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Comprehensive Pharmacogenetic Profiling of the Epidermal Growth Factor Receptor Pathway for Biomarkers of Response to, and Toxicity from, Cetuximab

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ABSTRACT

Background

Somatic mutations in the epidermal growth factor receptor (EGFR) intracellular signalling pathways predict non-response to cetuximab in the treatment of advanced colorectal cancer (aCRC). We hypothesized that common germline variants within these pathways may also play similar roles.

Methods

We analysed 54 potentially functional, common, inherited EGFR pathway variants in 815 aCRC patients treated with oxaliplatin-fluoropyrimidine chemotherapy +cetuximab. Primary endpoints were response and skin rash (SR). We had >85% power to detect ORs=1.6 for variants with minor allele frequencies >20%.

Results

We identified five potential biomarkers for response and four for SR, although none remained significant after correction for multiple testing. Our initial data supported a role for Ser313Pro in *PIK3R2* in modulating response to cetuximab - in patients with *KRAS* wild type CRCs, 36.4% of patients with one allele encoding proline responded, as compared to 71.2% of patients homozygous for alleles encoding serine (OR 0.23, 95% CI 0.09-0.56, P=0.0014) and this association was predictive for cetuximab ($P_{interaction}$=0.017); however, independent replication failed to validate this association. No previously proposed predictive biomarkers were validated.

Conclusions

Our study highlights the need to validate potential pharmacogenetic biomarkers. We did not find strong evidence for common germline biomarkers of cetuximab response and toxicity.

Key Words: Pharmacogenetics, colorectal cancer, cetuximab, biomarkers.

INTRODUCTION

The treatment of colorectal cancer (CRC) is improving with average survival for advanced CRC (aCRC) increasing from ~6 months with best supportive care alone, through 10-12 months with fluoropyrimidine-based regimens [1] and up to 16-21 months with oxaliplatin or irinotecan and a fluoropyrimidine.[2, 3] In addition, monoclonal antibodies (McAbs) against the epidermal growth factor receptor (EGFR) improve overall survival (OS) in patients with aCRC in whom other treatments have failed [4] and, in combination with first line therapy, in those with *RAS* wild type tumours.[5] EGFR acts as a gate-way for the Ras-Raf-MAP and PI3K-PTEN-Akt intracellular signalling pathways. The efficacy of cetuximab and panitumumab (anti-EGFR McAbs) is dependent upon an absence of somatic mutations in members of this signalling cascade such as *KRAS* [6] and *NRAS*,[5] and these predictive biomarkers help guide the treatment of aCRC.[7]

Inherited factors are also likely to affect response to, and side effects from, chemotherapy and biological therapy. Pro241 in *CCND1*,[8] 61A>G in *EGF*,[8, 9] His131Arg in *FCGR2A*,[10] Val158Phe in *FCGR3A*,[10, 11] 765G>C and +8473T>C in *PTGS2*,[12] and, Arg521Lys [13] and a $(CA)_n$ repeat [11, 14] in *EGFR* have all been suggested to predict response to cetuximab.

The United Kingdom MRC COIN trial (NCT00182715), which consists of 2445 aCRC patients treated with oxaliplatin-fluoropyrimidine chemotherapy ±cetuximab, serves as an important resource for the discovery of new, and validation of existing, genetic biomarkers.[15, 16] We used this resource, together with patients from the allied COIN-B trial of oxaliplatin-fluoropyrimidine chemotherapy +cetuximab

(NCT00640081) [17] to investigate the role of 54 potentially functional, common, inherited EGFR-related variants in predicting response to, and side effects from, cetuximab.

METHODS

Patients and treatments

All patients had metastatic or locally advanced colorectal adenocarcinoma and received no previous chemotherapy for advanced disease. All patients gave fully informed consent for this study (approved by REC [04/MRE06/60]). COIN patients were randomised 1:1:1 to receive continuous oxaliplatin and fluoropyrimidine chemotherapy (Arm A), continuous chemotherapy +cetuximab (Arm B), or intermittent chemotherapy (Arm C).[15, 16] COIN-B patients were randomised 1:1 to receive intermittent chemotherapy and cetuximab (Arm D) or intermittent chemotherapy and continuous cetuximab (Arm E) (Supplementary Figure).[17]

Selection and genotyping of potential pharmacogenetic variants

Potentially functional inherited variants were sought in 146 genes identified from literature reviews as likely to play a role in the EGFR signalling pathways. Variants were considered potentially functional if there was previous clinical or biological evidence for an effect on response or side effects, if they were nonsynonymous, or if they occurred in the promoter region. Variants were mined from dbSNP (v.129, http://www.ncbi.nlm.nih.gov/SNP/) and from exome re-sequencing germline data, and those with a minor allele frequency (MAF) >5% (Caucasian population) were considered for genotyping. Genotyping was carried out using a custom Illumina GoldenGate assay or by in-house assays (Supplementary Information).

Independent analysis of Ser313Pro in *PIK3R2*

We obtained germline DNA samples together with response data for 309 unrelated patients with *KRAS* wild-type CRCs that were treated with cetuximab alone or in combination with chemotherapy. These were previously collected as part of an international consortium study.[18] We carried out PCR amplification using the primers 5'-GGGCCGTAAATACTGATCCCT-3' and 5'-TCCAACATTGGGACTGCCGA-3' and directly sequenced the purified products. In total, 81.9% (n=253) of samples were successfully amplified and genotyped.

Clinical parameters assessed

The primary endpoints were: (i) 12-week response, defined as complete response or partial response *versus* stable disease or progressive disease at 12-weeks; and, (ii) grade ≥2 skin rash (SR) or cetuximab dose reduction or delay due to SR *versus* grade <2 SR with no cetuximab dose modification. Response was assessed using RECIST criteria and SR toxicity was graded using NCI Common Terminology Criteria version 3.0.[19] Secondary efficacy endpoints were OS and overall response rate (ORR), and secondary toxicity endpoints were grade ≥2 at any point *versus* grade <2 for lethargy, nausea or vomiting, diarrhoea, stomatitis, Hand-Foot Syndrome (HFS), hypomagnesaemia and nail changes.

Sample size and power considerations

Patients from COIN Arm B and COIN-B (those treated with cetuximab) had similar efficacy and toxicity outcomes at 12-weeks, so were combined to increase power, as were patients from COIN Arms A and C (no cetuximab). A total of 2183 patients

were genotyped, of which 815 received cetuximab (676 had a response outcome and 730 had a SR outcome) and 1368 did not receive cetuximab (1169 had a response outcome). Based on 676 patients (received cetuximab, genotyped and with data on response), we had >85% power (P<0.05) to detect an OR of 1.6, equating to a 12% difference in response or SR (45% responded or had SR) for a variant with a MAF>20%, and an OR of 2.3, corresponding to a 20% difference in response or SR, for a variant with a MAF>5%.

Statistical analyses

Genotypes were tested for deviation from the Hardy Weinberg Equilibrium (HWE) using a chi-squared test with $P<9.3\times10^{-4}$ (multiple testing for n=54 variants). Pharmacogenetic analyses were carried out using Stata 12.1 with a co-dominant model, and tested using the likelihood-ratio chi-squared statistic. For significant associations (P<0.05), subsequent analyses were carried out using logistic regression under the best-fitting allele model and adjusted for the type of fluoropyrimidine. Correction for multiple testing was by Bonferroni.

RESULTS

We extracted DNA from peripheral blood samples from 2183 unrelated patients with aCRC from the UK national trials COIN (2070 of the 2445 randomised) and COIN-B (113 of the 226 randomised). All patients received oxaliplatin and fluoropyrimidine chemotherapy ±cetuximab as continuous or intermittent regimens. For the first 12-weeks, at which point the primary pharmacogenetic analyses were carried out, treatments were identical in all patients apart from the choice of fluoropyrimidine (n=834, 38% received OxMdG and n=1349, 62% received Xelox) together with the

randomisation of ±cetuximab (n=815, 37% received cetuximab) (Supplementary Figure, Supplementary Table S1). Here, we focussed on the analysis of the 815 patients treated with cetuximab, to identify predictive biomarkers for this biological therapy (Figure).

Eighty potentially functional, common (MAFs >5%), inherited, coding and promoter-region variants were identified in the EGFR pathway. Of these, 71 passed *in silico* locus conversion on the GoldenGate platform and 51 were successfully assayed. Four variants were assayed 'in house' of which three were successfully genotyped. No genotypes deviated from the HWE. Therefore, in total, 54 variants were considered for the analyses of response to, and side effects from, cetuximab (Supplementary Table S2, Figure).

Primary analyses for response

Five variants were associated with response ($P<0.05$), the most significant being a nonsynonymous variant (Ser313Pro) in the phosphatidylinositol 3-kinase regulatory (PIK3R) subunit 2 (Table, Supplementary Table S3); 40.3% of patients with an allele encoding proline responded as compared to 60.4% of patients homozygous for alleles encoding serine (OR=0.44, 95% CI 0.26-0.75, $P=0.002$). We stratified by *KRAS* status and found that this association was only significant in patients with *KRAS* wild type CRCs (36.4% of patients with an allele encoding proline responded as compared to 71.2% of patients homozygous for alleles encoding serine, OR 0.23, 95% CI 0.09-0.56, $P=0.0014$; [as compared to 40.0% and 50.5% of patients with *KRAS* mutant CRCs respectively, OR 0.65 95% CI 0.30-1.43, $P=0.29$;

$P_{interaction}$=0.076], Supplementary Table S4). No associations remained significant after correction for multiple testing.

We analysed Ser313Pro in *PIK3R2* in *KRAS* wild-type patients who did not receive cetuximab (from Arms A and C of COIN), and observed a predictive effect for response to cetuximab ($P_{interaction}$=0.017, Supplementary Table S4).

We sought independent evidence for a predictive role of Ser313Pro by analysing germline DNA samples from 309 unrelated patients with *KRAS* wild-type CRCs that were treated with cetuximab. We had >90% power to observe an OR 0.23 equating to a 35% difference in response (found in COIN). We did not find any effect on objective response, with an allelic trend in the opposite direction: 45.8% (11/24) of patients with one allele encoding proline had a response, as compared to 32.2% (68/211) of patients homozygous for alleles encoding serine (*P*=0.18).

Table - Variants with *P*<0.05 for the primary endpoints

Endpoint	rs no.	Gene	Variant	Endpoint +/-	AA	AB	BB	X^2 (df) *P*-value[a]	OR (95% CI) *P*-value[b]	Predictive for cetuximab (YES/NO) OR (95% CI) & *P*-value for no cetuximab[c] *P* interaction	
										Any *KRAS* status	*KRAS* wild type
12-week response	rs1011320	*PIK3R2*	Ser313Pro	+	0	25	371	9.42 (1) 0.002	0.44 (0.26, 0.75) 0.002 (d)	NO 0.73 (0.50,1.07), 0.11 *P interaction* = 0.13	YES 0.82 (0.47, 1.45), 0.51 *P interaction* = 0.017
				-	0	37	243				
	rs17537869	*PLCG2*	Arg268Trp	+	1	61	336	8.13 (2) 0.017	1.66 (1.03, 2.67) 0.037 (d)	YES 0.64 (0.45, 0.89), 0.009 *P interaction* = 0.001	NO 0.68 (0.41, 1.11), 0.12 *P interaction* = 0.052
				-	3	25	253				
	rs4444903	*EGF*	c.1-382 A>G	+	135	218	45	7.54 (2) 0.023	0.56 (0.36, 0.86) 0.008 (r)	NO 0.91 (0.67, 1.25), 0.56 *P interaction* = 0.070	NO 0.73 (0.47, 1.14), 0.17 *P interaction* = 0.17
				-	94	135	52				
	rs78803121	*EREG*	Cys141Phe	+	1	34	363	7.44 (2) 0.024	0.57 (0.37, 0.89) 0.013 (a)	NO 0.85 (0.60,1.21), 0.38 *P interaction* = 0.16	NO 0.83 (0.50, 1.39), 0.49 *P interaction* = 0.15
				-	5	35	251				
	rs5275	*PTGS2*	c.1812+430 T>C	+	142	196	60	6.95 (2) 0.031	1.51 (1.10, 2.06) 0.010 (d)	YES 1.02 (0.80, 1.28), 0.90 *P interaction* = 0.046	NO 1.09 (0.78, 1.53), 0.60 *P interaction* = 0.21
				-	128	114	39				
SR	rs785467	*PIK3R3*	Asn283Lys	+	160	182	34	9.55 (2) 0.009	1.56 (1.17, 2.10) 0.003 (d)	YES 0.43 (0.16, 1.17), 0.099 *P interaction* = 0.014	n/a
				-	190	133	31				
	rs16858808	*IL8RA*	Arg335Cys	+	0	23	353	5.29 (1) 0.022	2.36 (1.10, 5.04) 0.027 (d)	NO 1.85 (0.42, 8.24), 0.42 *P interaction* = 0.81	n/a
				-	0	10	343				
	rs41292521	*EPS15*	Ser438Leu	+	0	25	351	5.17 (1) 0.023	2.26 (1.09, 4.68) 0.028 (d)	NO 1.24 (0.16, 9.47), 0.84 *P interaction* = 0.58	n/a
				-	0	11	342				
	rs602990	*VAV2*	Met584Val	+	83	163	130	6.85 (2) 0.033	n/a (od)	NO χ2 (df) = 0.33 (2), 0.85 *P interaction* = 0.91	n/a
				-	61	187	106				

Results shown using a co-dominant model[a] and, odds ratios and 95% confidence intervals using the best model that fitted the data[b] [models for (d) = dominant allele, (r) = recessive allele, (a) = additive allele, (od) = over-dominant allele]. [c]Patients not treated with cetuximab were from Arms A and C of COIN. For endpoints, + = patients that responded or had SR, - = patients that did not respond or have SR. A and B alleles were assigned by Illumina; the common allele encodes the wild type amino acid, so for Ser313Pro the B allele encodes Ser and for Asn283Lys the A allele encodes Asn. n/a, not applicable for over-dominant model and SR is unlikely to be related to the tumours molecular profile. No associations were significant after correction for multiple testing.

Arg268Trp in *PLCG2* was also associated with response in COIN/COIN-B (OR=1.66, 95% CI 1.03-2.67, *P*=0.037) and was predictive for cetuximab ($P_{interaction}$=0.001, Table); however, this effect was only significant in the *KRAS* mutant subset ($P_{interaction}$=0.034, Supplementary Table S5) and was not significant after correction for multiple testing.

Primary analyses for SR

Four variants were associated with SR (*P*<0.05), the most significant being Asn283Lys in *PIK3R3* (Table, Supplementary Table S3); 56.8% of patients with at least one allele encoding lysine had severe SR as compared to 45.7% of patients homozygous for alleles encoding asparagine (OR 1.56, 95% CI 1.17-2.10, *P*=0.003). This association was predictive for cetuximab ($P_{interaction}$=0.014, Table); however, no associations remained significant after correction for multiple testing. There was no interaction with the type of fluoropyrimidine used (*P*=0.66).

Previously proposed predictive biomarkers

Numerous germline variants in the EGFR pathway have been suggested to be predictive biomarkers for cetuximab response.[8-14] These were tested as part of our study and only c.1-382A>G (61A>G) in *EGF* and c.1812+430T>C in *PTGS2* were significantly associated with response (*P*=0.008 and 0.010, respectively), and trended towards ($P_{interaction}$=0.07), or had a significant ($P_{interaction}$=0.046), predictive effect for cetuximab (irrespective of *KRAS* status), respectively (Table). However, neither were predictive in the *KRAS* wild type subset ($P_{interaction}$ = 0.17 and 0.21, respectively; Table).

Secondary analyses

Ser313Pro in *PIK3R2* was associated with OS and ORR, Cys141Phe in *EREG* with ORR and Asp784Val in *EGF* with OS (Supplementary Table S6). Val906Ile in *MAP3K1* was associated with lethargy, His321Arg in *RASAL1* and Arg574Pro in *MMP9* with nausea/vomiting, Lys344Thr in *RPS6KA1* and Val906Ile in *MAP3K1* with diarrhoea, Arg298His in *PTGES2*, Met322Thr in *TSC1*, Phe212Val in *FCGR3A* and c.1-1671insA in *MMP3* with stomatitis, c.1-382 A>G in *EGF*, Pro1170Ala in *ERBB2*, Cys141Phe in *EREG* and Asp806Asn in *MAP3K1* with HFS, Tyr187His in *DUSP1* with hypomagnesaemia and Arg335Cys in *IL8RA*, Glu920Val in *EGF* and Lys220Arg in *PLAUR* with nail changes (Supplementary Table S7). None of the associations remained significant after correction for multiple testing.

DISCUSSION

In total, we analysed 54 inherited variants from genes in the EGFR-related pathways for a potential role in response to, or side effects from, cetuximab in the treatment of aCRC. Given the size of our cohort, we had considerable power to detect common alleles of small effects. Although, we identified five potential biomarkers for response and four for SR in our primary analyses, none remained significant after adjusting for multiple testing. Numerous common inherited biomarkers for cetuximab response have been proposed by others;[8-14] however, many of these have been derived from studies using small cohorts of patients and, consequently, the majority have failed,[14] or have been inconsistent upon independent replication.[12, 14, 18, 20] In our study, we analysed these variants and had limited evidence for c.1-382A>G (61A>G) in *EGF* and c.1812+430T>C (+8473T>C) in *PTGS2* in predicting response to cetuximab. However, neither effect was found in the important *KRAS* wild-type

subset (which had the potential to respond), and, our data did not support the proposed direction of allelic effect for c.1-382A>G.[12, 14] Therefore, we have no strong evidence for a predictive role for any of these variants.

Our study clearly highlights the need to validate potential pharmacogenetic biomarkers. Initial data from our study strongly supported a role for Ser313Pro in *PIK3R2* in modulating response to cetuximab and this association was only significant in those patients with CRCs that were wild type for *KRAS*, so had the potential to respond, and was not found in patients that did not receive cetuximab, regardless of their *KRAS* status, so was unlikely to be a prognostic effect. However, we carried out a well-powered independent analysis of unrelated patients and failed to validate our initial observations, suggesting that this was a chance event.

In conclusion, we have carried out a comprehensive, well-designed study to identify common germline biomarkers for cetuximab-related outcomes, but failed to establish strong evidence for their existence.

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COMPETING INTEREST

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AUTHOR CONTRIBUTIONS

JP Cheadle and TSM obtained funding for this study. The study was designed by JP Cheadle, AM, TSM, DF and RSK, and was carried out under the direction of JP Cheadle. AM carried out the literature searches and identified the variants for genotyping. TSM was CI of COIN, HW was CI of COIN-B and, RAA and AM were COIN trial fellows; all provided clinical advice and assistance, and supported the translational research. AMM and RSK managed the COIN and COIN-B trials and facilitated access to the clinical data. ST and BVdB provided samples and clinical data for the validation analyses. SI extracted the COIN and COIN-B blood DNA samples and, with RH, prepared them for genotyping at Illumina. VH and JM undertook the in-house genotyping under the direction of JP Colley. DF undertook all of the statistical analyses. AM and JP Cheadle interpreted the data with input from DF, RAA and TSM. JP Cheadle and AM wrote the paper with input from DF, and all authors provided comments.

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REFERENCES

1. Maughan TS, James RD, Kerr DJ, Ledermann JA, McArdle C, Seymour MT, Cohen D, Hopwood P, Johnston C, Stephens RJ; British MRC Colorectal Cancer Working Party. Comparison of survival, palliation, and quality of life with three chemotherapy regimens in metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2002;359:1555-63.

2. Tournigand C, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004;22:229-37.

3. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C, Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000;18:2938-47.

4. Jonker DJ, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, Price T, Simes RJ, Tebbutt NC, van Hazel G, Wierzbicki R, Langer C, Moore MJ. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;357:2040-8.

5. Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013;369:1023-34.

6. De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010;11:753-62.

7. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, Schilsky RL. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009;27:2091-6.

8. Zhang W, Gordon M, Press OA, Rhodes K, Vallböhmer D, Yang DY, Park D, Fazzone W, Schultheis A, Sherrod AE, Iqbal S, Groshen S, Lenz HJ. Cyclin D1 and epidermal growth factor polymorphisms associated with survival in patients with advanced colorectal cancer treated with cetuximab. *Pharmacogenet Genomics* 2006;16:475-83.

9. Hu-Lieskovan S, Vallbohmer D, Zhang W, Yang D, Pohl A, Labonte MJ, Grimminger PP, Hölscher AH, Semrau R, Arnold D, Dellas K, Debucquoy A, Haustermans K, Machiels JP, Sempoux C, Rödel C, Bracko M, Velenik V, Lenz HJ. EGF61 polymorphism predicts complete pathologic response to cetuximab-

based chemoradiation independent of KRAS status in locally advanced rectal cancer patients. *Clin Cancer Res* 2011;17:5161-9.

10. Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, Chang HM, Borucka E, Lurje G, Sherrod AE, Iqbal S, Groshen S, Lenz HJ. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. *J Clin Oncol* 2007;25:3712-8.
11. Pander J, Gelderblom H, Antonini NF, Tol J, van Krieken JH, van der Straaten T, Punt CJ, Guchelaar HJ. Correlation of FCGR3A and EGFR germline polymorphisms with the efficacy of cetuximab in KRAS wild-type metastatic colorectal cancer. *Eur J Cancer* 2010;46:1829-34.
12. Lurje G, Nagashima F, Zhang W, Yang D, Chang HM, Gordon MA, El-Khoueiry A, Husain H, Wilson PM, Ladner RD, Mauro DJ, Langer C, Rowinsky EK, Lenz HJ. Polymorphisms in cyclooxygenase-2 and epidermal growth factor receptor are associated with progression-free survival independent of K-ras in metastatic colorectal cancer patients treated with single-agent cetuximab. *Clin Cancer Res* 2008;14:7884-95.
13. Gonçalves A, Esteyries S, Taylor-Smedra B, Lagarde A, Ayadi M, Monges G, Bertucci F, Esterni B, Delpero JR, Turrini O, Lelong B, Viens P, Borg JP, Birnbaum D, Olschwang S, Viret F. A polymorphism of EGFR extracellular domain is associated with progression free-survival in metastatic colorectal cancer patients receiving cetuximab-based treatment. *BMC Cancer* 2008;8:169.

14. Graziano F, Ruzzo A, Loupakis F, Canestrari E, Santini D, Catalano V, Bisonni R, Torresi U, Floriani I, Schiavon G, Andreoni F, Maltese P, Rulli E, Humar B, Falcone A, Giustini L, Tonini G, Fontana A, Masi G, Magnani M. Pharmacogenetic profiling for cetuximab plus irinotecan therapy in patients with refractory advanced colorectal cancer. *J Clin Oncol* 2008;26:1427-34.

15. Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP; MRC COIN Trial Investigators. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;377:2103-14.

16. Adams RA, Meade AM, Seymour MT, Wilson RH, Madi A, Fisher D, Kenny SL, Kay E, Hodgkinson E, Pope M, Rogers P, Wasan H, Falk S, Gollins S, Hickish T, Bessell EM, Propper D, Kennedy MJ, Kaplan R, Maughan TS; MRC COIN Trial Investigators. Intermittent versus continuous oxaliplatin and fluoropyrimidine combination chemotherapy for first-line treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet Oncol* 2011;12:642-53.

17. Wasan H, Meade AM, Adams R, Wilson R, Pugh C, Fisher D, Sydes B, Madi A, Sizer B, Lowdell C, Middleton G, Butler R, Kaplan R, Maughan T; COIN-B investigators. Intermittent chemotherapy plus either intermittent or continuous cetuximab for first-line treatment of patients with KRAS wild-type advanced colorectal cancer (COIN-B): a randomised phase 2 trial. *Lancet Oncol* 2014;15:631-9.

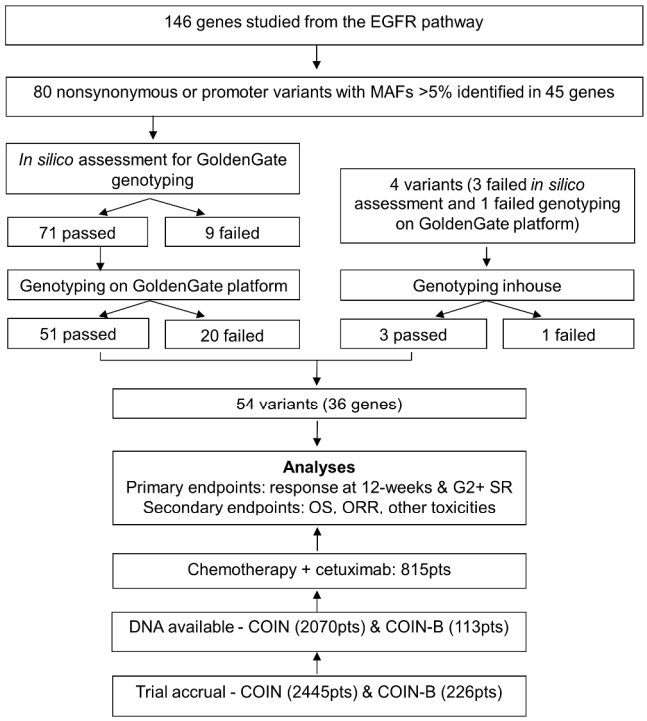
18. Geva R, Vecchione L, Kalogeras KT, Jensen BV, Lenz HJ, Yoshino T, Paez D, Montagut C, Souglakos J, Cappuzzo F, Cervantes A, Frattini M, Fountzilas G, Johansen JS, Høgdall EV, Zhang W, Yang D, Yamazaki K, Nishina T, Papamichael D, Vincenzi B, Macarulla T, Loupakis F, De Schutter J, Spindler KL, Pfeiffer P, Ciardiello F, Piessevaux H, Tejpar S. FCGR polymorphisms and cetuximab efficacy in chemorefractory metastatic colorectal cancer: an international consortium study. *Gut* 2015;64:921-8.

19. Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, Langer C, Murphy B, Cumberlin R, Coleman CN, Rubin P. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003;13:176-81.

20. Sclafani F, Gonzalez de Castro D, Cunningham D, Hulkki Wilson S, Peckitt C, Capdevila J, Glimelius B, Roselló Keränen S, Wotherspoon A, Brown G, Tait D, Begum R, Thomas J, Oates J, Chau I. FcγRIIa and FcγRIIIa polymorphisms and cetuximab benefit in the microscopic disease. *Clin Cancer Res* 2014;20:4511-9.

LEGEND TO FIGURE

Figure. CONSORT diagram of the study design and analyses. Shown are the numbers of variants analysed, together with the numbers of patients studied, and the primary and secondary endpoints. MAF, minor allele frequency; pts, patients; SR, skin rash; OS, overall survival; ORR, overall response rate.



CONSORT diagram of the study design and analyses. Shown are the numbers of variants analysed, together with the numbers of patients studied, and the primary and secondary endpoints. MAF, minor allele frequency; pts, patients; SR, skin rash; OS, overall survival; ORR, overall response rate.

Figure

254x190mm (300 x 300 DPI)

Supplementary Information for "Comprehensive Pharmacogenetic Profiling of the Epidermal Growth Factor Receptor Pathway for Biomarkers of Response to, and Toxicity from, Cetuximab"

Supplementary Methods

Genotyping

Most variants were single nucleotide polymorphisms (SNPs) genotyped using a custom Illumina GoldenGate assay. The Assay Design Tool (Illumina) was used to anticipate genotyping success. This was based on the designability rank and validation class for a given SNP. When two or more SNPs occurred within 60bp of one another, the SNP selected for submission was chosen based on its designability score, MAF and likelihood of being functional using *in silico* analyses (PolyPhen or align-GVGD). For the 51 SNPs successfully genotyped on the GoldenGate platform, the mean GC score was 0.83 (range 0.49-0.96), genotype success rate was 99.9% (41522/41565) and there was 100% concordance between duplicate samples.

Four variants were assayed 'in house' because they were not suitable for (n=3), or failed (n=1), GoldenGate genotyping. The $(CA)_n$ repeat in intron 1 of *EGFR* (rs11568315) was assayed using the primers 5'-GGCTCACAGCAAACTTCTCC-3' and 5'-TATGGTCGGTAGTCACGAAGC-3' and the c.1-1671 insertion A in the *MMP3* promoter (rs35068180) was assayed using the primers 5'-AGCTGCCACAGCTTCTACAC-3' and 5'-GTATTCTATGGTTCTCCATTC-3'. One of the primers for each pair was fluorescently labelled and PCR products were analysed on an ABI3100 using the GeneScan Analysis Software (ABI). Phe212Val in *FCGR3A* (rs396991) was assayed using a Taqman real time quantitative PCR assay

(ABI). The -216 G>T variant in the *EGFR* promoter (rs17288945) was analysed using a Taqman assay, allele-specific amplification and by direct sequencing without success.

Supplementary Figure: Treatment schedules for patients in COIN and COIN-B.

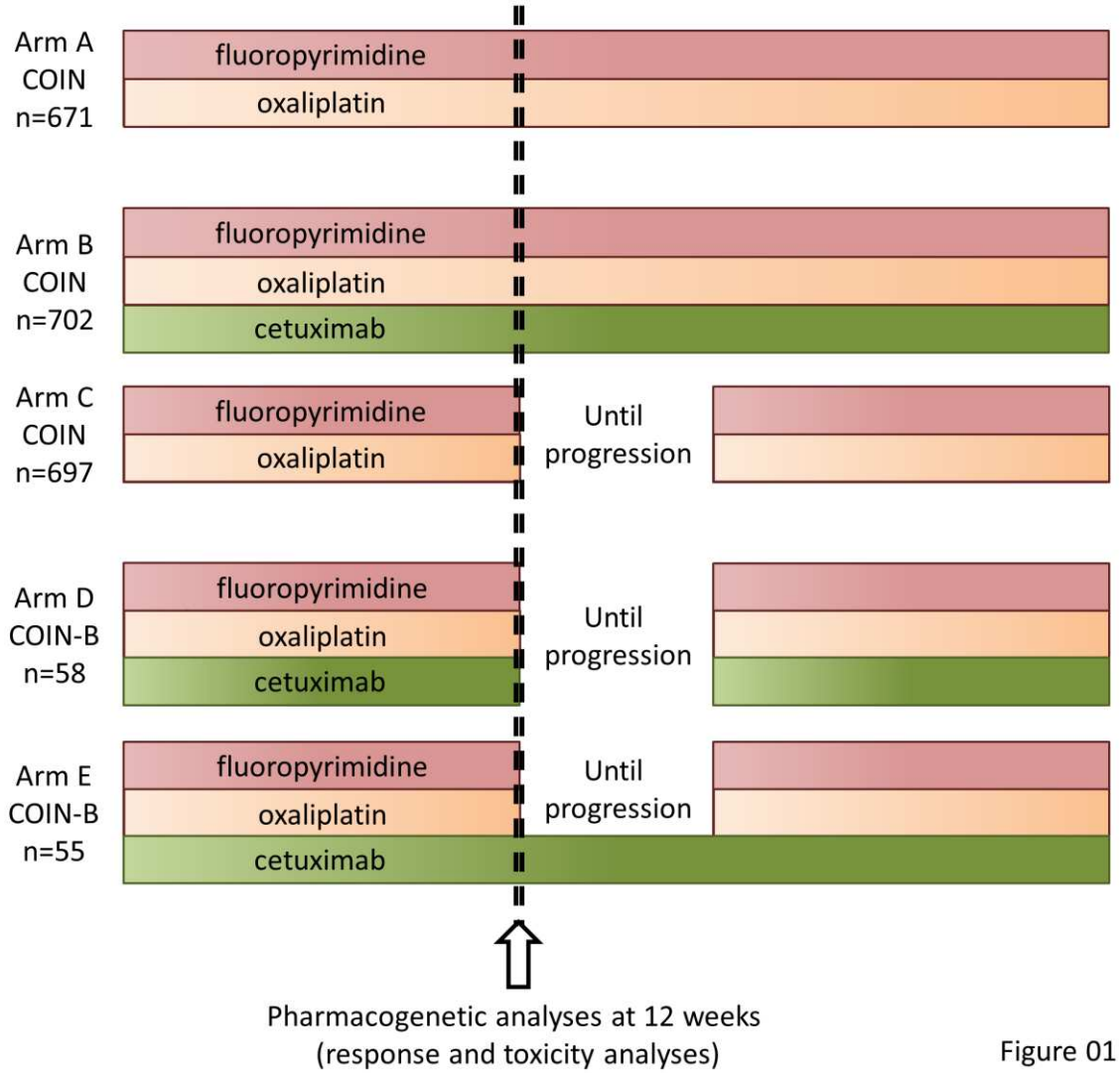


Figure 01

Patients received continuous oxaliplatin and fluoropyrimidine chemotherapy (Arm A), continuous chemotherapy +cetuximab (Arm B), intermittent chemotherapy (Arm C), intermittent chemotherapy with cetuximab (Arm D) and intermittent chemotherapy

with continuous cetuximab (Arm E). In all patients, treatment was identical for the first 12-weeks apart from the choice of fluoropyrimidine together with the randomisation of ±cetuximab. Primary pharmacogenetic analyses were carried out at 12-weeks. For arms with intermittent therapy, treatment was stopped from 12-weeks (apart from cetuximab in Arm E) if there was complete response, partial response or stable disease and re-initiated upon disease progression.

Supplementary Tables:

Supplementary Table S1 - Clinicopathological data for patients in COIN and COIN-B, and heterogeneity across analysis groups and their arms (genotyped patients)

		+ cetuximab		- cetuximab	*P*[1]	*P*	*P*
		COIN Arm B	COIN-B Arms D+E	COIN Arms A+C		D vs E	A vs C
n =		702	113	1368			
Age at randomisation	Mean (S.D.)	62.9 (9.8)	61.9 (10.5)	62.4 (9.8)	0.39	0.82	0.20
	<20	0 (0.0)	0 (0.0)	1 (0.1)	0.69	0.32	0.30
	20-49	74 (10.5)	12 (10.6)	133 (9.7)			
	50-59	147 (20.9)	25 (22.1)	329 (24.1)			
	60-69	289 (42.2)	50 (44.3)	563 (41.2)			
	70-79	186 (26.5)	24 (21.4)	335 (24.5)			
	80-89	6 (0.9)	2 (1.8)	7 (0.5)			
Sex	Female	231 (32.9)	48 (42.5)	465 (34.0)	0.14	0.77	0.92
	Male	471 (67.1)	65 (57.5)	903 (66.0)			
WHO-PS	0	330 (47.0)	58 (51.3)	639 (46.7)	0.76	0.89	0.99
	1	325 (46.3)	46 (40.7)	623 (45.5)			
	2	47 (6.7)	9 (8.0)	106 (7.8)			
Primary Site	Colon	377 (53.7)	69 (61.1)	739 (54.0)	0.85	0.009[2]	0.21
	Rectum	229 (32.6)	32 (28.3)	424 (31.0)			
	RSJ	95 (13.5)	12 (10.6)	202 (14.8)			
	Other	1 (0.1)	0 (0.0)	2 (0.2)			
	Missing	0 (0.0)	0 (0.0)	1 (0.1)			
Number of metastatic sites	0	5 (0.7)	1 (0.9)	9 (0.7)	0.37	0.41	0.99
	1	267 (38.0)	43 (38.1)	469 (34.2)			
	2	265 (37.8)	50 (44.3)	548 (40.1)			
	≥3	165 (23.5)	19 (16.8)	342 (25.0)			
Metastatic sites	Liver only	168 (23.9)	24 (21.2)	290 (21.2)	0.47	0.85	0.94
	Liver + others	356 (50.7)	56 (49.6)	738 (54.0)			
	No Liver	178 (25.4)	33 (29.2)	340 (24.9)			
Treatment details	Continuous OxFp	0 (0.0)	0 (0.0)	671 (49.1)	N/A	N/A	N/A
	Continuous OxFp+C	702 (100.0)	0 (0.0)	0 (0.0)			
	Intermittent OxFp	0 (0.0)	0 (0.0)	697 (50.9)			
	Intermittent OxFp C	0 (0.0)	58 (51.3)	0 (0.0)			
	Int. OxFp+maint C	0 (0.0)	55 (48.7)	0 (0.0)			
Fluoropyrimidine partner	Xelox	462 (65.8)	0 (0.0)	887 (64.8)	0.66[3]	N/A	0.88
	OxMdG	240 (34.2)	113 (100.0)	481 (35.2)			

KRAS result	Wild-type Mutated	319 (55.1) 260 (44.9)	60 (61.2) 38 (38.8)	671 (59.5) 456 (40.5)	0.17	0.083	0.35
NRAS result	Wild-type Mutated	551 (95.2) 28 (4.8)	53 (93.0) 4 (7.0)[4]	1087 (97.1) 33 (2.9)	N/A	N/A	N/A
BRAF result	Wild-type Mutated	545 (93.8) 36 (6.2)	44 (80.0) 11 (20.0)[4]	1006 (89.7) 116 (10.3)	N/A	N/A	N/A

[1]Comparing patients treated with cetuximab to those without. [2]Not significant after correction for multiple testing. [3]Excluding COIN-B (i.e. comparing COIN cetuximab vs non-cetuximab). [4]In COIN-B, only carried out on *KRAS* wild-type CRCs. N/A – not applicable. RSJ – Rectosigmoid junction. Percentages in parentheses, unless otherwise stated.

Supplementary Table S2 - Coding region and promoter variants and their associated genes analysed in this study

rs no.	Gene	Variant	MAF
rs3740199	*ADAM12*	Gly48Arg	0.45
rs459552	*APC*	Val1822Asp	0.22
rs11938093	*BTC*	Leu124Met	0.26
rs9344	*CCND1*	Pro241	0.43
rs2230804	*CHUK*	Val268Ile	0.47
rs34471628	*DUSP1*	Tyr187His	0.04
rs770087	*DUSP6*	Ser144Ala	0.20
rs4444903	*EGF*	promoter c.1-382 A>G	0.40
rs11568943	*EGF*	Arg431Lys	0.06
rs2237051	*EGF*	Ile708Met	0.38
rs11569017	*EGF*	Asp784Val	0.05
rs4698803	*EGF*	Glu920Val	0.21
rs2227983	*EGFR*	Arg521Lys	0.26
rs11568315	*EGFR*	intron 1 $(CA)_n$ repeat	0.45
rs17567	*EPS15*	Ile822Met	0.23
rs41292521	*EPS15*	Ser438Leu	0.02
rs1058808	*ERBB2*	Pro1170Ala	0.31
rs78803121	*EREG*	Cys141Phe	0.06
rs1801274	*FCGR2A*	His166Arg	0.48
rs396991	*FCGR3A*	Phe212Val	0.34
rs4073	*IL8*	promoter c.1-352 T>A	0.46
rs16858808	*IL8RA*	Arg335Cys	0.03
rs1870377	*KDR*	Gln472His	0.23
rs2305948	*KDR*	Val297Ile	0.11
rs702689	*MAP3K1*	Asp806Asn	0.28
rs832582	*MAP3K1*	Val906Ile	0.17
rs243865	*MMP2*	promoter c.1-2206 C>T	0.25
rs679620	*MMP3*	Lys45Glu	0.48
rs35068180	*MMP3*	promoter c.1-1671insA	0.48
rs17576	*MMP9*	Gln279Arg	0.35
rs2274756	*MMP9*	Arg668Gln	0.14
rs2250889	*MMP9*	Arg574Pro	0.04
rs41427445	*MMP9*	Asn38Ser	0.01
rs3729680	*PIK3CA*	Ile391Met	0.07
rs3730089	*PIK3R1*	Met326Ile	0.16
rs1011320	*PIK3R2*	Ser313Pro	0.05
rs785467	*PIK3R3*	Asn283Lys	0.30
rs2302524	*PLAUR*	Lys220Arg	0.16
rs4760	*PLAUR*	Leu317Pro	0.16
rs2228246	*PLCG1*	Ser279Gly	0.16
rs753381	*PLCG1*	Ile813Thr	0.46
rs17537869	*PLCG2*	Arg268Trp	0.07
rs13283456	*PTGES2*	Arg298His	0.20
rs1236913	*PTGS1*	Trp8Arg	0.7

rs5789	*PTGS1*	Leu237Met	0.03
rs20417	*PTGS2*	promoter c.1-899 C>G	0.16
rs5275	*PTGS2*	3'UTR c.1812+430 A>G	0.35
rs751019	*PTK2B*	Lys838Thr	0.45
rs1284879	*RASAL1*	His321Arg	0.22
rs2229712	*RPS6KA1*	Lys344Thr	0.22
rs61755579	*SOS2*	Ala208Thr	0.03
rs1073123	*TSC1*	Met322Thr	0.13
rs602990	*VAV2*	Met584Val	0.47
rs61751477	*VAV2*	Ile779Met	0.01

MAF – Minor allele frequencies in patients from COIN and COIN-B.

Supplementary Table S3 - Analyses of 12-week response and skin rash (SR) (primary endpoints)

	Response		SR	
rs no.	X^2 (df)	*P*-value	X^2 (df)	*P*-value
rs9344	0.18 (2)	0.91	1.35 (2)	0.51
rs1801274	2.41 (2)	0.30	0.08 (2)	0.96
rs396991	1.97 (2)	0.37	0.94 (2)	0.63
rs20417	0.87 (2)	0.65	2.72 (2)	0.26
rs5275	6.95 (2)	0.031	5.24 (2)	0.073
rs2227983	2.73 (2)	0.26	2.62 (2)	0.27
rs11568315	0.40 (2)	0.82	1.37 (2)	0.50
rs4444903	7.54 (2)	0.023	1.36 (2)	0.51
rs11568943	1.43 (2)	0.23	1.86 (2)	0.39
rs2237051	5.73 (2)	0.057	1.93 (2)	0.38
rs11569017	2.96 (2)	0.086	1.12 (1)	0.29
rs4698803	4.87 (2)	0.088	2.83 (2)	0.24
rs11938093	2.26 (2)	0.32	0.48 (2)	0.79
rs3729680	0.51 (2)	0.77	3.87 (2)	0.14
rs78803121	7.44 (2)	0.024	4.59 (2)	0.10
rs1011320	9.42 (1)	0.0021	3.59 (1)	0.058
rs17537869	8.13 (2)	0.017	1.85 (2)	0.40
rs2228246	1.99 (2)	0.37	2.27 (2)	0.32
rs2302524	1.06 (2)	0.59	1.37 (2)	0.50
rs4760	0.66 (2)	0.72	0.37 (2)	0.83
rs679620	1.76 (2)	0.41	0.10 (2)	0.95
rs751019	3.83 (2)	0.15	2.82 (2)	0.24
rs753381	3.16 (2)	0.21	1.15 (2)	0.56
rs13283456	0.99 (2)	0.61	0.56 (2)	0.76
rs1870377	5.02 (2)	0.081	0.66 (2)	0.72
rs2230804	0.13 (2)	0.94	1.50 (2)	0.47
rs2305948	0.52 (2)	0.77	0.91 (2)	0.63
rs4073	0.00 (2)	0.99	0.28 (2)	0.87
rs602990	1.27 (2)	0.53	6.85 (2)	0.033
rs702689	0.14 (2)	0.93	0.42 (2)	0.81
rs785467	0.37 (2)	0.83	9.55 (2)	0.0085
rs832582	0.92 (2)	0.63	0.77 (2)	0.68
rs1073123	1.56 (2)	0.46	2.89 (2)	0.24
rs1236913	0.32 (1)	0.57	0.22 (1)	0.64
rs1284879	0.09 (2)	0.96	0.72 (2)	0.70
rs17576	0.28 (2)	0.87	0.26 (2)	0.88
rs2274756	0.31 (2)	0.86	1.86 (2)	0.40
rs243865	2.74 (2)	0.25	2.54 (2)	0.28
rs3740199	3.48 (2)	0.18	3.33 (2)	0.19
rs459552	5.88 (2)	0.053	1.43 (2)	0.49
rs770087	1.07 (2)	0.59	4.28 (2)	0.12
rs1058808	2.28 (2)	0.32	3.30 (2)	0.19
rs2229712	0.64 (2)	0.73	1.73 (2)	0.42
rs16858808	0.60 (1)	0.44	5.29 (1)	0.022
rs17567	3.41 (2)	0.18	0.76 (2)	0.68
rs2250889	2.80 (1)	0.095	0.19 (1)	0.66
rs34471628	1.11 (1)	0.29	1.54 (1)	0.21
rs41427445	0.36 (1)	0.55	0.56 (1)	0.45
rs5789	0.12 (1)	0.73	1.23 (1)	0.27

rs41292521	1.00 (1)	0.32	5.17 (1)	0.023
rs61755579	0.07 (1)	0.79	0.13 (1)	0.72
rs61751477	0.63 (1)	0.43	0.20 (1)	0.65
rs3730089	0.32 (2)	0.85	1.93 (2)	0.38
rs35068180	2.01 (2)	0.37	0.10 (2)	0.95

Supplementary Table S4 - Association of Ser313Pro in *PIK3R2* with response to cetuximab

	All patients		*KRAS* mutant		*KRAS* wild type[1]	
Cetuximab	**+**	**-**	**+**	**-**	**+**	**-**
≥1 allele encoding proline homozygous for alleles encoding serine	25/62 (40.3%)	58/117 (49.6%)	12/30 (40.0%)	17/40 (42.5%)	8/22 (36.4%)	31/55 (56.4%)
	371/614 (60.4%)	602/1050 (57.3%)	110/218 (50.5%)	191/353 (54.1%)	210/295 (71.2%)	317/521 (60.8%)
OR (95% CI)	0.44 (0.26, 0.75)	0.73 (0.50, 1.07)	0.65 (0.30, 1.43)	0.63 (0.32, 1.22)	0.23 (0.09, 0.56)	0.82 (0.47, 1.45)
***P*-value**	0.002	0.11	0.29	0.17	0.001	0.51
Predictive for cetuximab?	NO *P* interaction=0.13		NO *P* interaction=0.94		YES *P* interaction=0.017	

Numbers represent patients with that genotype that responded to treatment over all patients for whom we had data on response, with percentages in parentheses. [1]On a *RAS* (*KRAS* and *NRAS*) wild-type background, 38.1% (8/21) of patients treated with cetuximab and with ≥1 allele encoding proline responded as compared to 74.0% (202/273) of patients homozygous for alleles encoding serine (OR 0.21, 95% CI 0.08-0.52, *P*=0.001 unadjusted; OR 0.22, 95% CI 0.09-0.58, *P*=0.002 adjusted for *BRAF* status). This was significantly predictive for cetuximab, $P_{interaction}$=0.027 unadjusted and 0.026 adjusted ($OR_{no\ cetuximab}$ 0.73, 95% CI 0.40-1.32, *P*=0.30 unadjusted, OR 0.80, 95% CI 0.44-1.46, *P*=0.46 adjusted). No associations were significant after correction for multiple testing.

Supplementary Table S5 - Association of Arg268Trp in *PLCG2* with response to cetuximab

	All patients		*KRAS* mutant		*KRAS* wild type	
cetuximab	**+**	**-**	**+**	**-**	**+**	**-**
≥1 allele encoding tryptophan	62/90 (69.9%)	72/154 (46.7%)	22/34 (64.7%)	24/52 (46.2%)	32/41 (78.1%)	38/73 (52.1%)
homozygous for alleles encoding arginine	336/589 (57.1%)	589/1015 (58.0%)	101/215 (47.0%)	184/341 (54.0%)	187/277 (67.5%)	311/504 (61.7%)
OR (95% CI)	1.66 (1.03, 2.67)	0.64 (0.45, 0.89)	2.05 (0.96, 4.40)	0.73 (0.41, 1.31)	1.70 (0.78, 3.73)	0.68 (0.41, 1.11)
***P*-value**	0.037	0.009	0.064	0.29	0.18	0.12
Predictive for cetuximab?	YES *P* interaction=0.001		YES *P* interaction=0.034		NO *P* interaction=0.052	

Numbers represent patients with that genotype that responded to treatment over all patients for whom we had data on response, with percentages in parentheses.

Supplementary Table S6 - Analyses of overall survival (OS) and overall response rate (ORR) (secondary endpoints)

	OS		ORR	
rs no.	X^2 (df)	*P*-value	X^2 (df)	*P*-value
rs9344	0.72 (2)	0.70	0.74 (2)	0.69
rs1801274	1.27 (2)	0.53	1.57 (2)	0.46
rs396991	0.63 (2)	0.73	1.91 (2)	0.39
rs20417	0.69 (2)	0.71	1.58 (2)	0.45
rs5275	1.26 (2)	0.53	5.04 (2)	0.080
rs2227983	1.00 (2)	0.61	3.48 (2)	0.18
rs11568315	0.41 (2)	0.81	0.35 (2)	0.84
rs4444903	3.33 (2)	0.19	5.08 (2)	0.079
rs11568943	2.73 (2)	0.26	0.46 (1)	0.50
rs2237051	1.87 (2)	0.39	4.34 (2)	0.11
rs11569017	3.91 (2)	0.048	3.03 (1)	0.082
rs4698803	1.46 (2)	0.48	1.42 (2)	0.49
rs11938093	4.68 (2)	0.096	0.68 (2)	0.71
rs3729680	0.75 (2)	0.69	0.85 (2)	0.65
rs78803121	0.77 (2)	0.68	6.71 (2)	0.035
rs1011320	7.34 (1)	0.0067	10.3 (1)	0.0014
rs17537869	2.09 (2)	0.35	5.11 (2)	0.078
rs2228246	2.23 (2)	0.33	2.31 (2)	0.31
rs2302524	3.02 (2)	0.22	1.41 (2)	0.49
rs4760	2.14 (2)	0.34	1.41 (2)	0.49
rs679620	0.82 (2)	0.66	1.06 (2)	0.59
rs751019	0.31 (2)	0.85	5.41 (2)	0.067
rs753381	2.03 (2)	0.36	2.49 (2)	0.29
rs13283456	1.42 (2)	0.49	2.98 (2)	0.23
rs1870377	1.25 (2)	0.54	1.77 (2)	0.41
rs2230804	0.34 (2)	0.84	0.46 (2)	0.79
rs2305948	0.41 (2)	0.82	0.39 (2)	0.82
rs4073	5.25 (2)	0.072	1.34 (2)	0.51
rs602990	1.21 (2)	0.55	0.98 (2)	0.61
rs702689	1.64 (2)	0.44	0.43 (2)	0.80
rs785467	0.83 (2)	0.66	0.31 (2)	0.85
rs832582	1.51 (2)	0.47	0.25 (2)	0.88
rs1073123	2.26 (2)	0.32	1.40 (2)	0.50
rs1236913	1.41 (1)	0.24	0.52 (1)	0.47
rs1284879	2.78 (2)	0.25	0.98 (2)	0.61
rs17576	2.32 (2)	0.31	0.39 (2)	0.82
rs2274756	0.88 (2)	0.64	0.32 (2)	0.85
rs243865	0.95 (2)	0.62	2.86 (2)	0.24
rs3740199	0.30 (2)	0.86	3.50 (2)	0.17
rs459552	0.17 (2)	0.92	5.24 (2)	0.073
rs770087	1.32 (2)	0.52	1.45 (2)	0.49
rs1058808	1.07 (2)	0.59	1.81 (2)	0.41
rs2229712	5.86 (2)	0.054	3.46 (2)	0.18
rs16858808	0.47 (1)	0.49	0.15 (1)	0.70
rs17567	2.45 (2)	0.29	0.13 (2)	0.94
rs2250889	1.96 (1)	0.16	2.90 (1)	0.089
rs34471628	0.42 (1)	0.52	1.48 (1)	0.22
rs41427445	0.30 (1)	0.58	1.62 (1)	0.20
rs5789	0.24 (1)	0.62	0.40 (1)	0.53

rs41292521	0.32 (1)	0.57	0.84 (1)	0.36
rs61755579	0.34 (1)	0.56	0.01 (1)	0.94
rs61751477	3.53 (2)	0.17	0.95 (1)	0.33
rs3730089	0.50 (2)	0.78	0.29 (2)	0.86
rs35068180	0.23 (2)	0.89	1.06 (2)	0.59

Supplementary Table S7 – Analyses of individual toxicities (secondary endpoints)

	Lethargy		Nausea/vomiting		Diarrhoea		Stomatitis		HFS		Hypomagnesaemia		Nail changes	
rs no.	X^2 (df)	*P*-value	χ^2 (df)	*P*-value	χ^2 (df)	*P*-value	X^2 (df)	*P*-value	χ^2 (df)	*P*-value	χ^2 (df)	*P*-value	χ^2 (df)	*P*-value
rs9344	1.36 (2)	0.51	4.83 (2)	0.089	0.29 (2)	0.87	0.12 (2)	0.94	1.01 (2)	0.60	0.32 (2)	0.85	0.21 (1)	0.64
rs1801274	2.13 (2)	0.34	2.52 (2)	0.28	5.40 (2)	0.067	2.84 (2)	0.24	4.84 (2)	0.089	2.24 (2)	0.33	4.62 (2)	0.099
rs396991	0.32 (2)	0.85	2.42 (2)	0.30	3.14 (2)	0.21	7.18 (2)	0.028	1.16 (2)	0.56	0.52 (2)	0.77	0.40 (1)	0.53
rs20417	0.20 (2)	0.91	1.01 (2)	0.60	2.36 (2)	0.31	0.35 (2)	0.84	0.10 (2)	0.95	0.91 (1)	0.34	0.31 (1)	0.58
rs5275	3.48 (2)	0.18	2.73 (2)	0.26	1.87 (2)	0.39	1.57 (2)	0.46	0.30 (2)	0.86	0.37 (1)	0.54	2.97 (2)	0.23
rs2227983	1.01 (2)	0.60	3.26 (2)	0.20	0.05 (2)	0.98	0.99 (2)	0.61	3.86 (2)	0.15	0.48 (1)	0.49	2.93 (1)	0.087
rs11568315	0.27 (2)	0.87	1.67 (2)	0.43	0.03 (2)	0.98	2.55 (2)	0.28	0.05 (2)	0.98	0.02 (1)	0.88	0.75 (2)	0.69
rs4444903	0.98 (2)	0.61	1.37 (2)	0.51	2.03 (2)	0.36	1.75 (2)	0.42	9.42 (2)	0.0090	0.86 (2)	0.65	0.65 (2)	0.72
rs11568943	0.01 (2)	0.99	0.82 (2)	0.66	0.18 (1)	0.67	0.79 (2)	0.67	0.23 (1)	0.63	0.06 (1)	0.81	0.11 (1)	0.74
rs2237051	1.05 (2)	0.59	2.14 (2)	0.34	3.76 (2)	0.15	3.23 (2)	0.20	3.94 (2)	0.14	1.14 (2)	0.56	1.10 (2)	0.58
rs11569017	0.01 (1)	0.94	0.08 (1)	0.78	0.56 (1)	0.45	1.45 (1)	0.23	0.11 (1)	0.74	0.21 (1)	0.64	0.00 (1)	0.97
rs4698803	1.03 (2)	0.60	1.01 (2)	0.60	1.44 (2)	0.49	2.65 (2)	0.27	2.81 (2)	0.25	0.18 (1)	0.67	10.6 (2)	0.0049
rs11938093	1.08 (2)	0.58	1.21 (2)	0.55	2.25 (2)	0.32	0.72 (2)	0.70	0.79 (2)	0.67	0.53 (2)	0.77	0.91 (2)	0.64
rs3729680	0.39 (2)	0.82	0.57 (1)	0.45	0.27 (1)	0.61	1.52 (1)	0.22	0.48 (2)	0.79	0.00 (1)	0.99	Cannot be fitted	
rs78803121	0.41 (2)	0.82	0.79 (2)	0.67	0.95 (2)	0.62	0.06 (2)	0.97	4.08 (1)	0.043	0.10 (1)	0.76	Cannot be fitted	
rs1011320	0.46 (1)	0.50	0.00 (1)	0.98	0.25 (1)	0.62	0.73 (1)	0.39	0.25 (1)	0.62	Cannot be fitted		0.68 (1)	0.41
rs17537869	1.69 (2)	0.43	2.39 (1)	0.12	4.09 (2)	0.13	0.14 (2)	0.93	2.29 (2)	0.32	1.02 (1)	0.31	0.27 (1)	0.60
rs2228246	0.84 (2)	0.66	0.55 (2)	0.76	2.19 (2)	0.34	0.79 (2)	0.67	1.10 (2)	0.58	Cannot be fitted		1.90 (1)	0.17
rs2302524	1.54 (2)	0.46	3.19 (2)	0.20	2.01 (2)	0.37	3.04 (2)	0.23	2.13 (2)	0.35	2.02 (1)	0.16	6.50 (2)	0.039
rs4760	1.84 (2)	0.40	1.37 (2)	0.50	0.30 (2)	0.86	1.06 (2)	0.59	0.97 (2)	0.62	0.47 (1)	0.49	1.60 (2)	0.45
rs679620	1.33 (2)	0.51	0.43 (2)	0.81	0.05 (2)	0.97	0.16 (2)	0.92	0.57 (2)	0.75	1.36 (2)	0.51	2.59 (2)	0.27
rs751019	2.23 (2)	0.33	0.62 (2)	0.73	0.38 (2)	0.83	0.42 (2)	0.81	3.79 (2)	0.15	2.00 (2)	0.37	2.89 (2)	0.24
rs753381	0.46 (2)	0.80	1.87 (2)	0.39	0.50 (2)	0.78	0.58 (2)	0.75	4.87 (2)	0.088	4.13 (2)	0.13	4.63 (2)	0.099
rs13283456	0.90 (2)	0.64	2.45 (2)	0.29	2.01 (2)	0.37	8.05 (2)	0.018	4.83 (2)	0.089	0.52 (1)	0.47	0.28 (1)	0.60
rs1870377	5.61 (2)	0.061	1.18 (2)	0.56	0.10 (2)	0.95	0.59 (2)	0.74	1.34 (2)	0.51	0.32 (1)	0.57	0.48 (1)	0.49
rs2230804	3.50 (2)	0.17	2.04 (2)	0.36	0.70 (2)	0.70	0.50 (2)	0.78	1.31 (2)	0.52	0.74 (2)	0.69	2.03 (1)	0.15
rs2305948	0.10 (2)	0.95	1.79 (2)	0.41	1.30 (2)	0.52	3.45 (2)	0.18	0.08 (1)	0.78	0.10 (1)	0.75	0.93 (1)	0.36
rs4073	2.20 (2)	0.33	0.92 (2)	0.63	1.50 (2)	0.47	0.43 (2)	0.81	0.88 (2)	0.64	0.29 (2)	0.86	3.20 (2)	0.20
rs602990	2.86 (2)	0.24	0.18 (2)	0.91	1.71 (2)	0.43	2.45 (2)	0.29	0.11 (2)	0.95	2.00 (2)	0.37	2.91 (2)	0.23
rs702689	3.76 (2)	0.15	1.37 (2)	0.50	5.58 (2)	0.061	1.59 (2)	0.45	6.08 (2)	0.048	0.33 (1)	0.57	0.01 (1)	0.93

rs785467	3.79 (2)	0.15	1.03 (2)	0.60	0.41 (2)	0.81	2.37 (2)	0.31	3.10 (2)	0.21	0.15 (2)	0.93	1.02 (1)	0.31
rs832582	8.72 (2)	0.013	2.21 (2)	0.33	6.98 (2)	0.030	0.96 (2)	0.62	2.43 (2)	0.30	2.43 (1)	0.12	0.28 (1)	0.60
rs1073123	0.11 (2)	0.95	0.26 (2)	0.88	0.70 (2)	0.70	7.41 (2)	0.025	0.41 (2)	0.82	Cannot be fitted		0.05 (1)	0.82
rs1236913	0.19 (1)	0.67	1.36 (1)	0.24	0.39 (1)	0.53	0.59 (1)	0.44	0.73 (1)	0.39	0.00 (1)	0.98	1.43 (1)	0.23
rs1284879	4.72 (2)	0.094	7.71 (2)	0.021	3.73 (2)	0.16	2.61 (2)	0.27	3.08 (2)	0.21	0.90 (2)	0.64	0.53 (1)	0.47
rs17576	5.60 (2)	0.061	5.70 (2)	0.058	2.15 (2)	0.34	5.26 (2)	0.072	4.19 (2)	0.12	1.75 (2)	0.42	2.26 (2)	0.32
rs2274756	2.15 (2)	0.34	0.09 (1)	0.77	3.52 (2)	0.17	0.10 (1)	0.75	2.92 (2)	0.23	2.73 (1)	0.098	2.20 (1)	0.14
rs243865	0.03 (2)	0.99	0.60 (2)	0.74	1.77 (2)	0.41	1.54 (2)	0.46	0.24 (2)	0.89	0.95 (2)	0.62	0.00 (1)	0.97
rs3740199	4.76 (2)	0.093	0.78 (2)	0.68	0.08 (2)	0.96	0.54 (2)	0.76	3.37 (2)	0.19	0.51 (2)	0.77	0.16 (2)	0.92
rs459552	2.64 (2)	0.27	3.37 (2)	0.19	4.68 (2)	0.096	1.86 (2)	0.39	5.34 (2)	0.069	2.09 (2)	0.35	0.51 (1)	0.48
rs770087	0.25 (2)	0.88	0.26 (2)	0.88	1.90 (2)	0.39	0.38 (2)	0.83	0.90 (2)	0.64	1.16 (1)	0.28	0.42 (2)	0.81
rs1058808	5.90 (2)	0.053	1.61 (2)	0.45	0.33 (2)	0.85	0.77 (2)	0.68	8.77 (2)	0.013	0.18 (2)	0.91	0.02 (2)	0.99
rs2229712	1.09 (2)	0.58	0.91 (2)	0.63	8.05 (2)	0.018	0.65 (2)	0.72	1.11 (2)	0.58	0.21 (1)	0.65	0.18 (2)	0.91
rs16858808	0.55 (1)	0.46	0.00 (1)	0.95	0.30 (1)	0.59	0.39 (1)	0.53	0.97 (1)	0.32	Cannot be fitted		12.6 (1)	0.00039
rs17567	1.89 (2)	0.39	2.58 (2)	0.28	2.57 (2)	0.28	5.69 (2)	0.058	4.64 (2)	0.098	0.06 (1)	0.80	2.33 (2)	0.31
rs2250889	0.05 (1)	0.82	4.62 (1)	0.032	0.01 (1)	0.92	0.19 (1)	0.66	2.44 (1)	0.12	0.24 (1)	0.62	1.11 (1)	0.29
rs34471628	0.98 (1)	0.32	1.63 (1)	0.20	0.04 (1)	0.83	0.54 (1)	0.46	0.00 (1)	0.99	6.62 (1)	0.010	0.03 (1)	0.86
rs41427445	3.16 (1)	0.075	0.04 (1)	0.84	1.05 (1)	0.30	0.15 (1)	0.70	0.10 (1)	0.76	Cannot be fitted		Cannot be fitted	
rs5789	0.39 (1)	0.53	1.94 (1)	0.16	0.13 (1)	0.72	0.90 (1)	0.34	2.36 (1)	0.12	Cannot be fitted		Cannot be fitted	
rs41292521	1.86 (1)	0.17	2.00 (1)	0.16	1.92 (1)	0.17	0.03 (1)	0.86	0.01 (1)	0.91	Cannot be fitted		0.35 (1)	0.56
rs61755579	0.00 (1)	0.99	1.89 (1)	0.17	0.00 (1)	0.98	0.03 (1)	0.86	1.66 (1)	0.20	1.09 (1)	0.30	Cannot be fitted	
rs61751477	1.47 (1)	0.23	0.12 (1)	0.73	0.18 (1)	0.67	0.14 (1)	0.71	0.22 (1)	0.64	Cannot be fitted		Cannot be fitted	
rs3730089	1.61 (2)	0.45	0.69 (2)	0.71	3.52 (2)	0.17	0.52 (2)	0.77	1.09 (2)	0.58	1.66 (2)	0.44	0.23 (1)	0.63
rs35068180	0.32 (2)	0.85	2.37 (2)	0.31	3.14 (2)	0.21	7.18 (2)	0.028	1.16 (2)	0.56	0.52 (2)	0.77	0.40 (1)	0.53