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Assessment of Tumor Genotypes in Metastatic Colorectal Cancer (mCRC): Mutations beyond KRAS Codons 12 and 13 as Predictive Biomarkers of Response to Panitumumab (pmab) in a Randomized, Phase 3 Monotherapy Study

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Introduction

General Background

- Panitumumab (Pmab)
  - a fully human monoclonal antibody
  - targeting the epidermal growth factor receptor (EGFR)
  - treatment for mCRC
- Retrospective subset analyses of mCRC trials have not shown a treatment benefit for Pmab in patients whose tumors had *KRAS* mutations in codon 12 or 13.
- Availability of massively parallel, next-generation sequencing
  - 454 Pyrosequencing is utilized
  - High sensitivity (5% mutant DNA detection)
  - Multiplexed gene analysis
  - Rapid data generation

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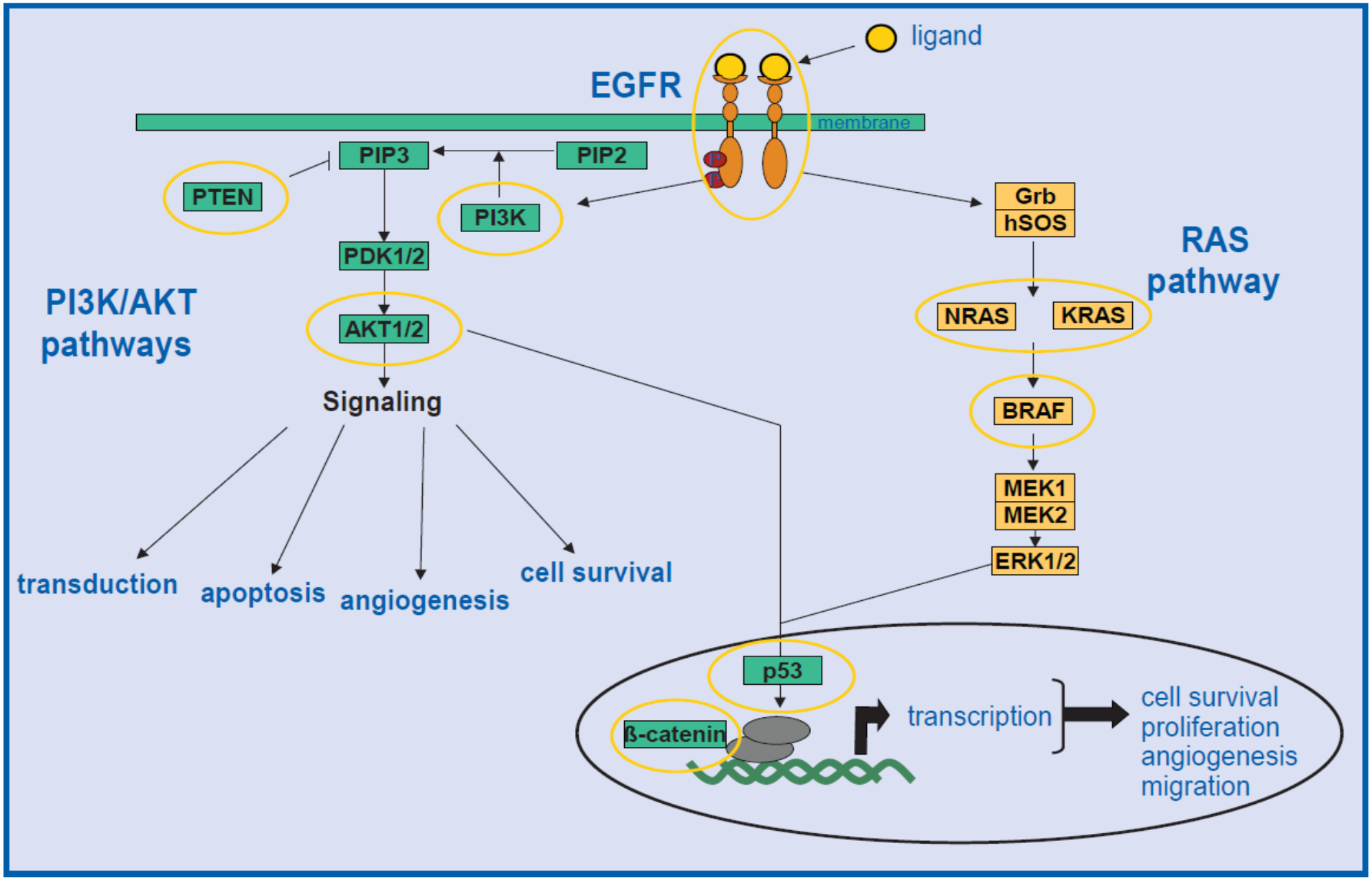
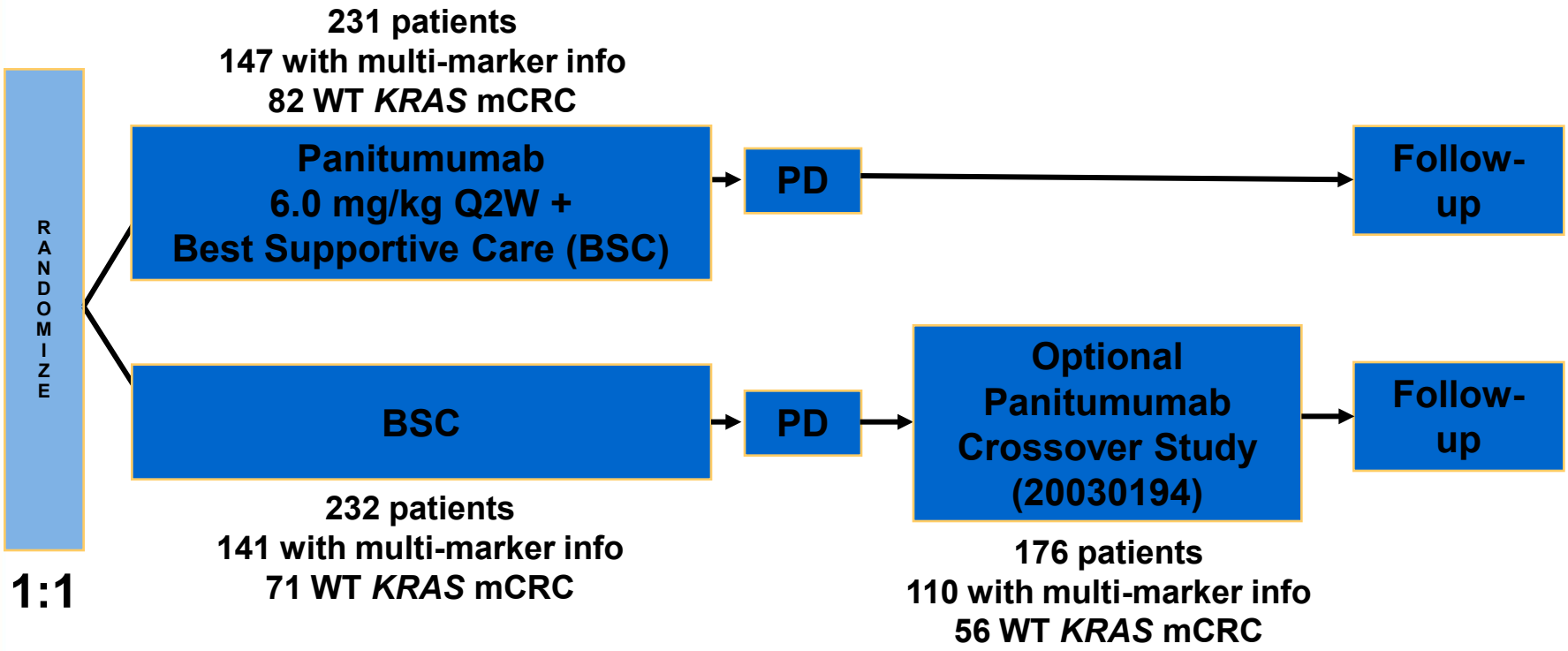
EGFR Signaling pathway



Schema for Pivotal Phase 3 Panitumumab Study (20020408) and Optional Crossover Study (20030194)



Primary Endpoint: Progression-free Survival (PFS)

PD, progressive disease

Adapted from Van Cutsem et al.[5]

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Objectives

- Investigate whether mutation of multiple genes known to be altered in mCRC are predictive of response to Pmab
  - Focus on codons 12, 13, and 61 of the *KRAS* gene
  - Assess clinical outcomes of patients with defined tumor genotypes treated with Pmab + BSC vs. BSC alone
  - Include patients treated on the BSC arm that enrolled in an optional crossover study to better understand response to Pmab

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Results

Tumor samples and DNA sequencing

- 320 archival tumor samples available
- 288 (90%) tumor samples yielded results
- 277 tumor samples yielded a definitive genotype (WT or MT) on codons 12, 13, and 61 for *KRAS*
  - 153 were WT *KRAS*
  - 71 patients with WT *KRAS* received BSC in the randomized study
  - 82 patients with WT *KRAS* received Pmab in the randomized study
  - 56 patients with WT KRAS received Pmab in the extension study
  - Total 138 patients with WT KRAS received Pmab
- 49 primer pairs to amplify 41 exons from 9 genes
- Data completeness range for each gene = 84% to 99%
- 5.7 million DNA sequence reads
- A total of 1.26 billion base pairs obtained

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Mutation rates in 288 mCRC tumors

Gene	Mutation Rate, %	Patients With Mutations, n	Patients With Data Available, n	Data Completeness %
KRAS (codons 12, 13, and 61)	46	124	277	96
KRAS (codons 12 and 13)†	42	117	280	97
KRAS (codon 61)	3	7	284	99
NRAS	5	14	282	98
EGFR	1	3	280	97
BRAF	7	18	243	84
PTEN	6	15	272	94
PIK3CA	9	24	255	89
AKT1	<1	1	250	87
TP53	60	167	277	96
CTNNB1	2	5	256	89

Joint distribution across all gene pairs

Gene - n	AKT1	BRAF	CTNNB1	EGFR	KRAS	NRAS	PIK3CA	PTEN	TP53
AKT1									
BRAF	1								
CTNNB1	–	–							
EGFR	–	–	–						
KRAS	–	2	1	3					
NRAS	–	–	–	–	3				
PIK3CA	–	2	1	–	13	–			
PTEN	–	4	–	1	6	1	1		
TP53	–	8	1	1	66	9	12	9	

- 109 tumors had mutations in more than one gene
- 20 tumors had more than one mutation in a single gene in this analysis

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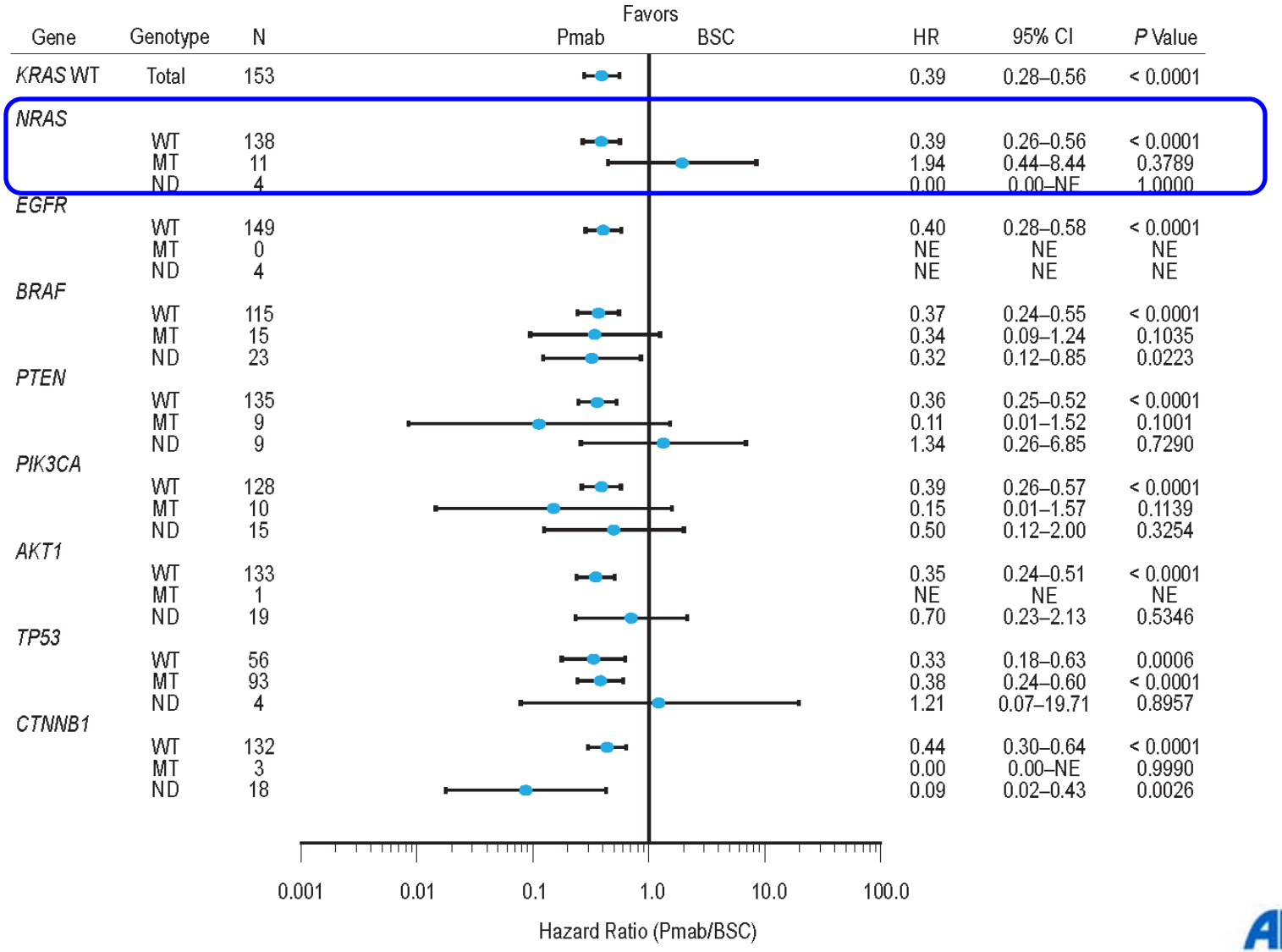
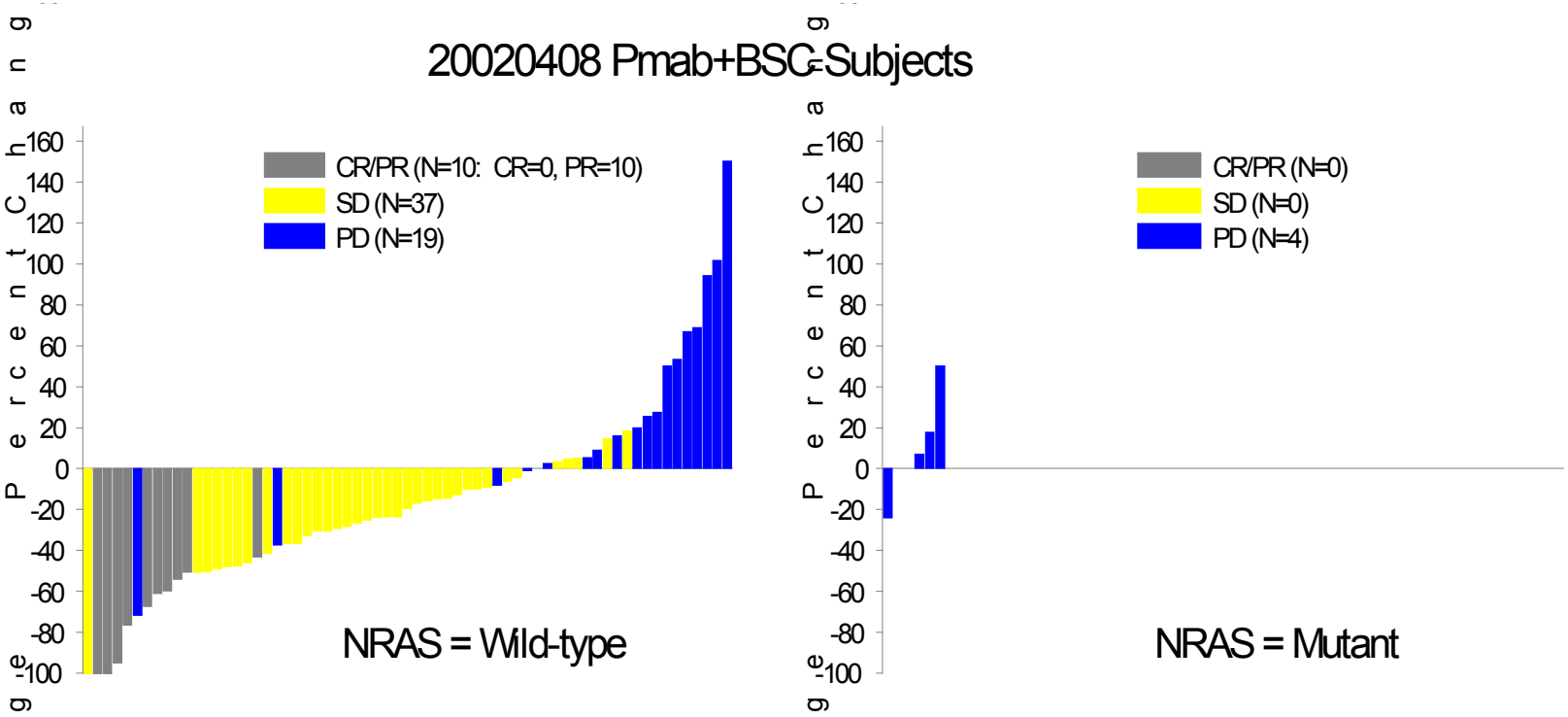
Treatment Effect on PFS within KRAS WT group



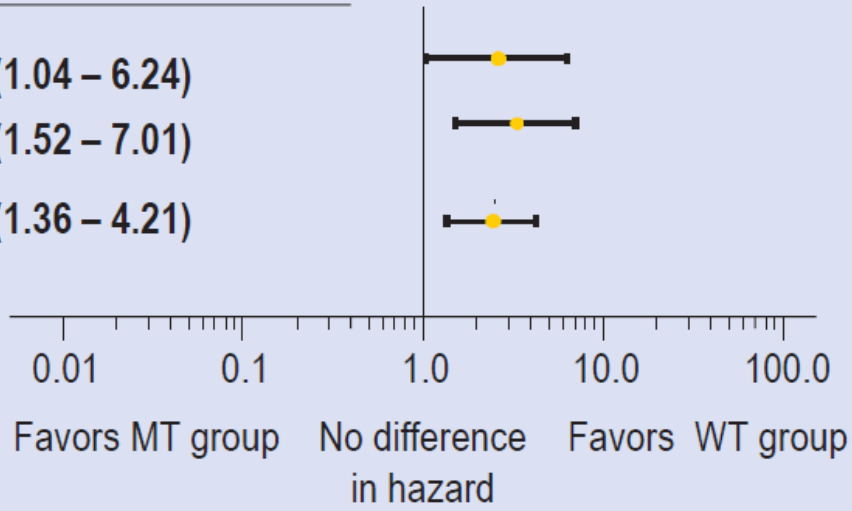
Gene	Genotype	N	HR	95% CI	*P* Value
KRAS WT	Total	153	0.39	0.28–0.56	< 0.0001
NRAS					
	WT	138	0.39	0.26–0.56	< 0.0001
	MT	11	1.94	0.44–8.44	0.3789
	ND	4	0.00	0.00–NE	1.0000
EGFR					
	WT	149	0.40	0.28–0.58	< 0.0001
	MT	0	NE	NE	NE
	ND	4	NE	NE	NE
BRAF					
	WT	115	0.37	0.24–0.55	< 0.0001
	MT	15	0.34	0.09–1.24	0.1035
	ND	23	0.32	0.12–0.85	0.0223
PTEN					
	WT	135	0.36	0.25–0.52	< 0.0001
	MT	9	0.11	0.01–1.52	0.1001
	ND	9	1.34	0.26–6.85	0.7290
PIK3CA					
	WT	128	0.39	0.26–0.57	< 0.0001
	MT	10	0.15	0.01–1.57	0.1139
	ND	15	0.50	0.12–2.00	0.3254
AKT1					
	WT	133	0.35	0.24–0.51	< 0.0001
	MT	1	NE	NE	NE
	ND	19	0.70	0.23–2.13	0.5346
TP53					
	WT	56	0.33	0.18–0.63	0.0006
	MT	93	0.38	0.24–0.60	< 0.0001
	ND	4	1.21	0.07–19.71	0.8957
CTNNB1					
	WT	132	0.44	0.30–0.64	< 0.0001
	MT	3	0.00	0.00–NE	0.9990
	ND	18	0.09	0.02–0.43	0.0026

Tumor Response by NRAS Mutation



Biomarker Effect on PFS: the BRAF Story

Gene	Treatment	WT N	MT N	Hazard Ratio (95% CI)
BRAF	BSC	52	6	2.55 (1.04 – 6.24)
	Pmab + BSC	63	9	3.27 (1.52 – 7.01)
	Total	115	15	2.39 (1.36 – 4.21)



*WT in codons 12, 13, and 61

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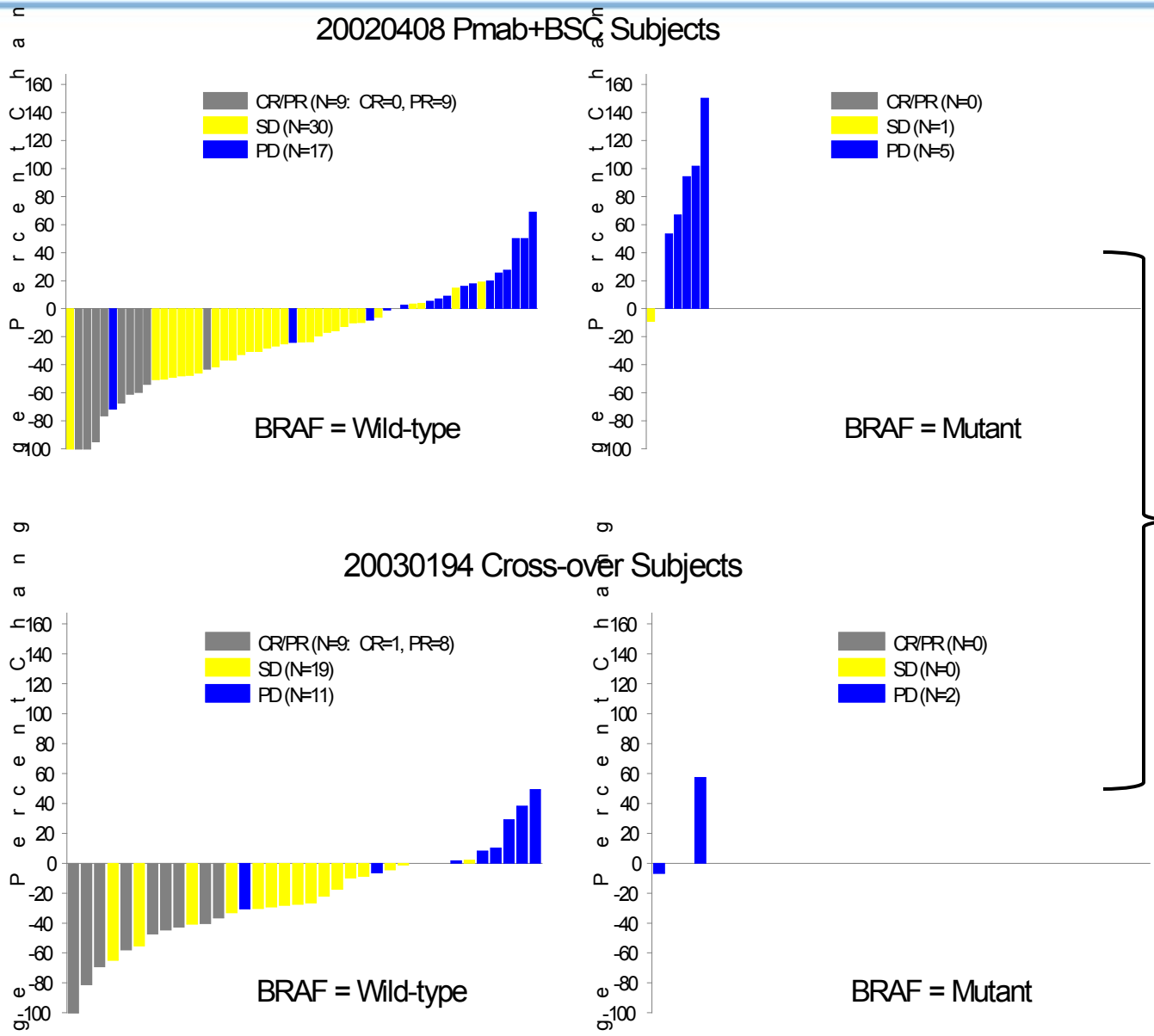
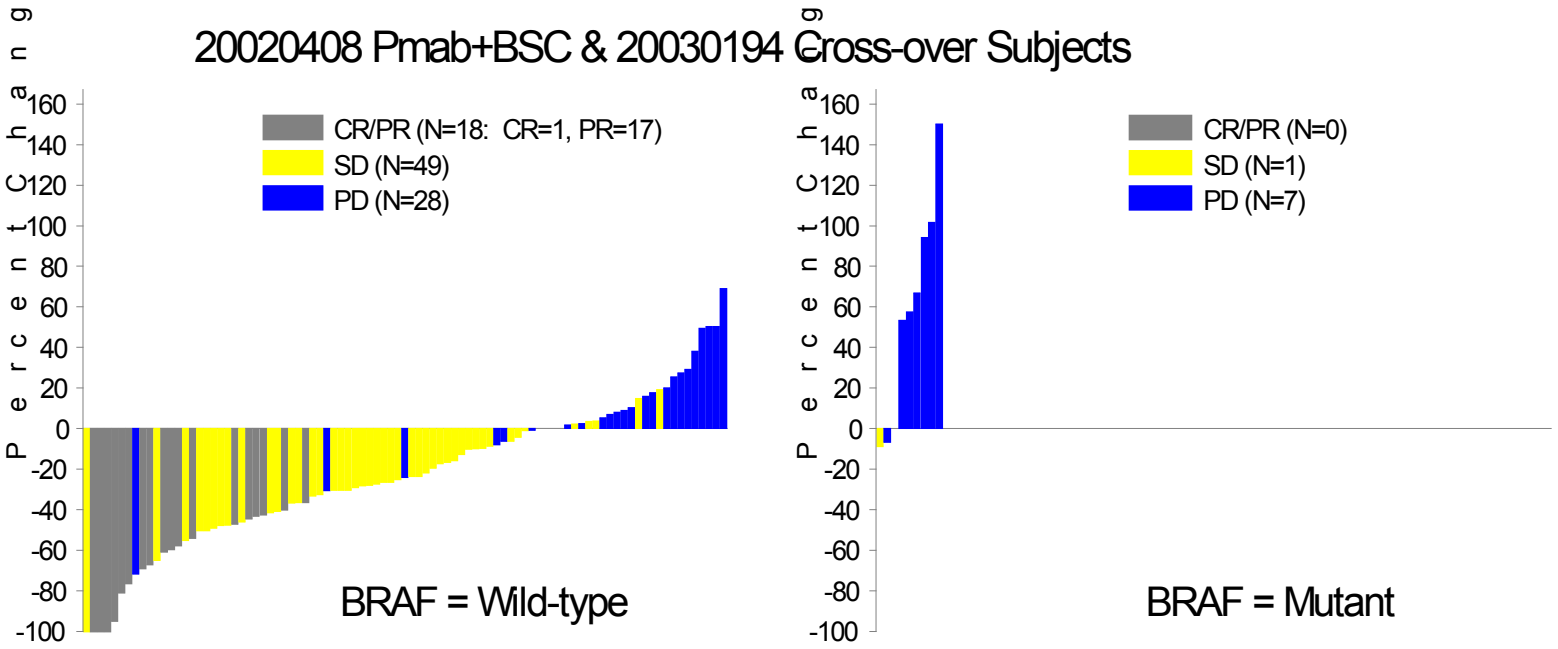
Tumor Response by BRAF Mutation



Combine together

Tumor Response by BRAF Mutation

combine the randomized and the cross-over studies



CR: Complete Response; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease.

Tumor Response

combine the randomized and the cross-over studies

Genotype		Randomized Phase 3 Study Panitumumab + BSC *n* = 82		Extension Study Panitumumab + BSC *n* = 56		Combined Panitumumab + BSC *n* = 138	
		n	Response Rate, % (95% CI)	n	Response Rate, % (95% CI)	n	Response Rate, % (95% CI)
NRAS	WT	76	13 (6–23)	50	24 (13–38)	126	17 (11–25)
	MT	4	0 (0–60)	5	0 (0–52)	9	0 (0–34)
EGFR	WT	82	12 (6–21)	52	23 (13–37)	134	16 (11–24)
	MT	0	NA	0	NA	0	NA
BRAF	WT	63	14 (7–25)	44	21 (10–35)	107	17 (10–25)
	MT	9	0 (0–34)	4	0 (0–60)	13	0 (0–25)
PTEN	WT	72	13 (6–22)	50	22 (12–36)	122	16 (10–24)
	MT	7	14 (0–58)	2	0 (0–84)	9	11 (0–48)
PIK3CA	WT	74	12 (6–22)	43	19 (8–33)	117	15 (9–22)
	MT	5	20 (1–72)	5	20 (1–72)	10	20 (3–56)

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Conclusion

- Next-generation sequencing technology can be employed to assess mutation in multiple genes in archival tumor samples from a randomized phase 3 study
- Observed mutation rates in this study were consistent with previous reports in mCRC
- Within the KRAS wild type population, patients whose tumors contain mutations in other RAS family members, such as NRAS, may not derive benefit from panitumumab
- Further investigation is required to determine the predictive value of KRAS codon 61, NRAS, and BRAF mutations due to the low prevalence of mutations

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Acknowledgements: It's a team work!

- Medical Science Biostatics
  - Ying Cheng, Lixia Zhu, Lisa Hendricks, Mike Hale, Li Chen
- Biomarker Group
  - Kelly Oliner, Alex Parker, Scott Patterson
- Clinical Development
  - Mike Wolf, Alan Rong, Kathy Zhang, Steve Dahlberg, Jeff Wiezorek, David Reese
- Medical Writing
  - Ali Hassan, Mee Rhan Kim, Jamie Ziobro

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