AMGEN®

Pioneering science delivers vital medicines™

Levels of evidence for retrospective analyses of banked samples

Scott D. Patterson, PhD
Executive Director, Medical Sciences

Outline

- Evidence-based biomarker qualification
- Phase 3 (20020408) case study – Vectibix® (Panitumumab)
  - Evidence for KRAS as a predictive biomarker
- Conclusions

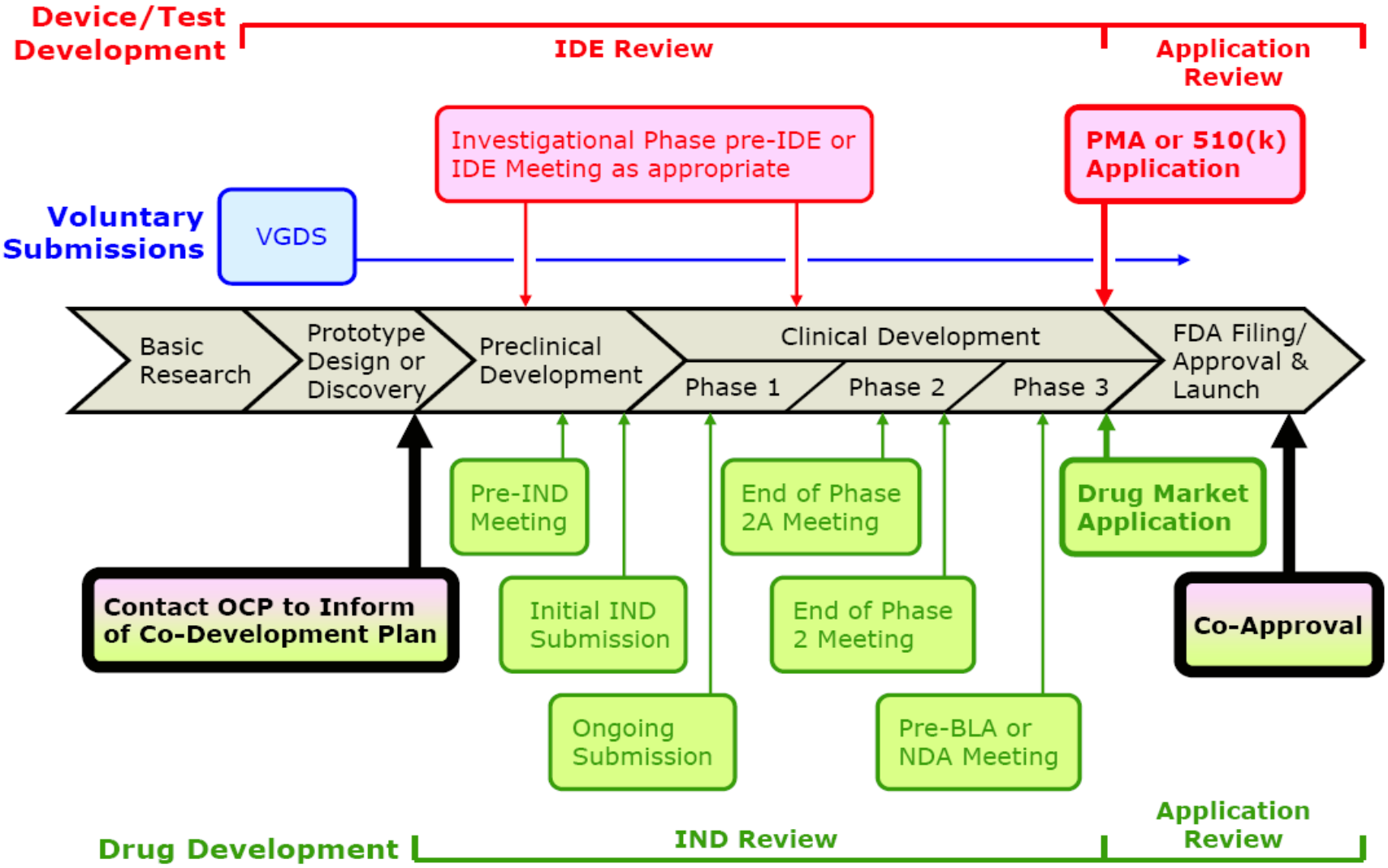
AMGEN

General patient selection biomarker considerations

- **Do you want a biomarker predictive of response to a therapeutic?**
  - **Can you separate out the effect of a prognostic biomarker (i.e., one that predicts the course of disease)?**
  - **Is the biomarker a positive or negative predictor?**
- **What is the clinical goal of the study?**
  - **Responders vs. non-responders? Are there really only two outcomes?**
- **Will it change the practice of medicine?**
  - **Engage physician key opinion leaders**
  - **Is there a health economic benefit to be realized (clear patient benefit)?**

AMGEN

The timeline issue:
Drug-Device Co-Development Process



Drug-Diagnostic Co-Development Concept Paper, Draft — Not for Implementation, HHS, FDA, April 2005

AMGEN

Biomarkers: The Promise of Personalized Medicine

- A fundamental challenge of personalized medicine is the development of predictive biomarkers
- Scientific data about biomarkers often becomes available late in the development of therapeutics
- A mechanism is required to assess biomarkers and diagnostics on retrospective data sets

What level of evidence (if any) is sufficient to validate biomarkers/diagnostics on retrospective data sets?

AMGEN

Critical Elements of Biomarker Validation

1. **Scientific plausibility**
   - Understanding of fundamental biology
   - Appropriate hypothesis-generating data
2. **Analytical validation of assay**
   - Performance characteristics, reproducibility, accuracy
   - Assessment against known ('gold') standards (if appropriate)
   - Practicality
3. **Rigorous demonstration of clinical utility**
   - Prospective vs retrospective data sets
   - Prespecified analysis plan (hypothesis testing)
   - Minimization of bias (e.g., ascertainment bias)
   - Generalizability

Were these levels of evidence met in the following case study?

Based on: FDA Critical Path Initiative 2004; FDA Pharmacogenomic Data Submissions Guidance 2005; FDA Drug-Diagnostic Co-Development Concept Paper 2005; Altar et al *Clin Pharmacol Ther* 2008.

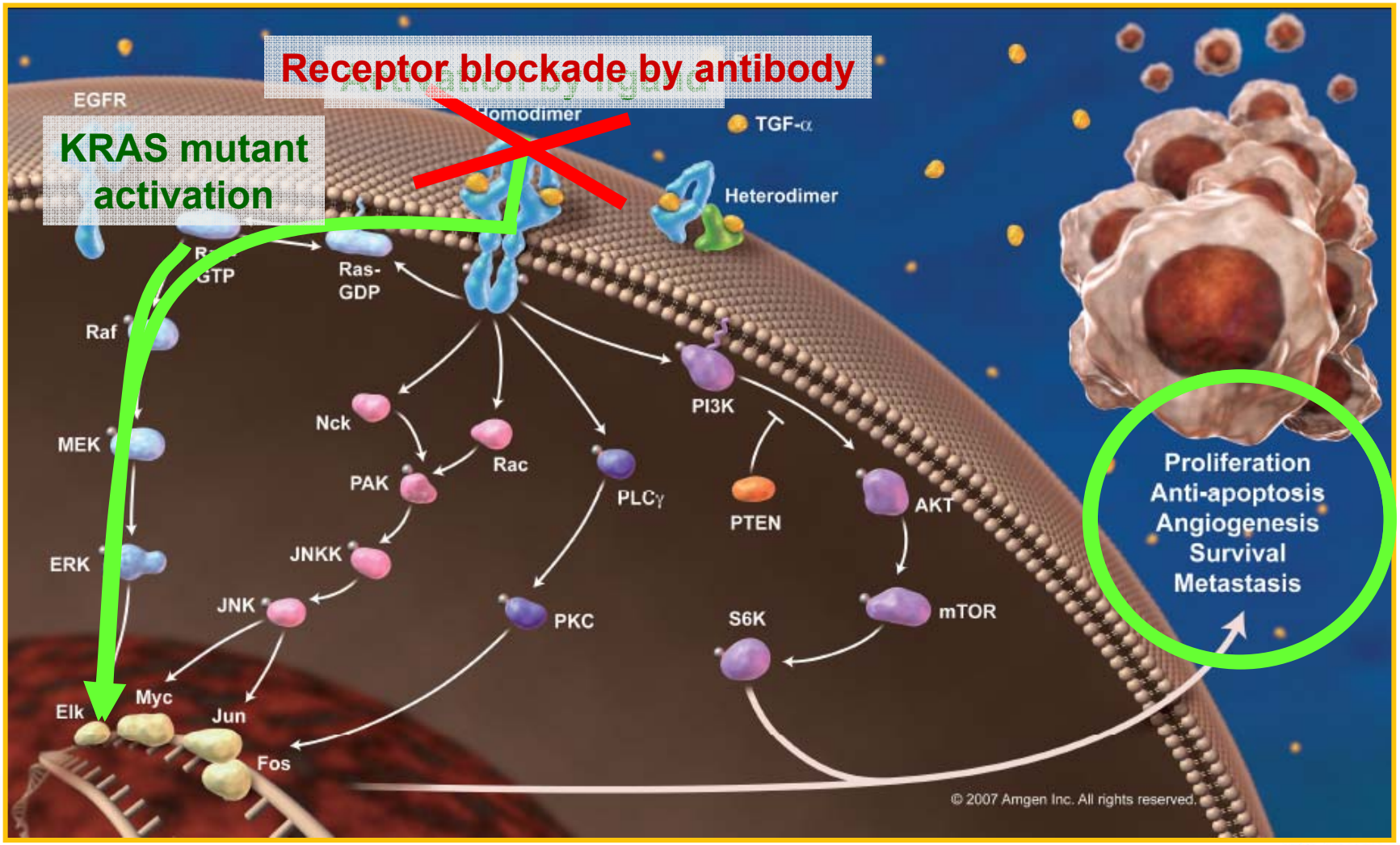
AMGEN

Outline

- Evidence-based biomarker qualification
- **Phase 3 (20020408) case study – Vectibix® (Panitumumab)**
  - **Evidence for KRAS as a predictive biomarker**
- **Conclusions**

AMGEN

RAS-RAF-MAP Kinase Pathway is part of the EGF Receptor Signaling Cascade



AMGEN

KRAS biomarker status pre-'408 study

- For more than 30 years known to be an oncogene
- Several studies indicate that the presence of mutant KRAS correlates with a poor prognosis
  - (Andreyev et al, 2001 *British J Canc* 85:692; Esteller et al, 2001 *J Clin Oncol* 19:299; Ince et al, 2005 *J Natl Cancer Inst* 97:981; Bazan et al, 2002 *Ann Oncol* 13:1438)
- Other studies refute prognostic value
  - (Bouzourene et al, 2000 *Eur J Cancer* 36:1008)
- However, preclinical data with xenografts showed variable response (+/- KRAS mutation) to anti-EGFR treatment
- Thus, we and others began to explore samples from single-arm panitumumab monotherapy phase 2 studies

#1 Scientific Plausibility
-Fundamental Biology

AMGEN

Single-arm Studies Support the Hypothesis for *KRAS* as a Biomarker for EGFr Inhibitors

Reference	Treatment (panitumumab or cetuximab)	No of patients (WT:MT)	Objective Response N (%)	
			WT	MT
A. Liévre, et al. *(AACR Proceedings, 2007)*	cmab ± CT	76 (49:27)	24 (49)	0 (0)
S. Benvenuti, et al. *(Cancer Res, 2007)*	pmab or cmab or cmab + CT	48 (32:16)	10 (31)	1 (6)
W. De Roock, et al. *(ASCO Proceedings, 2007)*	cmab or cmab + irinotecan	113 (67:46)	27 (40)	0 (0)
D. Finocchiaro, et al. *(ASCO Proceedings, 2007)*	cmab ± CT	81 (49:32)	13 (26)	2 (6)
F. Di Fiore, et al. *(Br J Cancer, 2007)*	cmab + CT	59 (43:16)	12 (28)	0 (0)
S. Khambata-Ford, et al. *(J Clin Oncol, 2007)*	cmab	80 (50:30)	5 (10)	0 (0)

WT, wild type; MT, mutant; cmab, cetuximab; CT, chemotherapy; pmab, panitumumab

AMGEN

KRAS Analysis of Single-Arm, Panitumumab Monotherapy Studies

- Patient samples from 3 Amgen panitumumab monotherapy, single-arm, phase 2 trials in metastatic colorectal cancer were obtained under a biomarker protocol
- The majority of patient samples were archived tumor samples from the primary resection
- *KRAS* mutational status was determined using cloning and sequencing of DNA isolated from paraffin-embedded tumor samples
- *KRAS* mutational status was correlated with clinical outcomes including response, progression-free survival, and overall survival

#1 Scientific Plausibility

-Appropriate hypothesis generating data

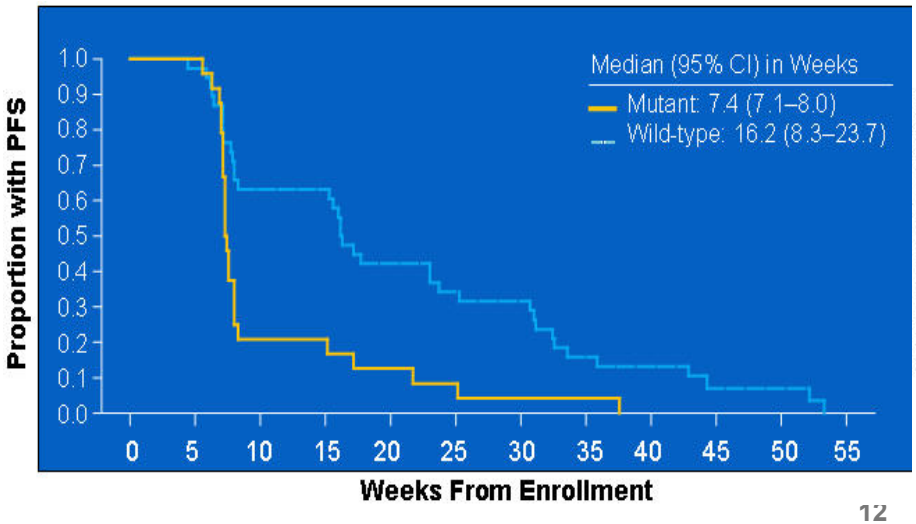
AMGEN

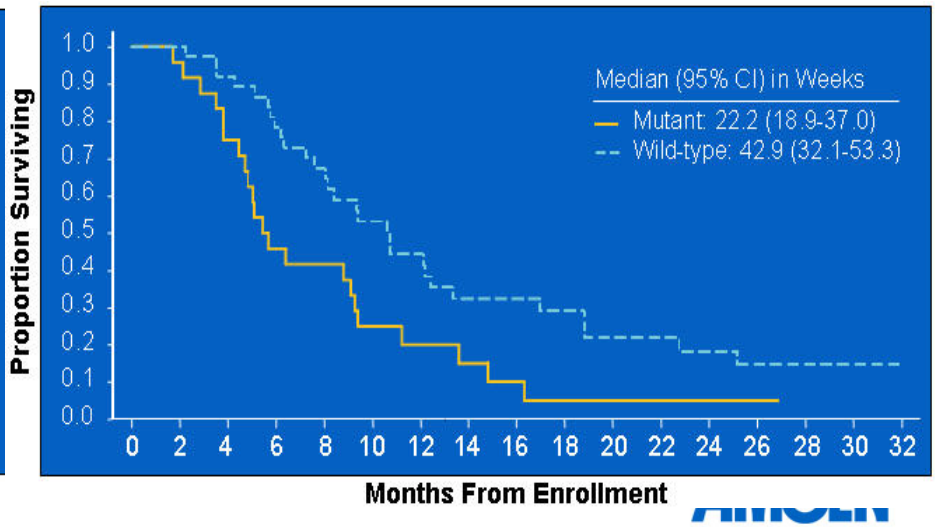
KRAS mutational status was correlated with clinical outcomes

Objective Response	All Patients Total (N = 62)	Wild-type *KRAS* Total (N = 38)	Mutant *KRAS* Total (N = 24)
Partial response, n (%)	4 (6.5)	4 (11)	0
Stable disease, n (%)	25 (40)	20 (53)	5 (21)
Disease progression, n (%)	33 (53)	14 (37)	19 (79)

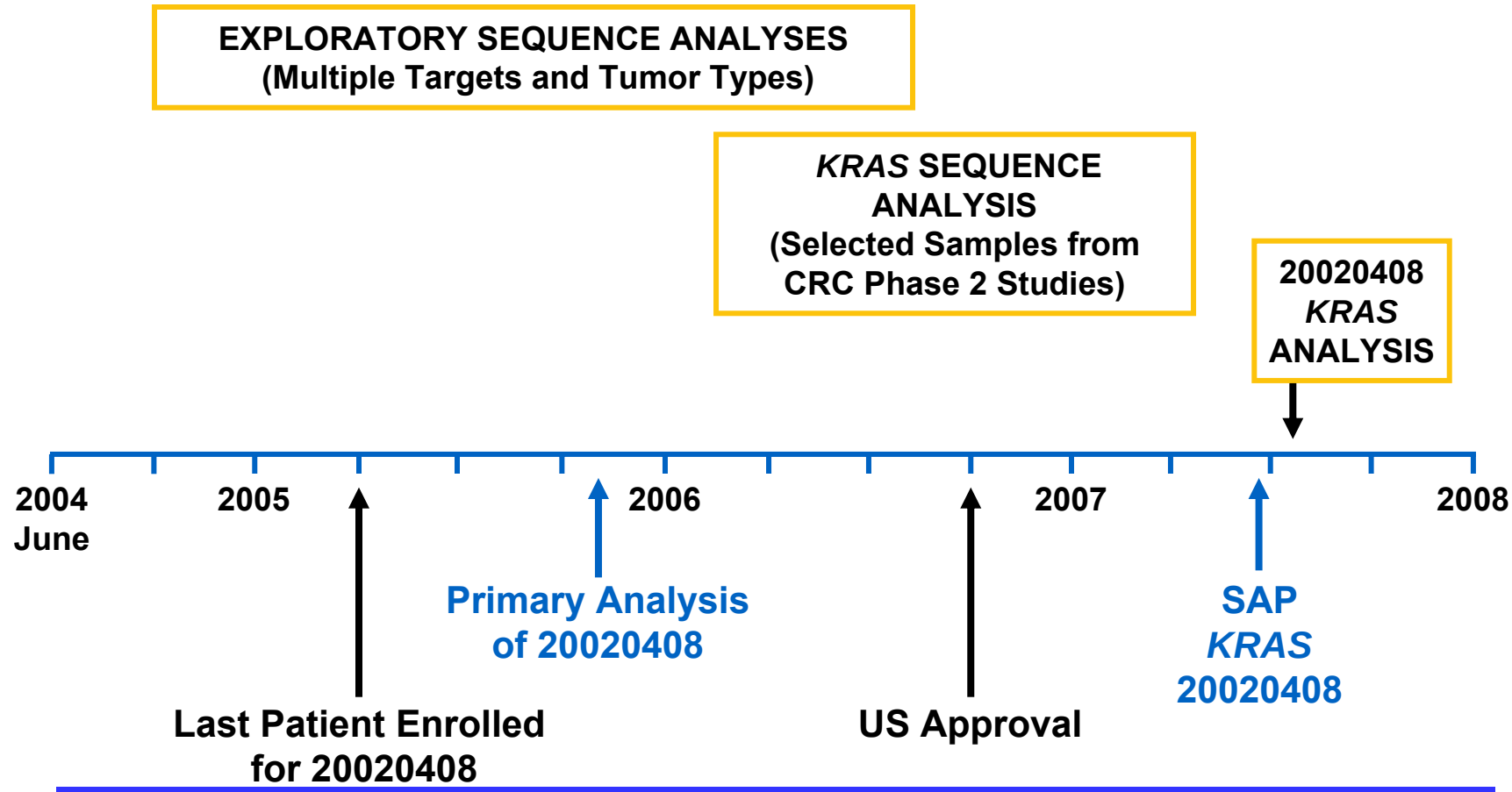
Panitumumab-Treated Patients by KRAS Status -

Progression-Free Survival & **Overall Survival**





KRAS Hypothesis in CRC Emerged in Parallel with Clinical Trial Data



FFPE samples collected to a single location

Beginning ~mid-2005 ending early 2007

CRC, colorectal cancer; SAP, statistical analysis plan

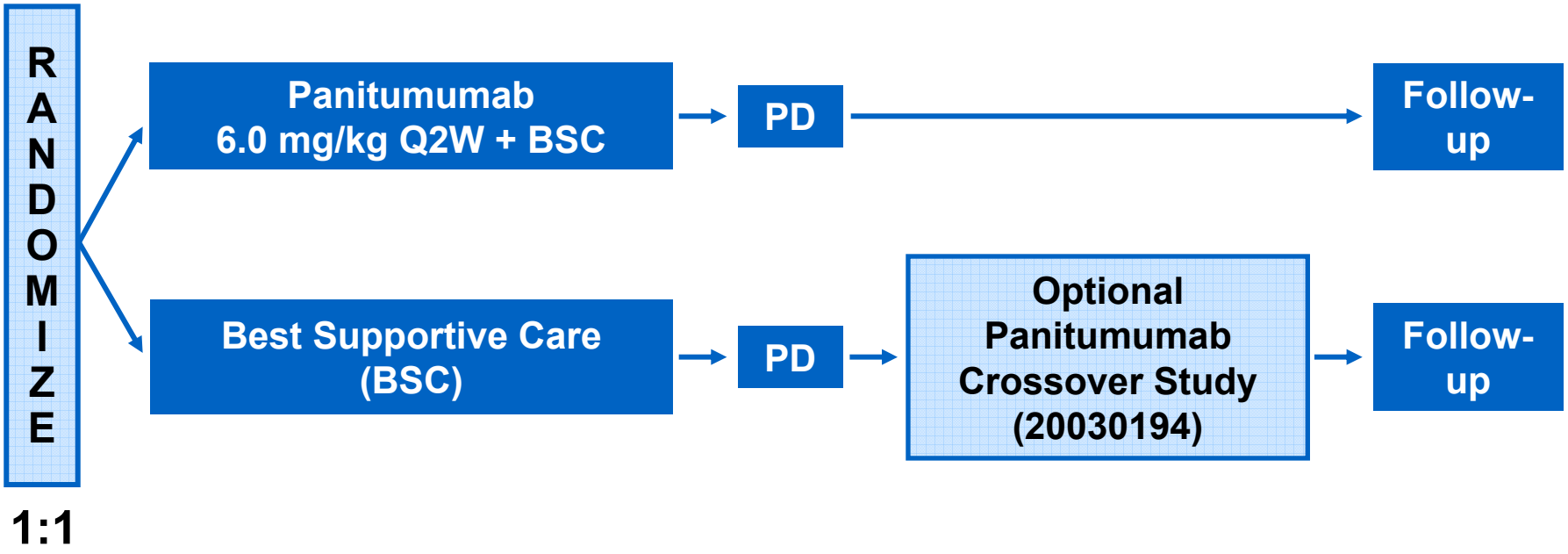
AMGEN

KRAS biomarker status pre-'408 study

- For more than 30 years known to be an oncogene
- Several studies indicate that the presence of mutant KRAS correlates with a poor prognosis
  - (Andreyev et al, 2001 *British J Canc* 85:692; Esteller et al, 2001 *J Clin Oncol* 19:299; Ince et al, 2005 *J Natl Cancer Inst* 97:981; Bazan et al, 2002 *Ann Oncol* 13:1438)
- Other studies refute prognostic value
  - (Bouzourene et al, 2000 Eur J Cancer 36:1008)
- However, preclinical data with xenografts showed variable response (+/- KRAS mutation) to anti-EGFR treatment
- Thus, we and others began to explore samples from single-arm panitumumab monotherapy phase 2 studies
- **It was then decided to determine *KRAS* mutational status in samples from our Phase 3 '408 study:**
  - **Completed evaluation of the assay/vendor to be employed**
  - **Pre-specified the statistical analysis plan**
  - **Executed the assay at HistoGeneX**
  - **Analyzed the data according to the pre-specified plan**

AMGEN

Phase 3 Study Design Leading to Accelerated Approval for Panitumumab (Study 20020408)



Primary Endpoint: Progression-free Survival (PFS)

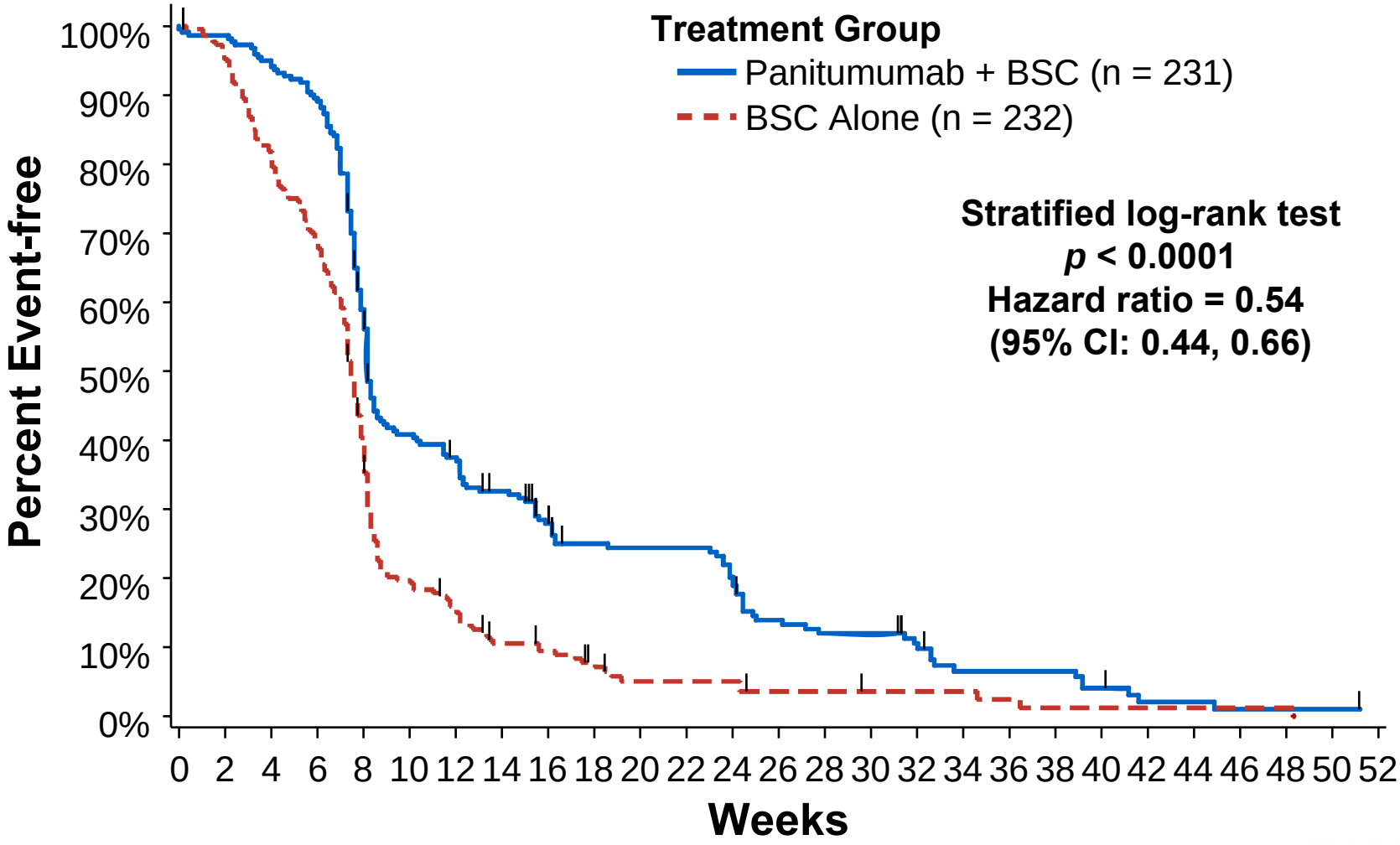
PD, progressive disease

Van Cutsem P, et al. *J Clin Onc.* 2007;25:1658-1664.

AMGEN

Panitumumab Improves Progression-free Survival in Advanced Colorectal Cancer

Primary Analysis, All Randomized Analysis Set, Central Radiology



Van Cutsem P, et al. *J Clin Onc.* 2007;25:1658-1664.

AMGEN

Phase 3 Trial (Study 20020408) Provided an Opportunity to Assess *KRAS*

- Protocol required tumor samples which were archived for potential biomarker correlative analyses
- Expected *KRAS* evaluable sample size was sufficient to provide balance between treatment arms
- *KRAS* was the only biomarker evaluated for correlation with clinical outcome
- High power (> 90%) to test whether *KRAS* was a predictive biomarker for progression-free survival (PFS)

AMGEN

Assay Used to Detect *KRAS* Mutational Status

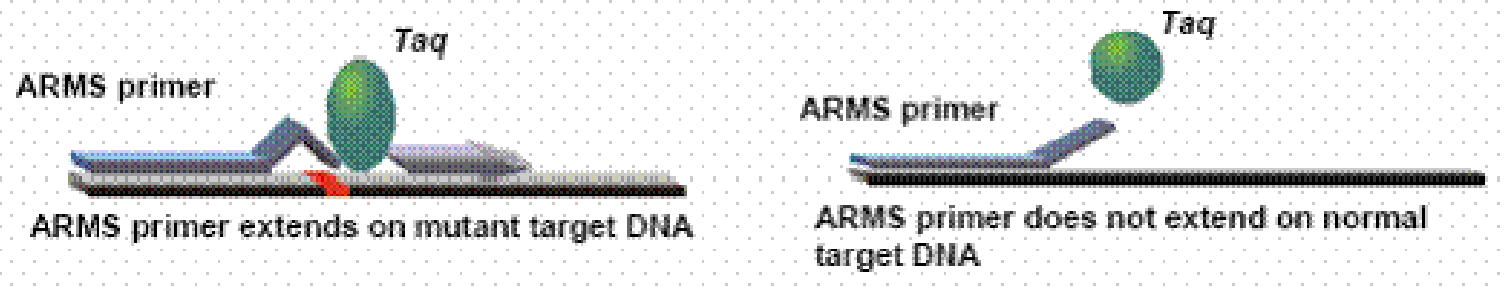
- DNA was isolated from fixed tumor samples
- Mutant *KRAS* was detected using a *KRAS* mutation kit (DxS Ltd, Manchester, UK) that used allele-specific, real-time PCR
  - The kit can detect approximately 1% of mutant DNA in a background of wild-type genomic DNA
  - The test identifies 7 somatic mutations in codons 12 and 13
    - Gly 12 Asp, Ala, Val, Ser, Arg, Gly, Cys and Gly 13 Asp
- Assay met CSLI performance characteristics of sensitivity (95%), specificity (100%*) and precision (<3%); and was performed by HistoGeneX (Belgium) under BelTest & CAP standards
- *@ LOD of 1% using 40 non-tumoral samples, all samples called WT

#2 Analytical validation of the assay
-Performance characteristics, Std, Practicality

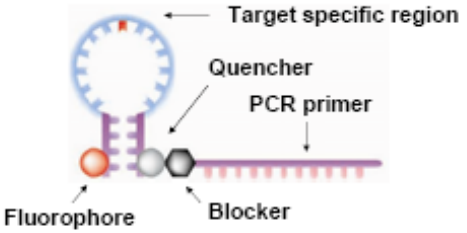
AMGEN

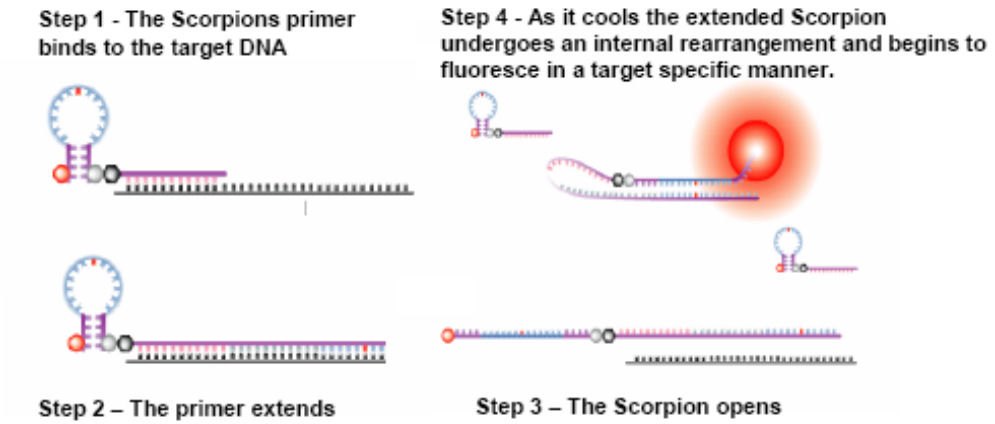
DxS KRAS Mutation Test Kit Overview

Allele-specific ARMS forward primer



Common Scorpions reverse primer





With permission: DxS Ltd, Manchester, UK

AMGEN

Critical Elements of Biomarker Validation

Scientific plausibility

- Understanding of fundamental biology
- Appropriate hypothesis-generating data

Analytical validation of assay

- Performance characteristics, reproducibility, accuracy
- Assessment against known ('gold') standards (if appropriate)
- Practicality

3. Rigorous demonstration of clinical utility

Prospective vs retrospective data sets
Prespecified analysis plan (hypothesis testing)
Minimization of bias (e.g., ascertainment bias)
Generalizability

Based on: FDA Critical Path Initiative 2004; FDA Pharmacogenomic Data Submissions Guidance 2005; FDA Drug-Diagnostic Co-Development Concept Paper 2005; Altar et al *Clin Pharmacol Ther* 2008.

AMGEN

Prospective Statistical Analysis Plan for Phase 3 Trial (Study 20020408)

- The Statistical Analysis Plan (SAP) was finalized prior to unblinding of *KRAS* status
- Objectives were to formally address the *KRAS* hypothesis:
  - To test that the relative improvement in progression-free survival (PFS) is larger in the wild-type vs. mutant *KRAS* stratum
  - To test the treatment effect on PFS, objective response and overall survival in *KRAS* wild-type stratum
  - Analysis designed to control overall type 1 error for the set of comparisons in the *KRAS* analysis

AMGEN

KRAS Results Obtained in > 90% of Patients from Phase 3 Trial (Study 20020408)

	Panitumumab + BSC	BSC alone	Total
Patients randomized, n	231	232	463
Tumor sample available, n (%)	220 (95)	225 (97)	445 (96)
KRAS* tests failed, n (%)	12 (5)	6 (3)	18 (4)
Patients included in *KRAS* analysis, n (%)	208 (90)	219 (94)	427 (92)
Wild-type *KRAS*, n (%)	124 (60)	119 (54)	243 (57)
Mutant *KRAS*, n (%)	84 (40)	100 (46)	184 (43)

**KRAS* tests failed due to insufficient DNA quality or quantity
BSC, Best Supportive Care

Amado, et al. *J Clin Onc.* 2008;26:1626-1634.

AMGEN

Baseline Characteristics were Balanced Between Treatment Arms

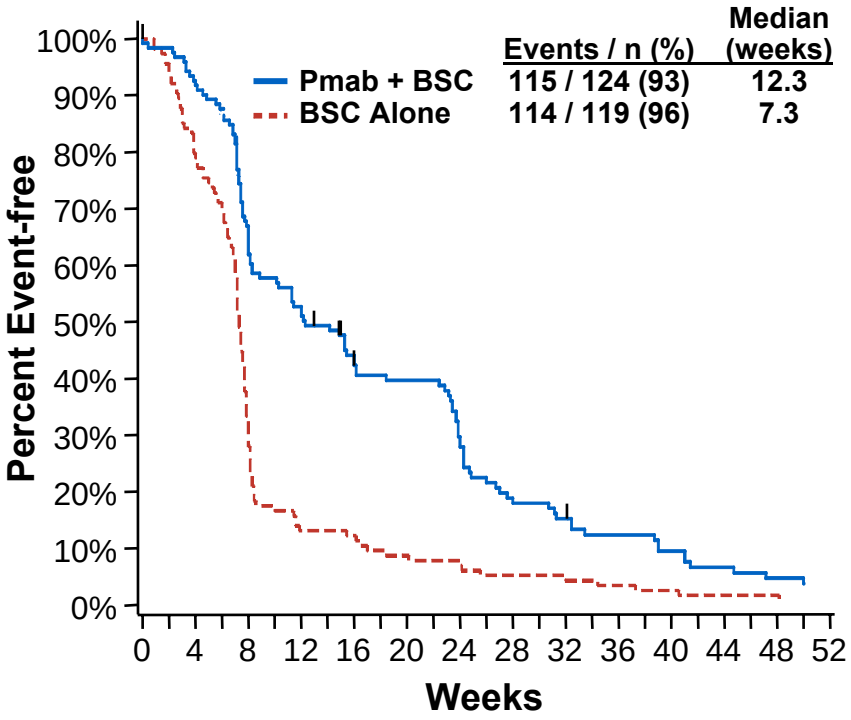
	Wild-type *KRAS*		Mutant *KRAS*	
	Panitumumab plus BSC (n = 124)	BSC alone (n = 119)	Panitumumab plus BSC (n = 84)	BSC alone (n = 100)
Sex, %				
Men	67	64	56	64
Baseline age, years				
Median (min, max)	62.5 (29, 82)	63.0 (32, 81)	62.0 (27, 79)	62.0 (27, 83)
Primary diagnosis, %				
Colon cancer	69	69	63	65
Months since primary diagnosis				
Median	25.2	25.0	23.5	25.5
ECOG performance status, %				
0–1	88	86	85	84
≥ 2	12	14	15	16
Prior adjuvant chemotherapy, %				
Yes	40	27	32	40
Cells with EGFR membrane staining, %				
1– < 10	25	24	24	23
10–100	75	75	75	77

Amado, et al. *J Clin Onc.* 2008;26:1626-1634.

AMGEN

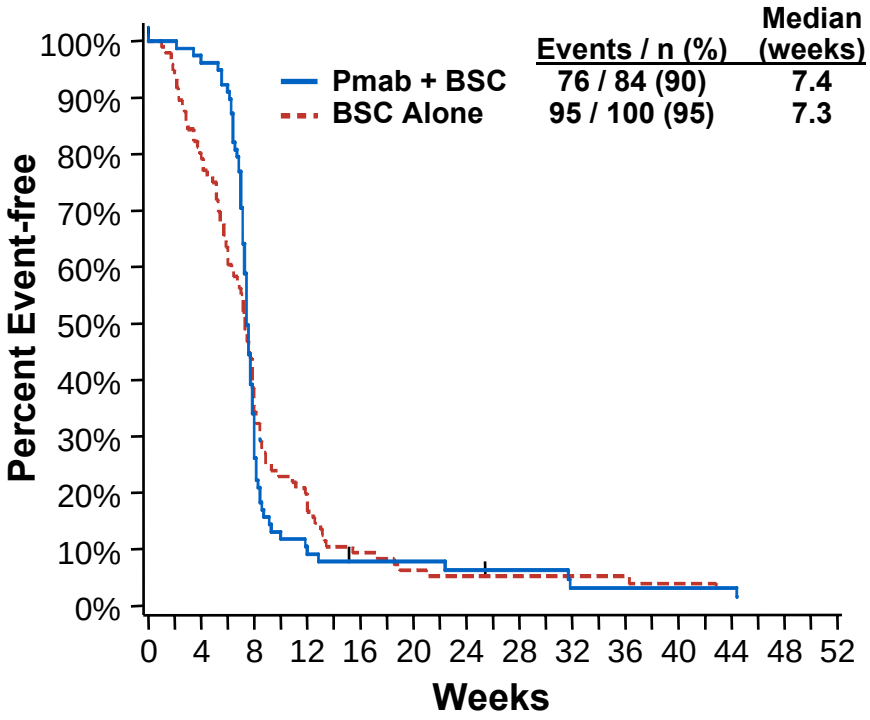
Increased PFS Observed in Patients with *KRAS* Wild-type Tumors

Wild-type



Hazard Ratio = 0.45 (95% CI: 0.34–0.59)
Stratified Log Rank Test *p* < 0.0001

Mutant



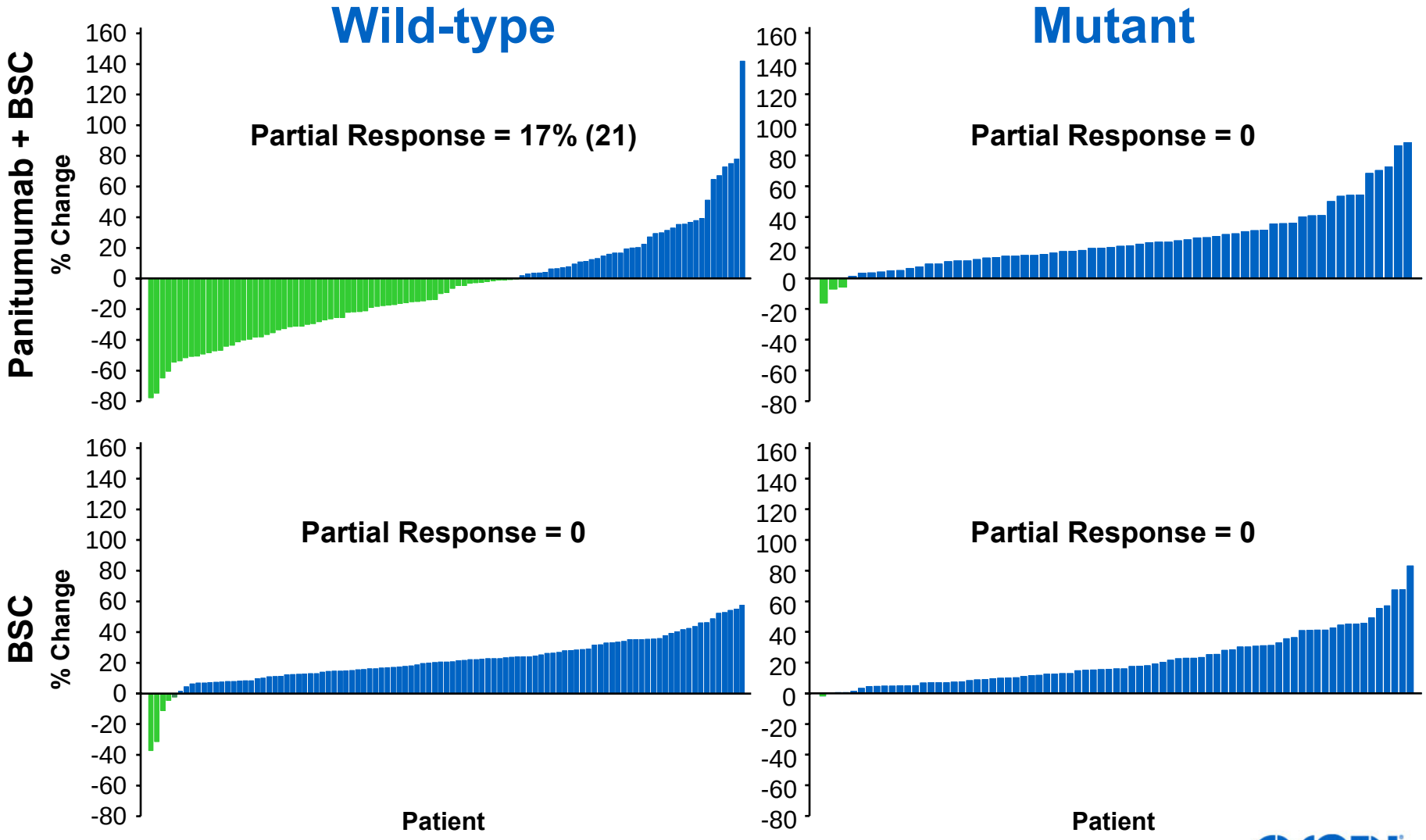
Hazard Ratio = 0.99 (95% CI: 0.73–1.36)

Quantitative interaction test *p* < 0.0001

Adapted from: Amado, et al. *J Clin Onc.* 2008;26:1626-1634.

AMGEN

Decreases in Target Lesions Observed in Patients with Wild-type *KRAS* Tumors Treated with Panitumumab



Adapted from: Amado, et al. *J Clin Onc.* 2008;26:1626-1634.

AMGEN

Key Aspects of KRAS Analysis

1. Hypothesis re: *KRAS* conferring primary resistance generated independently (from previous trials)
2. Only biomarker (in addition to EGFR) tested was *KRAS* – to avoid inflation of type-1 error
3. Analyses sufficiently powered and prespecified in statistical analysis plan before *KRAS* data known
4. Testing performed in an independent lab without patient-level knowledge of randomization or outcome
5. The magnitude of the interaction observed is substantial
6. There was a high ascertainment rate (92%)
7. Overall goal was to improve the utility of treatment through patient selection

Amado et al. *JCO.* 2008;26:1626-1634.

AMGEN

A Pooled Analysis of Panitumumab Monotherapy Studies Demonstrates Consistent Results

- Similar study designs in 4 monotherapy trials
- *KRAS* was tested with the same methodology independent of study outcomes (and treatment in the phase 3 study)
- A high rate of *KRAS* ascertainment was achieved in each study (84–96%)
- Each study had consistent outcomes by *KRAS* status

AMGEN

The Utility of *KRAS* as a Predictive Biomarker Consistently Seen in Panitumumab Monotherapy Studies

Study	*KRAS* Ascertainment	Objective Response	
		Wild-type	Mutant
20020408[1] (n = 208)	90%	17%	0%
20030194 (n = 168)	96%	22%	0%
20030167 (n = 168)	91%	6%	0%
20030250 (n = 171)	84%	9%	0%
Total (n = 715)	90%	14%	0%

No objective response from 320 patients with KRAS mutant tumors

[1]Panitumumab arm

Amado, et al. *Annal Onc.* 2008:19(8);359P (viii126)

AMGEN

Conclusions

- The efficacy of panitumumab monotherapy seems confined to patients with wild-type KRAS
- These data formed the basis for regulatory approval of panitumumab in the EU, Switzerland and Canada in patients with KRAS WT tumors who had developed disease progression after fluorouracil-, oxaliplatin-, and irinotecan-containing chemotherapy
- Ongoing studies in mCRC in 1st and 2nd lines will prospectively elucidate the role of KRAS mutational status in patient selection in the setting of panitumumab in combination with chemotherapy
- The DxS KRAS Kit is CE Marked (EU) and is in the process of being evaluated for a PMA (USA)

AMGEN

Critical Elements of Biomarker Validation

1. **Scientific plausibility**
   - Understanding of fundamental biology
   - Appropriate hypothesis-generating data
2. **Analytical validation of assay**
   - Performance characteristics, reproducibility, accuracy
   - Assessment against known ('gold') standards (if appropriate)
   - Practicality
3. **Rigorous demonstration of clinical utility**
   - Prospective vs retrospective data sets
   - Prespecified analysis plan (hypothesis testing)
   - Minimization of bias (e.g., ascertainment bias)
   - Generalizability

We believe these levels of evidence were met

Based on: FDA Critical Path Initiative 2004; FDA Pharmacogenomic Data Submissions Guidance 2005; FDA Drug-Diagnostic Co-Development Concept Paper 2005; Altar et al *Clin Pharmacol Ther* 2008.

AMGEN

Acknowledgments

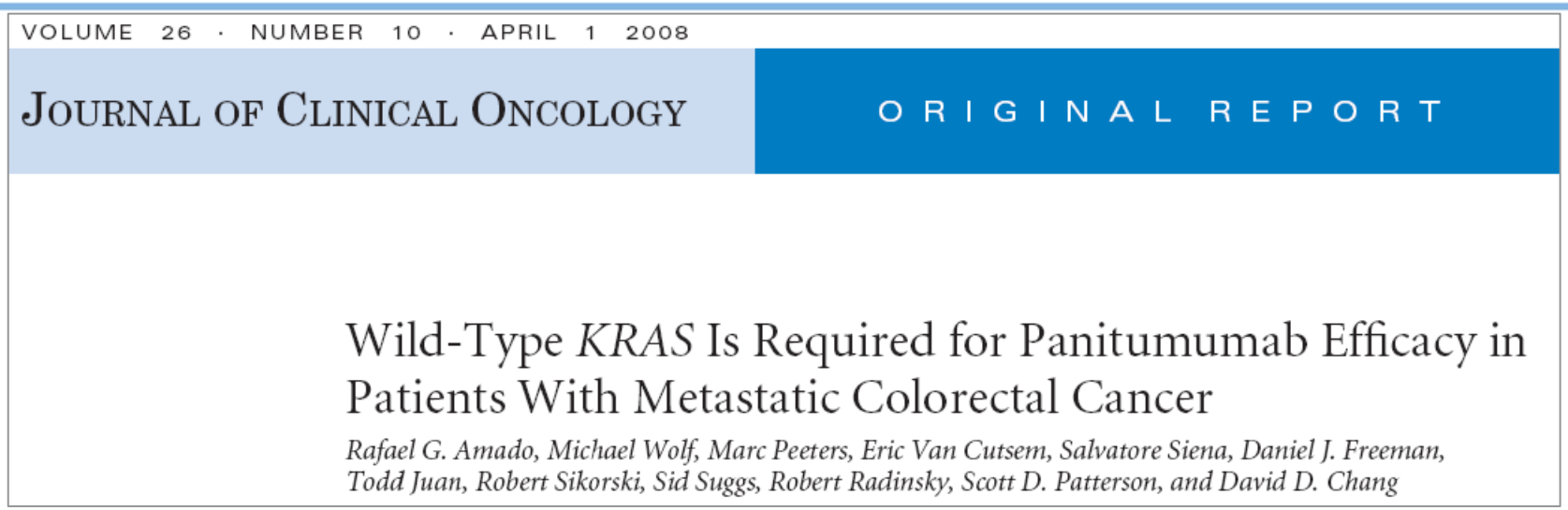
- We wish to thank the patients and their families for study participation
- We also thank all investigators and study personnel
- Portions of this talk were presented at the ECCO meeting in Barcelona, September 25th 2007, ASCO GI 2008 and December 16th ODAC, 2008

Disclosure

- This study was sponsored by Amgen Inc.
- R.G. Amado, M. Wolf, D. Freeman, S. Suggs, S. Patterson, D. Chang, T. Juan, M. Reiner, and R. Radinsky are employees of Amgen Inc. and own Amgen Inc. stock.
- M. Peeters has been on an advisory board for Amgen and has received honoraria from Amgen; E. Van Cutsem has been on an advisory board for Amgen and has received honoraria from Amgen; N.J. Meropol has been a consultant for Amgen, Genentech, and Imclone; and J. Berlin has been on advisory boards for Amgen, BMS, and Imclone.

AMGEN

References



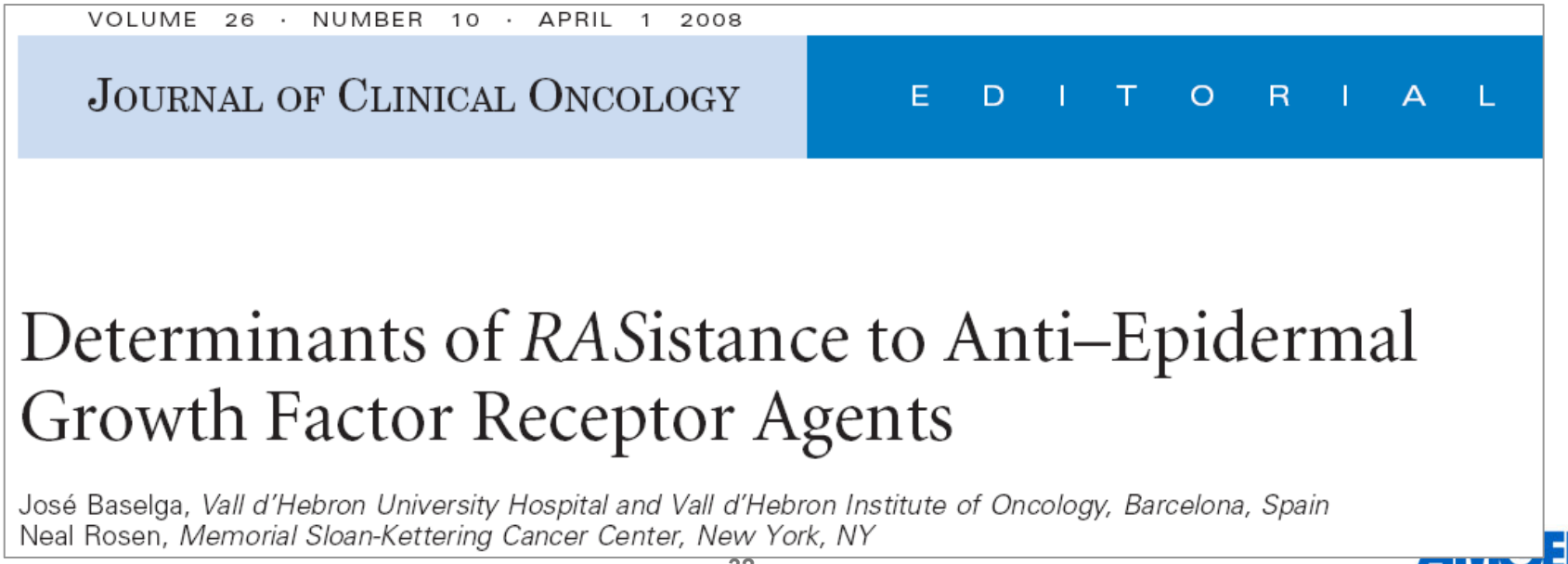
VOLUME 26 · NUMBER 10 · APRIL 1 2008

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Wild-Type *KRAS* Is Required for Panitumumab Efficacy in Patients With Metastatic Colorectal Cancer

Rafael G. Amado, Michael Wolf, Marc Peeters, Eric Van Cutsem, Salvatore Siena, Daniel J. Freeman, Todd Juan, Robert Sikorski, Sid Suggs, Robert Radinsky, Scott D. Patterson, and David D. Chang



VOLUME 26 · NUMBER 10 · APRIL 1 2008

JOURNAL OF CLINICAL ONCOLOGY

EDITORIAL

Determinants of *RA*Sistance to Anti–Epidermal Growth Factor Receptor Agents

José Baselga, *Vall d'Hebron University Hospital and Vall d'Hebron Institute of Oncology, Barcelona, Spain*
Neal Rosen, *Memorial Sloan-Kettering Cancer Center, New York, NY*