

BACKGROUND INFORMATION

FOR

THE ONCOLOGIC DRUGS ADVISORY COMMITTEE (ODAC) MEETING

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List of Abbreviations and Definition of Terms

Abbreviation/Term	Definition/Explanation
ABX-EGF	Panitumumab; Vectibix®
BELAC	Belgian Accreditation Body
BRAF	B-Raf proto-oncogene serine/threonine-protein kinase (murine sarcoma viral (v-raf) oncogene homolog B1)
BSC	best supportive care
CDRH	Center for Devices and Radiological Health
CE mark	Symbol used to indicate that a product conforms to relevant European health, safety, and environmental quality standards.
CI	confidence interval
CLSI	Clinical and Laboratory Standards Institute
CR	complete response
CRC	colorectal cancer
Ct	cycle threshold
CT	chemotherapy
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
ECOG	Eastern Cooperative Oncology Group
EGFR	epidermal growth factor receptor
erbB/HER	avian erythroblastic leukemia viral (v-erb-b) oncogene homolog
FDA	Food and Drug Administration
FFPE	formalin-fixed, paraffin-embedded
GDP, GTP	guanosine diphosphate, guanosine triphosphate
HER2	human epidermal growth factor receptor 2
HR	hazard ratio
HRM	high-resolution melt
ITT	intent-to-treat
IUO	Investigational-use only
IVRS	interactive voice response system
KRAS	Kirsten rat sarcoma virus oncogene
LCM	laser-capture microdissection
mCRC	metastatic colorectal cancer
MT	mutant
N	sample size
NSCLC	Non-small Cell Lung Cancer

List of Abbreviations and Definition of Terms (continued)

ODAC	Oncology Drugs Advisory Committee
OS	overall survival
PCR	polymerase chain reaction
PD	progressive disease
PFS	progression-free survival
PI3KCA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit
PMA	Pre-market Approval
PR	partial response
Q2W	every 2 weeks
Q3M	every 3 months
Q8W	every 8 weeks
RECIST	Response Evaluation Criteria in Solid Tumors
RUO	Research-use only
SAP	statistical analysis plan
SD	stable disease
VEGF	vascular endothelial growth factor
WHO	World Health Organization
WT	wild-type

Study Key

Study Number (Alias)	Title	Publications	Number of Subjects
20020408	An open-label, randomized, phase 3 clinical trial of ABX-EGF plus best supportive care versus best supportive care in subjects with metastatic colorectal cancer	Van Cutsem et al, 2007; Amado et al, 2008	463
20025405	An open label phase 2 clinical trial to evaluate the safety and efficacy of ABX-EGF in subjects with metastatic colorectal carcinoma	Hecht et al, 2007	150
20030167	A phase 2 multicenter single arm clinical trial of ABX-EGF monotherapy in subjects with metastatic colorectal cancer following treatment with fluoropyrimidine, irinotecan and oxaliplatin chemotherapy	Berlin et al, 2006	185
20030194	A multicenter open-label single-arm clinical trial to determine the safety of ABX-EGF extended therapy in subjects with metastatic colorectal cancer	Van Cutsem et al, 2008	177
20030250	A phase 2 multicenter single arm clinical trial of ABX-EGF monotherapy in subjects with metastatic colorectal cancer whose tumors express low or negative EGFR levels by immunohistochemistry following treatment with fluoropyrimidine, irinotecan, and oxaliplatin chemotherapy	Mitchell et al, 2007	203
20040249 (PACCE)	A multicenter, randomised, phase 3b study to evaluated panitumumab added to bevacizumab and chemotherapy (oxaliplatin- and irinotecan-based) as first-line treatment for metastatic colorectal cancer	JR Hecht, unpublished data	1053
20050181	A randomized, multicenter phase 3 study to compare the efficacy of panitumumab in combination with chemotherapy to the efficacy of chemotherapy alone in patients with previously treated metastatic colorectal cancer	Enrollment complete	1187

ABX-EGF, panitumumab

Study Key (continued)

Study Number (Alias)	Title	Publications	Number of Subjects
20050184 (STEPP)	An open-label phase 2 study to estimate the difference in incidence rates of specific \geq grade 2 skin toxicities of interest between patients with metastatic colorectal cancer receiving panitumumab + FOLFIRI or irinotecan-only chemotherapy as second-line treatment in the pre-emptive and reactive skin treatment	Mitchell et al, 2008	95
20050203 (PRIME)	A randomized, multicenter, phase 3 study to compare the efficacy of Panitumumab in combination with oxaliplatin/5-fluorouracil/leucovorin to the efficacy of oxaliplatin/5-fluorouracil/leucovorin alone in patients with previously untreated metastatic colorectal cancer	Enrollment complete	1183
20060277 (PRECEPT)	A multicenter, open-label, single-arm, phase 2 study evaluating panitumumab in combination with FOLFIRI therapy following first-line FOLFOX and bevacizumab treatment of metastatic colorectal cancer	Cohn et al, 2008	116

1. Executive Summary

In oncology, with the advent of targeted therapeutics, there has been increasing interest in the development of biomarkers that have the potential to optimize the benefit:risk of specific therapies. One challenge in developing new predictive biomarkers is translation of scientific advances that affect clinical decisions into actionable regulatory pathways that improve patient access to appropriate treatment. In some cases, for example, biomarker data may be obtained from retrospective analyses of clinical studies that have been previously completed and analyzed. In these instances, a question facing regulatory authorities, the clinical community, and sponsors is the level of evidence required to validate the clinical utility of a predictive biomarker. The appropriate level of evidence should be considered in the context of overall benefit:risk and may be influenced by pertinent safety issues and the availability of alternative therapies.

To inform discussion of the levels of evidence required for the validation of biomarkers in oncology, this briefing document provides data regarding the use of tumor *KRAS* status to predict clinical outcomes with panitumumab in metastatic colorectal carcinoma (mCRC). Panitumumab is a fully human monoclonal antibody directed against the epidermal growth factor receptor (EGFR) and is currently approved in the United States (US) as a single agent for the treatment of EGFR-expressing mCRC with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens.

KRAS, the human homolog of the Kirsten rat sarcoma-2 virus oncogene, encodes a small guanosine triphosphate (GTP)-binding protein that acts as a self-inactivating signal transducer by cycling from guanosine diphosphate (GDP)- to GTP-bound states in response to stimulation of a cell surface receptor, including EGFR. *KRAS* can harbor oncogenic mutations that yield a constitutively active protein. Such mutations are found in approximately 30% to 50% of colorectal cancer (CRC) tumors and are common in other tumor types as well. Since *KRAS* is a key signaling intermediate downstream of EGFR, it has been hypothesized that the presence of constitutively active (mutant) *KRAS* may confer resistance to anti-EGFR monoclonal antibody therapy in CRC.

This biological plausibility and emerging data from exploratory biomarker analysis of single-arm clinical studies, including panitumumab phase 2 studies, led to the hypothesis that tumor *KRAS* mutations correlate with lack of response to anti-EGFR monoclonal

antibodies in mCRC (*KRAS* hypothesis). At the time these initial *KRAS* data became available, the pivotal phase 3 study of panitumumab monotherapy in refractory mCRC had been completed and led to the accelerated approval of panitumumab in the US in 2006. During the conduct of this study, Amgen collected tumor samples with the specific intent of performing subsequent biomarker analysis. Based on the strength of data supporting the *KRAS* hypothesis, Amgen initiated a systematic evaluation of the utility of *KRAS* as a predictive biomarker for panitumumab.

Monotherapy Data

The pivotal phase 3 Study 20020408 was a multinational, randomized, controlled trial comparing panitumumab monotherapy with best supportive care (BSC) in subjects (n = 463) with EGFR-expressing mCRC after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. Key features from a retrospective analysis of outcomes according to *KRAS* status conducted subsequent to the US approval included the following:

- Methodologic aspects of the analysis provided a rigorous framework to evaluate *KRAS* as a biomarker.
 - Tissue samples were collected under appropriate informed consent prior to randomization during the initial study as part of the original study protocol.
 - Biomarker hypothesis testing was restricted to *KRAS*, ie, no other biomarkers were formally evaluated and the only stratification variable was presence or absence of mutant *KRAS*.
 - A pre-specified statistical analysis plan was finalized prior to *KRAS* testing. The primary objective was to assess a treatment-by-*KRAS* interaction on PFS.
 - Power calculations indicated the sample size was sufficient to detect a treatment-by-*KRAS* interaction.
 - *KRAS* testing was performed in a central laboratory under Belgian government test standards (BELAC) following validation according to Clinical and Laboratory Standards Institute (CLSI) Guidelines. The laboratory was blinded to treatment and clinical outcomes.
 - There was a high ascertainment rate (92% of all subjects) for *KRAS* status.

- The efficacy of panitumumab, based on progression-free survival (PFS) and objective response rate, was restricted to subjects with *KRAS* wild-type tumors.
 - The relative treatment effect on PFS was significantly greater among subjects with wild-type vs mutant *KRAS* tumors ($p < 0.0001$ quantitative interaction test).
 - Subjects whose tumors contained wild-type *KRAS* experienced significant improvements in PFS (hazard ratio, 0.45, $p < 0.0001$; median 12.3 vs 7.3 weeks) and response rate (17% vs 0%, $p < 0.0001$) in the panitumumab group compared to the BSC group.
 - There were no differences in PFS (hazard ratio = 0.99) or response rate (0% vs 0%) between the panitumumab and BSC groups in subjects whose tumors contained mutant *KRAS*.
 - The safety profile of panitumumab was similar in subjects regardless of tumor *KRAS* status when adjusted for treatment duration.

To further independently evaluate the association of *KRAS* status with efficacy of panitumumab seen in Study 20020408, additional retrospective analysis of all other studies of panitumumab monotherapy in mCRC was performed (uncontrolled Studies 20030167, 20030194, and 20030250). Each of these studies had consistent outcomes overall and within *KRAS* strata. In a pooled analysis ($n = 715$) including subjects randomized to panitumumab in Study 20020408, the objective response rate in subjects with wild-type *KRAS* tumors ($n = 395$) was 14%; there were no responses in subjects with *KRAS* mutant tumors ($n = 320$).

In summary, the monotherapy data are consistent and compelling, and indicate that the benefit:risk of panitumumab is optimized in subjects with *KRAS* wild-type tumors.

Combination Therapy Data

Preliminary investigations of *KRAS* as a predictive biomarker in the combination chemotherapy setting also have been performed.

- Preliminary results of uncontrolled phase 2 studies of panitumumab in combination with irinotecan-based therapy (20050184 [STEPP] and 20060277 [PRECEPT] trials)

demonstrated improved efficacy, as determined by response rate and PFS, in subjects with *KRAS* wild-type tumors compared to those with *KRAS* mutant tumors.

- In contrast, phase 3 Study 20040249 (PACCE), which investigated panitumumab in combination with bevacizumab and either oxaliplatin- or irinotecan-based chemotherapy, demonstrated inferior outcomes in the overall population and inconsistent results by *KRAS* status.

The available evidence from these clinical studies is not conclusive to generalize the findings from the panitumumab monotherapy studies to the combination setting.

Conclusion

Based on these data in the aggregate, **Amgen has concluded that the benefit:risk profile of panitumumab will be improved by restricting monotherapy use to those patients whose tumors have the wild-type *KRAS* gene.** This would not only restrict use to those patients likely to have improved clinical outcome with panitumumab, but would also prevent unnecessary exposure and potential toxicity in those highly unlikely to benefit. Discussions with FDA on the utility of these data to effect a change to the current panitumumab monotherapy label are ongoing.

Two large ongoing phase 3 studies examining panitumumab with chemotherapy in first- and second-line mCRC will provide more definitive evidence of the clinical utility of *KRAS* as a predictive biomarker in the combination therapy setting. These trials, which have completed enrollment, were amended prior to any *KRAS* testing and before the first efficacy analysis to allow primary analysis of the *KRAS* wild-type population, and will provide data in 2009.

2. Level of Evidence for Biomarkers

KEY POINTS

- Predictive biomarkers may be able to identify patients likely to benefit or suffer harm from specific therapies and thereby spare those unlikely to respond from unnecessary exposure to ineffective agents.
- The science leading to the identification of potential biomarkers may, in some instances, advance more quickly than trials designed to prospectively evaluate their clinical utility. In such cases, retrospective data analysis can contribute to the understanding of the clinical utility of biomarkers.
- Some important aspects of the scientific integrity of a biomarker analysis include assay validation, external hypothesis generation, high sample ascertainment, blinding to treatment and outcomes, and a prospective statistical analysis plan with controls for multiple testing.

2.1 Introduction

One of the fundamental challenges in realizing the goal of personalized medicine is the development of predictive biomarkers¹. Ultimately, the results of biomarker studies provide data that may affect the patient benefit:risk profile and could lead to changes in the practice of medicine. In oncology, there is currently substantial interest in identifying predictive biomarkers that will guide selection of therapies for individual patients.

Critical steps in the development of biomarkers and associated diagnostics² include establishment of biological plausibility of the target, development of a validated assay, and demonstration of the clinical utility of the biomarker in well-conducted studies.

¹ A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Biomarker Definitions Working Group 1998).

² Reagents, instruments, and systems intended for use in the diagnosis of disease or other conditions, including a determination of the state of health, in order to cure, mitigate, treat, or prevent disease or its sequelae. Such products are intended for use in the collection, preparation, and examination of specimens taken from the human body. These products are devices as defined in section 201(h) of the Federal Food, Drug, and Cosmetic Act, and may also be biological products subject to section 351 of the Public Health Service Act. [CFR 809.3(a)]

Ideally, co-development of a predictive biomarker assay and therapeutic in oncology would follow a step-wise process. First, preclinical studies would identify a disease target (or pathway), based on the biology of specific tumors or settings. Next, development of the diagnostic and early phase clinical development of the therapeutic would occur in parallel. Finally, prospective validation of the biomarker, using a validated assay, would be obtained in definitive randomized clinical trials that establish both the effectiveness of the therapeutic and clinical utility of the diagnostic ([Woolsey 2008](#); [Rifai et al, 2006](#); [Food and Drug Administration \[FDA\], 2005](#)).

In some cases, however, biomarker data may be obtained from retrospective analyses of clinical studies that were previously completed and analyzed. The challenge in these cases is rapid translation of scientific advances into clinical and regulatory guidances that improve patient access to appropriate treatment. This section provides a brief discussion of the levels of evidence required for the validation of biomarkers in oncology. The following sections provide, as an example, evidence about the utility of *KRAS* as a predictive biomarker for panitumumab use in mCRC.

2.2 Levels of Evidence

The FDA and others have previously developed criteria or levels of evidence required for a biomarker to be accepted as a diagnostic, whether as a prognostic marker, predictor of response or resistance, or as a marker useful in monitoring treatment ([Altar et al, 2008](#); [Woolsey, 2008](#); [Hayes, 1996](#); [FDA 2005](#); [FDA 2005a](#)). Broadly conceived, these development models all contain several essential elements, including the following:

Scientific foundation

Biological plausibility may be established through multiple lines of evidence. First, a comprehensive understanding of the pathway biology of the target, based on appropriate cellular and animal models, provides assurance that a potential biomarker is scientifically relevant. For example, the estrogen receptor signaling pathway is known to be a fundamental driver of pathogenesis and progression in a subset of human breast cancers. Numerous studies in the last three decades demonstrated that therapies targeting the estrogen receptor, such as tamoxifen and aromatase inhibitors, are effective in estrogen-receptor positive tumors. In this instance, the biomarker is the presence of estrogen receptor expression, and our confidence in the reliability of this biomarker is strengthened by the relatively thorough scientific understanding of the biology of estrogen receptor signaling.

As in the case of the estrogen receptor, biologic plausibility is strengthened by data obtained from the study of human tissue specimens. For example, HER2 receptor overexpression and gene amplification were identified in human breast tumors and correlated with poor clinical outcome ([Slamon et al, 1989](#), [Slamon et al, 1987](#)). Subsequently, preclinical experiments confirmed that HER2 overexpression resulted in aggressive tumor behavior, providing a biological explanation for the results obtained with human specimens.

Analytical Validation

Once plausible biologic evidence has been generated, and a candidate biomarker has been identified, the development of an appropriate assay is required. Analytical validation is a process for assessing an assay, including its performance characteristics under different conditions, reproducibility, and accuracy ([Chau et al, 2008](#)). In addition, preanalytical considerations are often critical, such as the stability of the analyte, sample handling or preparation requirements, and storage requirements. The greatest confidence is obtained when all major sources of technical variability are known and assay accuracy can be determined against well-determined standards ([Altar et al, 2008](#)).

Clinical Utility

Ultimately, the approval and clinical adoption of any diagnostic that will influence treatment decisions must be based on demonstration of clinical utility. In evaluating clinical utility, several important methodologic issues have been identified. As noted, prospective data obtained from a clinical trial expressly designed to test the biomarker/diagnostic affords the greatest degree of certainty. Other important methodologic considerations when evaluating data sets, whether prospective or retrospective, include the development of a prespecified analysis plan that precisely defines the hypotheses that will be tested. Type I error should be controlled for the key assessments. In addition, potential ascertainment bias should be minimized, for instance by achieving a very high ascertainment (successful test) rate in the trial population. To ensure generalizability, the study patient population should be representative of the target patient population. Finally, evidence for clinical utility is enhanced when multiple randomized studies provide consistent results about the value of the biomarker.

3. The *KRAS* Hypothesis

KEY POINTS

- *KRAS* encodes a self-inactivating G-protein that is a critical signaling intermediate downstream of receptors such as EGFR. In some human tumors, the *KRAS* oncogene harbors activating mutations yielding proteins with reduced GTPase activity (ie, without auto-off switch) that are constitutively active.
- Activating mutations in *KRAS* occur in 30% to 50% of colorectal tumors.
- Retrospective evaluation of single-arm studies with panitumumab or cetuximab generated the hypothesis that tumor *KRAS* mutations correlate with lack of response to anti-EGFR monoclonal antibodies in CRC.

3.1 EGFR Signal Transduction

EGFR is a transmembrane tyrosine kinase receptor of the ErbB (also known as HER) family. Receptor activation leads to recruitment and phosphorylation of several intracellular substrates, which in turn engage mitogenic signaling and other tumor-promoting activities ([Figure 1](#); [Hynes and Lane, 2005](#)).

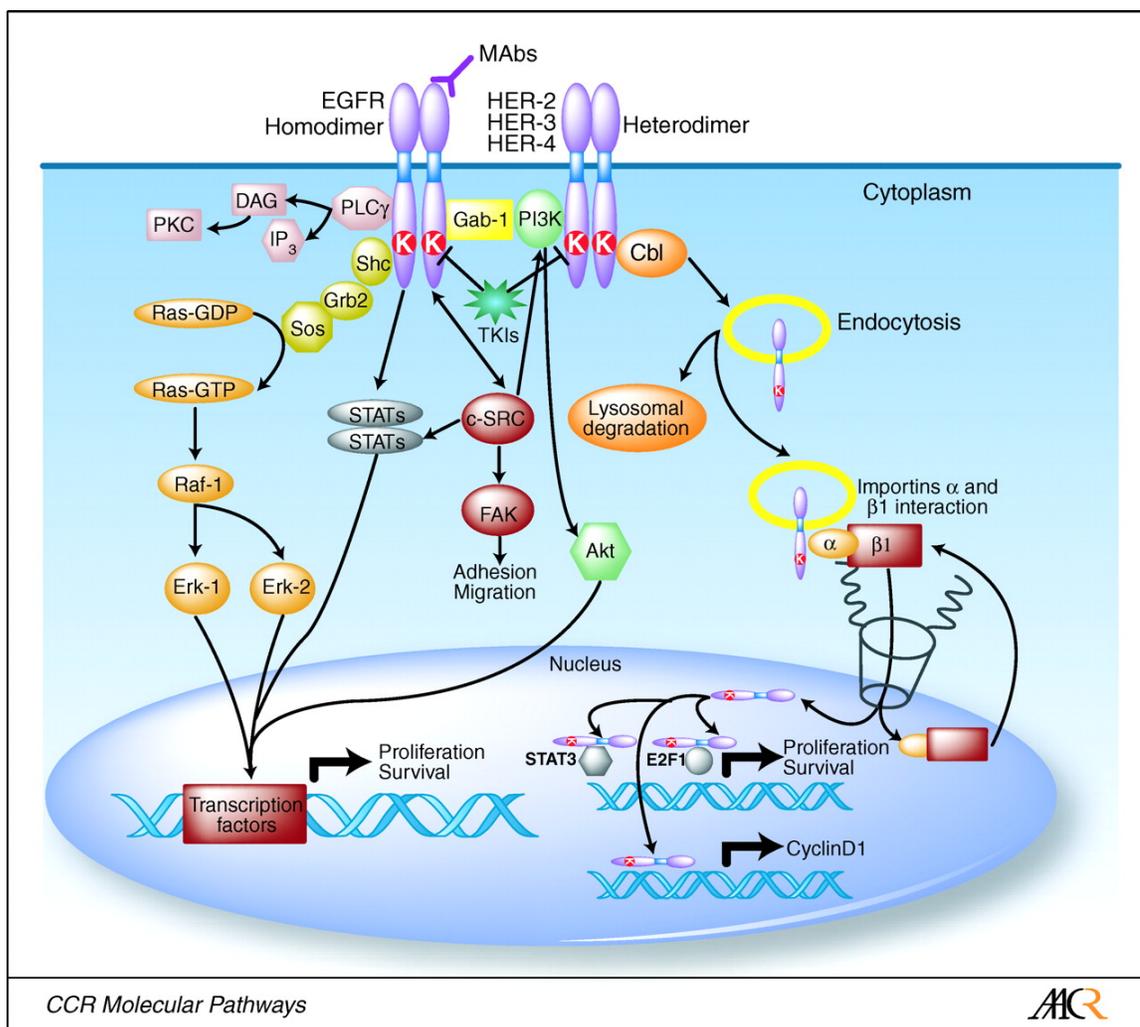
3.2 The *KRAS* Oncogene

Beginning in the 1960s, with the identification of retroviral oncogenes involved in the pathogenesis of rat sarcomas, RAS proteins have been recognized as key intracellular molecules involved in the regulation of cell growth ([Downward, 2003](#)). Three members of the RAS family (*HRAS*, *KRAS*, and *NRAS*) play a role in the pathogenesis and progression of human tumors, with mutations in *KRAS* being most frequent (approximately 85% of total RAS mutations). RAS proteins function as self-inactivating signal transducers by cycling from GDP to GTP bound states in response to stimulation of a cell surface receptor such as EGFR. When receptor tyrosine kinases such as EGFR bind ligand, the receptor dimerizes and undergoes a conformational change that results in phosphorylation of tyrosine residues within the intracellular domain; this in turn leads to activation of protein kinase signaling cascades including the Ras-Raf-MAP kinase (MAPK) pathway ([Malumbres and Barbacid, 2003](#)) ([Figure 1](#)).

Oncogenic RAS proteins have compromised GTPase activity, resulting in the accumulation of RAS in the GTP-bound, active form and subsequent activation of

growth-promoting signaling pathways (Schubbert, 2007). Aberrant activation of RAS therefore would result in the dysregulation of cancer cell proliferation, enhanced invasiveness, angiogenesis, and resistance to apoptosis (Malumbres and Barbacid, 2003).

Figure 1. EGFR Signal Transduction



Scaltriti and Baselga, 2006

RAS proteins are altered in approximately 20% of human tumors, usually through a point mutation that results in constitutive activation of the molecule. Activating mutations in *KRAS* have been observed in multiple malignancies, including CRC, pancreatic cancer, lung adenocarcinoma, gall bladder cancer, bile duct cancer, and thyroid cancer (Hilger 2002).

3.3 **KRAS in Colorectal Cancer**

CRC-activating mutations in the *KRAS* oncogene are present in approximately 30% to 50% of tumors ([Jones et al, 2008](#); [Andreyev et al, 2001](#); [Vogelstein et al, 1988](#); [Appendix 2](#)). In most cases, these mutations appear to arise relatively early in the course of the development of the disease ([Jones et al, 2008](#); [Vogelstein et al, 1988](#)). Based on the reported literature, > 95% of activating mutations in *KRAS* occur in codons 12 and 13 (coded by exon 2) through 1 of 7 different nucleotide changes ([Catalogue of Somatic Mutations in Cancer \[COSMIC\], 2008](#)).

In addition to its role in the pathogenesis of CRC, some studies have indicated that the presence of mutant *KRAS* may correlate with poor prognosis ([Andreyev et al, 2001](#); [Esteller et al, 2001](#); [Ince et al, 2005](#); [Bazan et al, 2002](#)), although the utility of *KRAS* status as a prognostic marker is uncertain ([Bouzourene et al, 2000](#)).

3.4 **KRAS Status and Response to Anti-EGFR Monoclonal Antibody Therapy**

Constitutive activation of downstream signaling pathways is one result of *KRAS* mutations. In theory, these downstream pathways should lose their dependence on upstream activators such as EGFR, with potential loss of responsiveness to anti-EGFR agents. Several retrospective studies conducted external to Amgen addressed this hypothesis by examining the relationship of *KRAS* status and response to anti-EGFR monoclonal antibodies in CRCs ([Table 1](#)).

[Moroni et al \(2005\)](#) reported an analysis of the mutation status of the EGFR catalytic domain and a number of downstream effectors, including *PI3KCA*, *KRAS* and *BRAF* in samples from 31 mCRC patients treated with panitumumab, cetuximab, or cetuximab and chemotherapy and concluded no correlation with disease response, although more *KRAS* mutations were observed in the non-responding group (8 vs 2). In 2006 an analysis of *KRAS*, *BRAF* and *PI3KCA* mutation status of 30 samples from mCRC patients treated with cetuximab with or without chemotherapy showed that no responders had *KRAS* mutations ([Lièvre et al, 2006](#)). In an extension of the study by Moroni et al, Benvenuti et al examined samples for *KRAS* and *BRAF* mutation status from 48 patients with mCRC treated with either panitumumab or cetuximab with or without chemotherapy. In this analysis 1 patient with mutant *KRAS* was classified as a responder compared with 15 non-responding patients who had *KRAS* mutations. The conclusion drawn in the paper was that mutations activating the RAS/RAF signaling pathway were inversely

correlated with response to anti-EGFR monoclonal antibodies (Benvenuti et al, 2007). A further analysis of the *KRAS* status of 59 mCRC patients receiving cetuximab with chemotherapy reported that *KRAS* mutations were only present in non-responding patients (Di Fiore et al, 2007).

In the largest single-arm study reported at the time, samples from a cetuximab monotherapy trial in patients with mCRC were examined for a range of potential biomarkers including *KRAS* status, with successful analysis of 80 samples from the 110 subjects originally enrolled in the study (Khambata-Ford et al, 2007). Of the 5 responders, none had *KRAS* mutations.

It should be noted that these initial studies were observational and lacked a control group. However, the consistency of these data suggested that response to anti-EGFR monoclonal antibodies is restricted to patients whose tumors contain wild-type *KRAS*.

Table 1. *KRAS* Status and Response to EGFR Antibodies in Colorectal Cancer

Publication	Treatment (panitumumab or cetuximab)	No. of Subjects (WT;MT)	Objective Response N (%)	
			Mutant	Wild-type
Moroni et al, 2005	Panitumumab, cetuximab or cetuximab + CT	31 (21;10) ^a	2 (20)	8 (38)
Benvenuti et al, 2007	Panitumumab, cetuximab or cetuximab + CT	48 (32;16) ^a	1 (6)	10 (31)
De Roock et al, 2007	Cetuximab +/- CT	37 (20;17) ^{a,b}	0 (0)	17 (46)
Di Fiore et al, 2007	Cetuximab + CT	59 (43;16) ^a (37 ;22) ^c	0 (0)	12 (28)
Finocchiaro et al, 2007	Cetuximab +/- CT	81 (49;32) ^d	2 (6.3)	13 (26.5)
Khambata-Ford et al, 2007	Cetuximab	80 (50;30) ^a	0 (0)	5 (10)
Lièvre et al, 2007	Cetuximab +/- CT	78 (49;27) ^a	0 (0)	24 (49)

CT, chemotherapy; MT, mutant *KRAS*; WT, wild-type *KRAS*

^a PCR sequencing

^b Reverse transcriptase PCR allelic discrimination analysis

^c *KRAS* status by SNaPshot analysis or PCR-ligase chain reaction

^d *KRAS* status SURVEYOR and Transgenomic WAVE HS system analysis (Cappuzzo et al, 2008)

4. Exploratory *KRAS* Analysis in Panitumumab Phase 2 Studies

KEY POINTS

- Selected archived tumor samples from 3 panitumumab monotherapy, single-arm, phase 2 trials in mCRC were used to evaluate *KRAS* status in relation to response.
- *KRAS* status was analyzed using cloning and sequencing of deoxyribonucleic acid (DNA) isolated from formalin-fixed paraffin-embedded (FFPE) tumor samples.
- *KRAS* status correlated with clinical outcomes, including response, PFS, and overall survival (OS).

Based on the preliminary data described above suggesting a potential role of *KRAS* in mediating response and resistance to anti-EGFR therapies (Table 1), Amgen initially evaluated the effect of *KRAS* status on response to panitumumab therapy in a subset of samples from subjects with mCRC who participated in phase 2, single-arm monotherapy studies (Studies 20025405, 20030167, and 20030250) (Berlin et al, 2006; Hecht et al, 2007, Mitchell et al, 2007). Tumor sections were analyzed from treated subjects who 1) provided informed consent, 2) had objective response data, and 3) had samples available for sequencing. The intent was to analyze a sampling of tumors from confirmed responders and non-responders. *KRAS* status of tumor samples from 62 subjects was determined using internal (ie, Amgen) clone-based DNA sequencing. Of the 62 samples, 38 (61%) were wild-type and 24 (39%) harbored a *KRAS* mutation.

All subjects with an objective response had *KRAS* wild-type tumors (Table 2).

In addition, the rate of stable disease was substantially higher in subjects with *KRAS* wild-type tumors. Although the subjects in this analysis are representative of the phase 2 populations from which they were drawn with respect to baseline demographics, disease characteristics, and tumor response rates (Freeman et al, 2008), these results were considered exploratory given the low sample ascertainment and potential bias due to selection of tumor samples. Nevertheless, the data provided a foundation to more rigorously assess *KRAS* as a predictive biomarker, using tumor samples from the panitumumab phase 3 pivotal trial.

Table 2. *KRAS* Analysis in Panitumumab Phase 2 Studies

Study	Disease	Sample Size	Response	Response According to <i>KRAS</i> Status N (%)	
				Wild-type (N = 38)	Mutant (N = 24)
Pooled Analysis (20025405, 20030167, 20030250)	CRC (Single Arm)	62 ^a	PR	4 (11)	0
			SD	20 (53)	5 (21)
			PD	14 (37)	19 (79)

CR, complete response; CRC, colorectal cancer; PD, progressive disease, SD, stable disease

^a 533 subjects were enrolled in the 3 studies

Source: [Freeman et al, 2008](#)

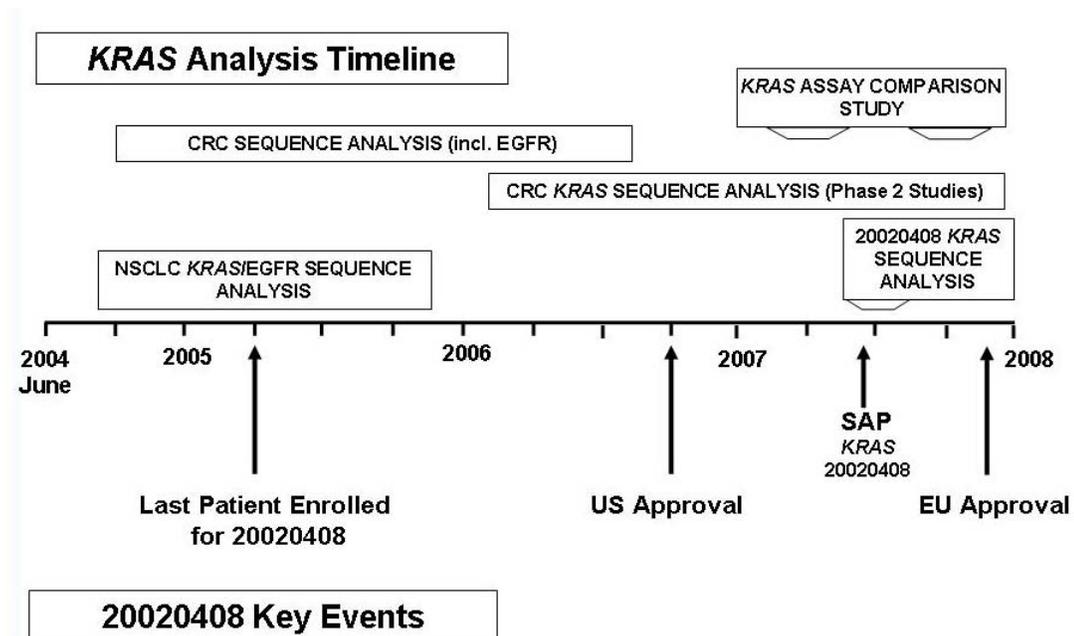
5. KRAS Testing

KEY POINTS

- The majority of *KRAS* mutations (> 95%) occur in codons 12 or 13. Most *KRAS* testing methods evaluate the sequence of these 2 codons.
- Tumor *KRAS* status can be determined from DNA isolated from archived, paraffin-embedded tissue. A comparability study was performed to establish a standard assay for *KRAS* analysis of Amgen studies and to select an external laboratory to perform the testing.
- One particular assay, the DxS K-ras Mutation test kit, which utilizes an allele-specific polymerase chain reaction (PCR), was found to have high sensitivity and specificity compared with direct sequencing. The DxS test was selected by Amgen as the standard assay for evaluation of *KRAS* in clinical studies.

Key events in the selection of *KRAS* testing methodology leading to the retrospective analysis of the pivotal phase 3 Study 20020408 are discussed in the following sections. The relative timing of these events and important milestones in the development of panitumumab are provided in [Figure 2](#).

Figure 2. Timeline of *KRAS* Analysis and Study 20020408



SAP, statistical analysis plan

5.1 **KRAS Analysis**

Detection of *KRAS* mutations can be achieved through direct DNA sequence analysis or with various PCR-based methods including allele-specific PCR. Because a high proportion (> 95%) of activating mutations in *KRAS* occur in codons 12 or 13, most methods evaluate the sequence of these 2 codons ([Appendix 2](#)).

5.2 **Selection of KRAS Assay Methodology**

Amgen examined alternate technologies that would allow the determination of *KRAS* status. Included in this evaluation were two non-sequencing based assays, high-resolution melt (HRM) analysis in-house and an allele-specific PCR-based approach (DxS K-ras Mutation test kit [DxS Ltd, Manchester, UK]) through a contract research organization ([Table 3](#), [Figure 2](#)). In addition, Amgen evaluated an external vendor who provided direct sequencing capability of *KRAS* codons 12 and 13 from FFPE samples (Gentris, Morrisville NC). To conduct this comparison, Amgen used 40 randomly selected CRC tumor blocks obtained from a commercial vendor. Subsequently, 2 additional methods (allele-specific primer extensions and allele-specific hybridization) were assessed as part of an ongoing study to evaluate different *KRAS* testing methodologies and vendors. Due to a high rate of uninformative results obtained by HRM analysis it was decided to not use this method for the prospective analysis of *KRAS* status in samples obtained from clinical trials.

As noted in [Table 3](#), a comparability study using these 40 samples indicated that the DxS test successfully assayed *KRAS* in all specimens and had high concordance (kappa statistic, 0.90) with in-house direct sequencing, which in this comparability study was considered the “gold standard.” Therefore, the DxS assay was selected for *KRAS* analysis.

Table 3. Comparison of *KRAS* Mutation Detection Methods

Vendor	Method	N	Test success (<i>KRAS</i> result obtained)	Kappa (95% CI)
Amgen	PCR <i>KRAS</i> exon 2/sequencing	40	40	-
HistoGeneX	DxS allele-specific PCR <i>KRAS</i> kit	40	40	0.90 (0.76, 1.00)
Gentris	PCR <i>KRAS</i> exon 2/sequencing	40	32	0.75 (0.52, 0.98)
Genzyme ^a	Allele-specific primer extensions	40	35	0.94 (0.83, 1.00)
Invitek ^a	PCR, restriction, PCR, allele-specific hybridization	40	27	0.13 (-0.15, 0.42)

CI, confidence interval; PCR, polymerase chain reaction

^a Evaluated following completion of the *KRAS* mutation testing of the 20020408 study samples.

The Kappa statistic measures agreement for presence of a *KRAS* mutation where a value of 1.0 represents perfect agreement, and -1.0 perfect disagreement with Amgen sequencing data.

[Juan et al, 2008](#)

The DxS K-ras Mutation test kit can detect approximately 1% of mutant DNA in a background of wild-type genomic DNA; the assay has a limit of detection between 5 and 10 copies of mutant *KRAS*. The assay recognizes the 7 most frequent mutations in codons 12 and 13 of the *KRAS* oncogene (Gly12Asp, Gly12Ala, Gly12Val, Gly12Ser, Gly12Arg, Gly12Cys and Gly13Asp) and can detect > 95% of known activating *KRAS* mutations in CRC. A description of the DxS K-ras Mutation test kit is provided in [Appendix 1](#).

6. KRAS Data in Panitumumab Monotherapy Studies

KEY POINTS

- Study 20020408 was a phase 3, randomized, controlled study that showed panitumumab improved PFS compared with BSC alone, and supported the current US label for treatment of EGFR-expressing mCRC in patients with disease progression on or following fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. A retrospective analysis of Study 20020408 to evaluate *KRAS* status in relation to outcomes was conducted.
- Methodologic aspects of the analysis provided a rigorous framework to evaluate *KRAS* as a biomarker.
 - Tissue samples were collected prior to randomization during the initial study as part of the original study protocol.
 - Biomarker hypothesis testing was restricted to *KRAS*, ie, no other biomarkers were formally evaluated and the only stratification variable was presence or absence of mutant *KRAS*.
 - A pre-specified statistical analysis plan was finalized prior to *KRAS* testing. The primary objective was to assess a treatment-by-*KRAS* interaction on PFS.
 - Power calculations indicated the sample size was sufficient to detect a treatment-by-*KRAS* interaction.
 - *KRAS* testing was performed in a central laboratory under BELAC test standards following validation according to CLSI Guidelines. The laboratory was blinded to treatment and clinical outcomes.
 - There was a high ascertainment rate (92% of all subjects) for *KRAS* status.
- The efficacy of panitumumab, based on PFS and objective response rate, was restricted to subjects with *KRAS* wild-type tumors.
 - The relative treatment effect on PFS was significantly greater among subjects with wild-type vs mutant *KRAS* tumors ($p < 0.0001$ quantitative interaction test).

- Subjects whose tumors contained wild-type *KRAS* experienced significant improvements in PFS (hazard ratio, 0.45, $p < 0.0001$; median 12.3 vs 7.3 weeks) and response rate (17% vs 0%, $p < 0.0001$) in the panitumumab group compared to the BSC group.
- There were no differences in PFS (hazard ratio = 0.99) or response rate (0% vs 0%) between the panitumumab and BSC groups in subjects whose tumors contained mutant *KRAS*.
- The safety profile of panitumumab was similar in subjects regardless of tumor *KRAS* status, when adjusting for treatment duration.
- Retrospective analyses of all other uncontrolled studies in the mCRC monotherapy setting independently support the conclusions of the 20020408 analysis. Each of these studies had consistent outcomes overall and within *KRAS* strata. In a pooled analysis ($n = 715$) including subjects randomized to panitumumab in Study 20020408, the objective response rate in subjects with wild-type *KRAS* tumors ($n = 395$) was 14%; there were no responses in subjects with *KRAS* mutant tumors ($n = 320$).

6.1 Phase 3 Study 20020408

Study 20020408 was a pivotal phase 3, randomized, controlled trial comparing panitumumab monotherapy with BSC in subjects with EGFR-expressing mCRC after failure of fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy regimens. Subjects in the BSC alone group were allowed to cross over to panitumumab treatment in Study 20030194 after disease progression. To test the *KRAS* hypothesis, Amgen performed a retrospective analysis of the *KRAS* status of tumor samples from subjects in Study 20020408. The tumor specimens were primarily obtained from resections, but also included some samples collected by endoscopy of the primary lesion or needle biopsies of liver metastases. All specimens were collected with the specific intent of performing biomarker analysis.

The *KRAS* analyses were conducted within a rigorous framework to confirm the clinical utility of *KRAS*. A statistical analysis plan was prepared prior to *KRAS* testing with the objective of evaluating only 1 stratification factor (ie, presence or absence of any

mutated *KRAS* gene). The sample size was shown prospectively to be sufficient to provide adequate power to assess clinical utility with a treatment-by-*KRAS* interaction test. *KRAS* was tested by a central laboratory blinded to treatment and clinical outcomes, using a validated assay. Furthermore, the overall false-positive error (type 1 error) was controlled for in a set of specific tests. No other attempt had been made using Study 20020408 to prospectively test a biomarker hypothesis, or a hypothesis related to any other factor for subject stratification.

Of the 463 subjects initially enrolled in the study, 427 (92%) had evaluable tissue samples and were included in the *KRAS* analyses. These 427 subjects were representative of all randomized subjects in the study in terms of baseline characteristics, disease status, and demographics ([Amado et al, 2008](#)).

Of the 427 subjects who had a tumor sample analyzed for *KRAS*, 243 (57%) were determined to have wild-type *KRAS* and 184 (43%) were determined to have mutant *KRAS* ([Table 4](#)). Among subjects who were randomized to receive panitumumab, 60% had wild-type and 40% had mutant *KRAS*; among subjects that were randomized to receive BSC alone, 54% had wild-type and 46% had mutant *KRAS*. Baseline demographic and disease-related characteristics were similar for subjects with mutant and wild-type tumors.

Table 4. KRAS Status by Treatment Group in Study 20020408

	Panitumumab Plus BSC	BSC Alone	Total
Subjects Randomized	231	232	463
<i>KRAS</i> Not Tested	11 (5%)	7 (3%)	18 (4%)
<i>KRAS</i> Tests Failed	12 (5%)	6 (3%)	18 (4%)
Subjects Included in <i>KRAS</i> Analysis	208 (90%)	219 (94%)	427 (92%)
Subjects Included in <i>KRAS</i> Analysis	208	219	427
Wild-type <i>KRAS</i>	124 (60%)	119 (54%)	243 (57%)
Mutant <i>KRAS</i>	84 (40%)	100 (46%)	184 (43%)

BSC, best supportive care
[Amado et al, 2008](#)

6.1.1 Overview of Efficacy

The primary objective of the analysis was to assess whether the relative effect of panitumumab on PFS in subjects with *KRAS* wild-type tumors was significantly greater than in subjects with mutant tumors. Conditional on the primary objective outcome, secondary objectives were to compare PFS, OS, and objective response rate by treatment in the wild-type stratum.

Progression-free Survival

Analyses by *KRAS* status demonstrated a statistically significantly larger panitumumab treatment effect on PFS in the wild-type *KRAS* stratum versus the mutant *KRAS* stratum (quantitative interaction test, $p < 0.0001$). Within the *KRAS* wild-type stratum, a 55% reduction in relative risk of disease progression or death was observed between subjects treated with panitumumab compared with those who received BSC alone (hazard ratio = 0.45, 95% confidence interval [CI]: 0.34, 0.59) ([Table 5](#) and [Figure 3](#)) ([Amado et al, 2008](#)).

The median (95% CI) PFS in the *KRAS* wild-type stratum was 12.3 (8.3, 16.1) weeks among subjects randomized to panitumumab versus 7.3 (7.0, 7.7) weeks for subjects randomized to BSC alone; an increase of 5.0 weeks ($p < 0.0001$, stratified log-rank test). The 95% CIs for the difference in Kaplan-Meier progression-free rates favored the

panitumumab group at all protocol-specified assessment time points from week 8 to week 32 (Amado et al, 2008).

In contrast, the hazard ratio for the mutant *KRAS* stratum was 0.99 (95% CI: 0.73, 1.36) (Table 5 and Figure 3), indicating no meaningful effect of panitumumab treatment on PFS among subjects with mutant *KRAS* tumor type (Amado et al, 2008). The median (95% CI) PFS was 7.4 (7.3, 7.9) weeks among subjects randomized to panitumumab versus 7.3 (6.3, 7.9) weeks for subjects randomized to BSC alone.

All prospectively defined sensitivity analyses confirmed the results of the primary analysis. These sensitivity analyses included re-calculation of the quantitative interaction test after adjusting the treatment hazard ratios within the *KRAS* strata with propensity scores to adjust for potential treatment group imbalances introduced through exclusion of subjects with non-evaluable *KRAS*, and also using an intention-to-diagnose principle in which subjects with non-evaluable *KRAS* were included in the wild-type *KRAS* stratum. Furthermore, the statistical significance of a treatment-by-*KRAS* interaction was evaluated in a proportional hazards model to have a test sensitive to potentially non-quantitative interactions.

The treatment effect on PFS observed in the wild-type *KRAS* stratum was consistent across all subsets defined by baseline demographic and disease characteristics (ie, age, sex, primary tumor diagnosis, Eastern Cooperative Oncology Group [ECOG] performance status, the number of prior lines of therapy, the number of sites of disease, and EGFR membrane staining or EGFR membrane staining intensity in tumor cells) (Figure 4).

Table 5. Summary of Efficacy Endpoints (Central Assessment)

	Wild-Type		Mutant	
	Panitumumab	BSC	Panitumumab	BSC
	N = 124	N = 119	N = 84	N = 100
Objective Response %				
Complete response	0	0	0	0
Partial response	17	0	0	0
Stable disease	34	12	12	8
Progressive disease	36	75	70	60
p-value ^a	< 0.0001		na	
PFS (median, weeks)	12.3	7.3	7.4	7.3
Hazard ratio	0.45		0.99	
p-value ^b	< 0.0001		na	
Test for quantitative difference in treatment PFS Hazard Ratios	p < 0.0001			
OS (median, months)	8.1	7.6	4.9	4.4
Hazard ratio	0.99		1.02	
p-value ^c	0.14		na	

BSC, best supportive care; OS, overall survival; PFS, progression-free survival

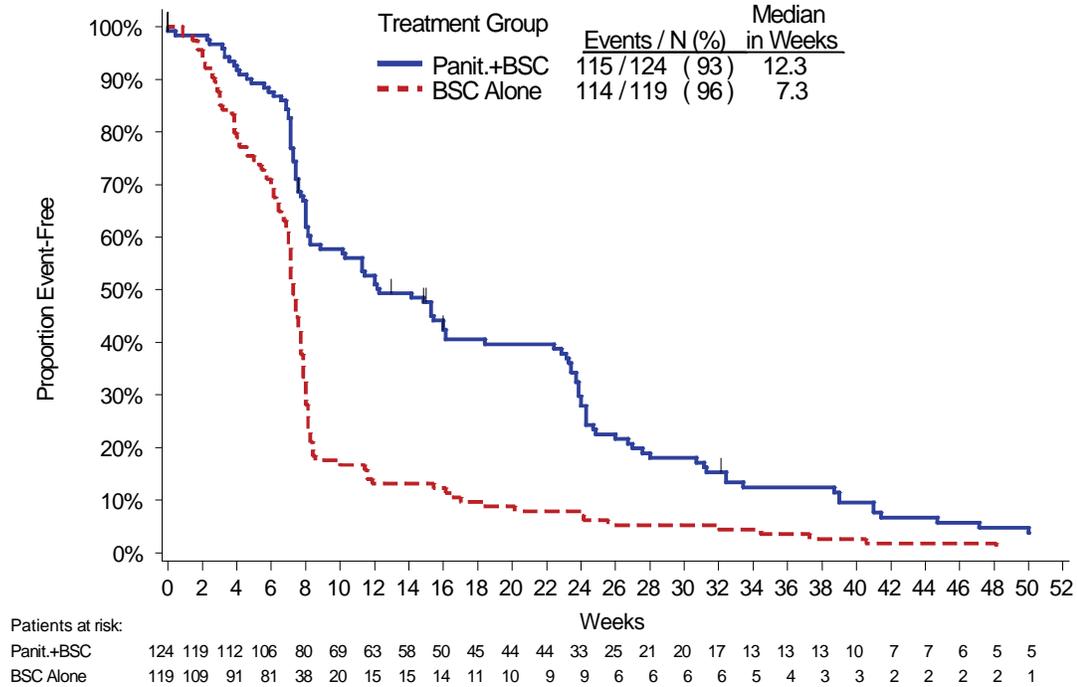
^a Cochran-Mantel-Haenzsel test stratified by ECOG and geographic region

^b Log-rank test stratified by ECOG and geographic region

^c Wilcoxon test stratified by ECOG and geographic region

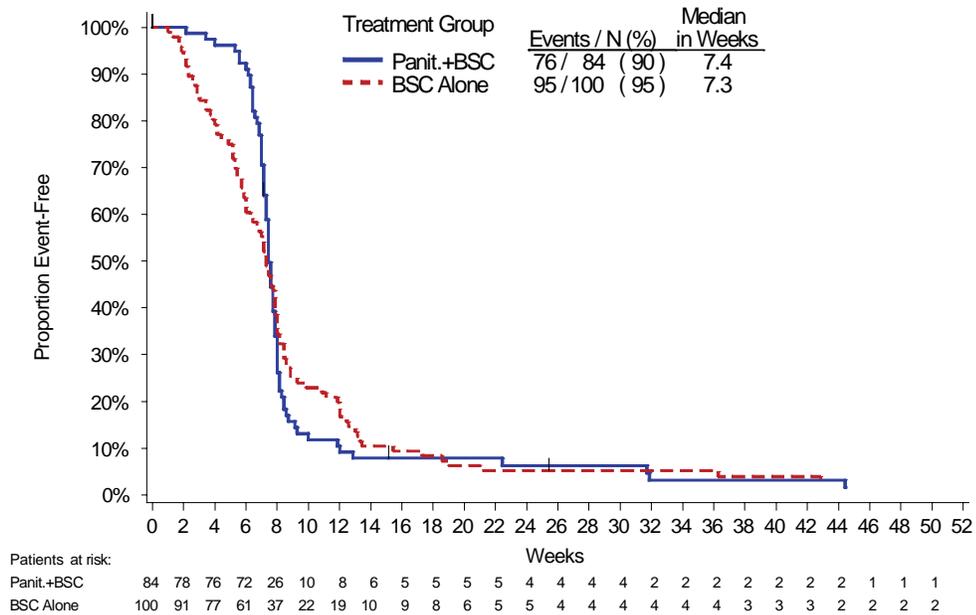
Figure 3. Kaplan-Meier Plot of Progression-free Survival Time (Central Assessment)

A. KRAS wild-type



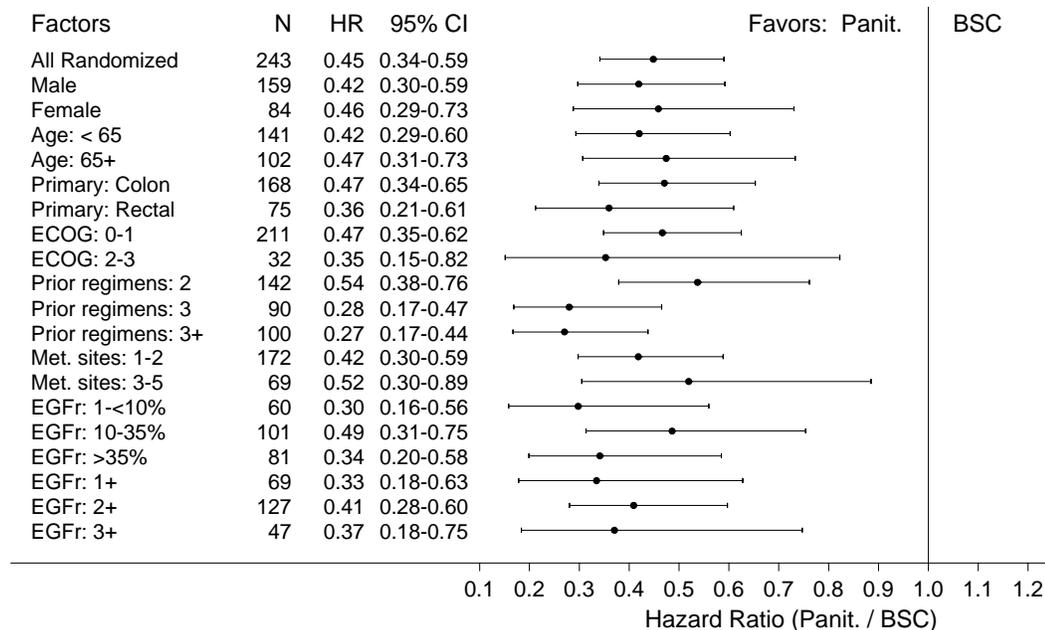
NOTE: hazard ratio = 0.45, 95% CI: 0.34, 0.59

B. KRAS mutant



NOTE: hazard ratio = 0.99, 95% CI: 0.73, 1.36

Figure 4. Forest Plot Showing Progression-free Survival (Central Assessment) Wild-type *KRAS* Stratum



Subset analyses of progression-free survival in the *KRAS* wild-type strata. Hazard ratio (HR; circle) and 95% CI (horizontal lines) adjusted for randomization factors for panitumumab (panit.) versus best supportive care (BSC). N, sample size; HR, hazard ratio; ECOG, Eastern Cooperative Oncology Group; Met, metastatic; EGFr, epidermal growth factor receptor; 1+, weak; 2+, moderate; 3+, strong. Hazard ratios estimated from Cox-proportional hazards model and are presented as panitumumab:BSC alone. A value < 1.0 indicates a lower average event rate longer time to event for panitumumab relative to BSC alone.

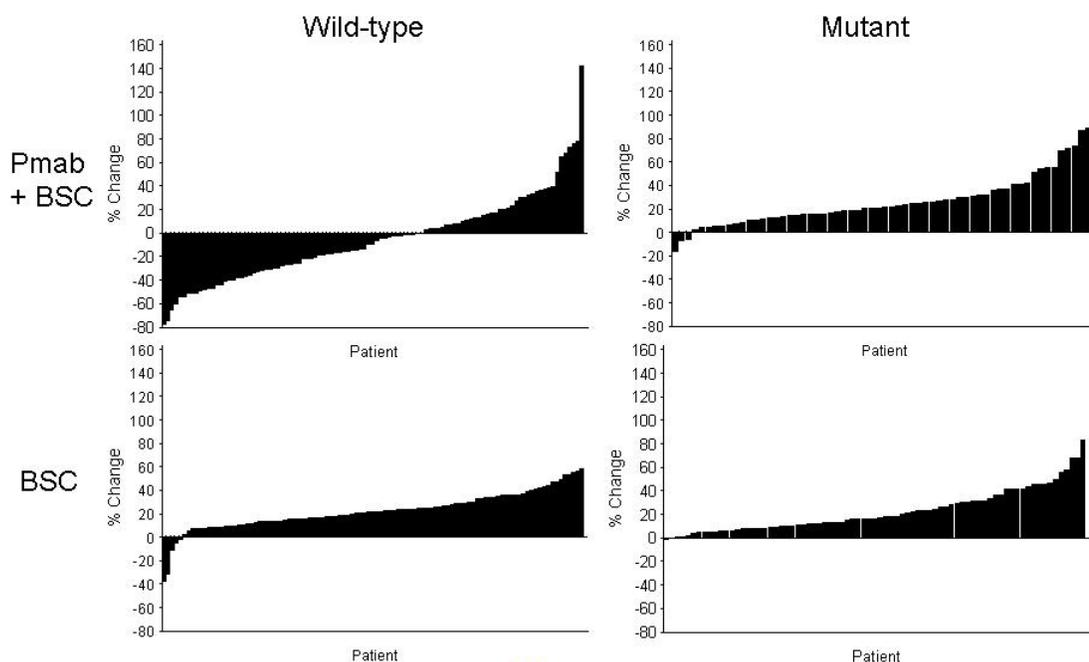
Tumor Response

Consistent with the PFS data, the objective response to panitumumab per modified RECIST (Response Evaluation Criteria in Solid Tumors) by central review was 17% (21/124) in the subjects whose tumors expressed wild-type *KRAS* ($p < 0.0001$, stratified generalized Cochran-Mantel-Haenszel test) and 0% (0/84) in those with mutant *KRAS* (Table 5). All responses were partial responses. No subject in the BSC alone group had an objective response. An additional 52 subjects (42 [34%] wild-type *KRAS*, 10 [12%] mutant *KRAS*) in the panitumumab group and 22 subjects (14 [12%] wild-type *KRAS*, 8 [8%] mutant *KRAS*) in the BSC alone group had a best response of stable disease (Table 5). The disease control rate, defined as the incidence of a confirmed objective response plus stable disease, in subjects that received panitumumab was 51% in the wild-type *KRAS* stratum and 12% in the mutant *KRAS* stratum.

Reduction in Tumor Size by *KRAS* Status

An analysis of the changes in target lesion size for individual subjects was consistent with the results observed for PFS and objective response. For the wild-type *KRAS* stratum, 62% of panitumumab subjects with post-baseline data had a target lesion decrease (Figure 5). In contrast, in the mutant *KRAS* stratum, 5% of panitumumab subjects had reductions in target lesions. Few subjects randomized to BSC alone with either wild-type (5%) or mutant *KRAS* (1%) had a tumor reduction.

**Figure 5. Maximum Reduction in Target Lesions (Central Radiology)
(Subjects Evaluable for *KRAS*)**



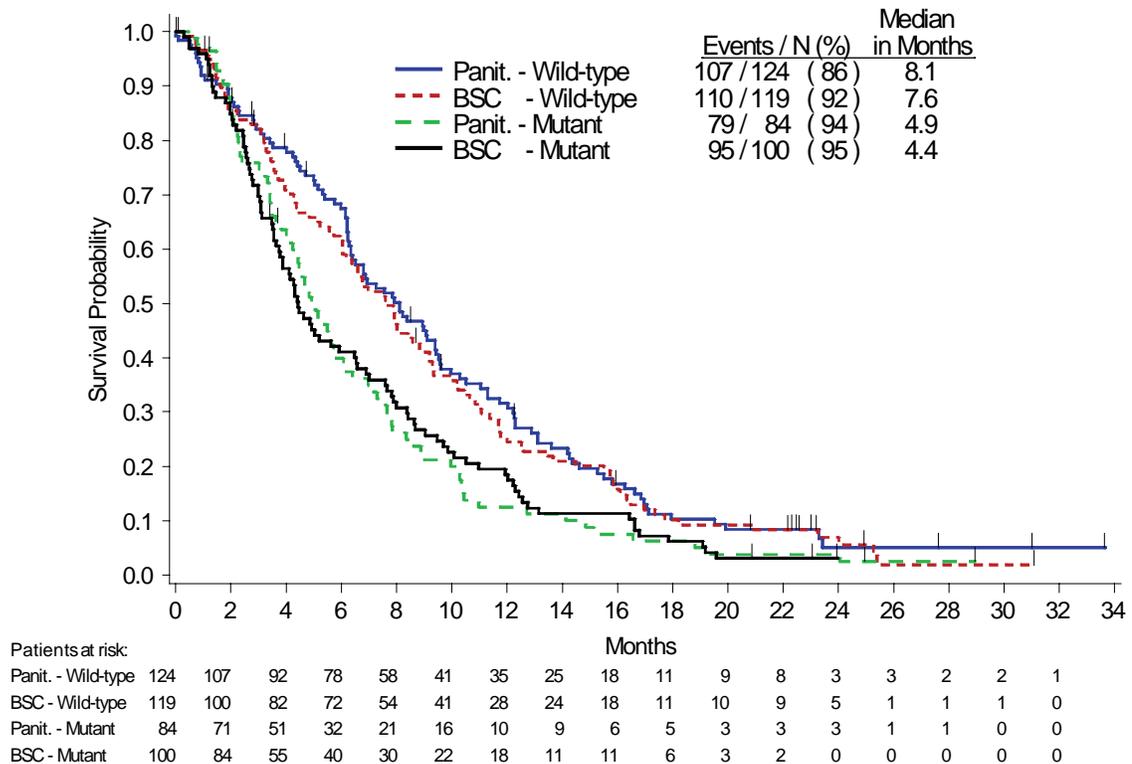
[Amado et al, 2008](#)

Overall Survival

As observed in the primary analysis for Study 20020408 ([Van Cutsem et al, 2008](#)), no statistically significant difference in survival was observed between treatment groups in either of the *KRAS* strata ([Table 5](#)). There was rapid and extensive crossover of BSC subjects into the open-label extension Study 20030194 (76% and 77% of subjects with wild-type mutant *KRAS*, respectively). No difference in survival was observed between treatment groups for subjects with wild-type *KRAS* (stratified Wilcoxon test p-value = 0.1395, hazard ratio = 0.99 [95% CI: 0.75, 1.29]). The hazard ratio for OS in the mutant group was 1.02 (95% CI: 0.75, 1.39). Subjects with wild-type *KRAS* had a median (95% CI) OS time approximately 3 months longer than subjects with mutant

KRAS, regardless of treatment group (panitumumab 8.1 (6.3, 9.4) months wild-type vs 4.9 (4.2, 6.1) months mutant, BSC 7.6 (6.2, 8.8) months wild-type vs 4.4 (3.7, 6.5) months mutant (Table 5, Figure 6).

Figure 6. Kaplan-Meier Curves for Overall Survival by Treatment and *KRAS* Status



BSC, best supportive care; events, deaths; N, sample size; Panit, panitumumab

Cross-over Study 20030194

Subjects enrolled to BSC alone in Study 20020408 were eligible to subsequently receive panitumumab monotherapy upon progression in crossover Study 20030194. In fact, there was extensive (76%) and rapid (median, 7.1 weeks) crossover from BSC alone to panitumumab (Van Cutsem et al, 2008). One hundred sixty-eight of 176 treated subjects (95%) who crossed over to receive panitumumab were evaluable for *KRAS*. Twenty (12%) experienced a response per modified-RECIST by investigator assessment (including 1 complete response). All responders had wild-type *KRAS* for a response rate of 22% in this group (20/91) (Table 6). Among cross-over subjects, PFS was also significantly longer among those with wild-type *KRAS* tumors (hazard ratio, 0.32; median PFS 17.0 vs 7.9 weeks for wild-type and mutant, respectively).

**Table 6. Summary of Efficacy Endpoints in Study 20030194
(Subjects with Evaluable *KRAS* Status)**

	<i>KRAS</i> Wild-type (N = 91)	<i>KRAS</i> Mutant (N = 77)
Progression-free survival		
Subjects who progressed/died - n (%)	89 (98)	77 (100)
Median time (weeks) (95% CI)	17.0 (13.1, 23.0)	7.9 (7.4, 8.1)
Min, Max	0,93	0,77
Overall survival		
Subjects who died - n (%)	85 (93)	73 (95)
Median time (months) (95% CI)	6.8 (5.9, 8.7)	4.5 (2.7, 6.6)
Min, Max	0,25	0,21
Objective tumor response		
Subject responding - n (%)	20 (22)	0 (0)
Response Rate - % (95% CI)	21.98 (13.97,31.88)	0.00 (0.00,4.68)
Min, Max	0,93	-

CI, confidence interval
[Amado et al, 2008](#)

6.1.2 Overview of Safety

Some differences in the overall adverse event profile were observed between the wild-type and mutant *KRAS* strata (Table 7). Compared with subjects in the mutant *KRAS* subset, subjects in the wild-type *KRAS* subset received more infusions of panitumumab (mean 10.0 infusions for wild-type *KRAS* subset compared with 4.9 infusions for the mutant *KRAS* subset), which may have contributed to the slightly increased subject incidence of adverse events in the wild-type *KRAS* subset. Integrated safety analyses have previously demonstrated a trend towards a higher incidence and severity of adverse events related to treatment (eg, integument toxicity, diarrhea, stomatitis/oral mucositis, and hypomagnesemia) with increased exposure.

All subjects who received panitumumab had at least 1 adverse event regardless of *KRAS* status. There was a higher subject incidence of \geq grade 3 adverse events in the wild-type *KRAS* subset (64% with wild-type *KRAS* subset compared with 55% with mutant *KRAS* subset) and a higher subject incidence of treatment-related grade 3 adverse events (25% in the wild-type *KRAS* subset compared with 12% in the mutant *KRAS* subset). No subjects in either subset had grade 4 or 5 treatment-related adverse events.

When adjusted for the increased panitumumab exposure in subjects with wild-type *KRAS*, no significant differences in the incidence of toxicities were observed within treatment groups between the wild-type *KRAS* stratum. Additionally, the exposure-adjusted subject incidence rates of adverse events \geq grade 3 (worst severity) were similar between the wild-type *KRAS* subset (1.5 subjects/year) and the mutant *KRAS* subset (1.4 subjects/year). However, the exposure-adjusted subject incidence for treatment-related adverse events of \geq grade 3 (worst severity) was higher in the wild-type *KRAS* subset (0.46 subjects/year) compared with the mutant *KRAS* subset (0.24 subjects/year).

A similar subject incidence of integument-related adverse events was observed in both *KRAS* subsets (93% in the wild-type *KRAS* subset and 90% in the mutant *KRAS* subset). Decreased magnesium values were associated with panitumumab administration, with larger mean decreases observed in the wild-type *KRAS* subset (0.2 mmol/L) than in the mutant *KRAS* subset (0.1 mmol/L). More subjects in the wild-type *KRAS* subset had a grade 3 or 4 (worst severity) laboratory finding of hypomagnesemia (6% and 2%, respectively) compared with the mutant *KRAS* subset (0% and 1%, respectively). A similar subject incidence of adverse events leading to withdrawal was observed in the wild-type (7%) and mutant (5%) *KRAS* subsets.

Table 7. Overview Summary of Adverse Events (KRAS Safety Analysis Set)

	Wild-type KRAS		Mutant KRAS		Total	
	Panitumumab Plus BSC (N = 123)	BSC Alone (N = 120)	Panitumumab Plus BSC (N = 84)	BSC Alone (N = 100)	Panitumumab Plus BSC (N = 207)	BSC Alone (N = 220)
Subjects with any adverse event – n (%)	123 (100)	108 (90)	84 (100)	84 (84)	207 (100)	192 (87)
Worst grade of 3 ^a	50 (41)	22 (18)	20 (24)	16 (16)	70 (34)	38 (17)
Worst grade of 4 ^a	4 (3)	1 (1)	3 (4)	2 (2)	7 (3)	3 (1)
Worst grade of 5 ^a	24 (20)	18 (15)	23 (27)	13 (13)	47 (23)	31 (14)
Any Serious	55 (45)	30 (25)	38 (45)	25 (25)	93 (45)	55 (25)
Leading to permanent discontinuation from treatment phase or study	9 (7)	4 (3)	4 (5)	2 (2)	13 (6)	6 (3)
Not Serious	3 (2)	1 (1)	0 (0)	0 (0)	3 (1)	1 (0)
Serious	6 (5)	3 (3)	4 (5)	2 (2)	10 (5)	5 (2)
Subjects with any treatment-related adverse event ^b – n (%)	112 (91)	1 (1)	76 (90)	1 (1)	188 (91)	2 (1)
Worst grade of 3 ^a	31 (25)	0 (0)	10 (12)	0 (0)	41 (20)	0 (0)
Worst grade of 4 ^a	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Worst grade of 5 ^a	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Any Serious	5 (4)	0 (0)	3 (4)	0 (0)	8 (4)	0 (0)
Leading to permanent discontinuation from treatment phase or study	2 (2)	0 (0)	1 (1)	0 (0)	3 (1)	0 (0)
Not Serious	1 (1)	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)
Serious	1 (1)	0 (0)	1 (1)	0 (0)	2 (1)	0 (0)

Adverse events were coded using the MedDRA dictionary V9.0

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This includes deaths occurring during treatment and deaths within 30 days of the decision to withdraw from the treatment period or the safety follow-up whichever is the later.

^aSeverity was graded using the National Cancer Institute Common Toxicity Criteria version 2.0, with the exception of some dermatology/skin adverse events (nail changes, erythema, pruritus/itching, rash acne/acneiform, desquamation and ulceration)

^bThe investigator considered there to be a reasonable possibility that the event may have been caused by study drug.

Source: Study 20020408 KRAS clinical study report Tables 14-6.1.1, 14-6.1.2, and 14-6.1.3

6.2 Extended Analysis of *KRAS* in Panitumumab Monotherapy Studies

To further evaluate the association of *KRAS* status with efficacy of panitumumab monotherapy, a retrospective analysis of all available samples was performed in 4 mCRC studies (Table 8) (Amado et al, 2008). This pooled analysis included all studies of panitumumab monotherapy in subjects with mCRC that had progressed after standard chemotherapy.

All studies included in the pooled analysis had similar designs, all but 1 required EGFR staining by immunohistochemistry (Hecht et al, 2007; Van Cutsem et al, 2007; Berlin et al, 2006), and each had a provision for collection of tumor tissue. *KRAS* status was determined using the DxS K-ras Mutation test kit, at the independent central laboratory blinded to outcomes (HistoGeneX). Tumor responses were assessed centrally in 3 studies.

Table 8. Panitumumab Monotherapy Studies Included in Pooled Analysis

	Van Cutsem et al, 2007		Berlin, et al, 2006	Hecht, et al, 2007
	Phase 3 Panitumumab	Crossover		
Amgen study number	20020408	20030194	20030167	20030250
Phase	3	3 ^a	2	2
Dose schedule	6 mg/kg Q2W	6 mg/kg Q2W	6 mg/kg Q2W	6 mg/kg Q2W
Response assessment	RECIST Central review	RECIST Local review	WHO Central Review	WHO Central Review
Assessment schedule per protocol	Q8W from weeks 8-48 ^b then Q3M	Q8W until disease progression	Q8W from weeks 8-48 ^b then Q3M	Q8W from weeks 8-48 ^b then Q3M
Tumor cells stained for membrane EGFR ^c	≥ 1%	≥ 1%	≥ 10%	< 1% or 1-9%

RECIST, Response Evaluation Criteria in Solid Tumors; Q2W, every 2 weeks; Q8W, every 8 weeks; Q3M, every 3 months; WHO, World Health Organization

^a Extension study. Subjects who developed progressive disease while receiving BSC in the phase 3 study were allowed to cross over to this extension study to receive panitumumab until disease progression or drug intolerability.

^b An additional scan was performed at week 12

^c By immunohistochemistry at central laboratory

A high rate of *KRAS* ascertainment was achieved in each study (84% to 95%). Across all studies, 795 subjects received panitumumab and had tumor samples available for

testing; 715 subjects (90%) had samples evaluable for *KRAS* and were included in the analysis and of these, 320 subjects (45%) had tumors with a *KRAS* mutation (Table 9).

Each of the 4 studies had consistent outcomes overall and within *KRAS* strata. Efficacy, as measured by objective tumor response, was restricted to subjects with wild-type *KRAS* tumors. The response rate in subjects with wild-type *KRAS* was 13.7%, whereas no responses were reported in subjects with mutant *KRAS*. As observed in the 20020408 study, PFS and OS favored subjects with wild-type *KRAS* receiving panitumumab. For PFS, the hazard ratio (wild-type vs mutant *KRAS* status) was 0.42 (95% CI: 0.36, 0.50); for OS, the hazard ratio was 0.63, (95% CI: 0.53, 0.74). Median PFS and OS times were longer in subjects with wild-type *KRAS* compared with subjects with mutant *KRAS* tumors.

Table 9. Efficacy Outcomes in Pooled Monotherapy Analysis

	<i>KRAS</i> Wild-type (n = 395 [55%])	<i>KRAS</i> Mutant (n = 320 [45%])
Objective Response Rate (95% CI)	13.7% (10.4, 17.5)	0% (0, 1.2)
Complete response - n (%)	1 (<1)	0 (0)
Partial response - n (%)	53 (13)	0 (0)
Stable disease - n (%)	151 (38)	46 (14)
Median Progression-free Survival (95% CI) weeks	14.1 (11.6, 15.4)	7.3 (7.1, 7.4)
Median Overall Survival (95% CI), months	8.3 (7.5, 9.1)	5.7 (5.0, 6.4)

CI, confidence interval
[Amado et al, 2008](#)

As expected from the results of the individual studies, there were no significant differences in the adverse event profile when adjusting for exposure (duration of therapy) between subjects with *KRAS* wild-type and subjects with mutant *KRAS*.

In summary, this extended analysis of panitumumab monotherapy data was consistent with the results observed in Study 20020408.

7. Evaluation of *KRAS* and Panitumumab in Combination With Chemotherapy

KEY POINTS

- Preliminary results of uncontrolled phase 2 studies of panitumumab in combination with irinotecan-based therapy (STEPP and PRECEPT trials) demonstrated improved efficacy, as determined by response rate and PFS, in subjects with *KRAS* wild-type tumors compared to those with *KRAS* mutant tumors.
- In contrast, Study 20040249 (PACCE), which investigated panitumumab in combination with bevacizumab and either oxaliplatin- or irinotecan-based chemotherapy, demonstrated inferior outcomes in the overall population and inconsistent results by *KRAS* status.
- Studies 20050203 (FOLFOX +/- panitumumab) and 20050181 (FOLFIRI +/- panitumumab) in the mCRC first- and second-line settings, respectively, will evaluate the clinical benefit of panitumumab in combination with chemotherapy in a *KRAS* wild-type population.
 - The statistical analysis plans of these studies were revised to focus the primary analysis on the wild-type *KRAS* subject population.
 - The trial protocols were amended before any *KRAS* testing was conducted and before the first efficacy analysis.
 - Pre-treatment tumor samples will be evaluated for *KRAS* by a central laboratory blinded to treatment and outcomes.

7.1 Preliminary Data for *KRAS* in Studies of Panitumumab in Combination With Chemotherapy

Panitumumab has been studied in clinical trials as combination therapy. Exploratory analyses were performed to assess outcomes by *KRAS* status.

Study 20050184 (STEPP)

STEPP is a phase 2, open-label study of pre-emptive versus reactive skin toxicity treatment in mCRC subjects (n = 95) receiving panitumumab plus FOLFIRI or irinotecan-only chemotherapy as second-line treatment. The primary objective of this study is to estimate the difference in incidence rates of specific \geq grade 2 skin toxicities of interest

between subjects in the pre-emptive versus reactive skin toxicity treatment arms during a 6-week skin treatment period.

An interim analysis of efficacy was conducted when all randomized subjects ($n = 95$) had the opportunity to complete the first tumor response assessment at week 13 or week 14. *KRAS* status could be determined for 87 of the 95 randomized subjects (92%). Of these 87 subjects, 49 subjects (56%) had wild-type *KRAS* and 38 subjects (44%) had mutant *KRAS* tumor status (Mitchell et al, 2008).

Numerical differences in favor of the subjects with wild-type *KRAS* were observed for the efficacy endpoints of overall response rate (14% vs 8%) and median PFS (5.7 vs 3.3 months). The STEPP study is continuing to further evaluate safety and efficacy.

Study 20060277 (PRECEPT)

PRECEPT is a phase 2, multicenter, open-label, single-arm study to prospectively estimate the effect of tumor *KRAS* status on efficacy endpoints in subjects with mCRC receiving panitumumab plus FOLFIRI treatment as second-line therapy. Tumor tissue for *KRAS* testing was collected at screening and mutant *KRAS* was detected using the DxS K-ras Mutation test kit.

A planned, exploratory, interim analysis occurred when the ~100th treated subject had the opportunity to complete the 17-week tumor evaluation. *KRAS* status could be determined for 109 of 115 randomized subjects (95%). Sixty-four subjects (59%) with wild-type *KRAS* and 45 subjects (41%) with mutant *KRAS* had received ≥ 1 dose of panitumumab and had a valid baseline *KRAS* status available and of these, 102 subjects (59 subjects wild-type *KRAS* and 43 subjects mutant *KRAS*) had the opportunity to complete the first scheduled tumor assessment (week 8) (Cohn et al, 2008). Numerical differences in favor of subjects with wild-type *KRAS* were observed in efficacy endpoints in this interim analysis. While response rates did not differ significantly between the wild-type and mutant *KRAS* strata (25% vs 26%), median (95% CI) PFS in the wild-type *KRAS* stratum was longer than in the mutant *KRAS* stratum (26 [15, 34] weeks and 16 [9, 24] weeks, respectively) (hazard ratio = 0.7). The PRECEPT study continues; final data collection and analyses are ongoing.

7.2 Panitumumab in Combination With Bevacizumab and Chemotherapy

The PACCE trial (Study 20040249) was a multicenter, randomized, phase 3b study that evaluated panitumumab added to bevacizumab and chemotherapy (oxaliplatin- or irinotecan-based) as first-line treatment for mCRC. In this study, 823 and 230 subjects were randomized to the oxaliplatin and irinotecan cohorts, respectively.

Panitumumab was discontinued after a planned interim analysis of 812 oxaliplatin subjects showed worse efficacy and increased toxicity (particularly diarrhea, infections, and pulmonary embolism) in the panitumumab group. Outcomes were inferior in the panitumumab groups in both the oxaliplatin and irinotecan cohorts in the overall intent-to-treat (ITT) population (JR Hecht, unpublished data; [Table 10](#)).

An exploratory analysis was performed to determine whether *KRAS* status influenced the relative efficacy and safety of panitumumab in the PACCE study. *KRAS* status was determined in 82% of subject tumor samples (81% in the oxaliplatin cohort; 87% in the irinotecan cohort). Mutations were found in 39% in the oxaliplatin cohort and 43% in the irinotecan cohort. In a combined analysis of both cohorts, PFS was inferior in the panitumumab group relative to the control group regardless of *KRAS* status; whereas OS was inferior primarily in subjects with *KRAS* wild-type tumors.

When evaluating the data by chemotherapy cohort, there were inconsistent results by *KRAS* status. Overall response rate in the irinotecan cohort favored the panitumumab group in the ITT as well as the *KRAS* wild-type stratum, but not the *KRAS* mutant stratum; however, an opposite trend was seen for the oxaliplatin cohort. In the oxaliplatin cohort, the median OS was longer in the control group in subjects with *KRAS* wild-type tumors (hazard ratio = 1.89), whereas there was no difference in OS in subjects with mutant *KRAS* tumors (hazard ratio = 1.02). In the irinotecan cohort, OS favored the control group regardless of *KRAS* status.

Table 10. Overview of Efficacy Outcomes From the PACCE Trial

	Oxaliplatin cohort		Irinotecan cohort	
	Control N = 410	Panitumumab N = 413	Control N = 115	Panitumumab N = 115
ITT Analysis				
Response rate	48%	46%	40%	43%
PFS (months)	11.4	10.0	11.7	10.1
OS (months)	24.5	19.4	20.5	20.7
KRAS Analysis				
<i>KRAS</i> ascertainment	81%		87%	
Response Rate				
<i>KRAS</i> wild-type	56%	50%	48%	54%
<i>KRAS</i> mutant	44%	47%	38%	30%
PFS (months)				
<i>KRAS</i> wild-type	11.5	9.8	12.5	10.0
HR (95% CI)	1.36 (1.04, 1.77)		1.50 (0.82, 2.76)	
<i>KRAS</i> mutant	11.0	10.4	11.9	8.3
HR (95% CI)	1.25 (0.91, 1.71)		1.19 (0.65, 2.21)	
OS (months)				
<i>KRAS</i> wild-type	24.5	20.7	19.8	-
HR (95% CI)	1.89 (1.30, 2.75)		1.28 (0.5, 3.25)	
<i>KRAS</i> mutant	19.3	19.3	20.5	17.8
HR (95% CI)	1.02 (0.67, 1.54)		2.14 (0.82, 5.59)	

HR, hazard ratio; ITT, intent-to-treat; OS, overall survival; PFS, progression-free survival
JR Hecht, unpublished data

The results of the PACCE trial raised the unexpected possibility of a negative interaction between panitumumab and chemotherapy plus bevacizumab. Interestingly, a similar negative interaction was observed in a phase 3 study of cetuximab in combination with oxaliplatin-based chemotherapy plus bevacizumab (Punt et al, 2008).

The difference in the outcome seen in the oxaliplatin versus irinotecan cohort further raised the possibility that the negative interaction may be dependent on the chemotherapeutic agent, although the data are limited to evaluate such a second-order interaction. While the exact explanation for the results of the PACCE trial is unknown, several hypotheses can be postulated.

- A physiologic interaction between the monoclonal antibodies and/or the monoclonal antibodies and chemotherapy is a potential explanation. Toxicity was exacerbated by dual pathway inhibition in combination with chemotherapy (JR Hecht, unpublished data). The presence of bevacizumab in the setting of anti-EGFR antibody and chemotherapy could have enhanced diarrhea and skin toxicity, by inhibiting tissue repair, and more complete inhibition of the vascular endothelial growth factor (VEGF) axis also could have increased the incidence of pulmonary embolism. There was an increase in dose delays and reductions, and decreases in dose intensity in the panitumumab groups of the PACCE trial, and excessive toxicity likely contributed significantly to these findings.
- A pharmacodynamic interaction induced by EGFR inhibition could have led to a blunting of the therapeutic effects of bevacizumab and/or chemotherapy. For example, EGFR-mediated alterations of primary targets or downstream molecules required for the activity of bevacizumab could have altered the response of tumor cells to this agent.
- Although pharmacokinetic interactions between monoclonal antibodies or between monoclonal antibodies and chemotherapy are extremely uncommon, the possibility cannot be excluded as drug concentration levels were not collected in the PACCE study.

Overall, the findings from PACCE indicate that adding panitumumab to standard chemotherapy and bevacizumab is not feasible using the regimens tested to date. The inconsistent results by *KRAS* status between the oxaliplatin and irinotecan cohorts are not fully understood, and may have been confounded by differences in toxicity and the dose intensity of chemotherapy delivered. One potential explanation is a chance association given the exploratory nature of the analysis. In addition, interpretation of the *KRAS* data from the PACCE study is limited by the early termination of the trial.

Ongoing phase 3 trials described in the following section will provide more definitive data about *KRAS* as a predictive biomarker when panitumumab is used in combination with chemotherapy (without bevacizumab).

7.3 Phase 3 Combination Chemotherapy Studies

Amgen is conducting 2 large phase 3 studies (20050203 and 20050181) in the first- and second-line CRC settings that will provide efficacy and safety data on panitumumab in combination with chemotherapy. These studies were ongoing at the time of the retrospective analysis of *KRAS* in Study 20020408. To date, no comparative interim analysis results from these phase 3 studies have been shared outside of the independent data monitoring committee (DMC).

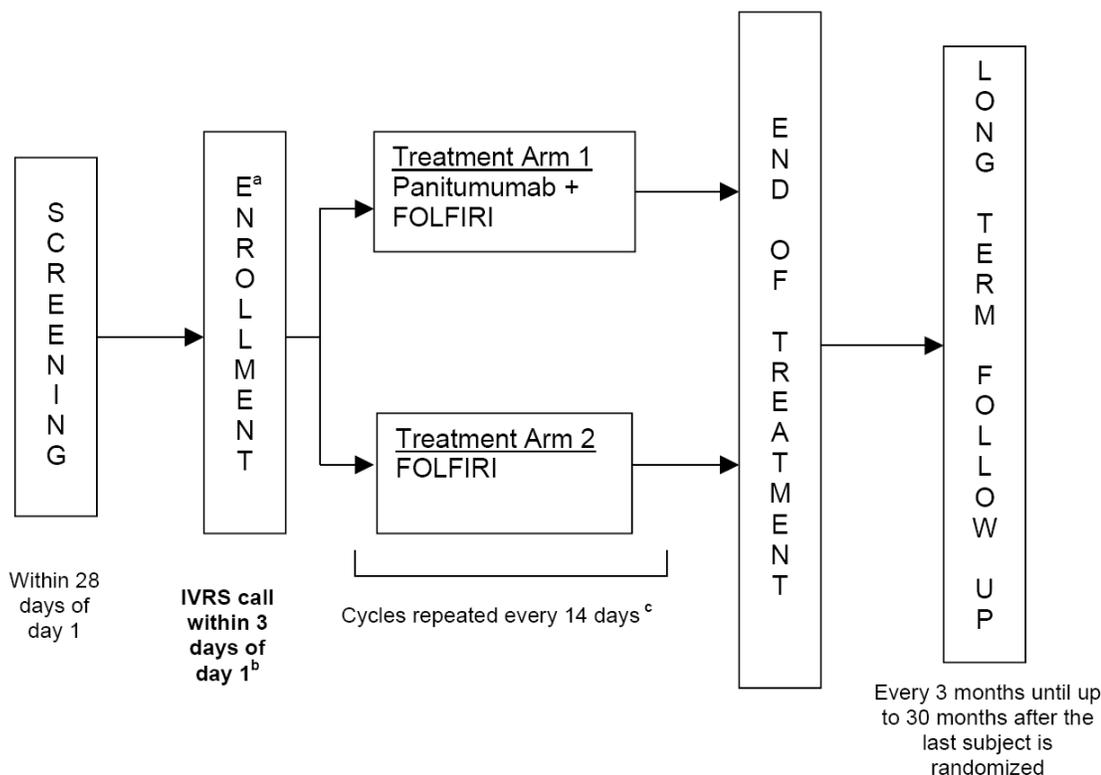
After the data from Study 20020408 became available, the primary analyses for the 20050203 and 20050181 studies were revised to prospectively evaluate the treatment effect of panitumumab in combination with chemotherapy within a wild-type *KRAS* population.

7.3.1 Study 20050181

Study 20050181, entitled “A Randomized, Multicenter Phase 3 Study to Compare the Efficacy of Panitumumab in Combination with Chemotherapy to the Efficacy of Chemotherapy Alone in Patients with Previously Treated Metastatic Colorectal Cancer,” is designed to evaluate the treatment effect of panitumumab plus FOLFIRI on OS and PFS compared to FOLFIRI alone as therapy for mCRC among subjects whose tumors express wild-type *KRAS* or whose tumors express mutant *KRAS* (n = 1,187; [Figure 7](#)).

The co-primary endpoints for this study are PFS and OS which will be compared in the wild-type and mutant *KRAS* populations.

Figure 7. Study Design and Treatment Schema for Study 20050181



IVRS, interactive voice response system

^a Subjects will be randomized to receive either panitumumab + FOLFIRI or FOLFIRI alone

^b Day 1 = day of first treatment administration

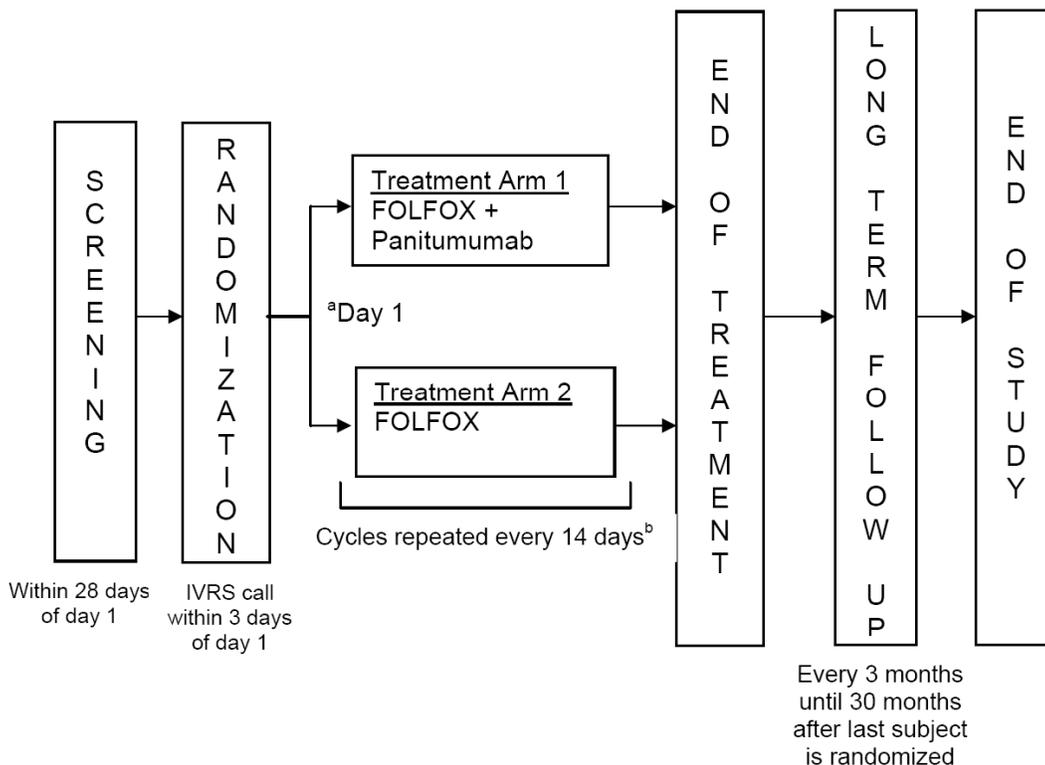
^c Subsequent cycles may be delayed due to panitumumab- or chemotherapy-associated toxicities

7.3.2 Study 20050203

Study 20050203, entitled “A Randomized, Multicenter, Phase 3 Study to Compare the Efficacy of Panitumumab in Combination with Oxaliplatin/ 5-fluorouracil/ leucovorin to the Efficacy of Oxaliplatin/ 5-fluorouracil/ leucovorin Alone in Patients with Previously Untreated Metastatic Colorectal Cancer” is designed to evaluate the treatment effect of panitumumab plus FOLFOX-4 chemotherapy on PFS compared to FOLFOX-4 alone as first-line therapy for mCRC among subjects whose tumors express wild-type *KRAS* or those whose tumors express mutant *KRAS* (n = 1,183; [Figure 8](#)).

The primary endpoint is PFS which will be compared in the wild-type and mutant *KRAS* populations.

Figure 8. Study Design and Treatment Schema for Study 20050203



IVRS, interactive voice response system

^a Day 1 = day of first treatment administration

^b Subsequent cycles may be delayed due to panitumumab- or chemotherapy-associated toxicities

7.3.3 Rationale for Modification of Ongoing Study Protocol and Statistical Analysis Plan

The amendment of studies 20050181 and 20050203 is based on the strength of the overall evidence suggesting that *KRAS* has clinical utility for selection of patients with mCRC for treatment with panitumumab. It is important to note that these trials were amended before any *KRAS* testing and before the first planned efficacy analysis. Trial integrity and scientific validity of the proposed protocol amendments are supported by the following:

- The hypothesis for the role of *KRAS* was generated from data external to the studies.
- For the second-line Study 20050181, adequate power to test for a PFS and OS effect can be achieved within the wild-type *KRAS* subset of the study with the original planned sample size.

- For the first-line Study 20050203, adequate power to test for a PFS effect within the wild-type *KRAS* subset of the study was achieved with an increase in planned sample size from 900 to 1,100.
- Follow-up time was extended in both trials to achieve adequate events for power in the wild-type *KRAS* stratum.
- The safety in both studies is overseen by an independent DMC comprising 3 specialists in CRC clinical research. Only the DMC has reviewed unblinded aggregate safety data. For Study 20050203, the DMC recommended continuation of the study after an interim PFS analysis which included a stopping rule for PFS inferiority in the all randomized subject population. No interim efficacy analyses of Study 20050181 have been performed.
- A revised statistical analysis plan will be finalized for each study prior to determination of *KRAS* status.
- It is anticipated that *KRAS* status will be determined for at least 90% of the planned randomized subjects, which will provide a representative sample of all randomized subjects and reduce the potential for ascertainment bias.
- *KRAS* status will be determined by a central laboratory blinded to treatment and study outcomes using the DxS K-ras Mutation test kit that was utilized in the analysis of samples from the pivotal 20020408 trial.
- The statistical analysis plans will achieve control of the overall type 1 error rate after accounting for primary endpoints (PFS and OS in Study 20050181, PFS in Study 20050203) and 2 primary analysis populations (wild-type *KRAS* and mutant *KRAS* subjects).

In summary, the ongoing phase 3 trials of panitumumab in combination with chemotherapy provide an opportunity to comprehensively evaluate *KRAS* as a predictive biomarker outside of the monotherapy setting.

8. Overall Conclusions

The development of predictive biomarkers may improve the benefit:risk of therapeutics, and consequently benefit patients and advance clinical medicine. Critical elements required for the validation of a biomarker include biological plausibility, analytic validation of the biomarker assay, and demonstration of clinical utility.

KRAS is a key downstream intermediate in the EGFR signaling pathway. The well-characterized role of *KRAS* in EGFR signaling generated the *KRAS* hypothesis that activating *KRAS* mutations would confer primary resistance to anti-EGFR monoclonal antibodies. This hypothesis was strengthened by consistent data from exploratory analysis of multiple clinical studies, including panitumumab phase 2 trials.

The pivotal monotherapy Study 20020408 provided an opportunity to comprehensively evaluate the clinical utility of *KRAS* as a predictive biomarker in patients with mCRC. The DxS K-ras Mutation test kit was used to determine *KRAS* status. In comparison to DNA sequencing methods, this assay was robust and reproducible.

Results of the 20020408 analysis confirmed the *KRAS* hypothesis, unequivocally demonstrating that improvements in response rate and PFS are observed only in patients with *KRAS* wild-type tumors in the monotherapy setting. The magnitude of the treatment-by-*KRAS* interaction for PFS was highly statistically significant ($p < 0.0001$). No patients with mutant *KRAS* tumors had a tumor response; the negative predictive value for response in patients with mutant *KRAS* was 100%. It is unlikely that such results could be generated by chance.

Based on these data, **Amgen has concluded that the benefit:risk profile of panitumumab will be improved by restricting monotherapy use to those patients whose tumors have the wild-type *KRAS* gene.** This would not only restrict use to those patients likely to have improved clinical outcome with panitumumab, but would also prevent unnecessary exposure and potential toxicity in those unlikely to benefit. Discussions with FDA on the utility of these data to effect a change to the current panitumumab monotherapy label are ongoing.

While mutant *KRAS* appears to predict resistance to panitumumab monotherapy in this population, wild-type *KRAS* alone may not be an adequate predictor of response. Thirty-six percent of subjects with wild-type *KRAS* in Study 20020408 had progressive disease.

Therefore, further investigation will be required to identify additional predictors of response and resistance to panitumumab therapy.

Additional studies are needed to extend the *KRAS* hypothesis into settings where panitumumab is used in combination with chemotherapy. Two large ongoing phase 3 studies examining panitumumab with chemotherapy in first- and second-line mCRC will provide more definitive evidence of the clinical utility of *KRAS* as a predictive biomarker in the combination therapy setting. These trials, which have completed enrollment, were amended prior to any *KRAS* testing and before the first efficacy analysis to focus the primary analysis on the *KRAS* wild-type population, and will provide data in 2009.

9. References

- Altar CA, Amakye D, Bounos D, et al. A prototypical process for creating evidentiary standards for biomarkers and diagnostics. *Clin Pharmacol Ther.* 2008;83:368-71.
- Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer: Results from a phase III trial of panitumumab compared to best supportive care. *J Clin Oncol.* 2008;26:1626-1634.
- Amado RG, Wolf M, Freeman D, et al. Association of KRAS mutational status and efficacy of panitumumab monotherapy for the treatment (tx) of metastatic colorectal cancer (mCRC): results of pooled data from 4 clinical studies. *Ann Oncol.* 2008;19(suppl 8):viii126.
- Andreyev, HJN, Norman AR, Cunningham D, et al. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Canc.* 2001;85:692-696.
- Bazan V, Migliavacca M, Zanna I, et al. Specific codon 13 K-ras mutations are predictive of clinical outcome in colorectal cancer patients, whereas codon 12 K-ras mutations are associated with mucinous histotype. *Ann Oncol* 2002;13(9):1438-46.
- Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, 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 Res.* 2007;67(6):2643-8.
- Berlin J, Neubauer M, Swanson P, et al. Panitumumab antitumor activity in patients (pts) with metastatic colorectal cancer (mCRC) expressing > 10% epidermal growth factor receptor (EGFr). *J Clin Oncol.* 2006;24(suppl 18): abstract 3548.
- Biomarker Definitions Working Group 1998. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther.* 2001;69:89-95.
- Bos JL, Fearon ER, Hamilton SR, et al. Prevalence of ras gene mutations in human colorectal cancers. *Nature* 1987;327(6120):293-7.
- Bouzourene H, Gervaz P, Cerottini J-P, et al. p53 and Ki-ras as prognostic factors for Dukes' stage B colorectal cancer. *Eur J Cancer.* 2000;36:1008-1015.
- Cappuzzo F, Varella-Garcia M, Finocchiaro G, et al. Primary resistance to cetuximab therapy in EGFR FISH-positive colorectal cancer patients. *Br J Cancer.* 2008;99:83-89.
- Catalogue of Somatic Mutations in Cancer (COSMIC). Wellcome Trust/Sanger Institute (<http://www.sanger.ac.uk/perl/genetics/CGP/cosmic?action=gene&ln=KRAS>). Accessed 20 October 2008

- Chau CH, Rixe O, McLeod H, Figg WD. Validation of analytic methods for biomarkers used in drug development. *Clin Cancer Res.* 2008;14:5967-76.
- Cohn AL, Smith DA, Neubauer MA, et al. Panitumumab regimen evaluation in colorectal cancer to estimate primary response to treatment (PRECEPT): effect of KRAS mutation status on second-line treatment with panitumumab and FOLFIRI. *J Clin Oncol.* 2008;26(May 20 suppl):4127.
- De Roock W, Piessevaux H, De Schutter J, et al. KRAS mutation status predicts survival in colorectal cancer treated with cetuximab. *J Clin Oncol.* 2007;25(18S):Abstract 4132.
- Di Fiore F, Blanchard F, Charbonnier F, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. *Br J Cancer* 2007;96(8):1166-9.
- Downward J. Targeting RAS signaling pathways in cancer therapy. *Nature Rev Cancer* 2003;3(1):11-22.
- Esteller M, Gonzalez S, Risques RA, et al. K-ras and p16 aberrations confer poor prognosis in human colorectal cancer. *J Clin Oncol* 2001;19(2):299-304.
- Finocchiaro G, Cappuzzo F, Jänne PA, et al. EGFR, HER2 and Kras as predictive factors for cetuximab sensitivity in colorectal cancer. *J Clin Oncol.* 2007;25(18S): Abstract 4021.
- Food and Drug Administration. Drug-diagnostic co-development concept paper. 2005; <http://www.fda.gov/Cder/genomics/pharmacoconceptfn.pdf>.
- Food and Drug Administration. Guidance for Industry: Pharmacogenomic Data Submissions. 2005a; <http://www.fda.gov/Cber/gdlns/pharmdntasub.pdf>.
- Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M. Detection of high incidence of K-ras oncogenes during human colon tumorigenesis. *Nature.* 1987;327:298-303.
- Fransén K, Klintenäs M, Osterström A, Dimberg J, Monstein HJ, Söderkvist P. Mutation analysis of the BRAF, ARAF and RAF-1 genes in human colorectal adenocarcinomas. *Carcinogenesis.* 2004;25:527-33.
- Freeman DJ, Juan T, Reiner M, et al. Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving panitumumab alone. *Clin Colorectal Cancer.* 2008;7:184-190.
- Freeman D, Juan T, Meropol NJ, et al. Association of somatic KRAS gene mutations and clinical outcome in patients (pts) with metastatic colorectal cancer (mCRC) receiving panitumumab monotherapy. *Eur J Cancer.* 2007; Suppl 5 (4):239.
- Hayes DF, Bast RC, Desch CE, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. *J Natl Cancer Inst.* 1996;88:1456-66.

- Hecht JR, Patnaik A, Berlin J, et al. Panitumumab monotherapy in patients with previously treated metastatic colorectal cancer. *Cancer*. 2007;110:980-988.
- Hilger RA, Scheulen ME, Strumberg D. The Ras-Raf-MEK-ERK pathway in the treatment of cancer. *Onkologie*. 2002;25:511-18.
- Hynes NE, Lane HA. ERBB receptors and cancer: The complexity of targeted inhibitors. *Nature Rev Cancer*. 2005;5:341-354.
- Ince WL, Jubb AM, Holden SN, et al. Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. *J Natl Cancer Inst*. 2005;97(13):981-9.
- Jones S, Chen W, Parmigiani G, et al. Comparative lesion sequencing provides insights into tumor evolution. *PNAS*. 2008;105:4283-4288.
- Juan T, Suggs S, Wolf M et al. A comparability study of 4 commercial *KRAS* tests. AACR Meeting Abstract, April 2008;1811.
- Khambata-Ford S, Garrett CR, Meropol NJ, et al. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol* 2007;25(22):3230-37.
- Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res*. 2006;66(8):3992-5.
- Lièvre A, Bachet J-B, Ychou M, et al. KRAS mutations in colorectal cancer is predictive factor of response and progression free survival in patients treated with cetuximab. *Proc Am Assoc Canc Res*. 2007. Abstract 5671.
- Malumbres M and Barbacid M. RAS oncogenes: the first 30 years. *Nature Rev. Cancer* 2003;3:7-13.
- Mitchell EP, LaCouture M, Shearer H et al. Updated results of STEPP, a phase 2, open-label study of pre-emptive versus reactive skin toxicity treatment in metastatic colorectal cancer (mCRC) patients receiving panitumumab + FOLFIRI or irinotecan-only chemotherapy as second-line treatment. *Ann Oncol*. 2008;19 (suppl 6):vi14.
- Mitchell EP, Hecht JR, Baranda J, et al. Panitumumab activity in metastatic colorectal cancer (mCRC) patients (pts) with low or negative tumor epidermal growth factor receptor (EGFr) levels: an updated analysis. *J Clin Oncol*. 2007;25(18S): abstract 4082a.
- Moroni M, Veronese S, Benvenuti S, et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to anti EGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol*. 2005;6(5):279-86.
- Punt CJ, Tol J, Rodenburg CJ, Cats A, Creemers G, Schrama JG, et al. Randomized phase III study of capecitabine, oxaliplatin, and bevacizumab with or without cetuximab in advanced colorectal cancer (ACC), the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). *J Clin Oncol*. 2008;26(May 20 suppl):LBA4011.

- Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature*. 2002;418:934.
- Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. 2006; doi:10.1038/nbt1235.
- Scaltriti M and Baselga J. The epidermal growth factor receptor pathway: A model for targeted therapy. *Clin Cancer Res*. 2006;12:5268-5272.
- Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. *Nature Rev Cancer*. 2007;7(4):295-308.
- Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science*. 1989;244:707-12.
- Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235:177-82.
- Van Cutsem E, Siena S, Humblet Y et al. An open-label, single-arm study assessing safety and efficacy of panitumumab in patients with metastatic colorectal cancer refractory to standard chemotherapy. *Annal Oncol*. 2008;19(1):92-98.
- Van Cutsem E, Peeters M, Siena S, et al. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol*. 2007;25:1658-1664.
- Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *NEJM*. 1988;319(9):525-32.
- Woolsey R. Drug-diagnostic co-development: a new paradigm. 2008; <http://www.c-path.org/Portals/0/PersonMedv2.pdf>

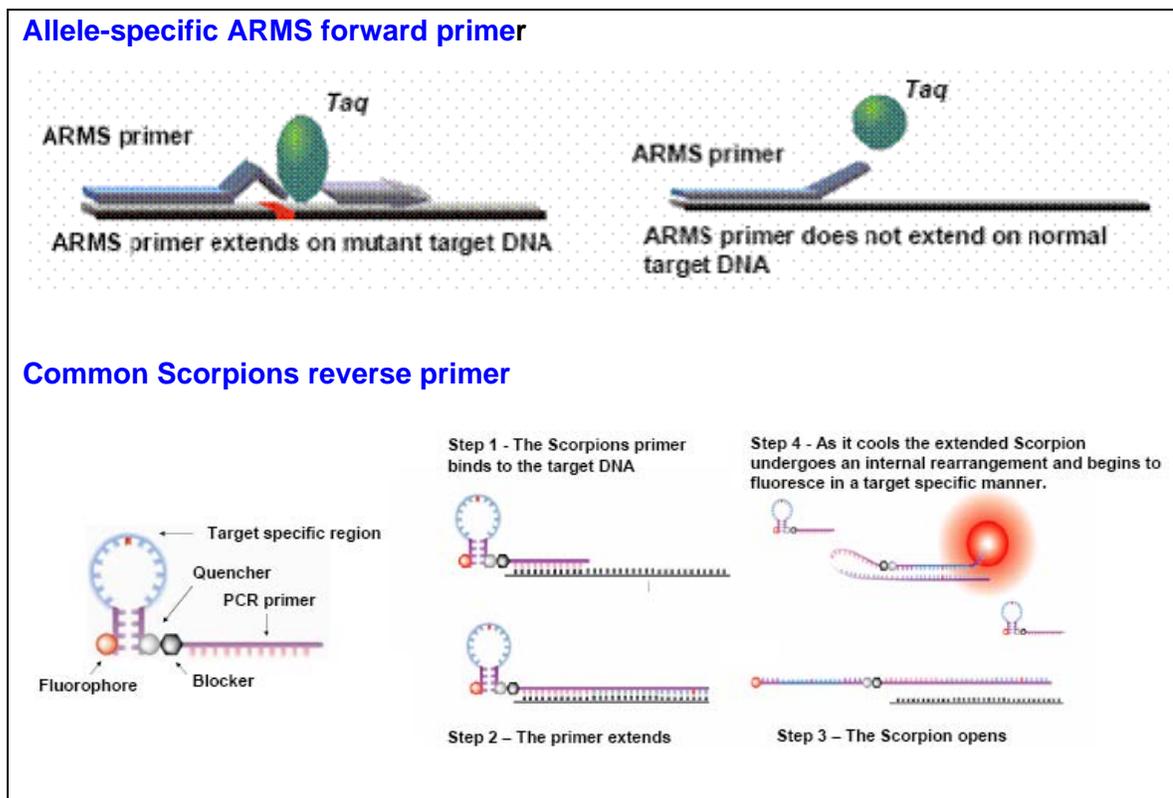
APPENDICES

Appendix 1. DxS K-ras Mutation Test Kit Description

The DxS K-ras test kit is intended for the detection of 7 somatic mutations in codons 12 and 13 of the *KRAS* gene (Gly12Ala, Gly12Asp, Gly12Arg, Gly12Val, Gly12Cys, Gly12Ser, and Gly13Asp). The kit is for use on DNA samples including DNA extracted from FFPE tissue and provides a qualitative assessment of mutation status. The assay utilizes allele-specific PCR amplification using amplification refractory mutation system technology (ARMS™) in combination with Scorpions® detection technology to measure the amplification products by means of real-time PCR.

The kit includes 7 primers that are specific for the most common mutations in codons 12 and 13 of the *KRAS* gene. The primers are complementary to the *KRAS* gene immediately adjacent to the sites of mutations, and each primer contains a unique sequence at its 3'-end that is specific for each mutation. During PCR amplification, the primers anneal to the DNA template strand and only the primer that contains the complementary nucleotide at its 3'-end will be able to extend the mutated target DNA. Taq DNA polymerase, which is used for PCR amplification, is extremely effective at distinguishing between a match and a mismatch at the 3' end of a PCR primer. To increase the efficiency of this reaction, a mismatch is included close to the specific base mutation. As indicated in [Figure 9](#), when the primer has only one mismatch, the amplification proceeds with high efficiency. When the 2 mismatches are close to the 3' end, efficient amplification does not occur. In this way specific mutated sequences can be selectively amplified, even in samples where the majority of the sequences are wild-type or do not carry that specific mutation. Real time PCR provides a means of monitoring the fluorescence produced by the Scorpions® technology during each cycle of the reaction. This dynamic approach allows the relative abundance of mutated DNA to be measured by comparing the efficiency of each ARMS reaction to a *KRAS* control reaction.

Figure 9. Schematic of the DxS K-RAS Test Kit ARMS/Scorpions Assay



The DxS K-ras test kit employs ARMS/Scorpions[®] real-time PCR technology to detect *KRAS* mutations. The forward primers are specific for the 7 *KRAS* mutations tested. They utilize an additional base mismatch to ensure PCR amplification of only the mutant sequence in a background of wild type DNA. The reverse primers are common for all of the tests and employ Scorpions[®] which are bi-functional molecules containing a PCR primer covalently linked to a fluorescent probe. The fluorophore in the probe interacts with a quencher, which reduces fluorescence. As shown in Figure 9, during a PCR reaction the fluorophore and quencher are spatially separated when the probe binds to the amplicon, which leads to increased fluorescence in the reaction tube.

Eight assays are supplied in the research-use only (RUO) kit. The control assay, labeled with FAM, is used to assess the total DNA in a sample. This Scorpions[®] assay amplifies a region of exon 4 of the *KRAS* gene. The primers and probe have been designed to avoid any known *KRAS* polymorphisms. The mutation assays are also labeled with FAM. They each contain one Scorpion[®] plus an ARMS primer for discrimination between the wild type DNA and the mutant DNA. All assays also contain a Scorpions[®] assay for an exogenous control labeled with HEX. This controls for the presence of inhibitors, which may lead to false negative results.

Scorpions[®] real time assays use the number of PCR cycles necessary to detect a signal above a background as a measure of the target molecules present at the beginning of the reaction. The threshold at which the signal is detected above background fluorescence is called the Cycle threshold (Ct). When using ARMS primers, some inefficient priming may still occur, giving a very late background Ct from DNA not containing the mutation. The difference between the Ct of the control assay and the background Ct gives the window into which positive samples fall. Sample $-\Delta Ct$ values are calculated as the difference between the mutation Ct and control Ct. If this difference is smaller than the difference between the background and control Ct (given as the cut-off point) the sample is classed as positive. The bigger the ΔCt (closer to the cut-off) the less mutation the sample contains. Beyond the cut-off point the sample is classed as mutation negative or beyond the limits of the test.

Status and commercial availability

The DxS K-ras Mutation test kit has been CE marked in accordance with the European IVD Directive (98/79/EC) and renamed the DxS TheraScreen: K-RAS Mutation Kit. Following distribution of notifications to the relevant Regulatory Authorities, the K-RAS Kit was placed on the market from January 2008 in the following European countries: United Kingdom, Germany, Austria, France, Spain, Italy, Sweden, Finland, Norway, Ireland, Denmark, Switzerland, Poland, Greece, Netherlands, Slovenia, Czech Republic, Slovakia, Belgium, Hungary, Lithuania, Latvia, Estonia, Ireland, Luxembourg and Portugal. Therefore, the DxS K-RAS Kit supplements the tests already commercially available to test for K-RAS status in Europe.

The DxS TheraScreen: K-RAS Mutation Kit is sold in the US as a RUO kit.

Amgen is currently working with DxS on their Pre-market Approval (PMA) submission for the DxS TheraScreen: K-RAS Mutation Kit. During this process, the Center for Devices and Radiological Health (CDRH) asked DxS to complete an abbreviated validation so that the existing product could be provided as an Investigational Use Only (IUO) device for use in nominated clinical trials; this validation was completed in October 2008 for the K-RAS Kit.

Appendix 2. Supplemental Tables

Table S1. KRAS Mutations in Colorectal Cancer: Representative Studies

Publication	KRAS Mutations	Tumor Material	Exons	Method of Analysis
Bos et al, 1987	12/27 (44%)	Frozen	2	PCR → oligonucleotide hybridization
Forrester et al, 1987	26/66 (39%)	Frozen	2	RNase mismatch assay
Vogelstein et al, 1988	43/92 (47%)	Frozen	2	PCR → oligonucleotide hybridization
Andreyev 2001	1197/3439 (35%)	Various	2	Multiple methods (registry study)
Rajagopalan et al, 2002	169/330 (51%)	Tumor, xenografts, cell lines	2, 3	Microdissection ^a → PCR → sequencing
Fransén et al, 2004	52/130 (40%)	Frozen	1, 2	SSCA → re-amplification → sequencing
Ince et al, 2005	88/255 (35%)	FFPE	2	LCM → nested PCR → sequencing
Moroni et al, 2005	10/31 (32%)	FFPE	2	Dissection → PCR → sequencing
Lièvre et al, 2006	13/30 (43%)	Frozen	2	PCR → sequencing (in duplicate)
Benvenuti et al, 2007	16/48 (33%)	FFPE	2	Dissection → PCR → sequencing
Di Fiore et al, 2007	16/59 (27%)	FFPE	2	Sequencing/allele-specific PCR/LCR ^b
Freeman et al, 2007	21/59 (36%)	FFPE	2	PCR → cloning → sequencing
Khambata-Ford et al, 2007	30/80 (38%)	RNALater	2	PCR → sequencing

FFPE, formalin-fixed, paraffin-embedded; LCM, laser-capture microdissection; PCR, polymerase chain reaction

^a 54 of the samples were from primary tumors and these tissues were microdissected.

^b Samples that were scored as wild-type after sequencing were re-analyzed by allele-specific PCR (SNaPshot) and LCR (Ligase Chain Reaction).

Appendix 3. Important Publications

Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman D, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson S, and Chang D. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer: Results from a phase III trial of panitumumab compared to best supportive care. *J Clin Oncol*. 2008;26:1626-1634.

Amado RG, Wolf M, Freeman D, et al. Association of KRAS mutational status and efficacy of panitumumab monotherapy for the treatment (tx) of metastatic colorectal cancer (mCRC): results of pooled data from 4 clinical studies. Poster presented at: 33rd ESMO Congress; September 12-16, 2008a; poster number 359P.

Freeman DJ, Juan T, Reiner M, et al. Association of K-ras mutational status and clinical outcomes in patients with metastatic colorectal cancer receiving panitumumab alone. *Clin Colorectal Cancer*. 2008;7:184-190.

Juan T, Suggs S, Wolf M et al. A comparability study of 4 commercial KRAS tests. AACR Meeting Abstract, April 2008;1811.

Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. 2006; doi:10.1038/nbt1235.