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Germline polymorphisms in genes involved in the IGF1-pathway predict efficacy of cetuximab in wild-type *KRAS* mCRC patients

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Abstract

Purpose—The insulin-like growth factor 1 (IGF1) signalling pathway is an important growth-regulatory pathway, which plays a crucial role in colorectal cancer (CRC) proliferation, differentiation, migration, angiogenesis, and apoptosis. Previous studies showed that hyperactivation of the IGF1-Receptor (IGF1R) may result in resistance to anti-EGFR targeted treatment. We tested whether germline variations within the IGF1-pathway are associated with clinical outcome in wild-type *KRAS* (wt *KRAS*) drug-refractory metastatic CRC (mCRC) patients who were treated with cetuximab monotherapy (IMC-0144).

Experimental Design—Formalin-fixed paraffin-embedded (FFPE) tissue samples of 130 drug-refractory mCRC patients enrolled in IMC-0144, a phase II clinical trial of cetuximab monotherapy, were analyzed. gDNA was extracted from dissected FFPE tumor tissue and *KRAS* mutation status and six potentially functional IGF1 and IGF1R polymorphisms were analyzed using direct DNA-sequencing or PCR-RFLP. Tumor response analysis was based on recursive partitioning and survival analyses were based on univariate and multivariate hazard regression models.

Results—In univariate and multivariate analyses five IGF-pathway SNPs were significantly associated with progression-free-survival (PFS) and/or overall survival (OS). In multivariate combined risk allele analysis the additive model for PFS and OS was significantly associated with the number of risk alleles in wt *KRAS* patients ($p=0.001$ and $p=0.02$, respectively). In addition, wt *KRAS* patients harbouring IGF1 rs2946834 A/A genotype had a 50 % ORR compared to 0% for A/G genotype.

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Conclusions—These results indicate that IGF1-pathway polymorphisms are potential predictive/prognostic molecular markers for cetuximab efficacy in wt *KRAS* mCRC patients. Prospective biomarker embedded clinical trials are warranted to validate our findings.

Keywords

KRAS; IGF1; IGF1R; Metastatic colon cancer; cetuximab

Introduction

CRC is the second most lethal malignancy in the United States. In 2009, 146,970 new cases of CRC and 49,920 death were recorded (1). Monoclonal antibodies (MoAbs) targeting the epidermal growth factor receptor (EGFR), including the chimeric immunoglobulin G1 (IgG1) anti-EGFR MoAb cetuximab, have been proven effective in combination with chemotherapy or as single agent for treatment of mCRC (2-5). Activating *KRAS* mutation has recently emerged as major predictor of resistance to the EGFR-targeted MoAbs and patient selection based on *KRAS* mutational status allows more accurate treatment selection with improved efficacy, reduction of unnecessary toxicities, and improved overall cost effectiveness (5,6). Although the *KRAS* mutation is a highly specific negative biomarker of response (93% specificity), it does lack sensitivity (47% sensitivity) (7). This indicates the existence of additional, but, as of yet, unknown determinants of efficacy to the anti-EGFR MoAbs. Previous studies have investigated additional determinants of EGFR MoAb sensitivity within the EGFR signaling network, including *BRAF* mutational status (8), epiregulin and amphiregulin mRNA expression (9), high EGFR gene copy number (10), loss of PTEN protein expression (11) and *PIK3CA* mutation status (12), in wt *KRAS* mCRC patients treated with cetuximab. Although several of these molecular markers appear promising, their utility as predictive determinants will require evaluation in prospective clinical trials.

Translational Relevance

KRAS mutation recently emerged as a highly specific negative biomarker of response to the EGFR-targeted antibodies in colorectal cancer. However, the presence of wildtype *KRAS* does not dictate response, indicating the existence of additional determinants of efficacy. Recently, the analysis of tumor receptor signaling pathways determined the presence of functional crosstalk between IGF1R and EGFR signal transduction events and reported that that IGF1R signaling is critical for EGFR activity and associated with resistance to EGFR-targeted therapy. Members of the IGF1 pathway possess a number of common polymorphic variants that may influence the activity of the IGF1R pathway and EGFR pathway crosstalk. The identification of functional IGF1 pathway polymorphisms could select patients with an increased likelihood of response or who are candidates for combined EGFR and IGF1R treatment. Furthermore, patient selection based on individual genetic profiling allows more accurate treatment selection with improved efficacy, reduced toxicities, and improved overall cost effectiveness.

IGF1 signaling mediated by IGF1R is an important growth regulatory pathway that plays a crucial role in CRC cell proliferation, migration, and apoptosis (13-17). IGF1 is a potent mitogenic activator via the Ras/Raf/MAPK signaling pathway and a powerful antiapoptotic molecule through the PI3K/Akt pathway (18). An analyses of functional cross-talk between IGF1R and EGFR has shown that activation of the IGF1 downstream signaling cascade is crucial for the mitogenic and transforming activity of EGFR (19). More specifically, the IGF1R pathway induces both transforming growth factor α (TGF α)-mediated activation of EGFR and stimulation of EGFR-independent PI3K/AKT activity (20). Both cetuximab and

the IgG2 EGFR-targeting MoAb panitumumab function principally by inhibiting ligand binding to EGFR, thereby suppressing downstream signaling. Consequently, IGF1-driven PI3K/Akt overstimulation due to hyperactivation and/or pathway aberrations provides a rational explanation, at least in part, for the lack of efficacy observed in a notable fraction of patients with wt *KRAS* CRC treated with EGFR-targeting MoAbs. Recently, Scartozzi *et al.* reported that high IGF1 protein expression correlates with poor clinical outcome in wt *KRAS* mCRC patients treated with cetuximab and Irinotecan (17). In addition, IGF1 and IGF1R polymorphisms have been associated with cancer risk (21,22) and increased IGF1 plasma levels (23), suggesting functional and clinical significance.

The current study sought to evaluate whether functional polymorphisms in the IGF1 and IGF1-R genes, alone or in combination, can augment the prediction of sensitivity to cetuximab treatment in drug-refractory wt *KRAS* mCRC patients treated with single-agent cetuximab in a phase II clinical trial (IMC-0144).

Patients and Methods

Patient characteristics

Formalin fixed paraffin embedded (FFPE) tumor tissue of one hundred thirty (38%) of 346 mCRC patients enrolled in a multicenter, multinational, open-label, phase II trial of cetuximab in patients with drug-refractory mCRC (IMCL-0144) was available for analysis of IGF1 and IGF1R polymorphisms. Patients were enrolled from November 2002 to December 2005. Cetuximab was administered as a 120-minute intravenous infusion at 400 mg/m² followed by weekly 60-minute infusions of 250 mg/m². Eligibility for the IMCL-0144 study required that mCRC patients failed chemotherapy consisting of oxaliplatin, irinotecan, and fluoropyrimidines (4). The tissue analysis presented in present study was conducted at the University of Southern California/Norris Comprehensive Cancer Center (USC/NCCC) following approval by USC Institutional Review Board for Medical Sciences. All patients provided their written informed consent for tissue and blood collection to allow study of molecular correlates.

Clinical evaluation of response criteria

Objective tumor response was assessed every 6 weeks during the course of the study and criteria were based on modified WHO guidelines (4). Response to cetuximab was determined by an independent response assessment committee that was blinded to the investigator-reported measurements and assessments were reported in the study as previously reported (4). A partial response (PR) required at least a 50% reduction in the sum of the bidimensional products of all measurable lesions documented at least 4 weeks apart. Treatment was continued in the absence of intolerable toxicity or progressive disease, defined as at least a 25% increase in measurable disease, unequivocal growth of existing nonmeasurable disease, the appearance of one or more new lesions, or reappearance of old lesions.

DNA Extraction, SNP Selection and Genotyping

DNA was extracted from FFPE tumor samples using the QIAamp kit (Qiagen, CA, USA). The genes, reference single nucleotide polymorphisms (SNP) identification numbers, location, function, forward and reverse primer and restriction enzymes are summarized in Table 2. The polymorphisms we tested were selected by an IGF1-pathway approach with the goal of selecting common and functional SNPs previously associated with other tumors. The following criteria were used to select the candidate polymorphisms: (a) a minor allele frequency (MAF) $\geq 10\%$ in Caucasians according to the HapMap Project database1, (b) potential functional polymorphisms located in the 3'UTR (untranslated region) and/or were

shown to be of biological significance according to the literature review; (c) were associated with cancer risk and/or IGF1 plasma level in previous studies (Table 2). *KRAS* mutation status was determined from microdissected tumor DNA by direct sequencing as previously described (24). IGF1 pathway polymorphisms were tested either by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique or direct sequencing as previously described. For quality control purposes, a total of 5% positive and negative duplicate-controls were matched for each polymorphism and were analyzed by direct DNA-sequencing where applicable. Genotype concordance was $\geq 99\%$.

Statistical analysis

IGF1 and IGF1R polymorphisms were related to parameters reflecting clinical outcome, including PFS, OS, and objective response as defined in the clinical trial.⁴ The PFS was calculated from the date of the first date of cetuximab treatment until disease progression or death from any cause or censored at the last follow up if the patients were still alive and not progressed. The OS time was the period from the first day of cetuximab infusion until death from any cause or the last follow up, at which point the data was censored. The objective response rate (RR) was defined as the total number of complete or partial responses divided by the number of evaluable patients.

The distributions of polymorphisms across baseline demographic and clinical characteristics were examined using Fisher's exact test. The association between each polymorphism with OS and PFS was analyzed using Kaplan-Meier curves and the log-rank test. RR was analyzed using contingency tables and the exact conditional test. Subgroup analysis was conducted in patients carrying wt *KRAS* only. The false discovery rate (FDR) of multiple testing was controlled using the Benjamini and Hochberg method (25). The FDR-adjusted *p* values $<15\%$ were considered as significant. In the multivariable Cox proportional hazards regression model, known prognostic, predictive variables, such as *KRAS* mutation status, and polymorphisms that had significant associations with clinical outcome in our previous study were included to investigate the independent effects of IGF1 and IGF1R polymorphisms. The genetic model of inheritance for IGF1 and IGF1R polymorphisms were unknown, we considered the dominant, recessive, co-dominant, or additive model whenever appropriate.

A regression tree method based on recursive partitioning (RP) was used to identify homogenous subgroups for tumor response from genetic markers we previously tested (24).

Allelic distribution of all polymorphisms in each race/ethnic group was tested for deviation from Hardy-Weinberg equilibrium (HWE). Linkage disequilibrium among polymorphisms in IGF1 and IGF1R was assessed using D' and r^2 values, and the haplotype frequencies of the two genes were inferred using HaploView version 4.12. All statistical tests were 2-sided and performed using the SAS statistical package version 9.2 (SAS Institute Inc. Cary, North Carolina, USA).

Results

DNA was extracted for analysis from 130 (38%) of 346 eligible tumor specimens. The median PFS (1.3 months; 95% confidence interval [CI], 1.3-1.5), OS (6.3 months; 95% CI, 4.3-7.7), and RR (9.2%; 95% CI, 4.9%-15.6%) of patients with eligible tumor samples were similar to those patients on trial without tissue and blood samples available ($n=216$); median PFS (1.5 mo; 95% CI, 1.4-2.6); OS (6.8 months; 95% CI, 5.8-8.1), and RR (13.0%; 95% CI,

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8.8%-18.2%) (4). The median follow-up for this translational study was 12.3 months (range: 2.2-17.3 months). Sixteen (12%) out of 130 patients were not evaluable for tumor response. In 114 patients assessable for tumor response, 77 patients had wt *KRAS* and 37 patients had mutant *KRAS*. The allelic frequencies observed for IGF1 and IGF1R polymorphisms analyzed were within the probability limits of HWE ($p>0.05$). The patient characteristics, *KRAS* mutation status and clinical outcome were described previously and summarized in table 1 (4,24).

Univariate analysis for IGF1 and IGF1R polymorphisms for PFS and OS

In univariate analysis, three IGF1 polymorphisms – IGF1 rs6214, IGF1 rs2946834, and IGF1 rs7136446 were significantly related to PFS in all patients ($p=0.048$, $p<0.001$ and $p=0.034$, respectively). Two of these polymorphisms, IGF1 rs2946834 and IGF1 rs713664, also related to PFS in the subgroup of wt *KRAS* patients ($p<0.001$ and $p=0.022$, respectively). Univariate analysis for OS was subsequently performed. One IGF1 (rs7136446) and the recessive model of two IGF1R polymorphisms (IGF1R rs2272037 and IGF1R rs2016347) were significantly associated with shorter OS in all patients ($p=0.026$, $p=0.039$ and $p=0.038$, respectively) and IGF1R rs2016347 was significantly associated with shorter OS in the subgroup of wt *KRAS* patients only ($p=0.004$) [Table 3].

Associations between IGF1 and IGF1R polymorphisms and tumor response to cetuximab treatment in wt *KRAS* patients only

Response to treatment with cetuximab monotherapy was related to SNPs in IGF1 and IGF1R genes. Three polymorphisms, IGF1 rs6214, IGF1 rs2946834, and IGF1R2016347, were significantly related to reduced responsiveness to cetuximab treatment (Table 4). RP was utilized to construct a decision tree as a predictive model to classify patients based on the presence of these molecular markers and the likelihood of response to cetuximab. This comprehensive RP analysis incorporated a total of 16 potential markers including the IGF1 and IGF1R polymorphisms in the current study, a panel of polymorphisms previously evaluated in this patient cohort (FCGR2Ars1801274 and FCGR3 Ars396991, EGFRrs11543848, CyclinD1rs17852153, IL8rs4073, VEGFRs3025039, COX-2rs20417, COX-2rs5275, EGFRs4444903, NRP-1rs3750733) (24) and the development of skin rash during cetuximab treatment. In the resultant decision tree, the most important factor that determined the RR in these patients was the IGF1 rs2946834 polymorphism (node 1). Patients carrying IGF1 rs2946834 A/A genotype had a RR of 50% to cetuximab treatment. Patients in node 2 or 3 segregated based on their COX2-765 genotype had RR of 18% versus 5%, respectively (Figure 2).

Multivariable model of IGF1 and IGF1R polymorphisms in all patients

The multivariable model was adjusted by skin rash severity, *KRAS* mutation, ECOG performance status, and three significant polymorphisms previously published by our group (24) (EGFRrs11543848, COX-2rs5275, EGFRs4444903) and stratified by race. IGF1 rs6214 (adjusted $p=0.008$), IGF1 rs6220 (adjusted $p=0.028$) and IGF1 rs2946834 (adjusted $p=0.002$) remained significantly associated with PFS and IGF1R rs2016347 (adjusted $p=0.033$) remained significantly associated with OS (Table 5). Because these four polymorphisms were consistently associated with either PFS or OS, an unfavorable genotype analysis was performed to determine the effect of the number of risk alleles (Table 5 and Figure 1). Both the additive model for PFS and OS were significantly associated with the number of risk alleles (Hazard Ratio (HR) 2.028; 95% CI, 1.478, 2.783; $p<0.001$ versus HR 1.828; 95% CI, 1.272, 2.627; $p=0.001$, respectively).

Multivariable model of IGF1 and IGF1R polymorphisms in wt *KRAS* only

The multivariable model for the wt *KRAS* subgroup was adjusted for the same factors mentioned above. IGF1 rs6214 (adjusted $p=0.044$), IGF1 rs2946834 (adjusted $p=0.009$), and the number of risk alleles ($p=0.004$) remained significantly associated with PFS (Table 4). No polymorphism was found to be significant for OS. Using unfavorable genotype analysis the additive model showed a significant association with the number of risk alleles for PFS (HR 1.895, 95% CI, 1.299, 2.765; $p=0.001$) and OS (HR 1.745, 95% CI, 1.092, 2.787; $p=0.02$; Figure 3a and 3b).

Multiple testing using Benjamini-Hochberg method

After adjusting for the FDR at <15% level, IGF1 rs6214 (FDR adjusted $p=0.022$), IGF1 rs2946834 (FDR adjusted $p=0.007$), and the number of risk alleles (FDR adjusted $p=0.001$) remained significant for PFS. IGF1 rs6214 (FDR adjusted $p=0.043$) and the number of risk alleles (FDR adjusted $p=0.009$) also remained significant for OS in all patients. In wt *KRAS* patients, IGF1 rs2946834 (FDR adjusted $p=0.038$), and the number of risk alleles (FDR adjusted $p=0.007$) remained significant for PFS.

Haplotype Analysis

IGF1 rs2946834 and IGF1 rs7136446 variants showed linkage disequilibrium, with D' value of 0.8 and r^2 value of 0.44. Haplotypes were constructed from these two polymorphisms. However, there were no significant relationships between these variants and the clinical outcome parameters PFS, OS and RR.

Discussion

The presence of activating mutations in *KRAS* has been associated with resistance to the anti-EGFR MoAbs in the treatment of mCRC (5). However, only 10-40% of chemorefractory mCRC with wt *KRAS* respond to anti-EGFR MoAbs, indicating the presence of additional determinants of sensitivity and resistance. The results of these translational studies using tumor tissues from chemorefractory mCRC patients receiving cetuximab monotherapy in a multicenter phase II clinical trial provide evidence that IGF1 and IGF1R polymorphisms are significantly associated with PFS, OS and RR, particularly in wt *KRAS* patients. These preliminary findings also provide support for the IGF1R – EGFR crosstalk hypothesis described by Hu and colleagues (20). These results remained significant after adjusting for other potential predictors of patients' outcome and adjusting the FDR for multiple comparisons. To the best of our knowledge, this is the first report demonstrating that IGF1 pathway polymorphisms may serve as potential predictive and/or prognostic determinants for mCRC patients undergoing cetuximab monotherapy, particularly those with wt *KRAS* tumors.

Several studies have suggested that the IGF1 pathway is a key mediator of resistance to cytotoxic chemotherapy and anti-EGFR treatment (26-28). In the present study, chemorefractory wt *KRAS* mCRC patients possessing the IGF1 rs2946834 variant A/A genotype had a significantly higher RR to cetuximab of 50 % compared to 0% RR for those with the A/G genotype. Moreover, the impact of IGF1 rs2946834 variant A/A genotype in determining response was further supported by subsequent RP analysis that included a total of 16 current and previously analyzed markers. The IGF1 rs2946834 polymorphism was identified as the most important determinant of response to cetuximab in the decision tree analysis. Collectively, these data provide strong evidence suggesting that the IGF1 rs2946834 variant A/A genotype is a predictive factor for cetuximab efficacy in wt *KRAS* mCRC patients (Figure 2). A potential explanation for these findings relates to the fact that the IGF1 rs2946834 polymorphism is located in the 3'UTR of the IGF1 gene and that 3'UTR

plays an important role in regulating mRNA stability via the presence of a number of regulatory elements, including selenocysteine insertion sequences, mRNA binding protein sites and microRNA binding sites. Alteration of mRNA sequence by the presence of a polymorphic variant in the 3'UTR can have measurable effects on mRNA stability and translational expression (29).

Previous investigations have demonstrated that the IGF1 rs2946834 A/G genotype is associated with the highest circulating IGF1 plasma level in breast cancer patients (30). In addition, high circulating level of IGF1 was correlated with increased risk (31), enhanced tumor growth and metastasis in CRC (32). Hyperactivation of the IGF1R pathway by IGF1 has been associated with resistance to several chemotherapeutics, particularly cisplatin and etoposide (27,28) and anti-EGFR (26) MoAbs through continued activation of PI3K signaling. Scartozzi *et al.* demonstrated that wt *KRAS* CRC with IGF1 positive protein expression had significantly lower response rates to cetuximab and irinotecan than tumors with IGF1 negative protein expression (22% versus 65%; $p=0.002$) (17). These findings are consistent with our results which show that the IGF1 rs2946834 A/G genotype is associated with resistance to cetuximab treatment in wt *KRAS* patients. We hypothesize that the IGF1 rs2946834

G allele leads to increased expression of IGF1 and the subsequent hyperactivation of IGF1R resulting in EGFR-independent stimulation of the PI3K pathway.

The association between IGF1 and IGF1R polymorphisms and clinical outcome has recently been investigated and established in various other cancer types. Zhang *et al.* showed that a 3'UTR polymorphism in IGF1 predicts survival of NSCLC patients (33). Dong *et al.* suggested that genetic variations in the IGF pathway predict worse survival in patients with pancreatic cancer (34). Additionally, the IGF1 and IGF1R polymorphisms evaluated in the present study were recently shown to be associated with an increased cancer risk and/or elevated IGF1 plasma levels (Table 2), further suggesting functional and clinical significance. We identified 5 out of 6 variants of IGF1 and IGF1R to be significantly associated with PFS and/or OS in univariate and multivariate analysis (Tables 3 and 5). In multivariate analysis wt *KRAS* patients treated with cetuximab had a significant increase in their risk for tumor progression with two IGF1 polymorphisms (rs6214 and rs2946834; HR 2.155 [95% CI; 1.023, 4.540] and HR 3.066 [95% CI; 1.316, 7.143], respectively) and in the combined risk allele analysis (additive model $p=0.001$; Figure 1b). Although these results require further validation, our findings also suggest a rationale for combination treatment with both EGFR and IGF1R inhibitors (35,36).

Several MoAbs and small molecules targeting IGF1R and related signal transduction pathways are currently undergoing preclinical and clinical evaluations (37). These therapeutics inhibit either the binding of IGF1 to its receptor or IGF1R tyrosine kinase activity. Clinical studies have demonstrated that the IGF1R is overexpressed in approximately 90% of CRC compared to normal tissues, suggesting a potentially broad therapeutic application for agents targeting this pathway (38). In addition, IGF1 signaling has been shown to protect tumor cells from EGFR targeted treatment. Indeed, recent evidence suggests that resistance to the anti-Her2 MoAb trastuzumab in breast cancer (39) and the anti-EGFR antibody AG1478 in human glioblastoma cells (26) is due to activation of IGF1R signaling and that blockage of IGF1R can restore sensitivity. These studies further support the notion of IGF1R - EGFR pathway cross-talk and the possibility that inhibition of both pathways may be required to achieve complete and sustained PI3K/AKT inhibition.

The retrospective design and relatively small numbers of patients involved in the present translational analysis indicate that the results should be considered hypothesis-generating

and confirmed in prospective randomized controlled clinical trials. Nevertheless, it should be stressed that the results remained significant even after adjusting for a total of 16 markers in RP and after adjusting the FDR for multiple comparisons in multivariate analysis. These data provide the first evidence that polymorphisms within the IGF1 pathway are significantly associated with RR, PFS and OS in wt *KRAS* mCRC patients treated with cetuximab monotherapy. Moreover, these observations may aid in the selection of patients with an increased likelihood of cetuximab response or who are candidates for combined EGFR and IGF1R treatment.

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Figure 1.

The number of IGF1 and IGF1R risk alleles correlate significantly with poorer progression free survival in all mCRC patients (A) and in wt *KRAS* mCRC (B) patients only (IMC-0144) in a Cox multivariate regression analysis adjusting for known prognostic factors of CRC. All mCRC patients with 4 risk alleles for IGF1 pathway polymorphisms are associated with a 2.028-fold increased risk for tumor progression compared to patients with 0-1 risk alleles (A). Wt *KRAS* mCRC patients only harboring 4 risk alleles of IGF1 pathway polymorphisms are associated with a 1.895-fold increased risk of tumor progression compared with patients with either zero or one risk alleles (B).

Figure 2.

This comprehensive recursive partitioning analysis for tumor response in wt *KRAS* mCRC patients only incorporated a total of 16 potential markers to define three distinct patient groups (node 1 to node 3) on the basis of tumor response to treatment with cetuximab monotherapy (IMC-0144). Patients carrying IGF1 rs2946834 A/A genotype had a RR of 50% to cetuximab treatment. Patients in node 2 or 3 had RR of 18% versus 5%, respectively. Abbreviations: IGF1, insulin like growth factor 1; COX2, cyclooxygenase-2.

Table 1

Baseline Patient Characteristics, KRAS mutation status and clinical outcome (n=130)

	N	Response*			Progression-Free Survival (PFS)			Overall Survival (OS)		
		PR	SD	PD	Median, m (95% CI)	Hazard Ratio (95% CI)	Median, m (95% CI)	Hazard Ratio (95% CI)	Median, m (95% CI)	Hazard Ratio (95% CI)
Age, years										
≤ 54	36	2 (6%)	11 (33%)	20 (61%)	1.2 (1.2, 1.5)	1 (Reference)	5.3 (3.6, 7.5)	1 (Reference)		
54-64	45	6 (16%)	12 (32%)	19 (51%)	1.4 (1.2, 2.5)	0.74 (0.48, 1.16)	7.0 (3.0, 11.5)	0.69 (0.42, 1.13)		
≥ 65	49	4 (9%)	14 (32%)	26 (59%)	1.4 (1.3, 2.4)	0.77 (0.50, 1.19)	6.6 (3.8, 8.8)	0.86 (0.54, 1.38)		
P value [†]			0.87			0.34		0.31		
Gender										
Female	66	7 (12%)	23 (38%)	30 (50%)	1.5 (1.3, 2.4)	1 (Reference)	7.9 (5.0, 8.9)	1 (Reference)		
Male	64	5 (9%)	14 (26%)	35 (65%)	1.3 (1.2, 1.4)	1.24 (0.88, 1.75)	4.8 (3.4, 7.0)	1.34 (0.91, 1.96)		
P value			0.22		0.21			0.13		
ECOG performance status score										
0	52	6 (12%)	19 (39%)	24 (49%)	1.4 (1.2, 2.4)	1 (Reference)	8.0 (5.3, 12.1)	1 (Reference)		
1	76	6 (9%)	18 (28%)	40 (63%)	1.3 (1.2, 1.8)	1.14 (0.80, 1.63)	4.9 (3.0, 7.0)	1.79 (1.19, 2.68)		
P value			0.21			0.44		0.003		
Tumor site										
Colon	99	10 (11%)	26 (30%)	51 (59%)	1.3 (1.2, 1.5)	1 (Reference)	6.3 (3.8, 8.2)	1 (Reference)		
Rectum	31	2 (7%)	11 (41%)	14 (52%)	1.4 (1.2, 2.5)	1.14 (0.76, 1.72)	5.5 (3.4, 8.7)	0.96 (0.61, 1.52)		
P value			0.87			0.51		0.86		
No. of prior chemotherapy regimens										
2-3	58	4 (8%)	16 (30%)	33 (62%)	1.3 (1.2, 1.3)	1 (Reference)	5.5 (3.6, 7.7)	1 (Reference)		
4-5	60	6 (12%)	18 (36%)	26 (52%)	1.5 (1.3, 2.6)	0.79 (0.54, 1.13)	5.9 (3.7, 8.2)	1.06 (0.71, 1.58)		
6-8	12	2 (18%)	3 (27%)	6 (55%)	1.4 (1.1, 6.6)	0.62 (0.33, 1.16)	12.5 (6.4, 17.7)	0.60 (0.29, 1.22)		
P value			0.29			0.18		0.26		
EGFR tumor immunostaining intensity										
1+	79	8 (12%)	19 (28%)	41 (60%)	1.3 (1.2, 1.5)	1 (Reference)	5.5 (3.8, 7.7)	1 (Reference)		
2-3+	50	4 (9%)	18 (40%)	23 (51%)	1.4 (1.3, 2.5)	0.89 (0.62, 1.27)	7.3 (3.6, 8.7)	0.97 (0.65, 1.43)		
P value			0.67			0.51		0.86		
Skin rash severity										

	Response*				Progression-Free Survival (PFS)			Overall Survival (OS)		
	N	PR	SD	PD	Median, m (95% CI)	Hazard Ratio (95% CI)	Median, m (95% CI)	Hazard Ratio (95% CI)		
Grade 0	17	0 (0%)	0 (0%)	7 (100%)	1.1 (0.9, 1.3)	1 (Reference)	2.0 (1.0, 3.4)	1 (Reference)		
Grade 1	57	6 (11%)	16 (30%)	31 (58%)	1.3 (1.3, 1.5)	0.37 (0.21, 0.66)	6.5 (3.6, 8.7)	0.27 (0.15, 0.48)		
Grade 2-3	56	6 (11%)	21 (39%)	27 (50%)	1.5 (1.2, 2.6)	0.35 (0.19, 0.61)	7.6 (5.4, 10.0)	0.21 (0.12, 0.39)		
P value			0.087			<.0001		<.0001		
KRAS mutation status										
Wild type	88	12 (16%)	26 (34%)	39 (51%)	1.4 (1.3, 2.4)	1 (Reference)	6.6 (4.3, 8.9)	1 (Reference)		
Mutant	42	0 (0%)	11 (30%)	26 (70%)	1.3 (1.2, 1.6)	1.49 (1.01, 2.20)	4.9 (2.8, 6.6)	1.59 (1.05, 2.40)		
P value			0.012			0.023		0.020		

Abbreviations: PR = partial response; SD = stable disease; PD = progressive disease; ECOG = Eastern Cooperative Oncology Group; EGFR = epidermal growth factor receptor.

[†] P values were based on the exact conditional test for response and for skin rash severity, and the log-rank test for PFS and OS

* 16 out of 130 patients (12%) were not evaluable for tumor response

Table 2

Analyzed polymorphisms within the IGF1 and IGF1-R genes and their functional significance, primer sequences, annealing temperatures, and restriction enzymes.

Gene (rs-number)	Location of Polymorphism	Function of Polymorphism	Forward-Primer (5'-3')	Reverse-Primer (5'-3')	Enzyme
IGF1 (<i>rs6214</i>)	3'UTR Ex4+2716 G>A	T variant associated with increased Risk for CRC (22)	TGAAGGAAATAAGTCATAGACACTCTT	TTCTTGTCCTCCAGTGTGTACC	NlaIII
IGF1 (<i>rs6220</i>)	3'UTR Ex4+1830 G>A	G variant associated with breast cancer risk and increased IGF1 plasma level (21)	GAAGGAATCATTTGTGTTTTTCAA	GCACTCACTGACTCTTCTATGCAG	MnlI
IGF1 (<i>rs2946834</i>)	3'UTR 91565 G>A	AG variant associated with increased IGF1 plasma level in breast cancer (21,30)	CATGCACATGTGGAAGAAGC	GGCACCTTTGAGTGATGACC	BclVI
IGF1-I (<i>rs7136446</i>)	Intron 1 40864C>T	C allele associated with increased IGF-1 plasma level in breast cancer (23)	AAGCTCAAGTCAATTAAAAACAAC	TCTCCTTACTGGGTCCCAA	n.a.*
IGF1-R (<i>rs2272037</i>)	Intron 7 -20 T>C	CT and TT associated with increased risk for Glioma (40)	TTGTTTATTAGACCTCCCAATTATAGA	GCACTCTGCCCATCATACTC	n.a.*
IGF1-R (<i>rs2016347</i>)	3'UTR 3129G>T	G allele associated with increased risk for Glioma (40)	TGAGGAGAGGAAGGTGTCCA	TGCTCAATGAATGCAGCAG	n.a.*
IGF1-R (<i>rs2229765</i>)	Exon 16 3129 G>A	A allele is associated with increased breast density (41)	TGAACCTGAAACCAGAGTGG	GTGCTGCATTTTGGCTTTTC	n.a. [†]

Abbreviations: IGF1, insulin like growth factor 1; IGF1-R, insulin like growth factor receptor; UTR, untranslated region; rs-number, reference identification number.

* direct sequencing with forward primer

[†] direct sequencing reverse primer

Table 3

Univariate analysis of IGF1 and IGF1R polymorphisms for progression-free survival (PFS) and overall survival (OS) in patients treated with cetuximab alone

	All patients				wt KRAS only			
	PFS		OS		PFS		OS	
	N	Hazard Ratio (95%CI) P [†]	Hazard Ratio (95%CI) P [†]	N	Hazard Ratio (95%CI) P [†]	Hazard Ratio (95%CI) P [†]	Hazard Ratio (95%CI) P [†]	N
IGF1 rs6214								
C/C	56	1 (Reference)	1 (Reference)	39	1.00 (Reference)	1.00 (Reference)		0.14
C/T	50	1.28 (0.86, 1.90)	1.26 (0.82, 1.93)	30	1.39 (0.84, 2.29)	1.46 (0.85, 2.52)		
T/T	16	1.90 (1.07, 3.36)	1.90 (1.07, 3.39)	12	1.98 (1.01, 3.87)	1.83 (0.92, 3.62)		
T/T [†]	16	1.71 (1.00, 2.90)	1.71 (1.00, 2.94)	12	1.74 (0.93, 3.25)	1.56 (0.83, 2.94)		0.16
IGF1 rs6220								
A/A	68	1 (Reference)	1 (Reference)	47	1.00 (Reference)	1.00 (Reference)		0.82
A/G	45	1.29 (0.88, 1.90)	0.89 (0.58, 1.35)	28	1.34 (0.82, 2.18)	0.85 (0.50, 1.44)		
G/G	11	0.69 (0.36, 1.32)	0.82 (0.39, 1.72)	8	0.66 (0.30, 1.43)	0.89 (0.38, 2.11)		
A/A, A/G	113	1.60 (0.84, 3.04)	1.16 (0.56, 2.40)	75	1.68 (0.78, 3.60)	1.05 (0.45, 2.45)		0.90
IGF1 rs2946834								
G/G	58	1 (Reference)	1 (Reference)	41	1.00 (Reference)	1.00 (Reference)		0.45
A/G	45	1.91 (1.27, 2.86)	1.36 (0.88, 2.09)	27	2.12 (1.27, 3.56)	1.21 (0.70, 2.08)		
A/A	18	0.71 (0.42, 1.21)	0.79 (0.43, 1.46)	13	0.65 (0.35, 1.21)	0.75 (0.36, 1.57)		
A/A [†]	18	0.56 (0.34, 0.94)	0.70 (0.39, 1.25)	68	1.96 (1.06, 3.60)	1.44 (0.71, 2.93)		0.30
IGF1R rs2272037								
T/T	34	1 (Reference)	1 (Reference)	25	1.00 (Reference)	1.00 (Reference)		0.46
C/T	47	0.91 (0.58, 1.42)	1.69 (1.01, 2.82)	32	0.87 (0.51, 1.48)	1.43 (0.78, 2.62)		
C/C	32	1.01 (0.62, 1.65)	1.51 (0.86, 2.64)	19	0.93 (0.51, 1.69)	1.35 (0.68, 2.66)		
C/T, C/C	79	0.95 (0.63, 1.42)	1.61 (1.00, 2.59)	51	0.89 (0.55, 1.45)	1.40 (0.80, 2.43)		0.22
IGF1R rs2016347								
T/T	46	1 (Reference)	1 (Reference)	33	1.00 (Reference)	1.00 (Reference)		0.011
G/T	35	1.26 (0.80, 1.96)	1.40 (0.84, 2.32)	23	1.21 (0.70, 2.08)	1.88 (1.01, 3.50)		
G/G	31	1.55 (0.98, 2.46)	1.76 (1.05, 2.94)	20	1.45 (0.82, 2.56)	2.39 (1.26, 4.55)		
G/T, G/G	66	1.38 (0.94, 2.02)	1.55 (1.00, 2.40)	43	1.31 (0.83, 2.08)	2.09 (1.22, 3.59)		0.004

All patients						wt KRAS only							
N	PFS			OS			N	PFS			OS		
	Hazard Ratio (95%CI)	<i>P</i> [†]		Hazard Ratio (95%CI)	<i>P</i> [†]			Hazard Ratio (95%CI)	<i>P</i> [†]		Hazard Ratio (95%CI)	<i>P</i> [†]	
IGF1R rs2229765													
G/G	56	1 (Reference)		0.52	0.98		37	1.00 (Reference)		1.00 (Reference)		0.90	
A/G	39	1.09 (0.72, 1.64)				1.01 (0.64, 1.59)	29	1.05 (0.64, 1.72)		0.89 (0.51, 1.54)			
A/A	24	1.31 (0.80, 2.12)				0.96 (0.56, 1.63)	14	1.27 (0.68, 2.38)		0.98 (0.50, 1.94)			
A/G, A/A	63	1.16 (0.81, 1.67)	0.40			0.99 (0.66, 1.48)	43	1.11 (0.71, 1.73)	0.62	0.92 (0.56, 1.50)	0.72		
IGF1 rs7136446													
T/T	51	1.00 (Reference)		0.034	0.026		36	1.00 (Reference)	0.022	1.00 (Reference)	0.13		
C/T	36	1.64 (1.06, 2.54)				1.84 (1.14, 2.96)	20	1.96 (1.11, 3.47)		1.78 (0.96, 3.30)			
C/C	30	1.01 (0.64, 1.59)				1.12 (0.68, 1.86)	21	1.01 (0.58, 1.74)		1.06 (0.58, 1.95)			
C/T, C/C	66	1.28 (0.88, 1.85)	0.17			1.43 (0.95, 2.16)	41	1.32 (0.84, 2.08)	0.21	1.32 (0.79, 2.19)	0.27		

* Based on log-rank test

[†]Recessive model

IGF1 and IGF1R polymorphisms and tumor response in wt KRAS mCRC patients treated with cetuximab monotherapy

Table 4

	Response*				P†
	N	PR	SD	PD	
IGF1 rs6214					0.026
C/C	34	8 (24%)	13 (38%)	13 (38%)	
C/T	26	1 (4%)	9 (35%)	16 (62%)	
T/T	12	1 (8%)	3 (25%)	8 (67%)	
C/C, C/T‡	60	9 (15%)	22 (37%)	29 (48%)	0.38‡
IGF1 rs6220					0.91
A/A	42	8 (19%)	14 (33%)	20 (48%)	
A/G	25	1 (4%)	11 (44%)	13 (52%)	
G/G	7	3 (43%)	0 (0%)	4 (57%)	
A/A, A/G‡	67	9 (13%)	25 (37%)	33 (49%)	0.60‡
IGF1 rs2946834					0.17
G/G	37	5 (14%)	14 (38%)	18 (49%)	
A/G	23	0 (0%)	6 (26%)	17 (74%)	
A/A	12	6 (50%)	3 (25%)	3 (25%)	
G/G, A/G‡	60	5 (8%)	20 (33%)	35 (58%)	0.002‡
IGF1R rs2272037					0.52
T/T	23	4 (17%)	9 (39%)	10 (43%)	
C/T	29	5 (17%)	8 (28%)	16 (55%)	
C/C	16	2 (13%)	5 (31%)	9 (56%)	
C/T, C/C‡	45	7 (16%)	13 (29%)	25 (56%)	0.50‡
IGF1R rs2016347					0.076
T/T	30	7 (23%)	12 (40%)	11 (37%)	
G/T	20	3 (15%)	3 (15%)	14 (70%)	
G/G	18	1 (6%)	7 (39%)	10 (56%)	
G/T, G/G‡	38	4 (11%)	10 (26%)	24 (63%)	0.034‡
IGF1R rs2229765					0.91

	Response [*]				<i>P</i> [†]
	<i>N</i>	PR	SD	PD	
G/G	32	5 (16%)	10 (31%)	17 (53%)	
A/G	27	4 (15%)	8 (30%)	15 (56%)	
A/A	12	1 (8%)	5 (42%)	6 (50%)	
A/G, A/A [‡]	39	5 (13%)	13 (33%)	21 (54%)	0.87 [‡]
IGF1 rs7136446					
T/T	33	6 (18%)	13 (39%)	14 (42%)	0.92
C/T	18	0 (0%)	3 (17%)	15 (83%)	
C/C	18	5 (28%)	6 (33%)	7 (39%)	
C/T, C/C [‡]	36	5 (14%)	9 (25%)	22 (61%)	0.26 [‡]

^{*} In patients carrying wildtype Kras only

[†] Based on the exact conditional test

[‡] Dominant or recessive model

Table 5

Multivariate Analysis of IGF1 and IGF1R polymorphisms for progression-free survival (PFS) and overall survival (OS) in patients treated with cetuximab alone

	All patients					wt KRAS only				
	PFS			OS		PFS			OS	
	N	Hazard Ratio (95%CI)	P [†]	Hazard Ratio (95%CI)	P [†]	N	Hazard Ratio (95%CI)	P [†]	Hazard Ratio (95%CI)	P [†]
IGF1 rs6214			0.008 (0.022)		0.011 (0.043)			0.044 (0.12)		0.28(0.45)
C/C, C/T	106	1 (Reference)		1 (Reference)		69	1 (Reference)		1 (Reference)	
T/T*	16	2.239 (1.230, 4.074)		2.282 (1.211, 4.299)		12	2.155 (1.023, 4.540)		1.537 (0.704, 3.359)	
IGF1 rs6220										
G/G	11	1 (Reference)	0.028 (0.057)	1 (Reference)	0.53 (0.60)	8	1 (Reference)	0.095 (0.19)	1 (Reference)	1.00 (1.00)
A/A, A/G*	113	2.378 (1.096, 5.162)		1.328 (0.554, 3.184)		75	2.360 (0.861, 6.473)		1.002 (0.340, 2.952)	
IGF1 rs2946834										
A/A	18	1 (Reference)	0.002 (0.007)	1 (Reference)	0.12 (0.20)	13	1 (Reference)	0.009 (0.038)	1 (Reference)	0.24 (0.45)
G/G, A/G*	103	2.943 (1.501, 5.771)		1.848 (0.846, 4.038)		68	3.066 (1.316, 7.143)		1.814 (0.677, 4.863)	
IGF1R rs2272037										
T/T	34	1 (Reference)	0.66 (0.75)	1 (Reference)	0.28 (0.38)	25	1 (Reference)	0.65 (0.86)	1 (Reference)	0.43 (0.49)
C/T, C/C	79	0.901 (0.566, 1.433)		1.346 (0.784, 2.312)		51	0.866 (0.469, 1.599)		1.312 (0.672, 2.563)	
IGF1R rs2016347										
T/T	46	1 (Reference)	0.27 (0.43)	1 (Reference)	0.033 (0.088)	33	1 (Reference)	0.63 (0.86)	1 (Reference)	0.060 (0.24)
G/T, G/G*	66	1.280 (0.824, 1.986)		1.734 (1.046, 2.874)		43	1.146 (0.655, 2.006)		1.934 (0.973, 3.843)	
IGF1R rs2229765										
G/G	56	1 (Reference)	0.41 (0.54)	1 (Reference)	0.70 (0.70)	37	1 (Reference)	0.92 (0.91)	1 (Reference)	0.35 (0.47)
A/G, A/A	63	1.188 (0.791, 1.785)		1.094 (0.697, 1.714)		43	1.027 (0.620, 1.701)		0.763 (0.432, 1.348)	
IGF1 rs7136446										
T/T	51	1 (Reference)	0.83 (0.83)	1 (Reference)	0.068 (0.14)	36	1 (Reference)	0.87 (0.92)	1 (Reference)	0.14 (0.38)
C/T, C/C	66	0.956 (0.632, 1.447)		1.550 (0.968, 2.481)		41	1.044 (0.615, 1.772)		1.566 (0.861, 2.846)	
Number of risk alleles*										
0-1	10	1 (Reference)		1 (Reference)		8	1 (Reference)		1 (Reference)	
2	39	3.622 (1.550, 8.466)	<.001	2.104 (0.710, 6.232)	0.003	27	3.321 (1.247, 8.848)	0.004	1.656 (0.505, 5.431)	0.098

All patients					wt <i>KRAS</i> only				
		PFS			OS			PFS	
	<i>N</i>	Hazard Ratio (95%CI)	<i>P</i> [‡]		Hazard Ratio (95%CI)	<i>P</i> [‡]		Hazard Ratio (95%CI)	<i>P</i> [‡]
3	54	4.884 (2.031, 11.746)			3.097 (1.071, 8.953)		34	4.098 (1.406, 11.943)	
4	7	17.630 (5.238, 59.333)			11.599 (2.759, 48.768)		6	12.888 (3.192, 52.040)	
Additive model		2.028 (1.478, 2.783)	<.001 (0.001)		1.828 (1.272, 2.627)	0.001 (0.009)		1.895 (1.299, 2.765)	0.001 (0.007)
								1.745 (1.092, 2.787)	0.020 (0.16)

‡ Additive model with number of risk alleles as a continuous variable.

FDR-adjusted P values are shown in parentheses

* Total number of risk alleles

[‡] Based on Wald test within Cox proportional hazards model adjusted by skin rash severity, *KRAS* mutation, ECOG performance status, and 3 polymorphisms (EGFR +497 G>A (rs11543848), COX-2 +8473 T>C (rs5275), EGF +61 A>G (rs4444903)) from our previous study, stratified by race with FDR-adjusted P values shown in parentheses.