Journal of Genetic Syndromes & Gene Therapy

Formica et al., J Genet Syndr Gene Ther 2013, 4:10
DOI: 10.4172/2157-7412.1000193

Review Article Open Access

Are Fragment C Gamma Receptor (FCGR) Germline Polymorphisms Predictive Biomarkers in Metastatic Colorectal Cancer?

Formica V*, Cereda V, Nardecchia A, Morelli C, Lucchetti J and Roselli M

Medical Oncology Unit, 'Tor Vergata' Clincal Center, University of Rome, Italy

Abstract

The treatment of metastatic colorectal cancer has undergone significant improvements over the last decade with the introduction of molecularly targeted monoclonal antibodies (mAbs). Among these, cetuximab, a chimeric IgG1 antibody directed against the extracellular domain of EGFR, has demonstrated a survival benefit for KRAS wild type (WT) tumors. Even though KRAS genotyping has significantly refined patient selection allowing an increase in tumor radiological response from 25% of the whole metastatic colorectal cancer (MCRC) population to 45% of the KRAS-WT subset, still for a significant proportion of patients anti-EGFR mAbs will be ineffective, thus resulting in unnecessary toxicity and cost.

Polymorphisms of Fragment C gamma receptor (FCGR) gene have the potential for being used as predictive biomarkers of cetuximab activity since part of this drug's tumoricidal effect is immune-mediated via FcγR-triggered ADCC (antibody-dependent cellular cytotoxicity). The aim of the present review is to summarize available data on the predictive/prognostic value of FCGR polymorphisms for cetuximab-treated MCRC patients and to discuss possible future directions in this area of research.

Keywords: Mcrc; Cetuximab; Anticancer mabs

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the fourth leading cause of cancer-related deaths worldwide [1]. When metastatic (MCRC), it is largely incurable and palliative chemotherapy represents the treatment of choice. Recently introduced monoclonal antibodies directed against the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) have produced substantial improvements in MCRC clinical outcome with a prolongation of median survival from 12 to 22-24 months [2].

Cetuximab, a chimeric immunoglobulin G 1 (IgG1) anti-EGFR monoclonal antibody (mAb), has been proven effective in MCRC without mutations in the downstream molecules of EGFR pathway such as KRAS.

However, even in the KRAS wild type enriched population, cetuximab produces, in combination with standard chemotherapy, a tumor overall response rate (ORR) ranging from 35-45% in the first-line setting.

Moreover, the action of this drug seems to be exerted beyond the mere interference with EGFR-related growth signal on cancer cell. Indeed, responses to cetuximab are also observed for tumors with constitutively activated EGFR pathway secondary to KRAS mutation. In particular, De Roock et al. [3] found in in vitro and in vivo mouse models that KRAS-G13D-mutated colorectal cancer cells were similarly killed by cetuximab as were KRAS wild-type cells. They confirmed this finding in the clinical setting by showing, in a pooled analysis of several randomized trials, a survival gain from the addition of cetuximab in patients carrying the KRAS-G13D tumor mutation.

Therefore, further reliable and predictive biomarkers are needed to more accurately select patients responsive to cetuxiamab and minimize unwanted toxicity and costs.

Since part of cetuximab activity is immune-mediated via activation of effector cell receptors binding the immunoglobulin fragment C (Fc), fuctional polymorphisms (single nucleotide polymorphisms, SNPs) of fragment c-γ receptors (FCGR2A and FCGR3A) are ideal candidate as predictive biomarkers. Moreover, the immune-mediated antitumor activity of cetuximab might also justify the benefit seen for some KRAS mutated tumors.

The immune response triggered by cetuximab via FcγR activation is defined Antibody dependent cellular cytotoxicity (ADCC). It relies on the bifunctional property of the IgG1 which can form a bridge by binding the EGFR expressed on tumor cell surface (with the idiotype moiety) and, at the same time, the FcγRs on immune cells (such as Natural Killer cells, Macrophages, Antigen Presenting Cells). The tumor cell-IgG1-FcγR interaction triggers the lytic attack or phagocytosis of tumor target cells by immune effector cells.

Three classes of closely related FcγR have been identified (FcγR1/CD64, FcγR2/CD32 and FcγR3/CD16) that are differentially encoded by a set of 10 genes located on chromosome 1 with high sequence homology (FCGR1A, FCGR1B, FCGR1C; FCGR2A, FCGR2B1, FCGR2B2, FCGR2B3, FCGR2C; FCGR3A and FCGR3B) [4]. Fuctional single nucleotide polymorphisms (SNPs) have been identified at position 131 of FCGR2A and at position 158 of FCGR3A which determine a substitution from histidine to arginine (H131R) and from valine to phenylalanine (F158V), respectively. In particular the FcγR IIIa 158 V has been demonstrated to possess a higher affinity for the IgG1 Fc and to induce an increased ADCC of cetuximab-coated tumor cells [5], while its expression level remains unchanged [6].

***Corresponding author:** Vincenzo Formica, MD, Medical Oncology Unit, 'Tor Vergata' Clincal Center University of Rome, Viale Oxford, 81 00133 Rome, Italy, Tel: +390620908190; Fax: +390620903804; E-mail: v.formica1@gmail.com

Received September 03, 2013; **Accepted** October 15, 2013; **Published** October 25, 2013

Citation: Formica V, Cereda V, Nardecchia A, Morelli C, Lucchetti J, et al. (2013) Are Fragment C Gamma Receptor (FCGR) Germline Polymorphisms Predictive Biomarkers in Metastatic Colorectal Cancer? J Genet Syndr Gene Ther 4: 193. doi:10.4172/2157-7412.1000193

Apart from cetuximab, the influence of ADCC has also been observed with other widely used monoclonal antibodies (mAbs) targeting surface cancer antigens, such as rituximab and trastuzumab. The aim of the present review is to briefly review the current knowledge on the influence of ADCC and FCGR polymorphisms for commonest used anticancer mAbs and to analyze in depth, and when possible meta-analyze, available data on the impact of FCGR polymorphisms on outcome of cetuximab-treated MCRC patients.

FCGR Snps and Commonly used Anticancer Mabs

ADCC has been identified as an effective immune-based mode of action for therapeutic mAbs of IgG1 class, as Fc-γ receptors display the highest affinity for this class of molecules and less or even absent binding to immunoglobulins of the IgG2 isotype.

Currently, the anti-CD20 rituximab, the anti-HER2 trastuzumab and the anti-EGFR cetuximab are IgG1 MAbs approved and widely used for B cell lymphomas and leukemias, HER2-positive breast and gastric cancer, and for head and neck and KRAS wild-type colorectal carcinomas, respectively. Rituximab and trastuzumab were initially investigated for differential activity on the basis of functional polymorphisms of FCGRs.

The possible influence of FCGR SNPs on trastuzumab efficacy was initially documented in two small retrospective series including, collectively, 89 metastatic and 15 locally advanced breast cancer patients. It was observed that FCGR-131 H/H and FCGR-158 V/V genotypes were associated with increased radiological response, pathological response and progression-free **survival [7,8].** However, Hurvits et al. reviewed the FCGR genotpypes in nearly 1200 patients enrolled in the BCIRG-006 phase III adjuvant trial, and found neither a prognostic nor a predictive role for FCGR SNPs as any disease free survival differences were noted according to different FCGR alleles, both in the trastuzumab and chemotherapy-only **arm** [**9**].

A number of retrospective studies including rituximab monotherapy in follicular lymphoma patients have shown an association between FCGR-158 V/V genotype and increased response to therapy. In particular, in a series of 49 patient's complete response at two months were 100% for FCGR-158 V/V and 68% for the other genotypes [10]. In another cohort of 87 patients, progression free survival at 2 years was 45%, 12% and 16% for FCGR-158 V/V, V/F and F/F patients, respectively [11]. However in the larger analysis of follicular lymphoma patients included in the PRIMA trial (n=460), a phase III randomized study exploring the role of rituximab maintenance after induction chemoimmunatherapy, no influence of FCGR polymorphisms was observed on progression-free survival (PFS), whilst FCGR-158 V/V genotype was associated with increased grade 3-4 neutropenia [12].

FCGR Snps and Cetuximab in Mcrc Patients

Methods

We searched pubmed for "cetuximab", "fragment c gamma receptor polymorphisms" and "colorectal cancer" key terms, relevant retrieved articles were scanned in their reference lists for additional studies of interest.

As of August 2013, we indentified 12 retrospective studies exploring the predictive/prognostic role of FCGR polymorphisms in cetuximab-treated MCRC patients (Table 1).

Results

There is still wide uncertainty on what extent cetuximab immune effects add to the functional inhibition of EGFR activation accomplished by this drug. Further complexity derives from the usual combination regimens applied for cetuximab as it is often administered in addition to the chemotereputic drugs fluorouracil, irinotecan or oxaliplatin. It has been documented that chemotherapy may alter EGFR expression on cancer cells. Having ascertained the functional effect of FCGR SNPs on ADCC, it remains to clarify on what extent they could be prognostic as determinants of a general antitumor immune response independent of cetuximab activity or rather predictive biomarker specific for cetuximab action.

Moreover, the concomitant administration of chemotherapy may significantly influence cetuximab-related ADCC, as it can either increase tumor antigen shedding (and hence adoptive immune response) via direct cancer cell killing or restrain immunostimulatory pathways because of its myelotoxic side effects.

Finally, the timing of cetuximab-driven immune reaction is still to be elucidated, as this could substantially influence the type of clinical endpoint that is most affected. An 'acute' effect of this immune reaction would mostly increase the radiological response rate whilst a chronic effect of immune-protection (vaccine-like) 'in the long run' would translate in survival advantage (PFS or OS). In the latter case, significant results would not be expected if FCGR SNPs were analysed in the third line setting of treatment where patient lifespan are anticipated to be relatively short.

Therefore, to unravel whether the line or combination of therapy would influence the predictive role of FCGR SNPs, in the present review retrieved data are classified according to the association or not with chemotherapy and the administration as first or subsequent line of treatment.

FCGR Polymorphisms and Single Agent Cetuximab

Zhang et al. were the first to report a possible influence of FCGR polymorphisms on cetuximab activity in colorectal cancer [13]. They evaluated the effect of FCGR2A-H131R and FCGR3A-F158V alleles in a sample of 39 subjects derived from the larger phase II single-arm trial of cetuximab monotherapy in heavily pre-treated EGFR-expressing MCRC patients [14]. To the best of our knowledge, this is the only published study reporting on FCGR polymorphisms in MCRC treated with cetuximab monotherapy.

The subgroup selected for FCGR analysis was considered adequately representative of the whole trial population as no differences in outcome were noted between the two patient cohorts (approximately, median progression free survival (PFS) 2 months and median overall survival (OS) 6 months for both). Authors found a shorter PFS for patients carrying the FCGR2A-131 R/R or FCGR3A-158 V/V genotype, Relative Risk 1.43 and 2.28, p values 0.037 and 0.055, respectively, thus stating that the two polymorphisms were independently associated with PFS. However the most significant difference was found for patients in whom the contemporary presence of at least one H and one F allele occurred, suggesting a possible functional interaction between the two receptors (stratified Relative Risk for PFS for patients with either R/R or V/V: 5.37, 95% CI 1.92-15.0, p=0.001). Moreover, Relative Risk for R/R and V/V genotypes evaluated singularly had 95% Confidence Intervals that crossed the unity, indicating a weak statistical significance when the two genes are considered separately. Finally tumor response to treatment was observed only in two cases making it difficult to discern a possible predictive value of the polymorphisms in addition to the prognostic effect of H+F allele combination. KRAS mutational status was not available.

Study	related clinical trial	N. of patients	Type of cetuximab treatment	line of therapy	main results
Zhang et al. [13]	phase II (ImClone 0144)	39	cetuximab monotherapy	3rd line	Shorter PFS for patients with FCGR2A R/R or FCGR3A V/V
Bibeau et al. [19]	None	69	cetuximab + irinotecan	3rd line	PFS advantage for FCGR3A V/V
Calemma et al. [20]	None	49	cetuximab + various chemotherapy	1st, 2nd and 3rd line	Response Rate and PFS advantage for FCGR3A V/V
Rodríguez et al. [21]	None	44	cetuximab+various chemotherapy	1st, 2nd and 3rd line	higher percentage of patients progressing within 6 months in the FCGR2A R/R and FCGR3A F/F groups
Etienne-Grimaldi et al. [15]	phase II (CETUFTIRI)	51	cetuximab + UFT + irinotecan	1st line	Shorter OS for FCGR3A F/F
Park et al. [22]	None	107	cetuximab+irinotecan	3rd line	no significant association of FCGR SNPs with outcome
Dahan et al. [23]	None	56	cetuximab+various chemotherapy	1st, 2nd and 3rd line	Shorter OS for FCGR3A V/V
Zhang et al. [24]	phase II (BOND-2)	65	cetuximab + bevacizumab ± irinotecan	3rd line	Increased response for FCGR3A F/F only in cetuximab + bevacizumab treated patients
Paez et al. [25]	None	104	cetuximab + various chemotherapy	1st, 2nd and 3rd line	no significant association of FCGR SNPs with outcome
Pander et al. [16]	phase III (CAIRO-2)	122	cetuximab + oxaliplatin + capecitabine + bevacizumab	1st line	PFS advantage for FCGR3A F/F
Graziano et al. [27]	None	110	cetuximab + irinotecan	3rd line	no significant association of FCGR SNPs with outcome
Negri et al. [26]	None	86	cetuximab + various chemotherapy	1st, 2nd and 3rd line	no significant association of FCGR SNPs with outcome

Abbreviation: PFS: progression Free Survival; OS: Overall Survival; SNPs: Single Nucleotide Polymorphisms

Table 1: Main characteristics of retrieved retrospective studies.

FCGR Polymorphisms and Cetuximab+Chemotherapy Combination in the First-line Setting

Two studies reported the effect of FCGR polymorphisms in MCRC patients treated with firstline cetuximab+chemotherapy.

In an ancillary study of the firstline phase II CETUFTIRI (cetuximab+tegafur-uracil+irinotecan) trial, including chemonaive metastatic patients with no KRAS selection, both FCGR2A-131 and FCGR3A-158 SNPs were evaluated. Patients with FCGR3A-158 V/F or V/V genotype have significantly longer survival compared with FCGR3A-158 F/F patients (Median overall survival 20.9 months (20 patients and 18 deaths) vs. 12.4 months (31 patients and 23 deaths), respectively, Log rank test: P=0.032) [15].

Pander et al reported the largest dataset regarding the impact of FCGR polymorphisms on outcome of cetuximab-treated MCRC patients. Included participants were from the large phase III trial, CAIRO-2, randomizing KRAS wild type chemo-naive patients to either capecitabine+oxalipaltin+bevacizumab or the same+cetuximab. In the cetuximab treated group (n=122), FCGR3A-158 F/F genotype (called by the authors FCGR3A 818 A/A) was associated with a significantly longer PFS compared to F/V or V/V, mPFS 12.8 v 8.2 months, HR 1.57, p=0.025 [16].

This is the only study derived from a large phase III trial and including a no-cetuximab control arm. FCGR3A 158 F/F genotype was found to correlate with a significantly longer PFS only in the cetuximab-treated arm, making this feature truly predictive of cetuximab effect. Surprisingly, the F variant produces a weaker binding of Fc gamma IIIA receptor to IgG1 antibodies resulting in reduced ADCC [17]. Pander motivated this apparent incongruity by speculating that a poor FcγR/IgG1 binding may also result in a reduced activation of tumor associated macrophages (TAMs). Since TAMs may facilitate tumor progression by releasing pro-angiogenic factors such as VEGF and metalloproteinases, the FCGR-158 F/F genotype will ultimately result in prolonged progression-free survival for patients on cetuximab.

These speculations were actually confirmed in an ex-vivo immunohistochemical and in vitro analysis by the same authors [18]. They analysed tumor tissue from 10 radically resected colon cancer patients who ultimately relapsed at follow-up and found an intense infiltration by tumor-promoting M2 macrophages (characterized by the CD68+ CD163+ immunophenotype) while nearly absent infiltration by antitumor natural killer cells. Moreover, coculture of M2 macrophages and EGFR-expressing tumor cell lines in presence of cetuximab, but not bevacizumab or rituximab, was associated with a significantly increased secretion of the pro-angiogenic and anti-inflammatory cytokines IL-8 and IL-10 and reduced secretion of the immunostimulatory IL-12 cytokine.

FCGR polymorphisms could further influence this immune response as IL-8 and IL-10 secretions were more prominent if M2 macrophages derived from healthy donors carrying the FCGR3A-158 V/V genotype as compared to M2 macrophages from FCGR3A-158 F/F subjects.

FCGR Polymorphisms and Cetuximab+Chemotherapy Combination in Second or Subsequent Line of Treatment

FCGR polymorphisms have been analysed in nine retrospective studies including MCRC patients treated with cetuximab in second or subsequent line of therapy.

In 2008, Bibeau et al. published a retrospective analysis on 69 patients treated with cetuximab+irinotecan in 3rd or higher line of therapy (only one patient received also fluorouracil). Both wild type and mutated KRAS tumors were included. FCGR3A-158 V/V genotype was significantly associated with longer PFS, while no association with tumor response was noted, confirming a role as prognostic factor,

rather than predictive, of this polymorphism. In further confirmation of that, the PFS advantage was seen for both wild type and mutant KRAS tumors [19].

In another dataset by Calemma et al., 49 KRAS wild-type MCRC patients were treated with different combinations of chemotherapy+cetuximab (or the other approved anti-EGFR MAb panitumumab) and at different phases of the disease (7 cases as firstline, 42 cases as subsequent line of treatment). Authors found a significant correlation of FCGR-158V allele with both tumor radiologic response (43% v 20%, for V/V or V/F genotype vs F/F genotype, respectively, p=0.035) and median PFS (18 vs. 9 months, respectively, p=0.04) [20].

Another retrospective analysis by Rodríguez et al. [21] avaluated FCGR polymorphisms in 44 patients treated with cetuximab+standard chemotherapy in first or subsequent lines of therapy. Only patients with mutation in genes involved in the downstream pathway of EGFR (namely KRAS, NRAS, BRAF or PI3K) were included. Results of note were the high percentage of early progressing patients (<6 months) within the group of FCGR2A-131 R/R genotype (83%, p=0.017) and within the FCGR3A-158 F/F group (77%, p=0.08).

In another cohort of 107 irinotecan-refractory patients published by Park et al., no significant correlation with outcome (ORR, PFS or OS) was found for both FCGR2A-131 and FCGR3A-158 polymorphisms [22]. No response was observed in the four patients with FCGR2A R/R genotype, whilst ORR was 24% and 39% for FCGR2A H/H ed H/R patients, respectively, but p value was not significant (p=0.08), presumably for the limited sample size.

In a retrospective analysis by Dahan et al., 56 patients treated in the majority of cases with cetuximab + irinotecan (71%) and in the third line setting (55%) were genotyped for both FCGR2A-131 and FCGR3A-158 loci. FCGR3A-158 V/V was carried by 9 patients and conferred a statistically significant worse prognosis (Median survival 9.8 months in F/F patients, 29 patients, 21 events vs. 9.0 months in F/V patients, 20 patients, 15 events vs. 2.6 months in V/V patients, 6 patients, 6 events; Log Rank test: p<0.001) [23].

Still from the group of Zhang et al., a subgroup of patients (n=65) included in the BOND-2 phase II trial were genotyped for several candidate genes involved in EGFR signalling pathway, ADCC, angiogenesis or drug-detoxification pathway. In the BOND-2 study, both wild-type and mutated KRAS patients, heavily-pretreated, were randomized to either cetuximab+bevacizumab (CB) or cetuximab +bevacizumab+irinotecan (CBI). FCGR3A-158 polymorphism was significantly associated with response rate in the CB arm (n=34), with a 56% response rate for the F/F genotype compared to 8% for V/V, p=0.054 [24].

Paez et al. included 104 patients treated with cetuximab (or panitumumab) + chemotherapy, in a retrospective study exploring the impact of clinical factors, skin toxicity, KRAS and FGCR genotypes on response rate (ORR) and progression free survival (PFS). Most of patients received cetuximab+irinotecan (69%) as second or third line of therapy (88%). They found no significant difference on clinical outcome across FCGR genotypes; in particular ORRs were 17% and 41% for FCGR2A-131 H/H and R/R and 27% and 22% for FCGR3A-158 V/V and F/F, respectively, p=0.13 and 0.5. Median PFS was 4 vs. 6 and 4 vs. 7 months, respectively, p=0.61 and 0.5 [25].

Finally, Negri et al. first demonstrated in vitro the enhanced ADCC mediated by peripheral blood mononuclear cells (PBMCs) of MCRC patients carrying the FCGR-158 V/V genotype; then they tried to translate in vitro findings into the clinical setting by correlating both FCGR2A and FCGR3A SNPs with ORR, PFS and OS. By using the EGFR-expressing LoVo colorectal cancer cell line and a Chromium-51 release assay, they found that PBMCs expressing the FCGR-158 V/V genotype lead to a two-fold increase in ADCC-mediated LoVo cell killing. However, this did not translate in increased clinical efficacy of cetuximab as no ORR, PFS or OS difference was observed for FCGR-158 V/V vs. F/F in 86 patients treated with cetuximab plus either irinotecan- or oxaliplatin-based chemotherapy, in both firstline and subsequent lines of therapy (ORR 16% vs. 26%, p 0.68, median PFS 3.1 vs. 4.6 months, p 0.18, median OS 9 vs. 13 months, p=0.16) [26].

FCGR were also assessed by Graziano et al. in a cohort of 110 patients treated with cetuximab+irinotecan in the third-line setting. Results were not explicitly reported, though it is presumable that no significant influence was found in terms of clinical outcome [27].

Meta-Analysis of Tumor Response Rate and FCGR3A 158 Genotype

Data on response rate by FGCR3A-158 genotype were reported in nine out of 12 retrieved studies and were meta-analysed to evaluate whether a definitive association between F/F or V/V genotype and cetuximab activity could be found (Table 2).

Altogether, a higher response rate was observed in presence of a V allele (29% vs. 27%) with a 8% increased probability of response for the F/V or V/V genotype, but this was not statistically significant: Total Odds Ratio (random effects) 1.08 (95% Confidence Interval 0.60-1.96) (Table 2 and Figure 1).

Conclusion

We found 12 retrospective studies exploring the possible prognostic/predictive role of FCGR polymorphisms on outcome of cetuximab-treated MCRC patient. Results were clearly conflicting and our attempted meta-analysis looking at tumor response failed to reach significant results. We focused on response rate, rather than progression free or overall survival, because it is an outcome measure related more to the drug activity than to the inherent tumor aggressiveness and prognosis. Apart from one study, all reported cohorts of patients were cetuximab-treated with no control arm and a significant impact of a variable on PFS or OS in single arm studies keeps open the question as to whether it represents a prognostic rather than predictive factor.

Contradictory results may be largely explained by the inter- and intra-study variety of chemotherapy combinations and phases of the disease, with treatments delivered as first, second, third and even forth line of therapy.

In particular, the pure effect of cetuximab (monotherapy) on ADCC, where no interference of chemotherapy is possible, has been investigated only in one study with a relatively small sample size, moreover only in two studies cetuximab was delivered in the firsline setting allowing acceptable observation on the effect of ADCC on survival outcome 'in the long run'.

The only study derived from a large phase III trial and including a no-cetuximab control arm was that by Pander et al, where KRAS-wild type patients with the FCGR3A 158 F/F genotype had a significantly longer PFS only in the cetuximab-treated arm, making this feature truly predictive of cetuximab effect. Surprisingly, the F variant produces a weaker binding of Fc gamma IIIA receptor to IgG1 antibodies resulting in reduced ADCC [28]. Pander motivated this apparent incongruity

Study	N. of responding patients with F/F genotype	Total n. of patients with F/F genotype	N. of responding patients with F/V or V/V genotype	Total n. of patients with F/V or V/V genotype
Calemma et al. [20]	1	4	19	44
Bibeau et al. [19]	2	15	9	53
Zhang et al. [13]	2	16	0	19
Rodriguez et al. [21]	3	13	16	31
Etienne-Grimaldi et al. [15]	5	20	19	31
Zhang et al. (2010) [24]	9	21	12	44
Negri et al. [26]	7	27	13	59
Park et al. [22]	14	36	18	71
Paez et al. [25]	10	46	11	53
TOTAL	53	198	117	405
Overall Response Rate	27%		29%	

Table 2: FCGR3A-158 genotype and tumour response rate in 9 retrospective studies.

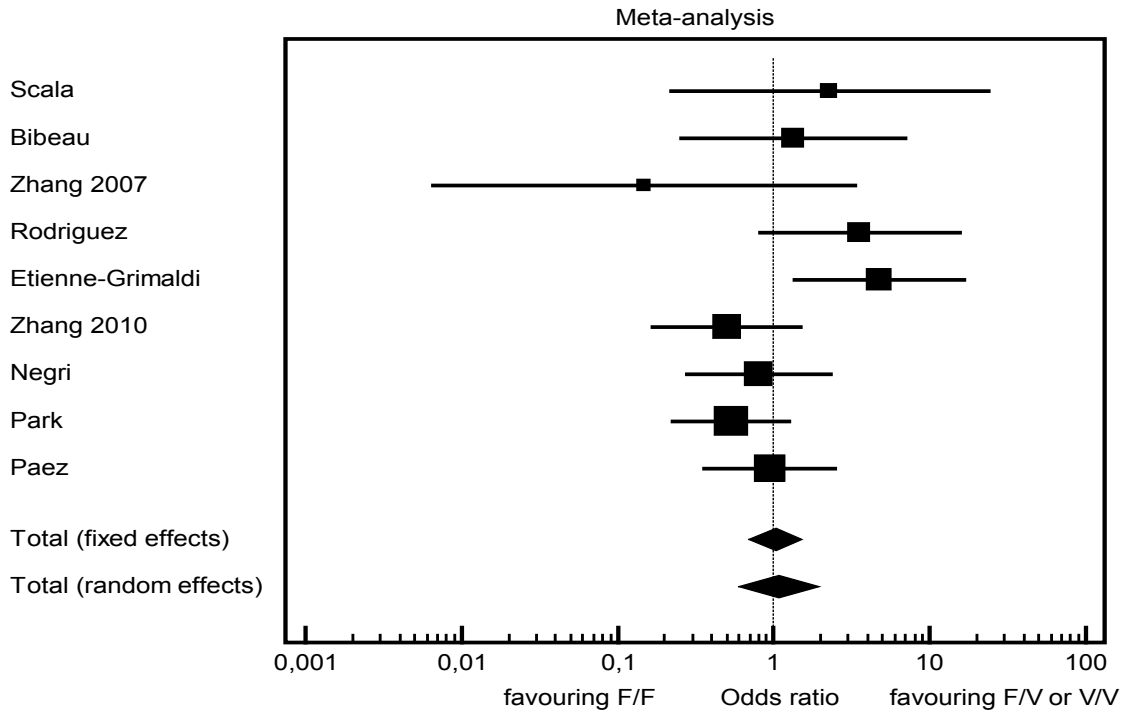

Figure 1: Meta-analysis of response rate of seven retrospective studies according to FCGR-158 genotype.

by speculating that a poor FcgammaR/IgG1 binding may also result in a reduced activation of tumor associated macrophages (TAMs) which facilitate tumor progression by releasing pro-angiogenic factors such as IL-8, IL-10 and VEGF [16]. This was confirmed by the same authors in in vitro tests. Authors justified with an enhanced M2 cetuximab-induced activation the failure of the phase III randomized CAIRO-2 trial aiming at improving outcome with the addition of cetuximab to the standard capecitabine+oxaliplatin+bevacizumab regimen [29]. In light of Pander findings, the way forward to optimize cetuximab combination would be, first of all, to extensively study in preclinical models the balance between M2 and ADCC activation. As an example, a robust engineered model of mouse expressing the human Fc-gamma-receptors is still lacking to fully explore immune effects of cetuximab. Such a model would be desirable also in order to evaluate the right sequence and combination with chemotherapy, since some chemotherapeutic agents may alter EGFR expression on cancer cells and macrophages and hence condition ADCC activation [30,31].

In conclusion FCGR genotyping, though it carries the potential for being an attractive predictive test of cetuximab activity, exploring the probability that an immune-mediated antitumor response is elicited, has clearly shown contradictory clinical results for MCRC patients. We think that such a wide inconsistency in research outcome may be largely explained by the study heterogeneity including different lines of therapy and different combination regimens. Currently FCGR polymorphisms cannot be considered reliable predictive biomarkers of cetuximab response and therefore it appears inappropriate to deny this drug on the basis of FCGR genotype. Furthermore, as the other side of the coin is the eventuality that genotypes enhancing ADCC may induce pro-inflammatory pro-tumorigenic macrophage activation, their role should be investigated prospectively in randomized trial and substantiate with the functional assessment of both immune and inflammatory responses.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69-90.
2. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, et al. (2010) Colorectal cancer. Lancet 375: 1030-1047.
3. De Roock W, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, et al. (2010) Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. JAMA 304: 1812-1820.
4. Gessner JE, Heiken H, Tamm A, Schmidt RE (1998) The IgG Fc receptor family. Ann Hematol 76: 231-248.
5. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, et al. (1997) Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood 90: 1109-1114.
6. Congy-Jolivet N, Bolzec A, Ternant D, Ohresser M, Watier H, et al. (2008) Fc gamma RIIIa expression is not increased on natural killer cells expressing the Fc gamma RIIIa-158V allotype. Cancer Res 68: 976-980.
7. Musolino A, Naldi N, Bortesi B, Pezzuolo D, Capelletti M, et al. (2008) Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. J Clin Oncol 26: 1789-1796.
8. Tamura K, Shimizu C, Hojo T, Akashi-Tanaka S, Kinoshita T, et al. (2011) FcÎ³R2A and 3A polymorphisms predict clinical outcome of trastuzumab in both neoadjuvant and metastatic settings in patients with HER2-positive breast cancer. Ann Oncol 22: 1302-1307.
9. Hurvitz SA, Betting DJ, Stern HM, Quinaux E, Stinson J, et al. (2012) Analysis of FcÎ³ receptor IIIa and IIa polymorphisms: lack of correlation with outcome in trastuzumab-treated breast cancer patients. Clin Cancer Res 18: 3478-3486.
10. Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, et al. (2002) Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood 99: 754-758.
11. Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 21: 3940-3947.
12. Ghesquieres H, Cartron G, Seymour JF, Delfau-Larue MH, Offner F, et al. Clinical outcome of patients with follicular lymphoma receiving chemoimmunotherapy in the PRIMA study is not affected by FCGR3A and FCGR2A polymorphisms. Blood 120: 2650-2657.
13. Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, et al. (2007) 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 25: 3712-3718.
14. Lenz HJ, Van Cutsem E, Khambata-Ford S, Mayer RJ, Gold P, et al. (2006) Multicenter phase II and translational study of cetuximab in metastatic colorectal carcinoma refractory to irinotecan, oxaliplatin, and fluoropyrimidines. J Clin Oncol 24: 4914-4921.
15. Etienne-Grimaldi MC, Bennouna J, Formento JL, Douillard JY, Francoual M, et al. (2012) Multifactorial pharmacogenetic analysis in colorectal cancer patients receiving 5-fluorouracil-based therapy together with cetuximab-irinotecan. Br J Clin Pharmacol 73: 776-785.
16. Pander J, Gelderblom H, Antonini NF, Tol J, van Krieken JH, et al. (2010) Correlation of FCGR3A and EGFR germline polymorphisms with the efficacy of cetuximab in KRAS wild-type metastatic colorectal cancer. Eur J Cancer 46: 1829-1834.
17. López-Albaitero A, Lee SC, Morgan S, Grandis JR, Gooding WE, et al. (2009) Role of polymorphic Fc gamma receptor IIIa and EGFR expression level in cetuximab mediated, NK cell dependent in vitro cytotoxicity of head and neck squamous cell carcinoma cells. Cancer Immunol Immunother 58: 1853-1864.
18. Pander J, Heusinkveld M, van der Straaten T, Jordanova ES, Baak-Pablo R, et al. (2011) Activation of tumor-promoting type 2 macrophages by EGFR-targeting antibody cetuximab. Clin Cancer Res 17: 5668-5673.
19. Bibeau F, Lopez-Crapez E, Di Fiore F, Thezenas S, Ychou M, et al. (2009) Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol 27: 1122-1129.
20. Calemma R, Ottaiano A, Trotta AM, Nasti G, Romano C, et al. (2012) Fc gamma receptor IIIa polymorphisms in advanced colorectal cancer patients correlated with response to anti-EGFR antibodies and clinical outcome. J Transl Med.
21. Rodríguez J, Zarate R, Bandres E, Boni V, Hernández A, et al. (2012) Fc gamma receptor polymorphisms as predictive markers of Cetuximab efficacy in epidermal growth factor receptor downstream-mutated metastatic colorectal cancer. Eur J Cancer 48: 1774-1780.
22. Park SJ, Hong YS, Lee JL, Ryu MH, Chang HM, et al. (2012) Genetic polymorphisms of FcÎ³RIIa and FcÎ³RIIIa are not predictive of clinical outcomes after cetuximab plus irinotecan chemotherapy in patients with metastatic colorectal cancer. Oncology 82: 83-89.
23. Dahan L, Norguet E, Etienne-Grimaldi MC, Formento JL, Gasmi M, et al. (2011) Pharmacogenetic profiling and cetuximab outcome in patients with advanced colorectal cancer. BMC Cancer 11: 496.
24. Zhang W, Azuma M, Lurje G, Gordon MA, Yang D, et al. (2010) Molecular predictors of combination targeted therapies (cetuximab, bevacizumab) in irinotecan-refractory colorectal cancer (BOND-2 study). Anticancer Res 30: 4209-4217.
25. Paez D, Paré L, Espinosa I, Salazar J, del Rio E, et al. (2010) Immunoglobulin G fragment C receptor polymorphisms and KRAS mutations: are they useful biomarkers of clinical outcome in advanced colorectal cancer treated with anti-EGFR-based therapy? Cancer Sci 101: 2048-2053.
26. Negri FV, Musolino A, Naldi N, Bortesi B, Missale G, et al. (2013) Role of immunoglobulin G fragment C receptor polymorphism-mediated antibody-dependant cellular cytotoxicity in colorectal cancer treated with cetuximab therapy. Pharmacogenomics J.
27. Graziano F, Ruzzo A, Loupakis F, Canestrari E, Santini D, et al. (2008) Pharmacogenetic profiling for cetuximab plus irinotecan therapy in patients with refractory advanced colorectal cancer. J Clin Oncol 26: 1427-1434.
28. López-Albaitero A, Lee SC, Morgan S, Grandis JR, Gooding WE, et al. (2009) Role of polymorphic Fc gamma receptor IIIa and EGFR expression level in cetuximab mediated, NK cell dependent in vitro cytotoxicity of head and neck squamous cell carcinoma cells. Cancer Immunol Immunother 58: 1853-1864.
29. Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, et al. (2009) Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med 360: 563-572.
30. Correale P, Marra M, Remondo C, Migali C, Misso G, et al. (2010) Cytotoxic drugs up-regulate epidermal growth factor receptor (EGFR) expression in colon cancer cells and enhance their susceptibility to EGFR-targeted antibody-dependent cell-mediated-cytotoxicity (ADCC). Eur J Cancer 46: 1703-1711.
31. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, et al. (2011) Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov 1: 54-67.

This article was originally published in a special issue, **Cancer Genetics** handled by Editor(s). Dr. Ahmed M Malki, Alexandria University, Egypt