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Correlation of *FCGR3A* and *EGFR* germline polymorphisms with the efficacy of cetuximab in *KRAS* wild-type metastatic colorectal cancer

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ABSTRACT

Background: Next to *KRAS* mutation status, additional predictive markers are needed for the response to cetuximab in patients with metastatic colorectal cancer (mCRC). Previous studies indicated that germline polymorphisms in specific genes may predict efficacy and toxicity of cetuximab in mCRC patients.

Methods: Germline DNA was isolated from 246 *KRAS* wild-type mCRC patients who were treated in the phase III CAIRO2 study with chemotherapy and bevacizumab alone or with the addition of cetuximab. Associations of epidermal growth factor (*EGF*) 61A > G, EGF receptor (*EGFR*) CA_{14-22}, cyclin D1 (*CCND1*) 932G > A, fragment-C gamma receptor (*FCGR*) 2A 535A > G and *FCGR3A* 818A > C polymorphisms with progression-free survival (PFS) and cetuximab-related skin toxicity were studied.

Results: In cetuximab-treated patients, the *FCGR3A* 818C-allele was associated with decreased PFS compared with the *FCGR3A* 818AA genotype (median PFS, 8.2 [95%CI, 6.7–10.3] versus 12.8 [95%CI, 10.3–14.7] months, respectively; HR, 1.57 [95%CI, 1.06–2.34]; $P = .025$). The *EGFR* $\geqslant 20$ genotype was associated with decreased PFS compared with the *EGFR* < 20 genotype (median PFS, 7.6 [95%CI, 6.7–10.0] versus 12.4 [95%CI, 10.3–13.4] months, respectively; HR, 1.58 [95%CI, 1.06–2.35]; $P = .024$). The *FCGR3A* and *EGFR* polymorphisms were not associated with PFS in patients treated without cetuximab. None of the polymorphisms were associated with the incidence of grades 2–3 skin toxicity.

Conclusion: *EGFR* and *FCGR3A* germline polymorphisms are associated with PFS in *KRAS* wild-type mCRC patients treated with cetuximab, bevacizumab and chemotherapy.

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1. Introduction

Cetuximab is an IgG_1-type chimeric monoclonal antibody that targets the epidermal growth factor receptor (EGFR). Its principal mechanism of action is the inhibition of ligand induced EGFR activation, resulting in reduced cell proliferation, cell survival and angiogenesis. Also, cetuximab may induce antibody-dependent cell cytotoxicity (ADCC) by recruitment of immune effector cells.[1]

Cetuximab is effective in patients with chemotherapy-refractory metastatic colorectal cancer (mCRC).[2,3] A modest clinical benefit was shown for cetuximab when added to first-line chemotherapy.[4–6] The indication for the use of cetuximab is restricted to patients with wild-type *KRAS* tumours.[7,8] However, the *KRAS* mutation status does not completely predict the response to cetuximab and other tumour characteristics such as *BRAF* mutation status have been investigated.[9,10] The severity of acneiform skin rash is also associated with the efficacy of cetuximab[2,3], but as this adverse event occurs after therapy has started, it cannot be used to predict response before start of treatment. Therefore, additional predictive markers are needed to better identify patients who will benefit from cetuximab.

Germline polymorphisms in genes involved in the mechanism of action of cetuximab have been investigated previously.[11–14] A CA-repeat polymorphism in intron 1 of the *EGFR* gene and the single nucleotide polymorphisms (SNPs) *EGF* c.61A > G, cyclin D1 (*CCND1*) c.932G > A and fragment-C gamma receptors 2A (*FCGR2A*) c.535A > G and 3A (*FCGR3A*) c.818A > C have previously been associated with the efficacy of cetuximab in chemotherapy-refractory mCRC patients who were treated with cetuximab either as monotherapy[11,12] or in combination with irinotecan.[13,14] However, these findings have been investigated in relation to *KRAS* mutation status in only one small study.[14] Furthermore, these former studies were hypothesis generating, and lacked a control group.

To provide more robust data, we investigated the association of these germline polymorphisms with the efficacy of cetuximab in a large cohort of *KRAS* wild-type mCRC patients who were treated in first-line with capecitabine, oxaliplatin, bevacizumab with or without cetuximab.

2. Patients and methods

2.1. Study population

Seven hundred and fifty five previously untreated mCRC patients participated in the multicentre phase III randomised CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG) and were treated with capecitabine, oxaliplatin and bevacizumab or the same regimen plus cetuximab.[15,16] Patient eligibility criteria are described in detail elsewhere.[15] Patients were stratified according to prior adjuvant chemotherapy, serum LDH, number of affected organs and per institution. Membrane expression of EGFR in the tumour was not required.

Cetuximab was administered intravenously at a dose of 400 mg/m^2 on the first day, followed by 250 mg/m^2 weekly thereafter. Dose reductions were carried out according to the study protocol. The duration of a treatment cycle was three weeks. Treatment was continued until disease progression, death or unacceptable toxicity, whichever occurred first.

The collection of a peripheral blood sample for pharmacogenetic research was pre-specified in the study protocol and required additional written informed consent. The protocol was approved by the local institutional review boards of all participating centres.

2.2. Clinical evaluation and toxicity criteria

Progression-free survival (PFS) was calculated using tumour response assessments every three cycles by CT scan according to RECIST 1.0 criteria.[15] PFS was defined as the interval from the date of randomisation to the date of disease progression, death or last follow-up, whichever occurred first. Toxicity was scored according to the National Cancer Institute Common Toxicity Criteria version 3.0. Cetuximab-related skin toxicity was defined as any skin toxicity with the exception of hand–foot syndrome.

2.3. Analysis of genetic variants

The *KRAS* mutation status was determined in 528 patients from whom primary tumour tissue was available. Tumour DNA was extracted and *KRAS* mutation status was analysed using a commercially available real-time PCR-based assay (DxS, Manchester, UK) and by direct sequencing.[17]

Of the 314 *KRAS* wild-type patients, a peripheral blood sample was available from 246 patients (127 and 119 from the treatment arms with and without cetuximab, respectively). For the germline polymorphisms, germline DNA was isolated from peripheral white blood cells by the standard manual salting-out method. Genotyping was performed on a TaqMan 7500 (Applied Biosystems, Foster City, CA, USA) with pre-designed assays for *EGF* c.61A > G (rs4444903), *CCND1* c.932G > A (rs9344; also referred to as 870G > A), *FCGR2A* c.535A > G (rs1801274; resulting in amino-acid change of histidine to arginine at position 131) and *FCGR3A* c.818A > C (rs396991; resulting in amino-acid change of phenylalanine to valine at position 158), according to the manufacturer's protocol. Negative controls (water) were included. In addition, genotypes were confirmed on the Biomark (Fluidigm, South San Francisco, CA, USA) according to the protocol provided by the manufacturer using the same TaqMan assays. The *FCGR3A* polymorphism was also analysed by Pyrosequencing for 15% of the samples, which confirmed the TaqMan results.

The *EGFR* $(CA)_n$ polymorphism was analysed by fragment analysis. Briefly, 10 ng of DNA was PCR amplified using primers FAM-5′-CCAAAATATTAAACCTGTCTT-3′ and 5′-AACCAGGGACAGCAATCC-3′. PCR products were run on an ABI PRISM® 3730xl Analyzer and analysed with Genemapper v3.5 software (Applied Biosystems). Plasmids with an *EGFR* insert containing 14–21 CA-repeats were used as a control.[18] For the purpose of this analysis, the *EGFR* CA-repeat polymorphism was dichotomised according to the criterion applied by Zhang and colleagues.[11] Patients with two alleles containing less than 20 CA-repeats were designated 'EGFR < 20', whereas patients with either one or two alleles with 20 CA-repeats or more were designated as 'EGFR ⩾ 20'.

All genotype frequencies were in Hardy–Weinberg equilibrium.

2.4. Statistical analysis

The primary objective was to assess the association of the *EGFR*, *EGF*, *CCND1*, *FCGR2A* and *FCGR3A* polymorphisms with PFS in *KRAS* wild-type mCRC patients treated with cetuximab added to chemotherapy and bevacizumab. The secondary objective was to assess the association between these polymorphisms and cetuximab-related skin toxicity (grades 0–1 versus 2–3).

The PFS of each polymorphism was analysed per treatment arm. Survival curves were estimated using the Kaplan–Meier method. The hazard ratios and 95% confidence intervals (95%CI) were estimated using a multivariate Cox proportional hazards model per treatment arm, using the most appropriate of a dominant or recessive model. The effects of the genotypes were assessed with the wild-type genotype as the reference, as this is the most frequent and therefore 'normal' genotype. Since age (<65 versus ⩾65 years) and gender potentially affect the influence of a genetic polymorphism[19], these factors were included in the multivariate analysis in addition to serum LDH (normal versus abnormal), which was an independent prognostic factor in the CAIRO2 study.[15]

For patients in the cetuximab arm, the association between the genotype and cetuximab-related skin toxicity (grades 0–1 versus 2–3) was analysed and odds ratios (ORs) and 95% CIs were estimated using a multivariate logistic regression model with age, gender and serum LDH as covariates.

All statistical analyses were performed using the Statistical Analysis Software version 9.1 (SAS Inc., Bethesda, Maryland, USA).

3. Results

In the cetuximab arm, the 65 patients who were carriers of the *FCGR3A* C-allele (AC and CC genotypes combined) had a significantly decreased PFS compared with the 57 patients with the *FCGR3A* AA genotype (median PFS, 8.2 [95%CI, 6.7–10.3] versus 12.8 [95%CI, 10.3–14.7] months, respectively; HR, 1.57 [95%CI, 1.06–2.34]; $P = .025$, Table 1 and Fig. 1A). The 51 patients with the *EGFR* ⩾ 20 genotype had significantly decreased PFS compared with the 72 patients with the *EGFR* < 20 genotype (median PFS, 7.6 [95%CI, 6.7–10.0] versus 12.4 [95%CI, 10.3–13.4] months, respectively; HR, 1.58 [95%CI, 1.06–2.35]; $P = .024$, Table 1 and Fig. 1B). The *FCGR3A* and *EGFR* polymorphisms were not associated with PFS in the arm without cetuximab ($P = .832$ and $P = .649$, respectively). The *FCGR2A*, *EGF* and *CCND1* polymorphisms were not significantly associated with PFS in the cetuximab arm ($P = .076$, $P = .999$ and $P = .111$, respectively), nor in the arm without cetuximab ($P = .111$, $P = .067$ and $P = .185$, respectively).

The *FCGR3A*, *EGFR*, *FCGR2A*, *EGF* and *CCND1* polymorphisms were not significantly associated with grades 2–3 cetuximab-related skin toxicity ($P = .203$, $P = .903$, $P = .338$, $P = .549$ and $P = .128$, respectively, Table 1).

4. Discussion

We demonstrate that the *FCGR3A* 818C-allele and the *EGFR* ⩾ 20 genotype were associated with a decreased PFS in a large group of *KRAS* wild-type mCRC patients treated with cetuximab, bevacizumab and chemotherapy in a phase III trial, compared with patients with the *FCGR3A* 818AA or *EGFR* < 20 genotype, respectively.

Table 1 – Analysis of progression-free survival and of the incidence of grades 2–3 cetuximab-related skin toxicity for *KRAS* wild-type metastatic colorectal cancer patients treated with first-line capecitabine, oxaliplatin, bevacizumab and cetuximab.

	n	Median PFS (months [95%CI])	HR[a] for PFS	95%CI	*P*	OR[b] for grade 2–3 skin toxicity	95%CI	*P*
EGFR CA-repeat								
EGFR < 20	72	12.4 [10.3–13.4]	1.00	–	–	1.00	–	–
EGFR ⩾ 20	51	7.6 [6.7–10.0]	1.58	1.06–2.35	.024	1.05	0.46–2.39	.903
FCGR3A 818A > C								
AA	57	12.8 [10.3–14.7]	1.00	–	–	1.00	–	–
AC or CC	65	8.2 [6.7–10.3]	1.57	1.06–2.34	.025	0.59	0.28–1.33	.203
FCGR2A 535A > G								
AA	34	12.6 [9.8–16.8]	1.00	–	–	1.00	–	–
AG or GG	92	10.0 [8.4–12.3]	1.50	0.96–2.36	.076	1.54	0.64–3.72	.338
EGF 61A > G								
AA	50	10.8 [8.5–12.7]	1.00	–	–	1.00	–	–
AG or GG	70	10.3 [8.4–13.2]	1.00	0.67–1.49	.999	1.29	0.56–2.95	.549
CCND1 870G > A								
GG or GA	91	10.8 [8.2–12.7]	1.00	–	–	1.00	–	–
AA	34	9.9 [8.4–12.7]	1.41	0.92–2.15	.111	0.50	0.21–1.22	.128

Abbreviations: PFS, progression-free survival; FCGR, immunoglobulin-G fragment C receptor; EGFR, epidermal growth factor receptor.

[a] Hazard ratios (HRs), 95% confidence intervals (95% CIs) and *P* values were calculated from the Cox proportional hazards model, with the wild-type genotype as the reference, and included age, gender and serum LDH as covariates.

[b] Odds ratios (ORs), 95% CIs and *P* values were calculated from the logistic regression model, with the wild-type genotype as the reference, and included age, gender and serum LDH as covariates.

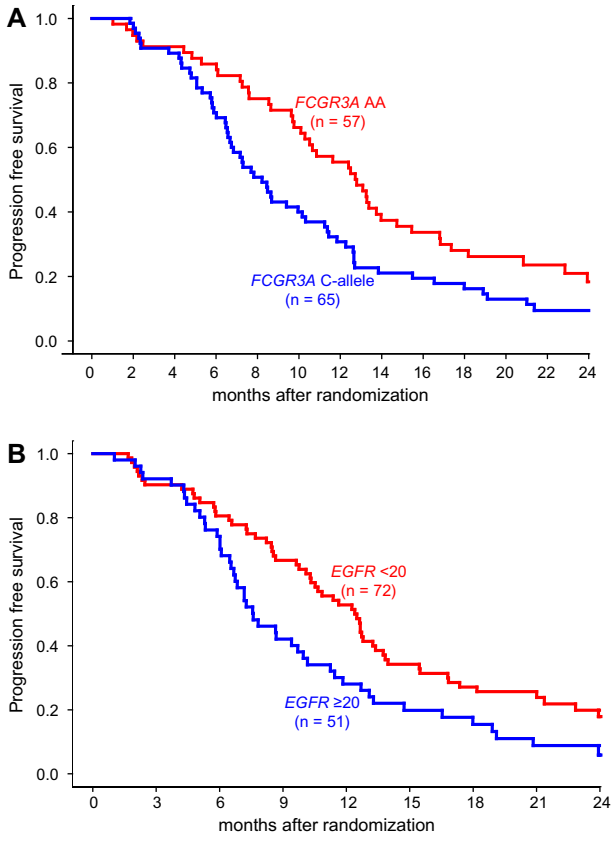

Fig. 1 – Progression-free survival for (A) the *FCGR3A* 818A > C polymorphism and (B) the *EGFR* CA-repeat polymorphism for patients with metastatic colorectal cancer treated with first-line capecitabine, oxaliplatin, bevacizumab and cetuximab (*P* = .025 and *P* = .024, respectively).

Bibeau and colleagues recently also reported that the *FCGR3A* polymorphism is associated with PFS in *KRAS* wild-type mCRC patients treated with cetuximab. However, in their study patients who were homozygous for the C-allele had longer PFS compared with carriers of the A-allele[14], which is not in agreement with our data. In one previous study the *FCGR3A* C-allele was also associated with decreased PFS in previously pretreated mCRC patients who were treated with cetuximab as a single agent[12], though this was not confirmed in an extended analysis of this study.[20] This indicates that the former finding was a false positive, making it not suitable for comparison with our study. Another study with 110 patients who received cetuximab monotherapy as salvage treatment for mCRC did also not find a significant association between the *FCGR3A* polymorphism and the efficacy of cetuximab.[13] A possible mechanism for the opposite association of the *FCGR3A* polymorphism could be that the high affinity C-allele[21–23] results in increased activation of tumour associated macrophages (TAMs) by cetuximab through cross-linking of the Fc gamma receptor[24], instead of increasing ADCC in our study. As a result of TAM activation, pro-angiogenic mediators are released in the tumour microenvironment, such as VEGF and matrix metalloproteinases (MMPs).[25,26] In our study, patients had not received palliative chemotherapy before, whereas patients in the other studies had been exposed to irinotecan and/or other lines of chemotherapy prior to cetuximab[13,14,20], which could have altered the infiltration of cells of the myeloid lineage, such as TAMs.[27] However, it must be noted that the *FCGR3A* C-allele was associated with increased efficacy of the IgG_1-type monoclonal antibodies rituximab in lymphoma[28,29] and trastuzumab in advanced breast cancer.[30] Therefore, fundamental research should be performed to support our highly speculative hypothesis.

Our finding that patients with a lower number of CA-repeats for the *EGFR* polymorphism experience longer PFS is in line with the study by Graziano and colleagues.[13] However, another study did not find a significant association between this *EGFR* polymorphism and PFS in cetuximab-treated mCRC patients.[20]

The biological mechanism for the association of the *EGFR* polymorphism is concordant with the finding that patients with the *EGFR* $\geqslant$ 20 genotype had shorter PFS. Transcription of the *EGFR* gene is lower for an increased number of CA-repeats.[31] Although *EGFR* expression, as measured by immunohistochemistry, is not a predictor of the efficacy of cetuximab[32,33], the number of *EGFR* gene copies is associated with the response to cetuximab treatment.[34]

Unexpectedly, we did not confirm previous findings that a lower number of CA-repeats is associated with increased incidence of skin toxicity during anti-EGFR therapy.[13,35] However, previous findings could have been biased by the correlation between the response to anti-EGFR therapy and the incidence of skin toxicity.[2,3] The mechanism underlying the development of cetuximab-related skin toxicity is poorly understood, and could be independent of EGFR expression and polymorphisms.

Even though the previous pharmacogenetic studies on cetuximab have used peripheral blood[12,13], normal tissue[14] or tumour tissue[20], this should not have influenced the results, because there is an almost perfect degree of concordance between germline genotype in tumour and normal tissue.[36]

Importantly, the polymorphisms in *FCGR3A* and *EGFR* are only predictive for the efficacy of cetuximab and do not influence the PFS in patients not treated with cetuximab.

Biomarker and genetic association studies are hampered by divergent and inconsistent results.[37] Retrospective pharmacogenetic studies must therefore be interpreted as hypothesis generating studies that require confirmation in an independent cohort.

Although our large study was set up to confirm previously published associations and included a control group[11–14], the results are conflicting and therefore remain inconclusive. It is likely that heterogeneity among the different studies, such as the stage and nature of the disease, previous treatment and concomitant medication may explain the discordance. These variables should therefore be carefully considered in retrospective biomarker studies, as these factors probably have large influence on the results.

In conclusion, we demonstrate that germline polymorphisms in *FCGR3A* and *EGFR* are associated with the efficacy of cetuximab. Confirmation and prospective studies are needed to define the predictive value of these markers. Due to inconsistent results among studies, our results require further confirmation before they can be applied in clinical practice.

5. Conflict of interest statement

None declared.

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