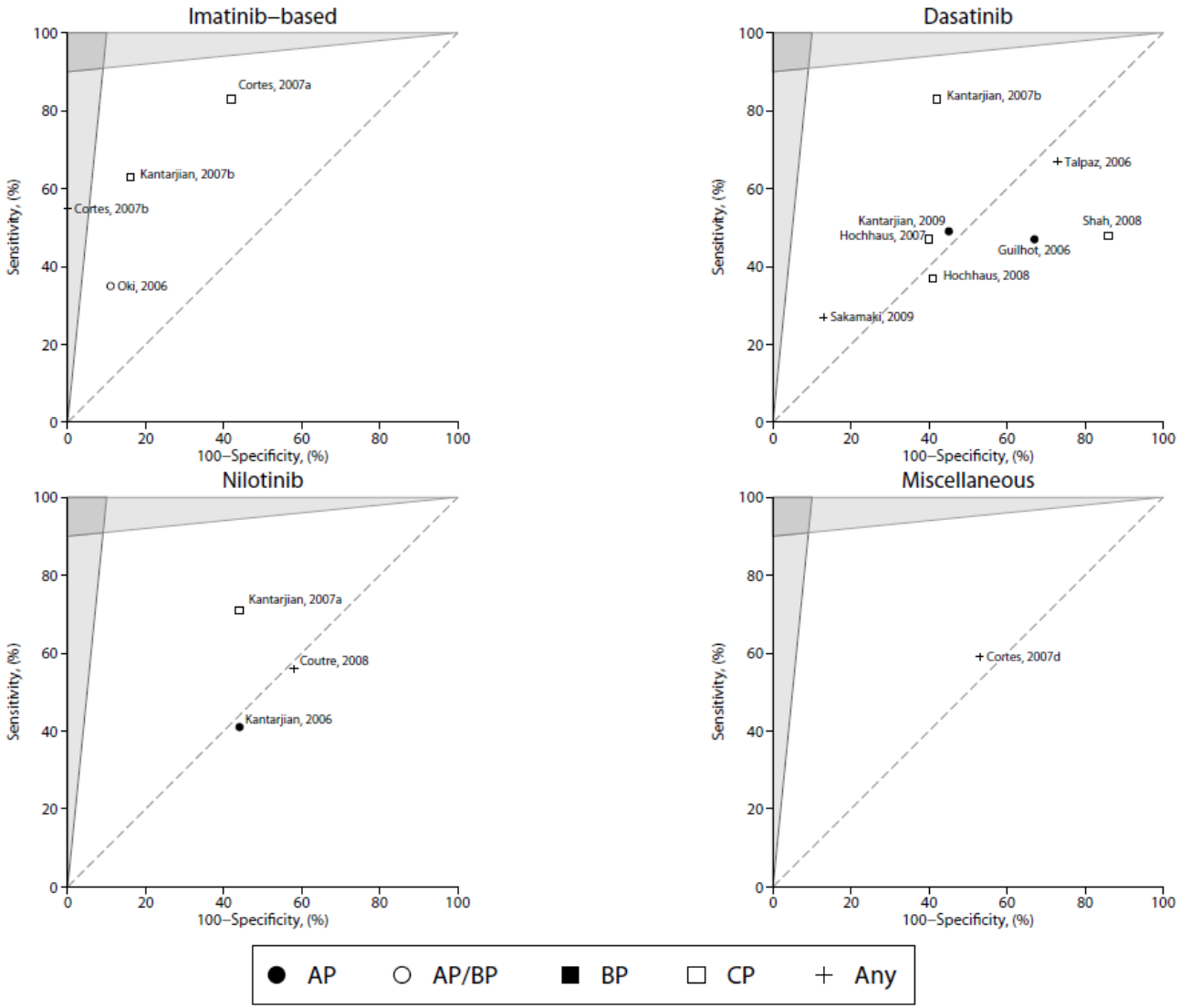
[b] 12 patients had no mutational analysis performed at baseline (9 from the dasatinib arm and 3 from the imatinib arm).
[c] CHR is reported only for patients who did not have CHR at study entry.

			T315I	4	0	30	86	0.12 (0.03, 0.27)	1.00 (0.96, 1.00)	>100	0.88
Kantarjian, 2006	AP	HR (Kantarjian NEJM 2002)	All identified mutations	14	23	20	29	0.41 (0.25, 0.59)	0.56 (0.41, 0.70)	0.93	1.05
			T315I	2	0	32	52	0.06 (0.01, 0.20)	1.00 (0.93, 1.00)	>100	0.94
Coutre, 2008	Any	Major HR + return to chronic phase[d] (ELN)	All identified mutations	15	14	12	10	0.56 (0.35, 0.75)	0.42 (0.22, 0.63)	0.95	1.07
			T315I	0	1	27	23	0.00 (0.00, 0.13)	0.96 (0.79, 1.00)	<0.01	1.04
Miscellaneous											
Cortes, 2007d	Any	HR (Kantarjian NEJM 2002)	All identified mutations	16	45	11	40	0.59 (0.39, 0.78)	0.47 (0.36, 0.58)	1.12	0.87
			T315I	8	2	19	83	0.30 (0.14, 0.50)	0.98 (0.92, 1.00)	12.59	0.72

Major HR

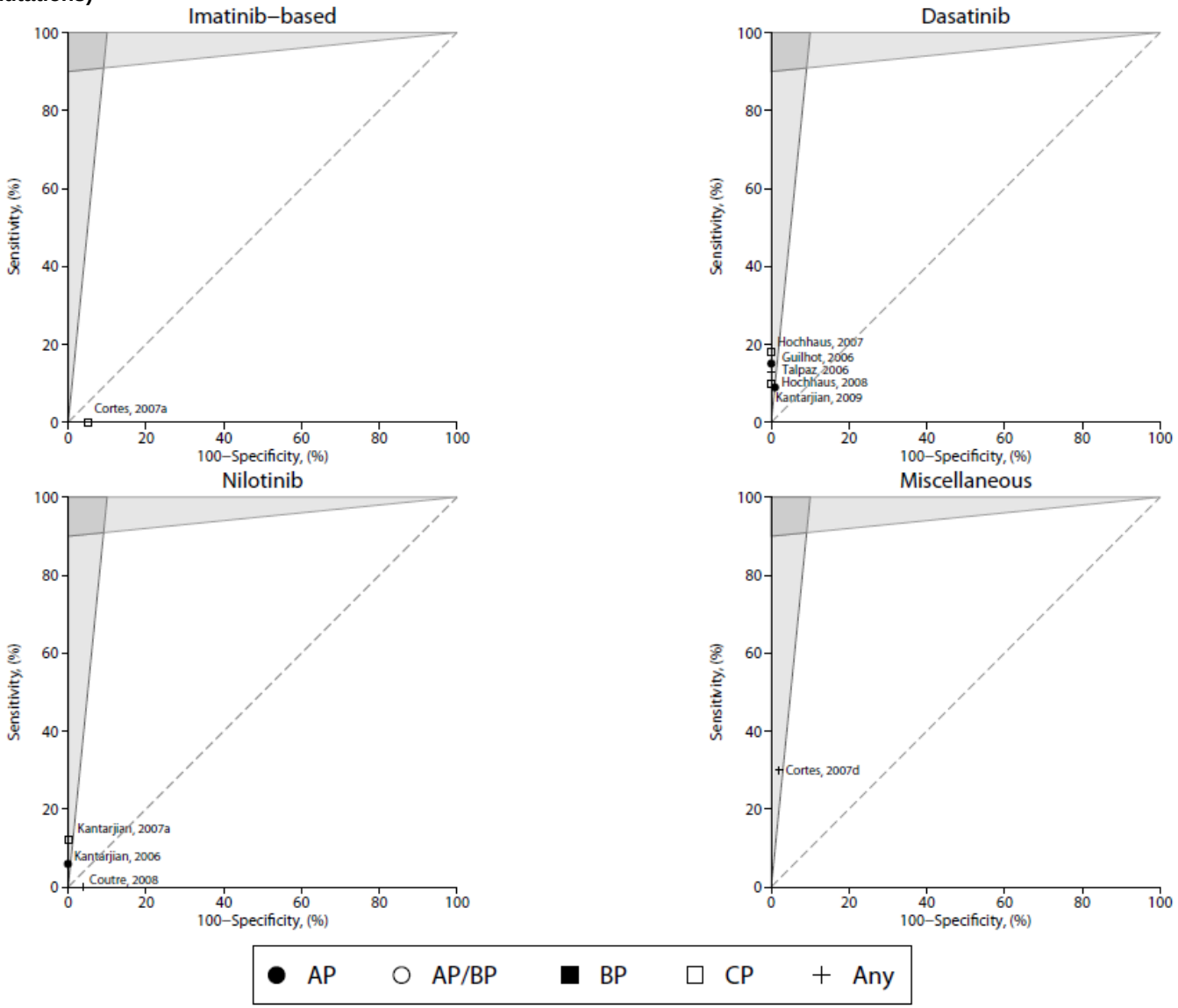
[d] Applicable only to accelerated and blastic phase.

Figure 14. *BCR-ABL1* and tyrosine kinase inhibitors for CML: receiver operating characteristic (ROC) plotting for treatment failure (hematologic response, all mutations)



Individual study estimates of sensitivity and 100-specificity (%) regardless of different definitions of failure in hematologic response are shown. “Imatinib-based” includes high dose imatinib and imatinib-based combination. See the method section for the definition of treatment categories. Abbreviations: AP = accelerated phase; BP = blastic phase; CP = chronic phase

Figure 15. *BCR-ABL1* and tyrosine kinase inhibitors for CML: receiver operating characteristic (ROC) plotting to predict treatment failure (hematologic response, T315I mutations)



Individual study estimates of sensitivity and 100-specificity (%) regardless of different definitions of failure in hematologic response are shown. “Imatinib-based” includes high dose imatinib and imatinib-based combination. See the method section for the definition of treatment categories. Abbreviations: AP = accelerated phase; BP = blastic phase; CP = chronic phase

Ability of BCR-ABL1 mutation testing to predict TKI treatment failure (no cytogenetic response)

In total 18 studies in 20 publications reported relevant data.(128;129;129;129;131-133;135-147) Studies defined treatment failure in non-uniform ways. Two most commonly employed definitions were absence of complete cytogenetic response or absence of major cytogenetic response. The latter was defined as absence of at least partial cytogenetic response. Only three studies(129;139;141) give details on the timing of patient failure assessments. **Table 28, Figure 16**, and **Figure 17** summarize sensitivities, specificities and likelihood ratios of any mutation or the T315I mutation to predict absence of cytogenetic response.

When any *BCR-ABL1* mutation was considered (**Figure 16**), almost all studies reported sensitivity and specificity values that are not suggestive of strong predictive ability (i.e., they fall near or below the diagonal line in the plots). Most studies do not fall on areas suggestive of high positive or low negative likelihood ratios and the few that do have small sample sizes and sparse numbers (**Table 28**). The following observations can be made for studies of different treatment types when it comes to the presence of any mutation, regardless of disease stage:

- The 4 high-dose imatinib and imatinib-based combination studies were scattered throughout the plot, with sensitivity and specificity ranging from 0.33 to 1.00 and from 0.56 to 1.00, respectively.(129;131-133) Two studies lie on regions suggestive of high positive or low negative likelihood ratios. However, these studies are small, the calculated sensitivity and specificity values have wide confidence intervals, and a range of different mutations was identified in each of them.
- Ten studies (12 publications(129;135-139;142-144)) pertained to dasatinib. As shown in **Figure 12** some have overlapping populations. Sensitivities and specificities ranged widely from 0 to 0.76 and from 0.33 to 0.87, respectively. However, studies were near or on the diagonal, suggesting lack of predictive ability.
- For nilotinib, three studies had relevant data.(145-147) Sensitivity ranged from 0.40 to 0.62 and specificity ranged from 0.54 to 0.59.

When we considered the T315I mutation (**Figure 17**) the general pattern is different. Again, the majority of data pertain to dasatinib treated patients. Across all treatment types, the majority of studies are located in an area of the plot suggestive of high positive likelihood ratio, with high specificity values, albeit low sensitivity values. This implies that presence of the relatively rare T315I mutation is strongly predictive of at least cytogenetic failure. Because the T315I mutation is relatively rare, the confidence intervals of the calculated sensitivity and specificity values are wide (**Table 28**).

Table 28. *BCR-ABL1* and tyrosine kinase inhibitors for CML: treatment failure (cytogenetic response)

Author, year	Stage	Response criteria	*BCR-ABL1* mutations	Patient, *n*				Sensitivity (95%CI)	Specificity (95%CI)	LR+	LR-
				Mutation +		Mutation –					
				TP	FP	FN	TN				
High-dose Imatinib											
Kantarjian, 2007b[a]	CP	Major CyR (ELN)	All identified mutations	8	3	23	12	0.26 (0.12, 0.45)	0.80 (0.52, 0.96)	1.29	0.93
			T315I	2	1	29	14	0.06 (0.01, 0.21)	0.93 (0.68, 1.00)	0.97	1
Jabbour, 2009	CP	Major CyR	All identified mutations	2	6	0	17	1.00 (0.16, 1.00)	0.74 (0.52, 0.90)	3.83	<0.01
			T315I	0	0	2	23	0.00 (0.00, 0.84)	1.00 (0.85, 1.00)	>100	1
Imatinib-based Combination											
Cortes, 2007a	CP	CyR (ELN)	All identified mutations	9	4	7	5	0.56 (0.30, 0.80)	0.56 (0.21, 0.86)	1.27	0.79
			T315I	0	1	16	8	0.00 (0.00, 0.21)	0.89 (0.52, 1.00)	<0.01	1.13
Oki, 2006	AP/BP	Major CyR	All identified mutations	7	0	14	8	0.33 (0.15, 0.57)	1.00 (0.63, 1.00)	>100	0.67
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Dasatinib											
Shah, 2008	CP	Major CyR	All identified mutations	116	89	138	220	0.46 (0.39, 0.52)	0.71 (0.66, 0.76)	1.59	0.76
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Hochhaus, 2008	CP	Major CyR	All identified mutations	59	80	83	123	0.42 (0.33, 0.50)	0.61 (0.54, 0.67)	1.05	0.96
			T315I	3	0	139	203	0.02 (0.00, 0.06)	1.00 (0.98, 1.00)	>100	0.98
Hochhaus, 2007	CP	Major CyR	All identified mutations	37	36	49	58	0.43 (0.32, 0.54)	0.62 (0.51, 0.72)	1.12	0.92
			T315I	3	0	83	94	0.03 (0.01, 0.10)	1.00 (0.96, 1.00)	>100	0.97
Quintas-Cardama, 2009	CP	CCyR (ELN)	All identified mutations	83	74	47	64	0.64 (0.55, 0.72)	0.46 (0.38, 0.55)	1.19	0.78
			T315I	4	0	126	138	0.03 (0.01, 0.08)	1.00 (0.97, 1.00)	>100	0.97
Kantarjian, 2007b[b]	CP	Major CyR (ELN)	All identified mutations	22	19	23	28	0.49 (0.34, 0.64)	0.60 (0.44, 0.74)	1.21	0.86

[a] Patients with specific mutations (L248V, G250E, Q252H/R, Y253H/F, E255K/V, T315I/D, F317L, and H396P/R) known to be associated with imatinib resistance were excluded from the study.

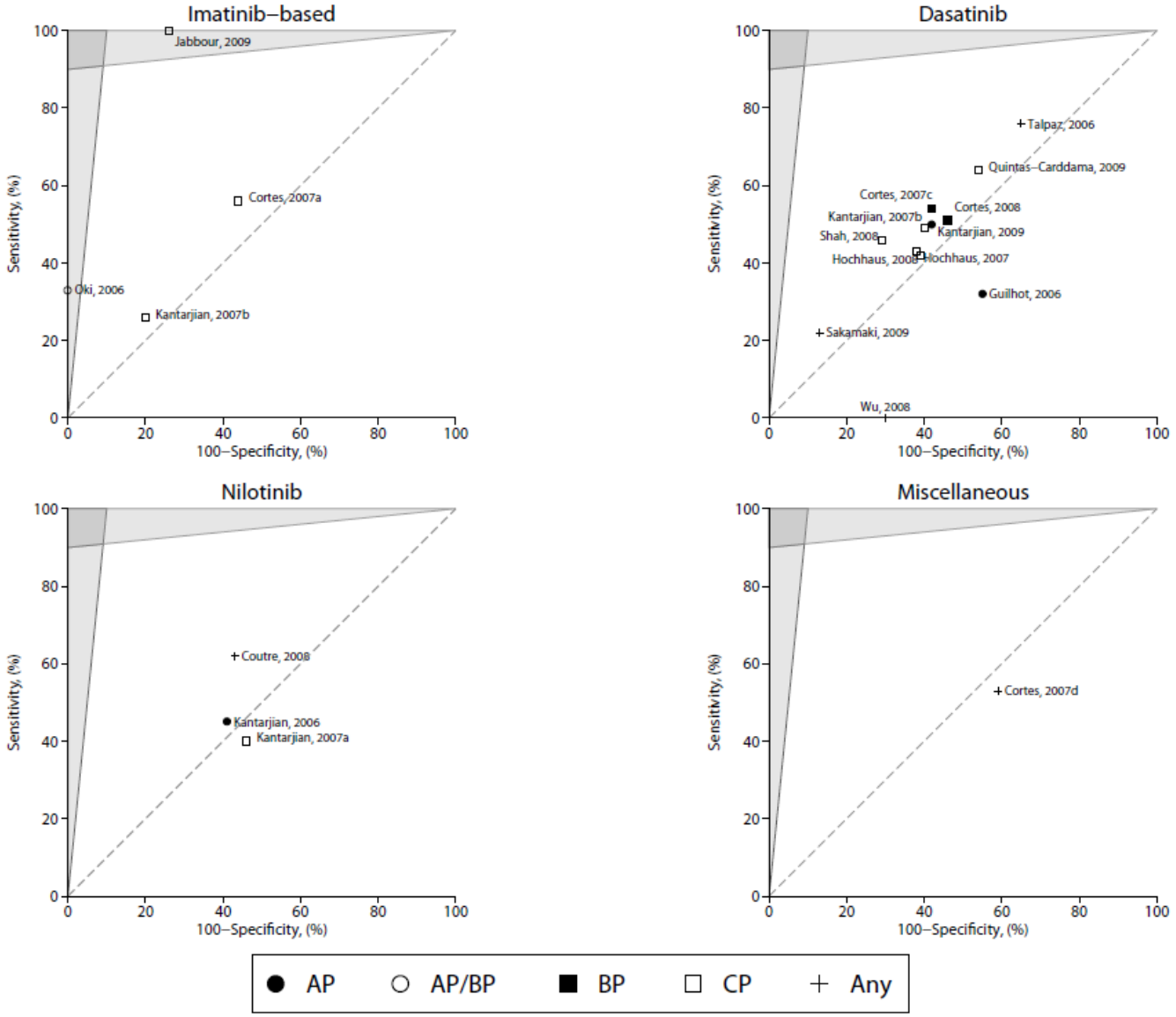
[b] 12 patients had no mutational analysis performed at baseline (9 from the dasatinib arm and 3 from the imatinib arm).

Author, year	Stage	Response criteria	*BCR-ABL1* mutations	Patient, *n*				Sensitivity (95%CI)	Specificity (95%CI)	LR+	LR-
				Mutation +		Mutation –					
				TP	FP	FN	TN				
			T315I	2	1	43	46	0.04 (0.01, 0.15)	0.98 (0.89, 1.00)	2.09	0.98
Kantarjian, 2009	AP	Major CyR (ELN)	All identified mutations	89	47	90	66	0.50 (0.42, 0.57)	0.58 (0.49, 0.68)	1.2	0.86
			T315I	11	0	168	113	0.06 (0.03, 0.11)	1.00 (0.97, 1.00)	>100	0.94
Guilhot, 2006	AP	Major CyR (ELN)	All identified mutations	12	18	25	15	0.32 (0.18, 0.50)	0.45 (0.28, 0.64)	0.59	1.49
			T315I	5	0	32	33	0.14 (0.05, 0.29)	1.00 (0.89, 1.00)	>100	0.86
Cortes, 2008	BP	Major CyR (ELN)	All identified mutations	46	25	44	29	0.51 (0.40, 0.62)	0.54 (0.40, 0.67)	1.1	0.91
			T315I	10	0	80	54	0.11 (0.05, 0.19)	1.00 (0.93, 1.00)	>100	0.89
Cortes, 2007c	BP	Major CyR (ELN)	All identified mutations	36	18	31	25	0.54 (0.41, 0.66)	0.58 (0.42, 0.73)	1.28	0.8
			T315I	8	0	59	43	0.12 (0.05, 0.22)	1.00 (0.92, 1.00)	>100	0.88
Talpaz, 2006	Any	Major CyR (ELN)	All identified mutations	34	22	11	12	0.76 (0.60, 0.87)	0.35 (0.20, 0.54)	1.17	0.69
			T315I	3	0	42	34	0.07 (0.01, 0.18)	1.00 (0.90, 1.00)	>100	0.93
		CCyR (ELN)	All identified mutations	40	16	15	8	0.73 (0.59, 0.84)	0.33 (0.16, 0.55)	1.09	0.82
			T315I	3	0	52	24	0.05 (0.01, 0.15)	1.00 (0.86, 1.00)	>100	0.95
Sakamaki, 2009	Any	Major CyR (ELN)	All identified mutations	4	3	14	20	0.22 (0.06, 0.48)	0.87 (0.66, 0.97)	1.7	0.89
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Wu, 2008	Any	Major CyR (ELN)	All identified mutations	0	3	8	7	0.00 (0.00, 0.37)	0.70 (0.35, 0.93)	<0.01	1.43
			T315I	NR	NR	NR	NR	NR	NR	NR	NR
Nilotinib											
Kantarjian, 2007a	CP	Major CyR (ELN)	All identified mutations	45	32	67	38	0.40 (0.31, 0.50)	0.54 (0.42, 0.66)	0.88	1.1
			T315I	4	0	108	70	0.04 (0.01, 0.09)	1.00 (0.95, 1.00)	>100	0.96
Kantarjian, 2006	AP	CyR[c] (ELN)	All identified mutations	19	18	23	26	0.45 (0.30, 0.61)	0.59 (0.43, 0.74)	1.11	0.93
			T315I	0	0	42	44	0.00 (0.00, 0.08)	1.00 (0.92, 1.00)	NE	1

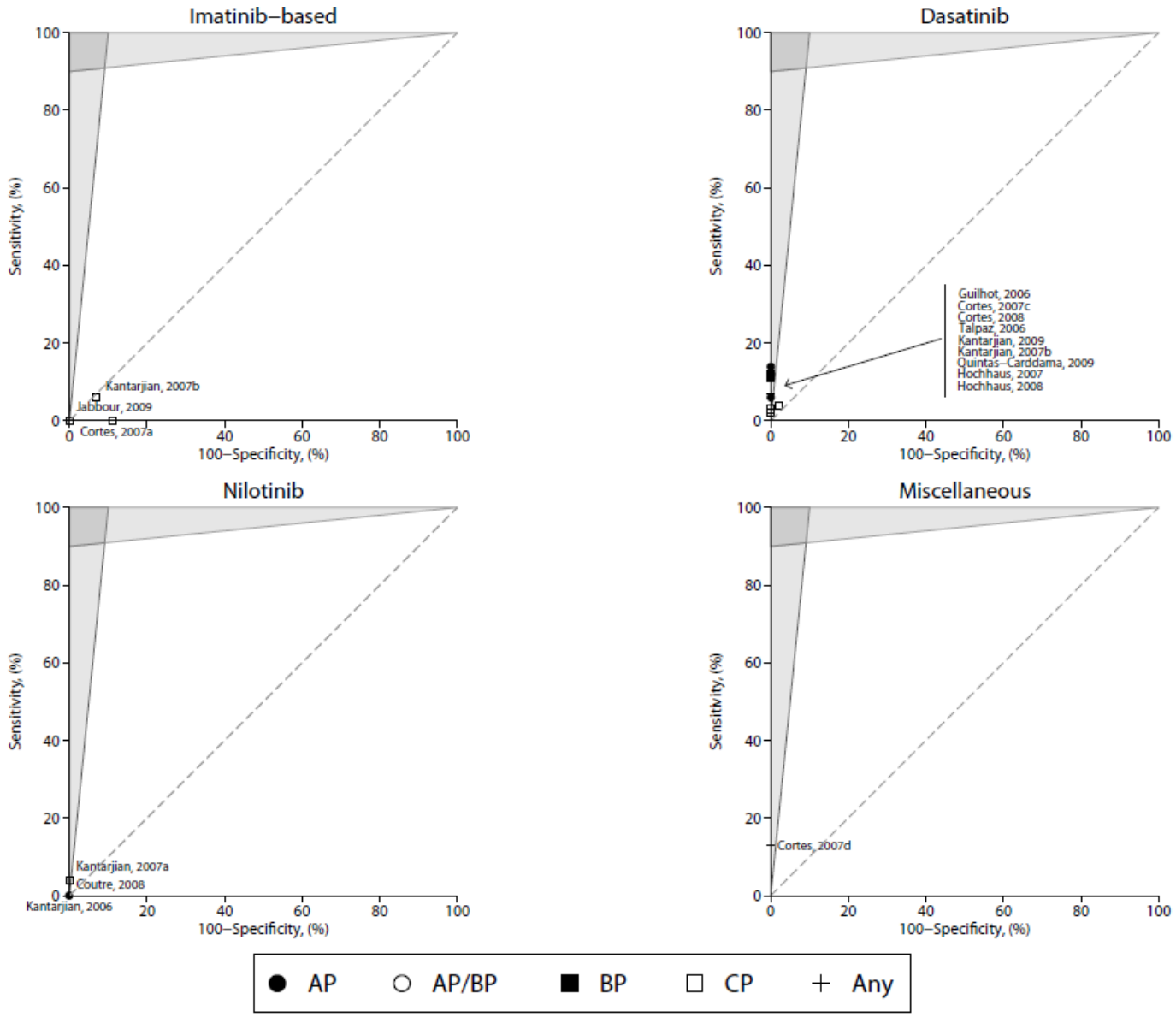
[c] Defined as minimal, minor, major, and complete CyR

Author, year	Stage	Response criteria	*BCR-ABL1* mutations	Patient, *n*				Sensitivity (95%CI)	Specificity (95%CI)	LR+	LR-
				Mutation +		Mutation –					
				TP	FP	FN	TN				
Coutre, 2008	Any	Major CyR (ELN)	All identified mutations	23	6	14	8	0.62 (0.45, 0.78)	0.57 (0.29, 0.82)	1.45	0.66
			T315I	1	0	36	14	0.03 (0.00, 0.14)	1.00 (0.77, 1.00)	>100	0.97
Miscellaneous											
Cortes, 2007d	Any	CyR (ELN)	All identified mutations	37	24	29	22	0.56 (0.43, 0.68)	0.48 (0.33, 0.63)	1.07	0.92
			T315I	10	0	56	46	0.15 (0.08, 0.26)	1.00 (0.92, 1.00)	>100	0.85
		CCyR (ELN)	All identified mutations	41	20	37	14	0.53 (0.41, 0.64)	0.41 (0.25, 0.59)	0.89	1.15
			T315I	10	0	68	34	0.13 (0.06, 0.22)	1.00 (0.90, 1.00)	>100	0.87

Figure 16. *BCR-ABL1* and tyrosine kinase inhibitors for CML: receiver operating characteristic (ROC) plotting to predict treatment failure (cytogenetic response, all mutation)



Individual study estimates of sensitivity and 100-specificity (%) regardless of different definitions of failure in cytogenetic response are shown. “Imatinib-based” includes high dose imatinib and imatinib-based combination. See the method section for the definition of treatment categories. Abbreviations: AP = accelerated phase; BP = blastic phase; CP = chronic phase**Figure 17. *BCR-ABL1* and tyrosine kinase inhibitors for CML: receiver operating characteristic (ROC) plotting to predict treatment failure (cytogenetic response, T315I mutation).**



Individual study estimates of sensitivity and 100-specificity (%) regardless of different definitions of failure in cytogenetic response are shown. “Imatinib-based” includes high dose imatinib and imatinib-based combination. See the method section for the definition of treatment categories. Abbreviations: AP = accelerated phase; BP = blastic phase; CP = chronic phase*Ability of BCR-ABL1 mutation testing to predict TKI treatment failure for molecular response*

Only Quintas-Cardama 2009 evaluated major molecular response (defined as ratio of BCR-ABL1 to ABL1 gene less than 0.1%) in 108 patients treated with dasatinib as second line TKI therapy (**Table 29**).(128) Estimated sensitivity was 0.69 and specificity 0.43 to predict failure in achieving molecular response.

Table 29. *BCR-ABL1* and tyrosine kinase inhibitors for CML: treatment failure (molecular response)

Author, year	Stage	Response criteria	*BCR-ABL1* mutations	Patient, *n*				Sensitivity (95%CI)	Specificity (95%CI)	LR+	LR-
				Mutation +		Mutation –					
				TP	FP	FN	TN				
Dasatinib											
Quintas-Cardama, 2009	CP	Major MolR (ELN)	All identified mutations	44	25	20	19	0.69 (0.56, 0.80)	0.43 (0.28, 0.59)	1.21	0.72
			T315I	NR	NR	NR	NR	NR	NR	NR	NR

CI, confidence interval; ENL, European Leukemia Net criteria compatible; Major MolR, major molecular response (defined as Ratio of *BCR-ABL1* to *ABL1* gene ≤0.1%);

Third line TKI therapy studies

One included study pertained to the use of dasatinib as third line therapy.(149) This study consisted of 23 patients with CML refractory to imatinib and nilotinib. It is likely that at least several patients in this study were included in at least some second line therapy studies (see legend of **Figure 12)**.

The majority of subjects had CML in the accelerated or blastic phase with a median tumor load of 72 percent (**Table 30**). Direct sequencing was performed on all but one patient. Sixteen patients had at least one detected mutation in *BCR-ABL1*. The T315I mutation was detected in two patients. There were 7 mutations in the P-loop region, and 2 mutations each in the C-loop and A-loop regions of the BCR-ABL1 fusion protein.

Association of BCR-ABL1 mutation test results and mortality

The study did not report mortality outcomes.

Ability of BCR-ABL1 mutation testing to predict response

Of the 16 patients with detected mutations, 13 showed hematologic response to dasatinib, and 8 showed cytogenetic response. None of the 6 patients who were negative for *BCR-ABL1* mutations responded to dasatinib. The two patients with the T315I mutation did not respond to treatment.

Because the sample size of this study is very small and there are cells with zero counts in **Table 31,** the estimates of sensitivity, specificity and likelihood ratios are not robust.

Table 30. *BCR-ABL1* and tyrosine kinase inhibitors for CML: characteristics of included studies (third line therapy)

Author, year (Country)	-Sample size, *n* -Study design -Sampling	Selection criteria	Median age, *y* [range] (% Men)	Staging distribution, *n (%)*	Disease status	Median Recent Tumor Load (range)	Prior therapy (n [%])	-Treatment -Adherence	Median followup, *mo* [range]
Quintas-Cardama, 2007 (USA)(132)	-23 -Retrospective single-center cohort -Convenience sampling	Failure to imatinib and nilotinib	58 [19-76] (NR)	Late CP 3 (13); second CP 1 (4); AP 10 (43); BP 9 (39)	Loss of response 15 (65); hematologic resistance 8 (35)	72% (0.03%-100%)	Imatinib 23 (100); nilotinib 23 (100); IFN 9 (39); allogeneic SCT 2 (9)	-Dasatinib 70 mg/d 13 (57); dasatinib 140 md/d 9 (39); dasatinib 240 mg/d 1 (4) -NR	4 [1-10][a]

AP, accelerated phase; BP, blastic phase; CP, chronic phase; IFN, interferon; SCT, stem cell transplantation

Table 31. *BCR-ABL1* and tyrosine kinase inhibitors for CML: treatment failure (third line therapy)

Author, year	Outcome (followup)	*BCR-ABL1* mutations	Mutation, no response	Mutation, response	No mutation, no response	No mutation, response	Sensitivity (95 %CI)	Specificity (95% CI)	LR+	LR-
Quintas-Cardama, 2007	Hematologic response (4 mo)	All identified mutations	3	13	6	0	0.33 (0.07, 0.70)	0.00 (0.00, 0.25)	0.36	18
		T315I	2	0	7	13	0.22 (0.03, 0.60)	1.00 (0.75, 1.00)	7.00	0.78
	Major cytogenetic response (4 mo)	All identified mutations	8	8	6	0	0.57 (0.29, 0.82)	0.00 (0.00, 0.37)	0.60	8
		T315I	2	0	12	8	0.14 (0.02, 0.43)	1.00 (0.63, 1.00)	3.00	0.88

[a] Duration of therapy

Studies of *BCR-ABL1* mutation monitoring

Seven studies reported relevant data (**Table 32**).(150-156) All but one(155) studies were retrospective, and the median sample size was 144 (range, 13 to 319). All studies generally included patients with chronic phase CML treated with standard- or high-dose imatinib as first line TKI therapy. In 3 studies, patients with accelerated phase CML were also included (up to 28 percent of total sample size).(153;154;156)

Five of seven studies assessed routine periodical (typically every 3 to 6 months) monitoring of mutation status.(150-154) Three of them were reported by a same group of investigators in the UK.(150-152) All studies typically performed direct sequencing with or without pyrosequencing in peripheral blood samples collected during routine monitoring of *BCR-ABL1* transcript levels (**Table 33**). Across the five studies the cumulative incidence of emerging mutations ranged from 4 to 23 percent or patients.

The remaining two studies assessed mutations only once during treatment course.(155;156) Only one study specified when the mutation testing was performed (as at 6 months or when imatinib resistance was suspected).(155) In one study mutations emerged at 1 percent frequency.(155)

The seven studies assessed various clinical outcomes suggestive of imatinib resistance in non-uniform followup periods (**Table 34)**. Generally, patients who developed mutations during treatment experienced higher imatinib resistance compared with those with no mutations detected during the followup.

Table 32. *BCR-ABL1* and tyrosine kinase inhibitors for CML: characteristics of included studies (monitoring)

Author, year (Country)	-Sample size, *n* -Study design - Sampling	Selection criteria	Median age, *y* [range] (% Men)	-Staging, *n (%)* & -Risk group, *n (%)*	Response status (median recent *BCR-ABL1* level [range], *%)*	Purpose and timing of mutation testing	Prior therapy	-Treatment -Adherence	Median followup, *mo* [range]
Routine monitoring									
Khorashad, 2008 (UK)	-319 -Retrospective analysis of two prospective trials -Convenience sampling	BCR-ABL+ CML CP treated with imatinib >6 mo followup Sequential samples available	47 [18-73] (55)	-Early (<6 mo) CP 171 (54); late (≥6 mo) CP 148 (46) -NR	NR	q 6 mo; more often when imatinib resistance suspected	INF 127 (39)	-Imatinib 400 mg/d -NR	51 [12-90][a]
De Lavallade, 2008 (UK)	-204 -Retrospective analysis of single-center cohort -"Consecutive"	BCR-ABL+ CML CP treated with imatinib	46 [18-79] (57)	-CP 204 (100) -Sokal score: low 59 (29); intermediate 86 (42); high 59 (29)	NR	q 6 mo; more often when imatinib resistance suspected	HU	-Imatinib 400 mg/d -54 (26%) discontinued imatinib at a median 16 mo.	38 [12-85][b]
Marin, 2009 (UK)	-145 (total samples NR) -Retrospective?; single-center -Consecutive sampling?	Adult BCR-ABL+ CML (CP) Achieved CCR	NR	-CP 145 (100) -NR	CCyR (0.4 [0.3-18])	q 6 mo; more often when imatinib resistance suspected	No 145 (100)	-Imatinib 400 mg qd adjusted per tolerance and response -NR	48 [13-95]
Branford, 2003 (Australia)	-144 (353 samples) -Retrospective; multi-center -Convenience sampling	Imatinib treatment At least one sample with nondegraded RNA with measurable level of bcr-abl	NR	-Early CP, 40 (28); Late CP 64 (44); AP 40 (28) [~ENL] -NR	No failure 142 (NR); Imatinib failure 2 (NR)	Available samples only[c]	No 104 (72); IFN 16 (11); HU 24 (17)	-Imatinib 400 mg qd (CP); Imatinib 600 mg qd (AP) NR	NR
Wei, 2006 (Sweden)	-40 -Retrospective single-center cohort	All CML treated with imatinib	NR [23-80] (53)	-Early (<12 mo) CP 30 (75); 7 late (≥12 mo) CP 7 (18); AP 3 (8) -NR	NR	Before therapy; q 3 mo until *BCR-ABL*1 transcripts undetectable; q 6	NR	-Imatinib 400 mg/d (CP); 600 mg/d (AP) NR	NR

[a] Surviving patients only.
[b] Surviving patients only.
[c] Samples were originally used for routine *BCR-ABL1* transcript level assessment (frequency of sampling not reported). Median 2 samples per patient (range, 1-18) with assessable RNA available for mutation testing.

	-NR					mo thereafter; q 3 mo if imatinib resistance suspected			
Single interim assessment									
Hughes, 2008 (Australia)	-103 -Prospective multi-center single arm trial -Unclear	BCR-ABL+ CML CP treated with only HU or anagrelide Diagnosed within the past 8 mo PS 0-2 (ECOG) Adequate renal and hepatic function	50 [19-76] (64)	-CP 103 (100) -Sokal score: good 26 (25); intermediate 30 (29); poor 28 (27) Hasford score: low 42 (41); Intermediate 21 (20); high 19 (18)	NR	At 6 mo (n=94) or when one met the criteria of mutation screening[d] (n=7)	HU or anagrelide	-Imatinib 600 mg/d; or 800 mg/d if no CHR at 3mo, no MCyR at 6mo, no CCyR at 9 mo, ≥0.01% BCR-ABL by RQ-PCR at 12 mo -NR	NR
Chu, 2005 (USA)	-13 -Retrospective; single-center -Convenience Sampling	BCR-ABL+ CML CP or AP Achieved CCyR with imatinib Adequate sample available	54 [37-70] (46)	-Early CP, 7 (54); Late CP, 5 (38); AP, 1 (8) -NR	NR[e]	1 available sample per patient[f]	IFN 6 (46)	-Imatinib 400 mg/d -NR	15 [2-30][g]

AP, accelerated phase; BP, blastic phase; BU, busulfan; CML, chronic myeloid leukemia; CCyR, complete cytogenetic response; CHR, complete hematologic response; CyR, cytogenetic response; Cr, creatinine; FISH, fluorescent in situ hybridization; GMCSF, granulocyte-macrophage-colony-stimulating factor; HHT, (semisynthetic) homoharringtonine; HU, hydroxyurea; IFN, interferon-α; L-PAM, melphalan; MCyR, major cytogenetic response; PS, performance status; RQ-PCR, real-time quantitative polymerase chain reaction; SCT, stem cell transplantation; TBil, total bilirubin; 6TG, 6-thioguanine; TopoII, topoisomerase II inhibitor; WHO, World Health Organization.

[d] Significant rise in RQ-PCR (defined as >2-fold rise).
[e] 7 out of 8 patients had detectable disease by FISH.
[f] Bone marrow mononuclear cells or G-CSF mobilized PBSCs were assessed at a median 6 months (range, 3-25) from the start of therapy (reason or timing of sampling not reported).
[g] No information on followup period for 4 patients.

Table 33. *BCR-ABL1* and tyrosine kinase inhibitors for CML: technical specification of mutation testing (monitoring)

Study (first author, year)	Assessed patients, *n (% of included patients)*	Mutation detection method	Source (Tube) [process and time between collection and RNA extraction, *h*]	Detection sensitivity *(%)*
Routine monitoring				
Khorashad, 2008	319 (100)	DS/PS	PB (NR) [NR]	NR
De Lavallade, 2008	204 (100)	DS/PS	PB (NR) [NR]	NR
Marin, 2009	145 (100)	DS/PS	PB (EDTA or heparin) [NR]	30/10
Branford, 2003	144 (100)	DS	PB (NR) [processed into Trizol RNA stabilizer and stored at -80°C, NR]	20
Wei, 2006	40 (100)	DS	PB (EDTA) [store at -20 °C]	20-30
Single interim assessment				
Hughes, 2008	100 (97)[a]	DS	NR	NR
Chu, 2005	13 (100)	SS	BM 11; PBSC 2 (NR) [NR]	NR

BM, bone marrow; DS, direct sequencing; EDTA, ethylenediaminetetraacetate; NR, not reported; PB, peripheral blood; PBSC, peripheral blood stem cell; PS, pyrosequencing; RNA, ribo nucleic acid; SS, subcloning and sequencing.

[a] Some patients who met the criteria of mutation screening may have undergone the mutation test multiple times. Two patients were not tested for no sample and one for no detectable level of BCR-ABL gene.

Table 34. *BCR-ABL1* and tyrosine kinase inhibitors for CML: reported outcomes by presence of mutations (monitoring)

Author, year	Evaluated patients, *n* (median followup, *mo*)	Main outcome assessed	Findings
Routine monitoring			
Khorashad, 2008	319 (51)	PFS[a]	Forty-nine (17%) out of 282 patients without mutations detected during treatment developed progression. In contrast, of 37 patients who developed detectable mutations during imatinib therapy, 17 patients (46%) progressed (RR =3.7; P<0.0001). In the final model in multivariate analyses based on a forward stepwise selection, the development of mutations was the only significant independent prognostic factor of progression (RR = 2.3; P=0.01) Subgroup analyses were performed for 250 patients who remained CHR at 2 years (RR=3.0; P=0.004).
De Lavallade, 2008	204 (38)	Loss of CCyR, loss of CHR; PFS; and OS	Eleven (5%) patients developed mutations during the followup. The development of mutations during treatment was a significant predictive factor for loss of CCyR (RR=13.4; P<0.001) but not for loss of CHR, PFS, or OS.
Marin, 2009	161 (48)	Loss of CCyR in CR[b]	Of 161 patients with CP who achieved CCyR in first-line treatment with imatinib, 6 (4%) developed mutations, all of who (100%) lost CCyR during the treatment. In those without mutations (n=155), only 12 patients (8%) lost CCyR. The median time from the detection of mutations to loss of CCyR was 17 months (range, 1-39).
Branford, 2003	144 (NR)	Imatinib resistance for CP[c] and for AP[d]	Twenty-seven (19%) out of 144 patients who had at least one test result developed detectable mutations. Mutations were detected at a median 8 months (range 3-18). None of the patients with early CP (n=40) developed mutations during first-line treatment with imatinib. Fourteen (22%) out of 64 patients with late CP developed mutations during imatinib therapy. Of these, 11 (79%) developed imatinib resistance, whereas only two (4%) experienced imatinib resistance out of 50 patients with late CP without mutations throughout the treatment period. All patients (n=13, 100%) with AP who developed mutations during imatinib therapy developed imatinib resistance, whereas only two (8%) experienced imatinib resistance out of 25 patients with AP without mutations throughout the treatment period.
Wei, 2006	40 (NR)	Acquired imatinib resistance[e]	Out of 9 patients who developed detectable mutations, 7 (78%) experienced an acquired imatinib resistance, whereas only one (3%) out of 31 patients without mutations throughout the followup experienced an acquired imatinib resistance. No patients with detectable mutations during treatment developed primary imatinib resistance (i.e., ≥minimal CyR at 6 mo; MCyR at 12 mo; persistent detectable BCR-ABL gene in blood). Subgroup analyses for early CP patients (n=30) had similar results.
Single interim assessment			
Hughes, 2008	100 (NR)	Not defined	No specific results were reported on those who met the mutation screening criteria and underwent the test[f]. Only one patient with a mutation out of 94 patients who did not meet the criteria and underwent the test at 6 mo had a significant rise in BCR-ABL during followup.
Chu, 2005	13 (15)	Relapse[g]	Two out of 5 patients with mutations experienced relapse, whereas none developed recurrent leukemia in 8 patients without detectable mutations (all in CCyR at last followup[h])

AP, accelerated phase; BC, blastic phase; CCyR, complete cytogenetic response; CP, chronic phase; CR, complete response; MCyR, major cytogenetic response; OS, overall survival; PFS, progression free survival;

[a] Survival without evidence of AP or BC.
[b] Druker et al, NEJM 2006.
[c] Kantarjian et al, NEJM 2002.
[d] Talpaz et al. Blood 2002.
[e] Loss of CHR; loss of MCyR/CCyR; and transformation to BC.
[f] Six (67%) out of 9 patients who lost response had mutations detected. Of these 6 patients, 2 progressed to BC at 3 and 6 months. Another two lost CHR and two additional patients lost CCyR.
[g] Morphologic or cytogenetic evidence of recurrent leukemia.
[h] Four out of 5 patients who had rising BCR-ABL gene level in peripheral blood developed mutations at last followup.

3.4 Discussion

In our systematic review of the literature, presence of any *BCR-ABL1* mutation does not appear to predict differential response to treatment in CML patients treated with imatinib-, dasatinib-, or nilotinib-based regimens. There is consistent evidence that presence of the relatively rare T315I mutation can predict TKI treatment failure, mainly in terms of hematologic and cytogenetic response. In contrast, there is no evidence that that presence of *any BCR-ABL1* mutation can differentiate response to TKI therapies. Further, the majority of evidence pertains to the short term surrogate outcomes of hematologic, cytogenetic or molecular response. Data on overall or progression-free survival are sparse. Finally, most evidence is on second line TKI treatments, especially dasatinib and nilotinib, and originates from a small number of referral cancer centers where those agents were first-tested before becoming more widely available.

Less than 9 percent of patients in any single included study had the relatively rare T315I mutation. Across all studies all, or almost all, patients with the T315I mutation have adverse response to treatment. This observation is in accordance with the prevailing knowledge in the field, and with the literature review performed for the updated European Leukemia Net recommendations, which suggests that patients with the T315I mutation consider allogeneic stem cell transplantation.(118) The ability of T315I mutation to predict TKI treatment failure seems to be similar across all studies, and thus applicable to all examined TKI-based treatments. However, most included studies pertain to second line TKI treatment with dasatinib, while the corresponding epidemiologic data on imatinib and nilotinib are sparse. Nevertheless, the updated European Leukemia Net recommendations state that mutation testing should be performed in all patients with suboptimal response or failure in first-line imatinib therapy before changing treatment, and that patients with the T315I mutation in particular should be considered for allogeneic stem cell transplantation.(118)

There is an apparent discrepancy between our findings on *any BCR-ABL1* mutation testing, and testing specifically for the T315I mutation. This can be explained by a dilution of the effect of the T315I mutation when other, more common mutations that do not confer resistance to treatment (or confer less resistance) are taken into account. This result is emblematic of the complexity of the topic: different mutations may confer different varying degrees of resistance to each of the three drugs. Exploring such relationships with systematic reviews of published aggregate data is extremely challenging. Other approaches, including collaborative registries of CML patients are much better suited to address such questions.[a] Collaborative international registries offer the advantages of standardized disease stage definitions, outcomes, treatments and assessment of mutations; allow the opportunity to analyze large numbers of patients, increasing the statistical power for analyzing associations of rare mutations; and may yield results that are widely applicable. After all, as described in the legend of **Figure 12**, most included CML studies originate from a limited number of world-leading referral centers.

We documented extensive between-study heterogeneity in treatments, the identified mutations, and the disease stages of the enrolled patients. This does not appear

[a] See for example http://www.eutos.org/content/registry/ (last accessed 12/07/2009).

to translate to differences in the predictive ability of included studies, either for any *BCR-ABL1* mutation testing or specifically for T315I mutation testing. Although we did not perform statistical tests for heterogeneity, all studies on any *BCR-ABL1* mutation testing fall into areas of poor prognostic performance, and all studies on T315I are consistent between them. This suggests that even if there is statistical heterogeneity, there is little clinically relevant information we can obtain from exploring it.

In conclusion, the results of our systematic review are in accordance with the prevailing knowledge in the field. Analyses of individual patient data rather than systematic reviews of aggregate data are better suited for exploring the complex relationships between various mutational patterns and conferred resistance to different TKIs. A pragmatic approach towards this goal is to support high quality registries of CML patients with detailed information on clinical and molecular variables, and well characterized outcomes.

Key Question 2: What patient- and disease-related factors affect the test results, their interpretation or their predictive response to therapy?

None of the included studies performed analyses for interaction between the aforementioned factors and *BCR-ABL1* mutation testing to predict response to therapy.

Key Question 3: How does the gene testing impact the therapeutic choice?

No study explicitly reported details on changes in treatment plans before and after testing.

Key Question 4: What are the benefits and harms or adverse effects for patients when managed with gene testing?

No study explicitly reported evidence on benefits or harms beyond what is covered in Key Question 1.

Section 4: Crosscutting methodological observations across the three topics

Here we summarize a range of methodological issues that we identified across the three topics. These issues are applicable to all three tests, which have quite different characteristics. In the first topic (*CYP2D6* polymorphisms and tamoxifen response modification) we evaluate germline polymorphisms, i.e., heritable common variations. In the other two cases we examine somatic mutations, i.e., genetic variations that are not heritable, and may evolve during the course of the disease. In the *KRAS* case mutations were relatively frequent, but the T315I mutation in *BCR-ABL1* is relatively rare. We comment only on methodological issues that we came across in the three topics; broader consideration of this literature is outside the scope of this work.

Study design issues

- By definition, a pharmacogenetic interaction implies that the genetic factor has differential effects on outcomes in treated versus untreated patients. One can test for interactions between treatment and genetic variants, by analyzing both treated and untreated patients. However, interaction tests were not reported in studies in the *CYP2D6* and *BCR-ABL1* topics (they were reported by some studies in the KRAS topic). Most studies analyze only treated patients, effectively assuming that effects in untreated patients are zero.[a] As we noted in Section 1.4, testing for gene by treatment interactions (when possible) is more than a formality; it presents the opportunity to triangulate results on the main effects, i.e., to perform a "reality check" on whether all analyses point to the same direction.
- It may not be necessary to design new studies to address pharmacogenetic associations. It is possible to "repurpose" already completed RCTs in which the drugs of interest are tested against a suitable comparator. One would perform genetic analyses in archival tissue from RCT enrollees, and associate them with the prospectively recorded clinical outcomes.
- Included studies often had small sample sizes. It is therefore likely that they will have low statistical power to detect modest or small effects (odds ratios less than 1.5 or 1.2, respectively). There are no empirical data on the typical effect sizes for pharmacogenetic associations, for germline or somatic variations, common or rare. The vast majority of genetic associations of complex diseases have association odds ratios less than 1.5,(157;158) and many independently replicated ones have even smaller effects (OR<1.10). If this is also true for most pharmacogenetic associations, it is likely that large sample sizes are necessary to attain sufficient statistical power.
- We found no evidence on whether patient or disease relevant factors affect the strength of the examined association between genetic factors and treatment effect modification. Such evidence would be obtained by examining interaction effects between the factors of interest and the genetic factors. However, no study performed interaction analyses. Several studies performed simple adjustments for patient level

[a] Otherwise one cannot distinguishif the genetic factor is prognostic (identifies heterogeneity in disease course irrespective of treatment) or predictive (identifies heterogeneity in treatment response)

factors. This is not only not informative, but also questionable from a methodological standpoint, because of mendelian randomization (see below).

Heterogeneity in the classification of genetic factors

- We documented extensive heterogeneity in the definitions of *CYP2D6*-derived metabolizer categories across the included studies (**Figure 3**), stemming from the large number of genotypes that can be studied. This is expected to be a common challenge, especially for multiallelic systems where many possible genotypes can be formed, and even more genetic contrasts (comparisons between genotypic groups) can be analyzed. Determining the clinically meaningful genetic comparisons in a multiallelic system is challenging, and offers opportunities for data dredging. It is not easy to select genetic contrasts solely based on biological rationale. After all, biological plausibility can be invoked retroactively and with relative ease(40) in support of even non-intuitive genotype comparisons.
- These and other challenges limit the usefulness of meta-analysis of aggregate level data. As was discussed in sections 1.4 and 3.4, meta-analyses of individual patient data are better suited to explore the complex relationships between genetic factors, treatments and outcomes.

Outcomes

- Most studies assessed surrogate short term outcomes of treatment failure, as defined by imaging or laboratory measurements. Data on the clinical outcomes of overall or progression-free survival are sparse.
- Further, no study reported details on changes in treatment decisions before and after testing. This absence of evidence may have different interpretation in the three settings. For example, *KRAS* testing is already used to guide treatment in several clinical settings, and thus affects treatment choice in these settings. This may not be the case in *CYP2D6* testing, which is not in clinical use. The absence of the information requested by Key Question 3 is essentially an issue of reporting.
- Finally, there was no direct evidence on benefits and harms associated with testing and its downstream effects beyond the evidence that was described in Key Question 1. This is hardly surprising: Like all tests, genetic testing exerts most of its effects in an indirect way: test results affect subsequent patient management decisions, which in turn impact on patient-relevant outcomes.(41) Harms are often reported inadequately in RCTs(42) and nonrandomized studies of interventions,(43) and reporting may be even worse for studies of medical tests.

Statistical analyses

- Finally, many studies followed poor analytic practices, by performing statistical adjustments for factors that cannot confound the relationship between the genetic factor and the outcome. This is particularly true for germline genetic variations, such as the ones in *CYP2D6*, because mendelian randomization (the natural randomization of genotypes during mitosis) protects the relationship between polymorphisms and outcomes from confounding. **Table 6** in section 1.4 discussed some examples of factors or design characteristics that can confound or bias relationships in classical

epidemiology, but not in genetic epidemiology. We remind the reader that associations of germline genetic variations are not immune to bias;(48) rather, a different set of considerations is applicable to such studies. Multiplicity of comparisons, data dredging, population stratification, and misclassifications of outcomes and genotypes and various biases (including publication and reporting biases) are the most common threats to the validity of associations between genetic factors and treatment effect modification.

- Theoretically, in repurposed RCTs, retrospective associations of both germline genetic variations and somatic mutations with outcomes should be unconfounded. A repurposed RCT would resemble a factorial randomized trial: the randomization process ensures that the treatment is allocated randomly. Mendelian randomization ensures that the germline genetic variations are allocated randomly across treatment arms. The randomization process itself ensures that somatic variations are randomly allocated.
- Adjustments for multiple comparisons were not documented in the included studies. This is a major issue in genetic epidemiology, because of the large number of possible hypotheses that can be examined.

Other issues

- Multiple studies on each topic frequently originated from a limited number of specialized centers, posing problems in identifying nonoverlapping populations, and potentially limiting the generalizability of the findings.

References

(1) Dezentje VO, Guchelaar HJ, Nortier JW, van d, V, Gelderblom H. Clinical implications of CYP2D6 genotyping in tamoxifen treatment for breast cancer. Clin Cancer Res 2009 January 1;15(1):15-21.

(2) Jimeno A, Messersmith WA, Hirsch FR, Franklin WA, Eckhardt SG. KRAS mutations and susceptibility to cetuximab and panitumumab in colorectal cancer. Cancer J 2009 March;15(2):110-3.

(3) Jimeno A, Messersmith WA, Hirsch FR, Franklin WA, Eckhardt SG. KRAS mutations and sensitivity to epidermal growth factor receptor inhibitors in colorectal cancer: practical application of patient selection. J Clin Oncol 2009 March 1;27(7):1130-6.

(4) Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood 2008 December 15;112(13):4808-17.

(5) Mandrekar SJ, Sargent DJ. Clinical trial designs for predictive biomarker validation: theoretical considerations and practical challenges. J Clin Oncol 2009 August 20;27(24):4027-34.

(6) Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002 June 15;21(11):1539-58.

(7) Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003 September 6;327(7414):557-60.

(8) Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. JAMA 1994 March 2;271(9):703-7.

(9) Chu H, Cole SR. Bivariate meta-analysis of sensitivity and specificity with sparse data: a generalized linear mixed model approach. J Clin Epidemiol 2006 December;59(12):1331-2.

(10) Reitsma JB, Glas AS, Rutjes AW, Scholten RJ, Bossuyt PM, Zwinderman AH. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005 October;58(10):982-90.

(11) Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005 May 14;365(9472):1687-717.

(12) Dowsett M, Cuzick J, Ingle J, Coates A, Forbes J, Bliss J et al. Meta-analysis of breast cancer outcomes in adjuvant trials of aromatase inhibitors versus tamoxifen. J Clin Oncol 2010 January;28(3):509-18.

(13) Ring A, Dowsett M. Mechanisms of tamoxifen resistance. Endocr Relat Cancer 2004 December;11(4):643-58.

(14) Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. Nat Rev Cancer 2009 September;9(9):631-43.

(15) Goetz MP, Suman VJ, Couch FJ, Ames MM, Rae JM, Erlander MG et al. Cytochrome P450 2D6 and homeobox 13/interleukin-17B receptor: combining inherited and tumor gene markers for prediction of tamoxifen resistance. Clinical Cancer Research 2008 September 15;14(18):5864-8.

(16) Hoskins JM, Carey LA, McLeod HL. CYP2D6 and tamoxifen: DNA matters in breast cancer. Nat Rev Cancer 2009 August;9(8):576-86.

(17) Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. J Pharmacol Exp Ther 2004 September;310(3):1062-75.

(18) Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenomics J 2005;5(1):6-13.

(19) Johnson MD, Zuo H, Lee KH, Trebley JP, Rae JM, Weatherman RV et al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. Breast Cancer Res Treat 2004 May;85(2):151-9.

(20) Nelson DR, Zeldin DC, Hoffman SM, Maltais LJ, Wain HM, Nebert DW. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. Pharmacogenetics 2004 January;14(1):1-18.

(21) Yu KD, Shao ZM. Genetic matters of CYP2D6 in breast cancer: copy number variations and nucleotide polymorphisms. Nat Rev Cancer 2009 November;9(11):842.

(22) Gaedigk A, Ndjountche L, Divakaran K, Dianne BL, Zineh I, Oberlander TF et al. Cytochrome P4502D6 (CYP2D6) gene locus heterogeneity: characterization of gene duplication events. Clin Pharmacol Ther 2007 February;81(2):242-51.

(23) Jin Y, Desta Z, Stearns V, Ward B, Ho H, Lee KH et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. J Natl Cancer Inst 2005 January 5;97(1):30-9.

(24) Bijl MJ, van Schaik RH, Lammers LA, Hofman A, Vulto AG, van GT et al. The CYP2D6*4 polymorphism affects breast cancer survival in tamoxifen users. Breast Cancer Res Treat 2009 November;118(1):125-30.

(25) Goetz MP, Rae JM, Suman VJ, Safgren SL, Ames MM, Visscher DW et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes.[see comment]. Journal of Clinical Oncology 2005 December 20;23(36):9312-8.

(26) Kiyotani K, Mushiroda T, Sasa M, Bando Y, Sumitomo I, Hosono N et al. Impact of CYP2D6*10 on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy.[see comment]. Cancer Science 2008 May;99(5):995-9.

(27) Lim HS, Ju LH, Seok LK, Sook LE, Jang IJ, Ro J. Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer.[see comment]. Journal of Clinical Oncology 2007 September 1;25(25):3837-45.

(28) Newman WG, Hadfield KD, Latif A, Roberts SA, Shenton A, McHague C et al. Impaired tamoxifen metabolism reduces survival in familial breast cancer patients. Clinical Cancer Research 2008 September 15;14(18):5913-8.

(29) Nowell SA, Ahn J, Rae JM, Scheys JO, Trovato A, Sweeney C et al. Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. Breast Cancer Research & Treatment 2005 June;91(3):249-58.

(30) Okishiro M, Taguchi T, Jin KS, Shimazu K, Tamaki Y, Noguchi S. Genetic polymorphisms of CYP2D6 10 and CYP2C19 2, 3 are not associated with prognosis, endometrial thickness, or bone mineral density in Japanese breast cancer patients treated with adjuvant tamoxifen. Cancer 2009 March 1;115(5):952-61.

(31) Ramon YC, Altes A, Pare L, Del RE, Alonso C, Barnadas A et al. Impact of CYP2D6 polymorphisms in tamoxifen adjuvant breast cancer treatment. Breast Cancer Res Treat 2009 February 3.

(32) Schroth W, Antoniadou L, Fritz P, Schwab M, Muerdter T, Zanger UM et al. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes.[see comment]. Journal of Clinical Oncology 2007 November 20;25(33):5187-93.

(33) Wegman P, Vainikka L, Stal O, Nordenskjold B, Skoog L, Rutqvist LE et al. Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients.[see comment]. Breast Cancer Research 2005;7(3):R284-R290.

(34) Wegman P, Elingarami S, Carstensen J, Stal O, Nordenskjold B, Wingren S. Genetic variants of cyp3a5, cyp2d6, sult1a1, ugt2b15 and tamoxifen response in postmenopausal patients with breast cancer. Breast Cancer Research 2007;9(1):R7.

(35) Xu Y, Sun Y, Yao L, Shi L, Wu Y, Ouyang T et al. Association between CYP2D6 *10 genotype and survival of breast cancer patients receiving tamoxifen treatment. Annals of Oncology 2008 August;19(8):1423-9.

(36) Toyama T, Yamashita H, Sugiura H, Kondo N, Iwase H, Fujii Y. No association between CYP2D6*10 genotype and survival of node-negative Japanese breast cancer patients receiving adjuvant tamoxifen treatment. Jpn J Clin Oncol 2009 October;39(10):651-6.

(37) Kiyotani K, Mushiroda T, Imamura CK, Hosono N, Tsunoda T, Kubo M et al. Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. J Clin Oncol 2010 March 10;28(8):1287-93.

(38) Schroth W, Goetz MP, Hamann U, Fasching PA, Schmidt M, Winter S et al. Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. JAMA 2009 October 7;302(13):1429-36.

(39) Visvanathan K, Chlebowski RT, Hurley P, Col NF, Ropka M, Collyar D et al. American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. J Clin Oncol 2009 July 1;27(19):3235-58.

(40) Ioannidis JP, Polyzos NP, Trikalinos TA. Selective discussion and transparency in microarray research findings for cancer outcomes. Eur J Cancer 2007 September;43(13):1999-2010.

(41) Bossuyt PM, McCaffery K. Additional patient outcomes and pathways in evaluations of testing. Med Decis Making 2009 September;29(5):E30-E38.

(42) Ioannidis JP, Lau J. Completeness of safety reporting in randomized trials: an evaluation of 7 medical areas. JAMA 2001 January 24;285(4):437-43.

(43) Papanikolaou PN, Christidi GD, Ioannidis JP. Comparison of evidence on harms of medical interventions in randomized and nonrandomized studies. CMAJ 2006 February 28;174(5):635-41.

(44) Lash TL, Ahern TP, Cronin-Fenton D, Garne JP, Hamilton-Dutoit S, Kvistgaard ME et al. Modification of tamoxifen response: what have we learned? J Clin Oncol 2008 April 1;26(10):1764-5.

(45) Ahern TP, Pedersen L, Cronin-Fenton DP, Sorensen HT, Lash TL. No increase in breast cancer recurrence with concurrent use of tamoxifen and some CYP2D6-inhibiting medications. Cancer Epidemiol Biomarkers Prev 2009 September;18(9):2562-4.

(46) Lash TL, Ahern TP, Cronin-Fenton D, Garne JP, Hamilton-Dutoit S, Sorensen HT. Comment on 'Impact of CYP2D6*10 on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy'. Cancer Sci 2008 August;99(8):1706-7.

(47) Lash TL, Lien EA, Sorensen HT, Hamilton-Dutoit S. Genotype-guided tamoxifen therapy: time to pause for reflection? Lancet Oncol 2009 August;10(8):825-33.

(48) Colhoun HM, McKeigue PM, Davey SG. Problems of reporting genetic associations with complex outcomes. Lancet 2003 March 8;361(9360):865-72.

(49) Hynes NE, Lane HA. ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 2005 May;5(5):341-54.

(50) Baselga J. Why the epidermal growth factor receptor? The rationale for cancer therapy. Oncologist 2002;7 Suppl 4:2-8.

(51) Chabner BA, Roberts TG, Jr. Timeline: Chemotherapy and the war on cancer. Nat Rev Cancer 2005 January;5(1):65-72.

(52) Goldstein NI, Prewett M, Zuklys K, Rockwell P, Mendelsohn J. Biological efficacy of a chimeric antibody to the epidermal growth factor receptor in a human tumor xenograft model. Clin Cancer Res 1995 November;1(11):1311-8.

(53) Schreiber AB, Lax I, Yarden Y, Eshhar Z, Schlessinger J. Monoclonal antibodies against receptor for epidermal growth factor induce early and delayed effects of epidermal growth factor. Proc Natl Acad Sci U S A 1981 December;78(12):7535-9.

(54) Siena S, Sartore-Bianchi A, Di NF, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst 2009 October 7;101(19):1308-24.

(55) Saltz LB, Meropol NJ, Loehrer PJ, Sr., Needle MN, Kopit J, Mayer RJ. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. J Clin Oncol 2004 April 1;22(7):1201-8.

(56) Chung KY, Shia J, Kemeny NE, Shah M, Schwartz GK, Tse A et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005 March 20;23(9):1803-10.

(57) Saltz L. Epidermal growth factor receptor-negative colorectal cancer: is there truly such an entity? Clin Colorectal Cancer 2005 November;5 Suppl 2:S98-100.

(58) Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, de BF et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer.[see comment]. Journal of Clinical Oncology 2009 February 10;27(5):663-71.

(59) Amado RG, Wolf M, Peeters M, Van CE, Siena S, Freeman DJ et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer.[see comment]. Journal of Clinical Oncology 2008 April 1;26(10):1626-34.

(60) Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007 November 15;357(20):2040-8.

(61) Van CE, Kohne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. New England Journal of Medicine 2009 April 2;360(14):1408-17.

(62) Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer 2003 June;3(6):459-65.

(63) Walther A, Johnstone E, Swanton C, Midgley R, Tomlinson I, Kerr D. Genetic prognostic and predictive markers in colorectal cancer. Nat Rev Cancer 2009 July;9(7):489-99.

(64) Porta M, Crous-Bou M, Wark PA, Vineis P, Real FX, Malats N et al. Cigarette smoking and K-ras mutations in pancreas, lung and colorectal adenocarcinomas: Etiopathogenic similarities, differences and paradoxes. Mutat Res 2009 September;682(2-3):83-93.

(65) Bengala C, Bettelli S, Bertolini F, Salvi S, Chiara S, Sonaglio C et al. Epidermal growth factor receptor gene copy number, K-ras mutation and pathological response to preoperative cetuximab, 5-FU and radiation therapy in locally advanced rectal cancer. Annals of Oncology 2009 March;20(3):469-74.

(66) Benvenuti S, Sartore-Bianchi A, Di NF, Zanon C, Moroni M, Veronese S et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Research 2007 March 15;67(6):2643-8.

(67) Bibeau F, Lopez-Crapez E, Di FF, Thezenas S, Ychou M, Blanchard F et al. Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. Journal of Clinical Oncology 2009 March 1;27(7):1122-9.

(68) Cappuzzo F, Varella-Garcia M, Finocchiaro G, Skokan M, Gajapathy S, Carnaghi C et al. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. British Journal of Cancer 2008 July 8;99(1):83-9.

(69) De RW, Piessevaux H, De SJ, Janssens M, De HG, Personeni N et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Annals of Oncology 2008 March;19(3):508-15.

(70) Debucquoy A, Haustermans K, Daemen A, Aydin S, Libbrecht L, Gevaert O et al. Molecular response to cetuximab and efficacy of preoperative cetuximab-based chemoradiation in rectal cancer.[see comment]. Journal of Clinical Oncology 2009 June 10;27(17):2751-7.

(71) Di NF, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer.[see comment]. Journal of Clinical Oncology 2008 December 10;26(35):5705-12.

(72) Di FF, Blanchard F, Charbonnier F, Le PF, Lamy A, Galais MP et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by Cetuximab plus chemotherapy.[see comment]. British Journal of Cancer 2007 April 23;96(8):1166-9.

(73) Frattini M, Saletti P, Romagnani E, Martin V, Molinari F, Ghisletta M et al. PTEN loss of expression predicts cetuximab efficacy in metastatic colorectal cancer patients. British Journal of Cancer 2007 October 22;97(8):1139-45.

(74) Freeman DJ, Juan T, Reiner M, Hecht JR, Meropol NJ, Berlin J et al. Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving panitumumab alone. Clinical Colorectal Cancer 2008 May;7(3):184-90.

(75) Garm Spindler KL, Pallisgaard N, Rasmussen AA, Lindebjerg J, Andersen RF, Cruger D et al. The importance of KRAS mutations and EGF61A>G polymorphism to the effect of cetuximab and irinotecan in metastatic colorectal cancer. Annals of Oncology 2009 May;20(5):879-84.

(76) Goncalves A, Esteyries S, Taylor-Smedra B, Lagarde A, Ayadi M, Monges G et al. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. BMC Cancer 2008;8:169.

(77) Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer.[see comment]. New England Journal of Medicine 2008 October 23;359(17):1757-65.

(78) Khambata-Ford S, Garrett CR, Meropol NJ, Basik M, Harbison CT, Wu S et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab.[see comment]. Journal of Clinical Oncology 2007 August 1;25(22):3230-7.

(79) Lievre A, Bachet JB, Boige V, Cayre A, Le CD, Buc E et al. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab.[see comment]. Journal of Clinical Oncology 2008 January 20;26(3):374-9.

(80) Lievre A, Bachet JB, Le CD, Boige V, Landi B, Emile JF et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Research 2006 April 15;66(8):3992-5.

(81) Loupakis F, Ruzzo A, Cremolini C, Vincenzi B, Salvatore L, Santini D et al. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. British Journal of Cancer 2009 August 18;101(4):715-21.

(82) Loupakis F, Pollina L, Stasi I, Ruzzo A, Scartozzi M, Santini D et al. PTEN expression and KRAS mutations on primary tumors and metastases in the prediction of benefit from

cetuximab plus irinotecan for patients with metastatic colorectal cancer. Journal of Clinical Oncology 2009 June 1;27(16):2622-9.

(83) Lurje G, Nagashima F, Zhang W, Yang D, Chang HM, Gordon MA et al. Polymorphisms in cyclooxygenase-2 and epidermal growth factor receptor are associated with progression-free survival independent of K-ras in metastatic colorectal cancer patients treated with single-agent cetuximab. Clinical Cancer Research 2008 December 1;14(23):7884-95.

(84) Molinari F, Martin V, Saletti P, De DS, Spitale A, Camponovo A et al. Differing deregulation of EGFR and downstream proteins in primary colorectal cancer and related metastatic sites may be clinically relevant. British Journal of Cancer 2009 April 7;100(7):1087-94.

(85) Moroni M, Veronese S, Benvenuti S, Marrapese G, Sartore-Bianchi A, Di NF et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study.[see comment]. Lancet Oncology 2005 May;6(5):279-86.

(86) Muro K, Yoshino T, Doi T, Shirao K, Takiuchi H, Hamamoto Y et al. A phase 2 clinical trial of panitumumab monotherapy in Japanese patients with metastatic colorectal cancer. Japanese Journal of Clinical Oncology 2009 May;39(5):321-6.

(87) Oden-Gangloff A, Di FF, Bibeau F, Lamy A, Bougeard G, Charbonnier F et al. TP53 mutations predict disease control in metastatic colorectal cancer treated with cetuximab-based chemotherapy. British Journal of Cancer 2009 April 21;100(8):1330-5.

(88) Perrone F, Lampis A, Orsenigo M, Di BM, Gevorgyan A, Losa M et al. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. Annals of Oncology 2009 January;20(1):84-90.

(89) Prenen H, De SJ, Jacobs B, De RW, Biesmans B, Claes B et al. PIK3CA mutations are not a major determinant of resistance to the epidermal growth factor receptor inhibitor cetuximab in metastatic colorectal cancer. Clinical Cancer Research 2009 May 1;15(9):3184-8.

(90) Sartore-Bianchi A, Martini M, Molinari F, Veronese S, Nichelatti M, Artale S et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Research 2009 March 1;69(5):1851-7.

(91) Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG et al. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer.[see comment]. New England Journal of Medicine 2009 February 5;360(6):563-72.

(92) Yen LC, Yeh YS, Chen CW, Wang HM, Tsai HL, Lu CY et al. Detection of KRAS oncogene in peripheral blood as a predictor of the response to cetuximab plus chemotherapy in patients with metastatic colorectal cancer. Clinical Cancer Research 2009 July 1;15(13):4508-13.

(93) Hecht JR, Mitchell E, Chidiac T, Scroggin C, Hagenstad C, Spigel D et al. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol 2009 February 10;27(5):672-80.

(94) Tabernero J, Cervantes A, Rivera F, Martinelli E, Rojo F, von HA et al. Pharmacogenomic and pharmacoproteomic studies of cetuximab in metastatic colorectal cancer: biomarker analysis of a phase I dose-escalation study. J Clin Oncol 2010 March 1;28(7):1181-9.

(95) Folprecht G, Gruenberger T, Bechstein WO, Raab HR, Lordick F, Hartmann JT et al. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. Lancet Oncol 2010 January;11(1):38-47.

(96) Yen LC, Uen YH, Wu DC, Lu CY, Yu FJ, Wu IC et al. Activating KRAS mutations and overexpression of epidermal growth factor receptor as independent predictors in metastatic colorectal cancer patients treated with cetuximab. Ann Surg 2010 February;251(2):254-60.

(97) Jacobs B, De RW, Piessevaux H, Van OR, Biesmans B, De SJ et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol 2009 October;27(30):5068-74.

(98) Laurent-Puig P, Cayre A, Manceau G, Buc E, Bachet JB, Lecomte T et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. J Clin Oncol 2009 December 10;27(35):5924-30.

(99) Paule B, Castagne V, Picard V, Saffroy R, Adam R, Guettier C et al. MDR1 polymorphism role in patients treated with cetuximab and irinotecan in irinotecan refractory colorectal cancer. Med Oncol 2009 October 28.

(100) Sartore-Bianchi A, Di NF, Nichelatti M, Molinari F, De DS, Saletti P et al. Multi-determinants analysis of molecular alterations for predicting clinical benefit to EGFR-targeted monoclonal antibodies in colorectal cancer. PLoS One 2009 October 2;4(10):e7287.

(101) Sohn BS, Kim TW, Lee JL, Ryu MH, Chang HM, Kang YK et al. The role of KRAS mutations in predicting the efficacy of cetuximab-plus-irinotecan therapy in irinotecan-refractory Korean metastatic colorectal cancer patients. Oncology 2009;77(3-4):224-30.

(102) Souglakos J, Philips J, Wang R, Marwah S, Silver M, Tzardi M et al. Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 2009 August 4;101(3):465-72.

(103) Chung CH, Seeley EH, Roder H, Grigorieva J, Tsypin M, Roder J et al. Detection of tumor epidermal growth factor receptor pathway dependence by serum mass spectrometry in cancer patients. Cancer Epidemiol Biomarkers Prev 2010 February;19(2):358-65.

(104) Graziano F, Canestrari E, Loupakis F, Ruzzo A, Galluccio N, Santini D et al. Genetic modulation of the Let-7 microRNA binding to KRAS 3'-untranslated region and survival of metastatic colorectal cancer patients treated with salvage cetuximab-irinotecan. Pharmacogenomics J 2010 February 23.

(105) Perkins G, Lievre A, Ramacci C, Meatchi T, de RA, Emile JF et al. Additional value of EGFR downstream signaling phosphoprotein expression to KRAS status for response to anti-EGFR antibodies in colorectal cancer. Int J Cancer 2010 January 4.

(106) Montagut C, Iglesias M, Arumi M, Bellosillo B, Gallen M, Martinez-Fernandez A et al. Mitogen-activated protein kinase phosphatase-1 (MKP-1) impairs the response to anti-epidermal growth factor receptor (EGFR) antibody cetuximab in metastatic colorectal cancer patients. Br J Cancer 2010 March 30;102(7):1137-44.

(107) Scartozzi M, Mandolesi A, Giampieri R, Pierantoni C, Loupakis F, Zaniboni A et al. Insulin-like growth factor 1 expression correlates with clinical outcome in K-RAS wild type colorectal cancer patients treated with cetuximab and irinotecan. Int J Cancer 2010 January 22.

(108) Personeni N, Fieuws S, Piessevaux H, De HG, De SJ, Biesmans B et al. Clinical usefulness of EGFR gene copy number as a predictive marker in colorectal cancer patients treated with cetuximab: a fluorescent in situ hybridization study. Clin Cancer Res 2008 September 15;14(18):5869-76.

(109) Dworak O, Keilholz L, Hoffmann A. Pathological features of rectal cancer after preoperative radiochemotherapy. Int J Colorectal Dis 1997;12(1):19-23.

(110) Wheeler JM, Warren BF, Mortensen NJ, Ekanyaka N, Kulacoglu H, Jones AC et al. Quantification of histologic regression of rectal cancer after irradiation: a proposal for a modified staging system. Dis Colon Rectum 2002 August;45(8):1051-6.

(111) Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009 April 20;27(12):2091-6.

(112) Linardou H, Dahabreh IJ, Kanaloupiti D, Siannis F, Bafaloukos D, Kosmidis P et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol 2008 October;9(10):962-72.

(113) Hebbar M, Fournier P, Romano O. KRAS mutational status assessment in patients with metastatic colorectal cancer: are the clinical implications so clear? Eur J Cancer Care (Engl) 2009 August 20.

(114) Mittmann N, Au HJ, Tu D, O'Callaghan CJ, Isogai PK, Karapetis CS et al. Prospective cost-effectiveness analysis of cetuximab in metastatic colorectal cancer: evaluation of National Cancer Institute of Canada Clinical Trials Group CO.17 trial. J Natl Cancer Inst 2009 September 2;101(17):1182-92.

(115) Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009 July;59(4):225-49.

(116) Deininger MW. Milestones and monitoring in patients with CML treated with imatinib. Hematology Am Soc Hematol Educ Program 2008;419-26.

(117) Ross DM, Hughes TP. Current and emerging tests for the laboratory monitoring of chronic myeloid leukaemia and related disorders. Pathology 2008 April;40(3):231-46.

(118) Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J et al. Chronic Myeloid Leukemia: An Update of Concepts and Management Recommendations of European LeukemiaNet. J Clin Oncol 2009 November 2.

(119) Hochhaus A, O'Brien SG, Guilhot F, Druker BJ, Branford S, Foroni L et al. Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. Leukemia 2009 June;23(6):1054-61.

(120) Quintas-Cardama A, Kantarjian HM, Cortes JE. Mechanisms of primary and secondary resistance to imatinib in chronic myeloid leukemia. Cancer Control 2009 April;16(2):122-31.

(121) Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 2001 August 3;293(5531):876-80.

(122) Jones D, Kamel-Reid S, Bahler D, Dong H, Elenitoba-Johnson K, Press R et al. Laboratory practice guidelines for detecting and reporting BCR-ABL drug resistance mutations in chronic myelogenous leukemia and acute lymphoblastic leukemia: a report of the Association for Molecular Pathology. J Mol Diagn 2009 January;11(1):4-11.

(123) Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood 2006 July 1;108(1):28-37.

(124) Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood 2006 September 15;108(6):1809-20.

(125) Ernst T, Erben P, Muller MC, Paschka P, Schenk T, Hoffmann J et al. Dynamics of BCR-ABL mutated clones prior to hematologic or cytogenetic resistance to imatinib.[see comment]. Haematologica 2008 February;93(2):186-92.

(126) Marin D, Kaeda JS, Andreasson C, Saunders SM, Bua M, Olavarria E et al. Phase I/II trial of adding semisynthetic homoharringtonine in chronic myeloid leukemia patients who have achieved partial or complete cytogenetic response on imatinib. Cancer 2005 May 1;103(9):1850-5.

(127) Willis SG, Lange T, Demehri S, Otto S, Crossman L, Niederwieser D et al. High-sensitivity detection of BCR-ABL kinase domain mutations in imatinib-naive patients: correlation with clonal cytogenetic evolution but not response to therapy. Blood 2005 September 15;106(6):2128-37.

(128) Quintas-Cardama A, Cortes JE, O'Brien S, Ravandi F, Borthakur G, Liu D et al. Dasatinib early intervention after cytogenetic or hematologic resistance to imatinib in patients with chronic myeloid leukemia. Cancer 2009 July 1;115(13):2912-21.

(129) Kantarjian H, Pasquini R, Hamerschlak N, Rousselot P, Holowiecki J, Jootar S et al. Dasatinib or high-dose imatinib for chronic-phase chronic myeloid leukemia after failure of first-line imatinib: a randomized phase 2 trial. Blood 2007 June 15;109(12):5143-50.

(130) Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 2002 February 28;346(9):645-52.

(131) Jabbour E, Kantarjian HM, Jones D, Shan J, O'Brien S, Reddy N et al. Imatinib mesylate dose escalation is associated with durable responses in patients with chronic myeloid leukemia after cytogenetic failure on standard-dose imatinib therapy. Blood 2009 March 5;113(10):2154-60.

(132) Cortes J, Quintas-Cardama A, Garcia-Manero G, O'Brien S, Jones D, Faderl S et al. Phase 1 study of tipifarnib in combination with imatinib for patients with chronic myelogenous leukemia in chronic phase after imatinib failure. Cancer 2007 November 1;110(9):2000-6.

(133) Oki Y, Kantarjian HM, Gharibyan V, Jones D, O'Brien S, Verstovsek S et al. Phase II study of low-dose decitabine in combination with imatinib mesylate in patients with accelerated or myeloid blastic phase of chronic myelogenous leukemia. Cancer 2007 March 1;109(5):899-906.

(134) Cortes J, Jabbour E, Daley GQ, O'Brien S, Verstovsek S, Ferrajoli A et al. Phase 1 study of lonafarnib (SCH 66336) and imatinib mesylate in patients with chronic myeloid leukemia who have failed prior single-agent therapy with imatinib. Cancer 2007 September 15;110(6):1295-302.

(135) Shah NP, Kantarjian HM, Kim DW, Rea D, Dorlhiac-Llacer PE, Milone JH et al. Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. Journal of Clinical Oncology 2008 July 1;26(19):3204-12.

(136) Hochhaus A, Baccarani M, Deininger M, Apperley JF, Lipton JH, Goldberg SL et al. Dasatinib induces durable cytogenetic responses in patients with chronic myelogenous leukemia in chronic phase with resistance or intolerance to imatinib. Leukemia 2008 June;22(6):1200-6.

(137) Hochhaus A, Kantarjian HM, Baccarani M, Lipton JH, Apperley JF, Druker BJ et al. Dasatinib induces notable hematologic and cytogenetic responses in chronic-phase chronic myeloid leukemia after failure of imatinib therapy.[erratum appears in Blood. 2007 Sep 1;110(5):1438]. Blood 2007 March 15;109(6):2303-9.

(138) Kantarjian H, Cortes J, Kim DW, Dorlhiac-Llacer P, Pasquini R, DiPersio J et al. Phase 3 study of dasatinib 140 mg once daily versus 70 mg twice daily in patients with chronic myeloid leukemia in accelerated phase resistant or intolerant to imatinib: 15-month median follow-up. Blood 2009 June 18;113(25):6322-9.

(139) Guilhot F, Apperley J, Kim DW, Bullorsky EO, Baccarani M, Roboz GJ et al. Dasatinib induces significant hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in accelerated phase. Blood 2007 May 15;109(10):4143-50.

(140) Cortes J, Kim DW, Raffoux E, Martinelli G, Ritchie E, Roy L et al. Efficacy and safety of dasatinib in imatinib-resistant or -intolerant patients with chronic myeloid leukemia in blast phase. Leukemia 2008 December;22(12):2176-83.

(141) Cortes J, Rousselot P, Kim DW, Ritchie E, Hamerschlak N, Coutre S et al. Dasatinib induces complete hematologic and cytogenetic responses in patients with imatinib-resistant or -intolerant chronic myeloid leukemia in blast crisis. Blood 2007 April 15;109(8):3207-13.

(142) Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias.[see comment]. New England Journal of Medicine 2006 June 15;354(24):2531-41.

(143) Sakamaki H, Ishizawa K, Taniwaki M, Fujisawa S, Morishima Y, Tobinai K et al. Phase 1/2 clinical study of dasatinib in Japanese patients with chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia. International Journal of Hematology 2009 April;89(3):332-41.

(144) Wu J, Meng F, Kong LY, Peng Z, Ying Y, Bornmann WG et al. Association between imatinib-resistant BCR-ABL mutation-negative leukemia and persistent activation of LYN kinase.[see comment]. Journal of the National Cancer Institute 2008 July 2;100(13):926-39.

(145) Kantarjian HM, Giles F, Gattermann N, Bhalla K, Alimena G, Palandri F et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood 2007 November 15;110(10):3540-6.

(146) le CP, Ottmann OG, Giles F, Kim DW, Cortes J, Gattermann N et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is active in patients with imatinib-resistant or -intolerant accelerated-phase chronic myelogenous leukemia. Blood 2008 February 15;111(4):1834-9.

(147) Kantarjian H, Giles F, Wunderle L, Bhalla K, O'Brien S, Wassmann B et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL.[see comment]. New England Journal of Medicine 2006 June 15;354(24):2542-51.

(148) Cortes J, Jabbour E, Kantarjian H, Yin CC, Shan J, O'Brien S et al. Dynamics of BCR-ABL kinase domain mutations in chronic myeloid leukemia after sequential treatment with multiple tyrosine kinase inhibitors. Blood 2007 December 1;110(12):4005-11.

(149) Quintas-Cardama A, Kantarjian H, Jones D, Nicaise C, O'Brien S, Giles F et al. Dasatinib (BMS-354825) is active in Philadelphia chromosome-positive chronic myelogenous leukemia after imatinib and nilotinib (AMN107) therapy failure. Blood 2007 January 15;109(2):497-9.

(150) Khorashad JS, de LH, Apperley JF, Milojkovic D, Reid AG, Bua M et al. Finding of kinase domain mutations in patients with chronic phase chronic myeloid leukemia responding to imatinib may identify those at high risk of disease progression. Journal of Clinical Oncology 2008 October 10;26(29):4806-13.

(151) de LH, Apperley JF, Khorashad JS, Milojkovic D, Reid AG, Bua M et al. Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis.[see comment]. Journal of Clinical Oncology 2008 July 10;26(20):3358-63.

(152) Marin D, Khorashad JS, Foroni L, Milojkovic D, Szydlo R, Reid AG et al. Does a rise in the BCR-ABL1 transcript level identify chronic phase CML patients responding to imatinib who have a high risk of cytogenetic relapse? British Journal of Haematology 2009 May;145(3):373-5.

(153) Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by

clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood 2003 July 1;102(1):276-83.

(154) Wei Y, Hardling M, Olsson B, Hezaveh R, Ricksten A, Stockelberg D et al. Not all imatinib resistance in CML are BCR-ABL kinase domain mutations. Annals of Hematology 2006 December;85(12):841-7.

(155) Hughes TP, Branford S, White DL, Reynolds J, Koelmeyer R, Seymour JF et al. Impact of early dose intensity on cytogenetic and molecular responses in chronic- phase CML patients receiving 600 mg/day of imatinib as initial therapy. Blood 2008 November 15;112(10):3965-73.

(156) Chu S, Xu H, Shah NP, Snyder DS, Forman SJ, Sawyers CL et al. Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment.[see comment]. Blood 2005 March 1;105(5):2093-8.

(157) Castaldi PJ, Cho MH, Cohn M, Langerman F, Moran S, Tarragona N et al. The COPD genetic association compendium: a comprehensive online database of COPD genetic associations. Hum Mol Genet 2009 December 2.

(158) Ioannidis JP, Trikalinos TA, Khoury MJ. Implications of small effect sizes of individual genetic variants on the design and interpretation of genetic association studies of complex diseases. Am J Epidemiol 2006 October 1;164(7):609-14.