

## *FcγRIIa* and *FcγRIIIa* Polymorphisms and Cetuximab Benefit in the Microscopic Disease

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### Abstract

**Purpose:** *FcγR* polymorphisms have been reported to enhance the immune-mediated effects of cetuximab in metastatic colorectal cancer. There are no data on the relationship between these polymorphisms and cetuximab in the early-stage setting. We performed a pharmacogenomic analysis of EXPERT-C, a randomized phase II trial of neoadjuvant CAPOX followed by chemoradiotherapy, surgery, and adjuvant CAPOX ± cetuximab in high-risk, locally advanced rectal cancer.

**Experimental Design:** *FcγRIIa*-H131R and *FcγRIIIa*-V158F polymorphisms were analyzed on DNA from peripheral blood samples. Kaplan–Meier method and Cox regression analysis were used to calculate survival estimates and compare treatment arms.

**Results:** Genotyping was successfully performed in 105 of 164 (64%) patients (CAPOX = 54, CAPOX-C = 51). No deviation from the Hardy–Weinberg equilibrium or association of these polymorphisms with tumor *RAS* status was observed. *FcγRIIa*-131R (HR, 0.38;  $P = 0.058$ ) and *FcγRIIIa*-158F alleles (HR, 0.21;  $P = 0.007$ ) predicted improved progression-free survival (PFS) in patients treated with cetuximab. In the CAPOX-C arm, carriers of both 131R and 158F alleles had a statistically significant improvement in PFS (5 years: 78.4%; HR, 0.22;  $P = 0.002$ ) and overall survival (OS; 5 years: 86.4%; HR, 0.24;  $P = 0.018$ ) when compared with patients homozygous for 131H and/or 158V (5-year PFS: 35.7%; 5-year OS: 57.1%). An interaction between cetuximab benefit and 131R and 158F alleles was found for PFS ( $P = 0.017$ ) and remained significant after adjusting for prognostic variables ( $P = 0.003$ ).

**Conclusion:** This is the first study investigating *FcγRIIa* and *FcγRIIIa* polymorphisms in patients with early-stage colorectal cancer treated with cetuximab. We showed an increased clinical benefit from cetuximab in the presence of 131R and 158F alleles. *Clin Cancer Res*; 20(17): 4511–9. ©2014 AACR.

### Introduction

Cetuximab is a chimeric IgG1 monoclonal antibody which targets the extracellular domain of the EGF receptor (EGFR; ref. 1). Its antitumor activity is largely secondary to the competitive inhibition of EGFR–ligand interactions, prevention of receptor dimerization and autophosphorylation, and blockage of EGFR signaling through the MAPK and PI3K/AKT/mTOR pathways. Following binding to EGFR,

cetuximab exerts an inhibitory effect on tumor proliferation, survival, motility, invasion, and angiogenesis (2). Studies showed that the activity of this agent is mainly limited to those patients whose tumors lack mutations of the *RAS* genes and cetuximab is currently used for the treatment of *RAS* wild-type metastatic colorectal cancer (3–5).

In contrast with the metastatic setting, cetuximab failed to improve the outcome of patients treated with standard adjuvant chemotherapy (6, 7) and screening for *KRAS* or *RAS* mutations did not appear to identify early-stage tumors with increased sensitivity to EGFR inhibition (6–8). In a recent update of the EXPERT-C trial, we showed a nonsignificant improvement in survival with cetuximab in both the *KRAS/BRAF* wild-type and molecularly unselected population. Moreover, in a retrospective biomarker analysis of this study, patients with *TP53* wild-type tumors had significantly better survival outcomes when treated with cetuximab and the association between *TP53* status and cetuximab benefit was independent of *RAS* (9). We hypothesized that antibody-dependent cell-mediated cytotoxicity (ADCC) may have accounted for most of the beneficial effects of cetuximab observed in this trial.

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### Translational Relevance

The relationship between *FcγRIIa* and *FcγRIIIa* polymorphisms and cetuximab benefit in metastatic colorectal cancer has been investigated with controversial results. However, according to preclinical studies, the mechanism of antibody-dependent cell-mediated cytotoxicity (ADCC) may be more relevant in the presence of micrometastatic disease. We found that in a prospective series of patients with locally advanced rectal cancer, carriers of 131R and 158F alleles had an increased clinical benefit from cetuximab and this effect appeared to be independent of RAS. These results suggest the hypothesis of a dual mechanism of action of cetuximab (i.e., inhibition of EGFR downstream signaling pathways prevalent for macroscopic disease and ADCC prevalent for microscopic foci of disease) and may trigger further research into the role of the immune-mediated effects of cetuximab and other monoclonal antibodies in the adjuvant setting.

ADCC is an alternative mechanism whereby IgG1 monoclonal antibodies can exert their antitumoral properties (10). ADCC is initiated when the antigen-binding fragment (Fab) and the crystalline fragment (FC) of the monoclonal antibody engage the tumor cell antigen and an FC gamma receptor (FcγR) on an effector cell, respectively (11). As a result, antibody-coated tumor cells are attacked and eliminated by activated natural killer (NK) cells, monocytes, and macrophages.

Three distinct classes of FcγRs (FcγRI, FcγRII, and FcγRIII) have been identified and shown to modulate the antitumor activity of monoclonal antibodies by enhancing or inhibiting their immune-mediated, cytotoxic potential (12–14). Moreover, SNPs in the coding regions of the activating *FcγRIIA* (C>T substitution at position 131 which changes the amino acid from histidine to arginine) and *FcγRIIIA* genes (T>G substitution at position 158 which changes the amino acid from valine to phenylalanine) have been reported to correlate with the *in vitro* antitumor activity and *in vivo* clinical benefit from rituximab, trastuzumab, and cetuximab (11, 15–17). However, there are currently no available clinical data on the potential role of ADCC when these monoclonal antibodies are administered in the adjuvant setting.

In this study, we analyzed the association between *FcγRIIIa*–*FcγRIIIa* polymorphisms and cetuximab benefit in a population of patients with high-risk, locally advanced rectal cancer treated within the EXPERT-C trial.

### Materials and Methods

EXPERT-C was an international, multicenter, randomized phase II trial investigating the addition of cetuximab to a sequential treatment strategy with neoadjuvant capecitabine and oxaliplatin (CAPOX) chemotherapy followed

by chemoradiotherapy (CRT), total mesorectal excision (TME), and adjuvant CAPOX (18). Patients were randomized in a 1:1 ratio to receive cetuximab throughout the study treatment or not. Eligibility criteria for EXPERT-C have been described in detail previously (18). Briefly, patients had to have a locally advanced rectal adenocarcinoma with high-risk features according to a baseline MRI. Baseline high-risk features included circumferential resection margin involved or at risk (tumor within 1 mm of mesorectal fascia), T3 distal tumor (tumor at/below levators), extramural extension  $\geq 5$  mm (T3c/T3d), T4 tumor, or presence of extramural vascular invasion. Study procedures, including treatment design, chemotherapy and radiotherapy regimens, timing of surgery, pathologic assessment of surgical specimens, and clinical follow-up, have been previously reported (18).

This study was approved by local ethics committees, and written informed consent was obtained from each patient before study entry.

### *FcγRIIa* and *FcγRIIIa* polymorphisms analysis

DNA was extracted from peripheral blood mononuclear cells (PBMC) with QIAamp DNA Blood Mini Kit on QIAcube (Qiagen). Polymorphism analyses of *FcγRIIa* and *FcγRIIIa* were done as previously described by Bibeau and colleagues, (19), with modifications. Briefly, sequencing analysis of the *FcγRIIa* was done on Genetic Analyser 3730 (Life Technologies), and results were analyzed using Mutation Surveyor Software (Softgenetics). Multiplex reaction with specific *FcγRIIIa*-V and *FcγRIIIa*-F reverse primers were amplified in combination with *FcγRIIIa*-specific forward primer. Given the high degree of sequence homology between *FcγRIIIa* and *FcγRIIIb*, an *FcγRIIIa*-specific forward primer was used whose 3' end of the primer is at a one of the regions where the 2 genes differ in exon 4 (i.e., forward primer for *FcγRIIIa*: TCCAAAAGCCACACTCAAAGAC; homologous sequence in *FcγRIIIb*: TCCAAAAGCCACACTCAAAGAT). Genetic Analyser 3500 (Life Technologies) was used for the fragment analysis of the *FcγRIIIa* amplification products in the presence of Hi-Di Formamide (Life Technologies) and GeneScan 500 LIZ Size Standard (Life Technologies). Results were analyzed using GeneMapper Software (Life Technologies).

### Tumor molecular analyses

Mutational analyses of *KRAS* (exons 2–4), *NRAS* (exons 2–4), and *TP53* (exons 4–9) were performed centrally on genomic DNA extracted from formalin-fixed, paraffin-embedded tissue from pretreatment biopsy and/or primary resection samples as previously described (8, 9, 18).

### Statistical considerations

The primary endpoint of the EXPERT-C trial was complete response (CR) in patients with *KRAS*/*BRAF* wild-type tumors. Radiological response to treatment, pattern of failure, progression-free survival (PFS), and overall survival (OS) were secondary endpoints and calculated as previously reported (18). Distant PFS (DPFS) was

measured from date of randomization to date of extrapelvic progression.

The  $\chi^2$  test was used to assess whether the polymorphic variants of *FcγRIIIa* and *FcγRIIIa* in the study population were in Hardy–Weinberg equilibrium. The Kaplan–Meier method was used to calculate survival estimates, and comparison of the treatment arms was carried out using a log-rank analysis. HRs and 95% confidence intervals (CI) were obtained from Cox regression.

An interaction term between treatment arm and polymorphism status was included in the Cox regression to test for a significant interaction. Multivariate Cox regression was used to assess whether a significant interaction remained significant after addition of known prognostic variables. Variables were included in the multivariate model using forward selection if  $P < 0.1$ .

## Results

Of 164 eligible patients enrolled into the EXPERT-C trial, 106 (64.6%) had a baseline blood sample available for genotyping. One patient was not assessable for both *FcγRIIIa* and *FcγRIIIa* polymorphisms and was not included in this analysis. Of 105 assessable patients, 54 were treated in the CAPOX arm and 51 in the CAPOX-C arm. The study population was representative of the overall trial population (data not shown).

The *FcγRIIIa* and *FcγRIIIa* genotype frequencies are reported in Table 1. Twenty-five patients (23.8%) were homozygous for *FcγRIIIa*-131H allele, 52 (49.5%) were heterozygous (131H/R), and 28 (26.7%) were homozygous for 131R allele. Thirteen patients (12.4%) were homozygous for *FcγRIIIa*-158V allele, 48 (45.7%) were heterozygous (158V/F), and 44 (41.9%) were homozygous for 158F allele. The *FcγRIIIa* and *FcγRIIIa* genotype frequencies were in line with those expected and no deviation from the Hardy–Weinberg equilibrium (*FcγRIIIa*:  $\chi^2 = 0.928$ ; *FcγRIIIa*:  $\chi^2 = 0.987$ ) or association with tumor RAS or TP53 status was observed (data not shown). Baseline

patient characteristics by *FcγR* allelic variants were overall balanced between the treatment arms (Supplementary Table S1).

In the group of patients treated with cetuximab, no statistically significant differences in response to neoadjuvant chemotherapy, CRT, or CR based on the *FcγRIIIa*-H131R or *FcγRIIIa*-V158F polymorphisms were observed (Table 2). However, after a median follow-up of 67.4 months, an increased benefit from cetuximab in terms of PFS was found in favor of *FcγRIIIa*-131R carriers (5-year PFS: 72.5% vs. 45.5%; HR, 0.38; 95% CI, 0.14–1.03;  $P = 0.058$ ) and *FcγRIIIa*-F carriers (5-year PFS: 71.7% vs. 20.0%; HR, 0.21; 95% CI, 0.07–0.66;  $P = 0.007$ ) when compared with 131H/H and 158V/V patients, respectively. Patients with *FcγRIIIa*-131R allele had also a significantly better 5-year DPFS (76.4%) than patients homozygous for *FcγRIIIa*-131H (45.5%; HR, 0.30; 95% CI, 0.11–0.85;  $P = 0.023$ ). A similar difference in OS between the above-mentioned groups was also found; however, this did not reach the statistical significance. In particular, 5-year OS was 82.4% in *FcγRIIIa*-131R carriers versus 63.6% in patients homozygous for 131H allele (HR, 0.42; 95% CI, 0.12–1.45;  $P = 0.171$ ) and 80.0% in *FcγRIIIa*-158F carriers versus 60.0% in patients homozygous for 158V allele (HR, 0.33; 95% CI, 0.07–1.55;  $P = 0.162$ ; Table 3).

Fourteen patients (27.4%) in the CAPOX-C arm were homozygous for 131H and/or 158V allele, whereas 37 (72.6%) carried both 131R and 158F alleles. When these 2 groups were compared, a statistically significant improvement in PFS (HR, 0.22; 95% CI, 0.08–0.57;  $P = 0.002$ ) and OS (HR, 0.24; 95% CI, 0.07–0.78;  $P = 0.018$ ) was observed in favor of the 131R and 158F allele carriers (5-year PFS: 78.4%; 95% CI, 65.1–91.7 vs. 35.7%; 95% CI, 10.6–60.8; 5-year OS: 86.4%; 95% CI, 75.2–97.6 vs. 57.1%; 95% CI, 31.2–83.0; Fig. 1). Moreover, 131R and 158F allele carriers were also found to have a lower incidence of distant tumor recurrence at 5 years (21.6%; 95% CI, 8.3–34.9 vs. 58%; 95% CI, 30.0–86.1; HR 0.27;  $P = 0.012$ ; Table 3).

When we also analyzed the outcome of patients treated in the CAPOX control arm according to the *FcγRIIIa* and *FcγRIIIa* polymorphisms, an interaction between cetuximab benefit and presence of 131R and 158F alleles was found for PFS ( $P = 0.017$ ) and DPFS ( $P = 0.032$ ). After adjusting for prognostic variables, including World Health Organization (WHO) performance status, T4, RAS status, TP53 status, and skin rash, this interaction remained significant for both PFS ( $P = 0.003$ ) and DPFS ( $P = 0.028$ ). An interaction, although not statistically significant, was also observed for OS ( $P = 0.08$ , adjusted  $P$  value = 0.09; Table 4).

Tumor mutational status of RAS was available for 98 of 105 (93.3%) patients. Tumor mutational status of TP53 was available for 94 of 105 (89.5%) patients. Although further stratification by RAS and TP53 status reduced significantly the number of assessable patients in each group, the beneficial effect of cetuximab in the presence of 131R and 158F alleles did not appear to be significantly influenced by the either RAS or TP53 tumor status (Table 5).

**Table 1.** *FcγRIIIa* and *FcγRIIIa* polymorphisms in the study population by treatment arm

<b>FcγR polymorphism</b>	<b>CAPOX n (%)</b>	<b>CAPOX-C n (%)</b>
<i>FcγRIIIa</i>		
HH	14 (25.9)	11 (21.6)
HR	27 (50.0)	25 (49.0)
RR	13 (24.1)	15 (29.4)
<i>FcγRIIIa</i>		
FF	22 (40.7)	22 (43.1)
VF	24 (44.4)	24 (47.1)
VV	8 (14.8)	5 (9.8)
<i>FcγRIIIa</i> / <i>FcγRIIIa</i>		
HH and/or VV	17 (31.5)	14 (27.5)
R and F	37 (68.5)	37 (72.5)

**Table 2.** Response to neoadjuvant chemotherapy, CRT, and complete response rate by *FcγRIIIa* and *FcγRIIIb* polymorphisms and treatment

<i>FcγR</i> polymorphism	Response rate after neoadjuvant chemotherapy (%)		Response rate after CRT (%)		Treatment by <i>FcγR</i> SNP interaction		Complete response rate (%)		Treatment by <i>FcγR</i> SNP interaction	
	CAPOX (n = 54)	CAPOX-C (n = 51)	CAPOX (n = 54)	CAPOX-C (n = 51)	P	P	CAPOX (n = 54)	CAPOX-C (n = 51)	P	P
HH	63.6	63.6	75.0	80.0	0.735	0.944	7.1	18.2	0.944	0.434
HR	50.0	65.2	72.0	82.6			14.8	12.0		
RR	41.7	64.3	83.3	85.7			7.7	26.7		
HH	63.6	63.6	75.7	83.8	0.475	0.854	12.5	17.5	0.854	0.645
HR/RR	47.4	64.9	75.0	80.0			7.1	18.2		
VV	33.3	100	50.0	100	0.546	0.976	0	20.0	0.976	0.808
VF	59.1	56.5	72.7	78.3			8.3	16.7		
FF	47.6	68.2	85.7	86.4			18.2	18.2		
VV	33.3	100	50.0	100	0.999	0.999	0	20.0	0.999	0.999
VF/FF	53.5	62.2	79.1	82.2			13.0	17.4		
HH and/or VV	57.1	66.7	73.3	81.8	0.815	0.957	5.9	14.3	0.957	0.686
R and F	48.6	63.9	76.5	83.3			13.5	18.9		

**Table 3.** Survival outcomes by *FcγRIIIa* and *FcγRIIIb* polymorphisms in the group of patients treated with cetuximab (n = 51)

<i>FcγR</i> SNPs	5-y PFS (95% CI)		HR (95% CI)		5-y DPFS (95% CI)		HR (95% CI)		5-y OS (95% CI)		HR (95% CI)		P	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P		
HH	45.5 (16.1–74.9)	0.100	1.0	[0.100]	45.5 (16.1–74.9)	0.058	1.0	[0.052]	63.6 (35.2–92.0)	0.171	1.0	[0.252]		
HR	80.0 (64.3–95.7)	0.032	0.27 (0.08–0.90)	0.032	83.5 (68.6–98.4)	0.331	0.21 (0.06–0.74)	0.016	88.0 (75.3–100)	0.100	0.28 (0.06–1.27)	0.100		
RR	60.0 (35.3–84.7)	0.331	0.57 (0.18–1.77)	0.331	64.3 (39.2–89.4)	0.058	0.46 (0.14–1.51)	0.198	72.7 (49.8–95.6)	0.567	0.66 (0.16–2.67)	0.567		
HH	45.5 (16.1–74.9)	0.100	1.0	[0.100]	45.5 (16.1–74.9)	0.058	1.0	[0.052]	63.6 (35.2–92.0)	0.171	1.0	[0.252]		
HR/RR	72.5 (58.6–86.4)	0.032	0.38 (0.14–1.03)	0.032	76.4 (62.9–89.9)	0.007	0.30 (0.11–0.85)	0.023	82.4 (70.6–94.2)	0.100	0.42 (0.12–1.45)	0.100		
VV	20.0 (0–55.1)	0.017	1.0	[0.017]	33.3 (0–86.7)	0.007	1.0	[0.250]	60.0 (17.1–100)	0.162	1.0	[0.271]		
VF	62.5 (43.1–81.9)	0.039	0.28 (0.09–0.94)	0.039	62.5 (43.1–81.9)	0.005	0.54 (0.12–2.53)	0.438	74.2 (59.9–88.5)	0.308	0.43 (0.09–2.16)	0.308		
FF	81.8 (65.7–97.9)	0.005	0.14 (0.03–0.55)	0.005	81.8 (65.7–97.9)	0.007	0.25 (0.05–1.41)	0.117	86.4 (72.1–100)	0.107	0.23 (0.04–1.37)	0.107		
VV	20.0 (0–55.1)	0.017	1.0	[0.017]	33.3 (0–86.7)	0.007	1.0	[0.250]	60.0 (17.1–100)	0.162	1.0	[0.271]		
VF/FF	71.7 (58.8–84.6)	0.002	0.21 (0.07–0.66)	0.002	71.7 (58.8–84.6)	0.002	0.41 (0.09–1.81)	0.012	80.0 (68.6–91.8)	0.018	0.33 (0.07–1.55)	0.018		
HH and/or VV	35.7 (10.6–60.8)	0.002	1.0	[0.002]	42.0 (13.9–70.0)	0.002	1.0	[0.002]	57.1 (31.2–83.0)	0.018	1.0	[0.018]		
R and F	78.4 (65.1–91.7)	0.002	0.22 (0.08–0.57)	0.002	78.4 (65.1–91.7)	0.002	0.27 (0.10–0.75)	0.012	86.4 (75.2–97.6)	0.018	0.24 (0.07–0.78)	0.018		

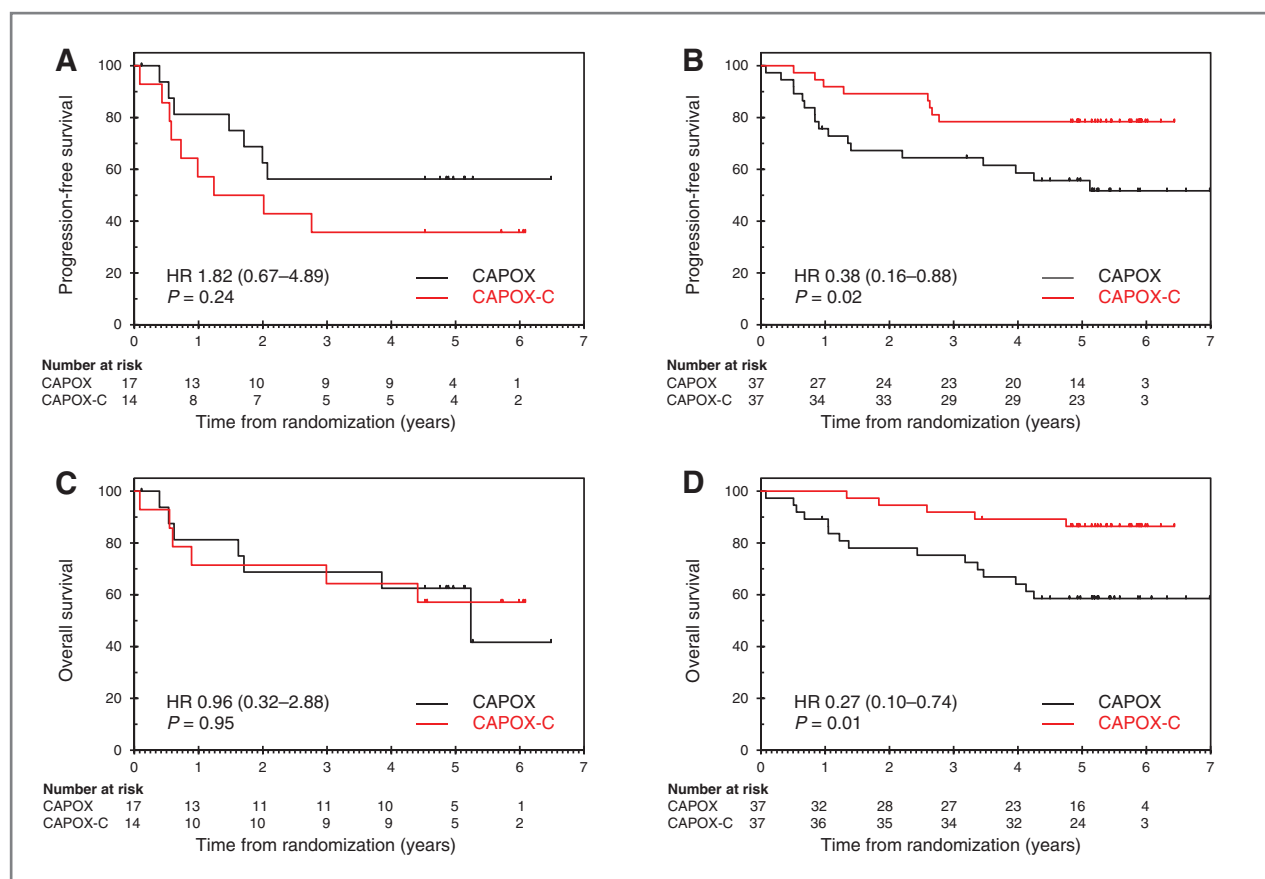


Figure 1. Kaplan-Meier curves for PFS and OS in patients homozygous for 131H and/or 158V (A and C) and in carriers of 131R and 158F alleles (B and D).

## Discussion

To our knowledge, this is the first study that investigated the potential role of *FcγRIIIa* and *FcγRIIIa* polymorphisms in predicting cetuximab benefit in the perioperative setting of colorectal cancer. We showed that in patients with locally advanced rectal cancer but without radiologic evidence of metastatic disease, polymorphic variants of *FcγRIIIa* and *FcγRIIIa* were associated with increased clinical benefit from cetuximab. In particular, we found that patients carrying 131R and 158F alleles had better survival than patients homozygous for the 131H and/or 158V alleles when cetuximab was administered in association with systemic chemotherapy and CRT in the perioperative setting.

On the basis of the *in vitro* data suggesting an increased binding affinity of *FcγRIIIa* and *FcγRIIIa* for IgG when amino acid changes occur at specific positions in the IgG binding domain, several studies have investigated the predictive role of genetic polymorphisms of these *FcγRs* in patients treated with cetuximab for metastatic colorectal cancer (19–31). Possibly due to the retrospective design, small numbers, variable endpoints, heterogeneity of patient populations and treatments received in association with cetuximab, and methodological issues, the findings of these studies have been inconsistent (11). As a result, although ADCC may

account for some of the antitumor effects of cetuximab, inhibition of the EGFR signaling pathway is generally considered the main mechanism of action of this monoclonal antibody and mutations downstream of EGFR are the only validated markers used to predict treatment outcome and guide patient selection (32).

However, it is legitimate to hypothesize that the main reason for the failure to consistently replicate *in vivo* the association observed *in vitro* between genetic polymorphisms of *FcγRs* and increased activity of cetuximab may be the influence of tumor burden on ADCC against cancer cells. The ability of the immune effector cells to kill antibody-coated tumor cells is dependent on the tumor microenvironment and ADCC may be more effective against micro-metastases than large tumor lesions, which are less accessible to immune effector cells and are more likely to have acquired resistance to the mechanisms of immune-mediated cytotoxicity. In support of this contention, a study conducted in intrinsically trastuzumab-resistant, HER-2-positive breast cancer xenografts, the ability of trastuzumab to inhibit tumor growth by ADCC was maintained only in the presence of microscopic disease which simulated the general conditions of adjuvant therapy (33). Moreover, the immune-mediated effects of IgG1 monoclonal antibodies are highly dependent on the functional activity of NK cells

**Table 4.** Survival outcomes by *FcγRIIIa* and *FcγRIIIa* polymorphisms and treatment

<i>FcγR</i> polymorphism	Treatment by <i>FcγR</i> SNP interaction			Treatment by <i>FcγR</i> SNP interaction			Treatment by <i>FcγR</i> SNP interaction		
	5-y PFS (95% CI)	CAPOX (n = 54)	CAPOX-C (n = 51)	5-y DPFS (95% CI)	CAPOX (n = 54)	CAPOX-C (n = 51)	5-y OS (95% CI)	CAPOX (n = 54)	CAPOX-C (n = 51)
HH	61.5 (35.0-88.0)	45.5 (16.1-74.9)	45.5 (16.1-74.9)	80.0 (55.3-100)	80.0 (44.9-100)	45.5 (16.1-74.9)	69.2 (44.1-94.3)	63.6 (35.2-92.0)	0.186
HR	45.9 (26.5-65.3)	80.0 (64.3-95.7)	63.5 (44.3-82.7)	63.5 (44.3-82.7)	63.5 (44.3-82.7)	83.5 (68.6-98.4)	50.2 (30.9-69.4)	88.0 (75.3-100)	
RR	69.2 (44.1-94.3)	60.0 (35.3-84.7)	81.8 (59.1-100)	81.8 (59.1-100)	84.3 (39.2-89.4)		69.2 (44.1-94.3)	72.7 (49.8-95.6)	
HH	61.5 (35.0-88.0)	45.5 (16.1-74.9)	45.5 (16.1-74.9)	80.0 (55.3-100)	45.5 (16.1-74.9)		69.2 (44.1-94.3)	63.6 (35.2-92.0)	0.232
HR/RR	53.9 (38.2-69.6)	72.5 (58.6-86.4)	69.2 (53.9-84.5)	69.2 (53.9-84.5)	76.4 (62.9-89.9)		56.6 (41.1-72.1)	82.4 (70.6-94.2)	
VV	42.9 (6.3-79.6)	20.0 (0-55.1)	33.3 (0-86.6)	33.3 (0-86.6)			42.9 (6.2-79.6)	60.0 (17.1-100)	0.880
VF	50.0 (30.0-70.0)	62.5 (43.1-81.9)	70.7 (50.9-90.5)	62.5 (43.1-81.9)			58.3 (38.5-78.1)	74.2 (56.4-92.0)	
FF	67.3 (47.3-87.3)	81.8 (65.7-97.9)	70.8 (51.0-90.6)	81.8 (65.7-97.9)			67.0 (47.0-86.9)	86.4 (72.1-100)	
VV	42.9 (6.3-79.6)	20.0 (0-55.1)	33.3 (0-86.6)	33.3 (0-86.6)			42.9 (6.2-79.6)	60.0 (17.1-100)	0.666
VF/FF	57.9 (43.4-72.4)	71.7 (58.8-84.6)	70.7 (56.8-84.6)	71.7 (58.8-84.6)			62.4 (48.3-76.5)	80.2 (68.6-91.8)	
HH and/or VV	56.3 (32.0-80.6)	35.7 (10.6-60.8)	75.5 (51.4-99.6)	42.0 (14.0-70.0)			62.5 (38.8-86.2)	57.1 (31.2-82.9)	0.080
R and F	55.7 (38.8-72.6)	78.4 (65.1-91.7)	69.8 (54.1-85.5)	78.4 (65.1-91.7)			58.5 (42.4-74.6)	86.4 (75.2-97.6)	

**Table 5.** Survival outcomes by *FcγRIIIa* and *FcγRIIIa* polymorphisms and treatment in *RAS*-mutant versus *RAS* wild-type patients and *TP53*-mutant versus *TP53* wild-type patients

<i>FcγR</i> polymorphism	Biomarker status	Treatment by <i>FcγR</i> SNP interaction			Treatment by <i>FcγR</i> SNP interaction			Treatment by <i>FcγR</i> SNP interaction		
		5-y PFS (95% CI)	CAPOX	CAPOX-C	5-y DPFS (95% CI)	CAPOX	CAPOX-C	5-y OS (95% CI)	CAPOX	CAPOX-C
HH and/or VV	<i>RAS</i> mutant	44.4 (11.9-76.9)	0 (0-0)	0 (0-0)	66.7 (29.1-100)	0 (0-0)	0 (0-0)	55.6 (23.1-88.1)	50.0 (0-100)	0.349
R and F	(n = 46)	58.2 (32.5-83.9)	70.6 (48.8-92.3)		74.3 (48.8-99.8)	70.8 (49.0-92.6)		57.8 (31.9-83.7)	81.9 (63.3-100)	
HH and/or VV	<i>RAS</i> wild-type	71.4 (37.9-100)	50.0 (10.0-89.9)	0.064	83.3 (53.5-100)	50.0 (10.0-89.9)	0.076	71.4 (37.9-100)	66.7 (29.1-100)	0.182
R and F	(n = 52)	55.0 (32.9-77.1)	84.2 (67.7-100)		68.6 (47.8-89.4)	84.2 (67.7-100)		60.0 (38.4-81.6)	89.5 (75.8-100)	
HH and/or VV	<i>TP53</i> mutant	50.0 (10.0-90.0)	14.3 (0-40.2)	0.070	80.0 (44.9-100)	17.1 (0-47.7)	0.059	50.0 (10.0-90.0)	42.9 (6.2-79.6)	0.281
R and F	(n = 49)	47.6 (21.9-73.3)	63.2 (41.4-84.9)		54.7 (27.3-82.1)	63.2 (41.4-84.9)		54.2 (29.1-79.3)	78.9 (60.5-97.3)	
HH and/or VV	<i>TP53</i> wild-type	60.0 (29.6-90.4)	66.7 (13.4-100)	0.322	75.0 (45.0-100)	66.7 (13.4-100)	0.382	70.0 (41.6-98.4)	100 (100-100)	0.987
R and F	(n = 45)	53.5 (29.4-77.6)	92.9 (79.4-100)		81.6 (62.8-100)	92.9 (79.4-100)		61.1 (38.6-83.6)	93.3 (80.0-100)	

which is known to be significantly impaired in patients with advanced disease when compared with patients with early-stage disease or healthy individuals (34, 35).

Altogether, these data suggest the hypothesis of a dual mechanism of action for cetuximab (i.e., inhibition of EGFR downstream signaling pathways prevalent for macroscopic tumor lesions and ADCC prevalent for microscopic foci of disease). We acknowledge that our *post hoc*, retrospective analysis of a relatively small phase II trial cannot definitively confirm this hypothesis but provides further supportive evidence. Intriguingly, we observed a disconnect between radiologic response of the primary tumor and long-term outcome. Although *FcγRIIIa*-131H and *FcγRIIIa*-158V polymorphisms predicted favorable survival outcomes with cetuximab, they did not identify patients with a higher rate of tumor response during preoperative treatment or pathologic complete response at the time of surgery. In contrast, we have previously shown that the mutational status of *KRAS* (or *RAS*) maintained its predictive value for response of the primary tumor to cetuximab but did not significantly correlate with the potential, long-term beneficial effect of this drug (8, 9). Interestingly, and consistently with the hypothesis of ADCC as an important mechanism of action of cetuximab against micrometastases, we found that carriers of 131R and/or 158F alleles had a significantly lower risk of distant failure than patients homozygous for 131H and/or 158V when cetuximab was added to the study sequential treatment. It is worth noting that additional signaling pathways beyond EGFR (i.e., IGF-1R pathway) which have been shown to cross-talk with EGFR downstream effectors and mediate the ADCC-independent therapeutic effects of cetuximab are more frequently activated and potentially more clinically relevant in rectal cancer than in colon cancer (36–38). Therefore, our findings could be potentially influenced by the specific location of the primary tumor and may not apply to a population of patients with colon cancer.

To assess the predictive value of *FcγRs* polymorphisms independent of the inhibitory effects of cetuximab on the EGFR signaling pathways, we analyzed treatment outcomes by *RAS* status (including *KRAS* exon 2–4 and *NRAS* exon 2–4). The ability of *FcγRIIIa*-131R and *FcγRIIIa*-158V alleles to predict cetuximab benefit was largely independent of the status of *RAS*. Moreover, and in contrast with our initial hypothesis, the predictive value of these polymorphic variants seemed to be independent of *TP53*. However, the limited number of patients in these subgroup analyses precludes any definitive conclusion. Larger studies are needed to evaluate the role of ADCC in the adjuvant setting in relation to *RAS* mutations. Moreover, further investigation is necessary to assess whether enhanced ADCC may be the main mechanism underlying the increased beneficial effects of cetuximab previously observed in patients with *TP53* wild-type tumors treated in this trial (9).

Our results outlining the predictive role of the 131R allelic form of *FcγRIIIa* are in line with *in vitro* experiments showing that the presence of arginine at amino acid position 131 is associated with increased binding affinity for

IgG1 (12). In contrast, IgG binding studies with innate immune effector cells and the anti-CD20 monoclonal antibody rituximab show that the polymorphic variant 158V has the strongest interaction with IgG and the highest ability to promote the ADCC-related therapeutic response to this agent (39, 40). However, it is reasonable to argue that given the interference from several confounding factors, including the immune-suppression effect of the accompanying chemotherapy agents, the interaction between immune effector cells, monoclonal antibodies, and cancer cells *in vivo*, may be by far more complex than that simulated in *in vitro* conditions (41, 42). Moreover, it is worth noting that preclinical studies demonstrated that the different binding affinity of the *FcγRIIIa* genotypes was maintained at low concentrations but abolished at saturating concentrations of IgG (15, 43), and the 158F allele was found to be predictive of cetuximab benefit in previous retrospective and prospective series (20–22, 28).

For the purpose of this study, an *FcγRIIIa*-specific primer was used to minimize the risk of amplifying *FcγRIIIb*. Indeed, the high degree of sequence homology between these genes has represented one of the major difficulties in analyzing the *FcγR* polymorphisms and likely one of the most important reasons of the large inconsistency between studies. Importantly, the frequencies of *FcγR* haplotypes and alleles found in our study population were in line with those described in 2 large datasets of the Single Nucleotide Polymorphism Database (dbSNP) of the National Center for Biotechnology Information (NCBI; ss491608261 CSAGilent and ss342007110 ESP cohort populations; ref. 44), and no deviation from the Hardy–Weinberg equilibrium was observed.

We recommend caution in the interpretation of the results of this study, which remain hypothesis-generating. Moreover, the proposed explanation of our findings is speculative and requires confirmation in future pharmacogenomic studies of cetuximab in a similar patient population. Indeed, our analysis has several limitations, including the retrospective design, the limited number of trial patients eligible for genotyping, the overall small number of patients included in the analysis, and the lack of a validation set. Moreover, the inclusion of radiotherapy into the multimodality treatment strategy may have potentially introduced significant biases in the ascertainment of the true predictive effect of *FcγR* polymorphisms. However, this is the first time that the clinical relevance of cetuximab-associated ADCC in early-stage colorectal cancer has been analyzed in a prospective randomized trial. The data from this study could inform and encourage further investigation of the role of the immune-mediated effects of cetuximab and other monoclonal antibodies in the adjuvant setting.

#### Disclosure of Potential Conflicts of Interest

D. Cunningham reports receiving commercial research grants from Amgen, Merck Serono, Roche, and Sanofi-Aventis. I. Chau is a consultant/advisory board member for Bristol Myers Squibb, Eli-Lilly, Gilead Science, Merck-Serono, Novartis, Roche, and Sanofi Oncology; reports receiving research grants from Merck-Serono, Novartis, Roche, and Sanofi Oncology; and speakers bureau honoraria from Eli-Lilly, Roche, Sanofi-Oncology, and Taiho. No potential conflicts of interest were disclosed by the other authors.

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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** F. Sclafani, D. Gonzalez de Castro, S. Hulkki Wilson, C. Peckitt, J. Capdevila, I. Chau

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# Clinical Cancer Research

## ***FcγRIIa* and *FcγRIIIa* Polymorphisms and Cetuximab Benefit in the Microscopic Disease**

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