Chapter 7

L.L. Krens, M. Fiocco, H. Piessevaux, S. Tejpar, J. Rodriguez, C.J.A. Punt, H. Gelderblom, H.J. Guchelaar and R.J.H.M. van der Straaten

Submitted

Effect of the Fc gamma receptor polymorphism V158F status on the survival of metastatic colorectal cancer patients treated with cetuximab: a meta-analysis

Abstract

Background

The use of cetuximab in metastatic colorectal cancer (CRC) is limited to patients with wild type *KRAS* tumors and more recently to *RAS* wild type tumors only. Antibody-dependent cellular cytotoxicity (ADCC), mediated by the Fc gamma receptor (FCGR) is assumed to be an important mechanism for induction of tumor cell death by cetuximab. Several studies explored the role of FCGR3A (rs396991) genetic polymorphism in cetuximab efficacy in mCRC patients, but the results from these studies are discordant.

Method

An individual patient data meta-analysis was performed, to better understand the effect of FCGR3A FF versus non FF (FV and VV) polymorphism on progression free survival (PFS) and overall survival (OS) in patients with *KRAS* mutant or wild type metastatic CRC, treated with cetuximab. Three studies were included in this meta-analysis.

Results

The hazard ratio (HR) for the primary endpoint progression free survival for FCGR3A non FF, adjusted for *KRAS* and the interaction between FCGR3A and *KRAS* was equal to 1.07 (95% confidence interval 0.89 - 1.29, p = 0.45). For overall survival, the HR for FCGR3A non FF, adjusted for *KRAS* and the interaction between FCGR3A and *KRAS* was equal to 0.91 (95% confidence interval 0.77 - 1.07).

Conclusion

The results of the present analysis suggest that FCGR3A rs396991 is not associated with progression free or overall survival in cetuximab treated mCRC patients.

Introduction

Cetuximab is an IgG1-type chimeric monoclonal antibody (MoAb) that targets the epidermal growth factor receptor (EGFR). Cetuximab is mainly used for treatment of metastatic colorectal cancer. Blocking of EGFR results in decreased proliferation, cell survival and angiogenesis. However, about 40% of colorectal cancers harbor a mutation in *KRAS* and these tumors do not respond to anti-EGFR therapy[1-3]. For this reason, the use of cetuximab is limited to patients with wild type *KRAS* tumors and more recently to patients with *RAS* wild type tumors only[4].

Antibody-dependent cellular cytotoxicity (ADCC), mediated by the Fc gamma receptor (FCGR) is assumed to be an important mechanism for induction of tumor cell death by cetuximab[5]. MoAbs are molecules of the IgG class and have an antigen-binding fragment (Fab) and a constant fragment (Fc). Fc gamma receptors (FCGRs) are expressed on immune effector cells, such as macrophages and natural killer lymphocytes. ADCC is induced when FCGRs bind to the monoclonal Fc fragment, since this interaction leads to the activation and degranulation of the effector cells and the subsequent lysis of the tumor[6].

Several germline single nucleotide polymorphisms (SNP) in the *FCGR* gene have been identified that confer a different binding affinity of the FCGR to the Fc fragment of the MoAb. The polymorphism in the Fc gamma receptor 3A gene (*FCGR3A*) c.818A>C results in a change of phenylalanine (F) to valine (V) at position 158 (rs396991)[6,7]. The C allele coding for valine of FCGR3A has a much higher affinity for binding to Fc than the wild type A allele coding for phenlyalanine. Importantly, the V phenotype has been related with a more extensive IgG1-induced ADCC[8,9]. Several studies, especially in large B-cell and follicular lymphoma patients treated with the MoAb rituximab, show a better clinical outcome for patients with FCGR3A VV phenotype, a finding that might be explained by the higher binding affinity conferred by this V phenotype[5,10-12].

The possible advantage of FCGR3A VV phenotype is less clear in mCRC patients treated with cetuximab. Indeed, the results from several studies investigating the association between *FCGR3A* genotype and cetuximab efficacy in mCRC patients are discordant (Table 1). A total of 13 published studies have investigated the association between *FCGR3A* and response, progression free or overall survival in mCRC patients treated with cetuximab. Seven studies[13-19] did not find any significant association between *FCGR3A* and outcome. The studies by Bibeau et al.[20] and Calemma et al.[21] reported that patients with the FCGR3A VV phenotype had a longer PFS. In contrast, four other studies reported a higher likelihood of cetuximab induced progression free or overall survival for patients with the FCGR3A F phenotype. In the study of Zhang et al.[22] a significantly higher response rate (RR) was seen in cetuximab plus bevacizumab treated patients with the FF group (RR = 56%) compared to FV (RR = 25%) and VV (RR = 8%) phenotypes. Dahan et al. [23] (58 patients) reported a decreased overall survival for patients with the *FCGR3A* VV phenotype whereas Pander et al. showed that the C allele coding for valine was associated with a shorter progression free survival[24]. Finally, a small study performed by Zhang et al. in 2007 with only 39 mCRC patients showed that those with the F-containing phenotype (FF or FV) had a longer PFS [31]. These conflicting results could be explained by a limited sample size of the different studies, genotyping errors (distribution of the genotypes is not always consistent with the Hardy-Weinberg equilibrium)[25-26] and different clinical scenarios.

As mentioned, most studies did have some drawbacks regarding the number of patients per study. The studies of Bibeau et al.[27], Zhang et al.[22] and Park et al.[28] reported genotype distributions of *FCGR3A* which deviated from Hardy-Weinberg equilibrium. Some of these

studies reported an altered outcome for the different *FCGR3A* genotypes, however, deviation from the Hardy-Weinberg equilibrium raises concerns in interpreting the outcome of these studies. All studies conducted retrospective analysis and positive/significant associations were found in relatively small patient cohorts, ranging from 32 to 270 patients. In these small studies many associations may have been studied and due to multiplicity, false positive associations may have occurred.

In an earlier study of our research group, the study of Pander et al.[24], we showed that the V-phenotype was associated with worse progression. In addition, in an *in vitro* study we showed that an extensive binding of *FCGR3A* with the C allele coding for valine, expressed by type 2 macrophages, resulted in the release of tumor promoting factors[29]. This effect of the *FCGR3A* genotype appeared independent of the *KRAS* mutation status of the tumor[24]. This preclinical finding, resulting in extensive release of tumor promoting factors after extensive binding by the C allele coding for V, was the basis to further investigate the association between *FCGR3A* polymorphisms, *KRAS* mutational status and survival. A dominant model was used to study the differences between FCGR3A wild type FF versus FCGR3A heterozygous mutant FV plus homozygous mutant VV. Interestingly, in both our preclinical study and the CAIRO2 study an effect of FCGR3A was seen independent of *KRAS* mutational status. Interestingly, the effect of the FCGR3A polymorphism was substantial and in patients with a KRAS mutant tumor and a favourable FCGR3A polymorphism survival was comparable to patients with a KRAS wild type tumor but unfavourable FCGR3A polymorphism. For this reason, we aimed to study the effect of the *FCGR3A* (rs396991) polymorphism in patients with *KRAS* wild type and mutant CRC despite the fact that cetuximab is nowadays only used in RAS wild type patients. Consequently, for our meta-analysis we selected studies in which patients were included with KRAS mutant and wild type tumors, performed at the time when the use of cetuximab was not yet restricted to (K) RAS wild type tumors.

We conducted an individual patient data meta-analysis combining 1,301 patients from three independent studies, to study and FCGR3A FF versus non FF (FV and VV) phenotypes on the progression free survival (PFS) and overall survival (OS) of metastatic colorectal cancer in patients with a *KRAS* mutant or wild type tumors treated with cetuximab. The other studies were excluded due to unknown *KRAS* status, *KRAS* wild type patients only, genotyping method, missing survival data or inability to provide the data.

Table 1: Overview of previous published studies, which studied the association between *FCGR3A* polymorphisms and cetuximab response in metastatic colorectal cancer.

Study	Patients	Distribution of FCGR3A phenotypes [1]	Treatment	*KRAS* status of tumor	Results
Zhang et al. 2007	39	FF: 16 FV: 14 VV: 5 $X^2 = 0.44$ p = 0.51	cetuximab	Unknown	Patients with any F phenotype showed favourable response (median PFS 3.7 vs. 1.1 months p = 0.004).
Graziano et al. 2008	110	FF: 38 FV: 50 VV: 22 $X^2 = 0.56$ p = 0.45	Irinotecan + cetuximab	Whole population	No association found on progression free or overall survival.
Bibeau et al. 2009	68	FF: 15 FV: 43 VV: 10 $X^2 = 5.02$ P = 0.03	Irinotecan + cetuximab	Whole population and subgroup analysis in *KRAS* wild type and *KRAS* mutant	VV phenotype associated with longer PFS. VV phenotype 6.9 months vs. FV or VV phenotype 3.2 months in whole population. VV phenotype 5.5 months vs. FV or VV phenotype 2.8 months in *KRAS* mutant patients
Zhang et al. 2010	31	FF: 11 FV: 9 VV:11 $X^2 = 5.45$ p = 0.02	Cetuximab + bevacizumab + Irinotecan	In whole population	No association found on response rate.
	32	FF: 10 FV: 12 VV: 12 $X^2 = 2.89$ p = 0.09	Cetuximab + bevacizumab	Independent of *KRAS* status. Effect seen in whole population and *KRAS* wild type patients.	FF associated with a better response rate (56%) compared to FV (25%) and VV (8%) p = 0.05
Pander et al. 2010	270	FF: 119 FV and VV: 157	CAPOX + bevazizumab + cetuximab	Whole population and subgroup analysis in *KRAS* wild type and *KRAS* mutant	V allele associated with decrease in PFS (VV and FV 8.2 vs 12.8 months in FF and HR 1.56, p = 0.006) regardless of *KRAS* status
Paez et al. 2010	104	FF: 47 FV: 41 VV: 16 $X^2 = 1.89$ p = 0.17	Chemotherapy + cetuximab or panitumumab or panitumumab alone	Whole population and subgroup analysis in *KRAS* wild type and *KRAS* mutant	No association found for response rate or PFS.

Study	Patients	Distribution of FCGR3A phenotypes [1]	Treatment	*KRAS* status of tumor	Results
Dahan et al. 2011	58	FF: 30 FV: 20 VV: 6 $X^2 = 0.88$ $p = 0.34$	Irinotecan + cetuximab	Whole population and in subgroup of *KRAS* wild type patients	Median OS was 9.8 months in FF vs. 9.0 in FV vs. 2.6 in VV patients $p < 0.001$
Calemma et al. 2012	49	FF: 5 FV: 26 VV: 18 $X^2 = 0.98$ $p = 0.32$	Cetuximab or panitumumab	*KRAS* wild type patients only	Unfavourable prognosis for FF phenotypeMedian PFS VV, FV,FF; 18.2 vs. 17.3 vs. 9.4 months $p = 0.04$.
Rodriguez et al. 2012	44	FF: 13 FV and VV: 31	cetuximab	In mutated phenotype population (any *KRAS*, *BRAF*, *NRAS* or *PI3CA* mutation)	No association found, adjusted odds ratio for VV +FV was 3.8 (95% CI 0.5 -26)
Park et al. 2012	118	FF: 36 FV: 65 VV: 6 $X^2 = 10.86$ $p < 0.001$	Chemotherapy + cetuximab	Whole population and subgroup analysis in *KRAS* wild type, and *KRAS* mutant	No significant differences between RR, OS or PFS
Negri et al.	86	FF: 27 FV: 40 VV: 19 $X^2 = 0.33$ $p = 0.85$	cetuximab	*KRAS* wild type only	No significant differences between response rate or time to tumor progression
Kjersem et al. 2014	328	FF: 162 FV: 131 VV: 35 $X^2 = 1.19$ $p = 0.27$	FLOX +cetuximab	Subgroup analysis in patients with *KRAS* wild type and *KRAS* mutant	None of the FCGR3A phenotype were associated with altered response
Geva et al. 2014	1024	FF: 391 FV: 466 VV: 167 $X^2 = 1.99$ $p = 0.16$	Chemotherapy + cetuximab or cetuximab monotherapy	Whole population and subgroup analysis in *KRAS* wild type, exploratory analysis in *KRAS* mutant	No differences between median PFS between VV vs. FF +FV, better DCR and median OS in *KRAS* mutant subgroup in exploratory setting

Abbreviations: PFS, progression free survival; OS, overall survival; DCR, disease control rate; RR, response rate; CAPOX, capecitabine and oxaliplatin; FLOX, fluorouracil and oxaliplatin
1. If $p < 0.05$ not consistent with Hardy-Weinberg equilibrium.

Material and Methods

Individual patient data acquisition

To study the association between survival times and *FCGR3A* polymorphism, a literature search was performed in June 2014 on PubMed, by using the keywords cetuximab, FCGR polymorphisms, KRAS and (metastatic) colorectal cancer. We used the following criteria to select publications:

1. Treatment with cetuximab in mCRC;

2. Individual patient data regarding overall survival (OS) and progression free survival (PFS) or the individual patient data reconstruction using Kaplan Meier curves;

3. Availability of *FCGR3A* (rs396991) genotype;

4. Genotyping methods of eligible studies were reviewed to prevent inclusion of patients with an unreliable *FCGR3A* genotype. (Several methods do not discriminate between *FCGR3A* and *FCGR3B*, which may result in genotyping errors[26]);

5. Availability of *KRAS* mutational status (KRAS codon 12, 13 and if possible 61) of the tumor (both *KRAS* wild type and mutant patient were included in this study).

This resulted in the inclusion of patients from three studies, the CAIRO2 [24], Rodriguez et al.[15] and the study of the European colorectal cancer consortium [30]. The FCGR3A polymorphism data from the European colorectal cancer consortium, was not published at time of analysis but the authors provided us with the data. Recently, the FCGR3A polymorphisms data from the European colorectal cancer consortium was published by Geva et al.[18]. All studies were approved by the local ethics committees and all included patients gave informed consent. Articles were excluded due to unknown *KRAS* status[13,31]. *KRAS* wild type patients only[19,21], genotyping method[16,20,22,23], missing survival data[14] and inability to provide the data[17].

Study 1: cohort CAIRO2 study

Data from 193 patients were available from the CAIRO2 study, which started in the pre-*KRAS* era. These patients with mCRC were treated with firstline capecitabine, oxaliplatin and bevacizumab (CAPOX-B) or the same regimen plus cetuximab. Cetuximab was administered intravenously at a dose of 400 mg/m2 on the first day, followed by 250 mg/m2 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.

Study 2: cohort Rodriguez et al.

Data were available from 99 patients. Patients with mCRC were treated with cetuximab administered on an every-second week schedule at a dose of 500 mg/m2 combined with standard irinotecan or oxaliplatin based chemotherapy. Patients were treated in either first (31%) or second line therapy

Study 3: cohort European colorectal cancer consortium

From the European colorectal cancer consortium data were available from 1009 patients. Patients with mCRC were treated with irinotecan or oxaliplatin based chemotherapy and cetuximab or cetuximab monotherapy.

KRAS tumor status and FCGR3A rs396991 polymorphism

In all three studies, genotyping of the *FCGR3A* was performed on a validated realtime PCR system with a predesigned assay for *FCGR3A* rs396991 (C__25815666_10). Details about the used methods are described elsewhere [15,30,32].

Outcome measures

The association between *FCGR3A* rs396991 genotype and the primary endpoint PFS and the secondary endpoint OS were investigated. PFS was calculated as time from randomisation to the first documented progression, death or loss to follow up, whichever occurred first. OS was estimated from time since randomisation to death or loss to follow up.

Statistical analysis

Meta-analysis based on the survival outcomes coming from the three studies described above was performed, for two studies individual patient data were available while for the third study individual patient data were reconstructed from the estimated PFS and OS. Reconstruction of the relevant data is discussed by Fiocco et al. [33,34]. Further details on data analysis are described in appendix 1. A multivariate mixed effects Cox proportional hazard model with study as random effects was employed to investigate the effects of *FCGR3A*, *KRAS* mutation status and the interaction between FCGR3A and KRAS on the primary endpoint PFS and secondary endpoint OS.

Results

Individual patient data meta-analysis

A total of 1,301 patients were included in the analysis. In table 2 an overview of the incidence of *FCGR3A* polymorphism and *KRAS* tumor status is shown. For all three studies, the reported *FCGR3A* genotypes were in Hardy-Weinberg equilibrium. To study the effect of *FCGR3A* polymorphisms, we used a dominant genetic model (FF vs non FF). Table 3 shows an overview of the median PFS and OS for the three different studies for *FCGR3A* FF and non FF.

Progression free survival

The hazard ratio (HR) for FCGR3A non FF, adjusted for *KRAS* and the interaction between FCGR3A and *KRAS* was equal to 1.07 (95% confidence interval 0.89 – 1.29, p = 0.45). The estimated pooled Kaplan Meier curves, for patients with *KRAS* mutant and wild type tumors and FCGR3A FF and non FF status, are shown in Figure 1. A small, non-significant effect is seen between FCGR3A FF and non FF, stratified for KRAS status of the tumor. For patients with *KRAS* wild type tumors, median PFS was equal to 14.0 (95% CI interval 12.5 – 15.9) and 15.2 (95% CI interval 14.0 – 17.1) months for FCGR3A FF and non FF respectively. For patients

with a *KRAS* mutant tumor PFS was 10.6 (95% CI interval 9.0 – 13.1) and 9.2 (95% CI interval 8.0 – 11.3) months for FCGR3A FF and non FF, respectively.

Table 2: distribution of FCGR3A polymorphisms

Polymorphism or mutation	Study 1: CAIRO2 N (%)	Study 2: Rodriguez N (%)	Study 3: European colorectal cancer consortium N (%)	Total
total	193	99	1009	1301
KRAS wild type	125 (64.8)	56 (56.7)	676 (67.0)	857 (65.9)
KRAS mutant	68 (35.2)	43 (43.4)	333 (33.0)	444 (34.1)
FCGR3A – FF	84 (43.5)	43 (43.4)	384 (38.1)	511 (39.3)
FCGR3A – VF	83 (43.0)	41 (41.4)	459 (45.5)	583 (44.8)
FCGR3A – VV	26 (13.5)	15 (15.1)	166 (16.5)	207 (15.9)
HWE p-value[1]	0.45	0.32	0.15	0.06

Abbreviation: HWE: Hardy-Weinberg Equilibrium.
1. If $p < 0.05$ not consistent with Hardy-Weinberg Equilibrium.

Table 3: Median PFS and OS for cetuximab treated KRAS wild type and mutant patients

Study	Treatment	FCGR3A	Median PFS	Median OS
1: CAIRO2	CAPOX + bevacizumab and cetuximab	FF non FF	11.6 months 8.1 months	21.7 months 21.9 months
2: Rodriguez	Cetuximab 2-weekly	FF non FF	5.0 months 4 months	44.5 months 25.9 months
3: European colorectal cancer consortium	Irinotecan/ oxaliplatin based chemotherapy +cetuximab or cetuximab alone.	FF non FF	4.1 months 3.8 months	9.9 months 10.1 months

Overall survival

The HR for FCGR3A non FF, adjusted for *KRAS* and the interaction between FCGR3A and *KRAS* was 0.91 (95% confidence interval 0.77 – 1.07). In figure 2 the pooled Kaplan Meier curves for patients with KRAS mutant and wild type tumors, and FCGR3A FF and non-FF status are depicted. A difference between patients with a KRAS wild type and KRAS mutant tumor was observed in the plots, although this difference is not significant. For patients with KRAS wild type tumor, median OS was equal to 37.3 (95% CI interval 33.1 – 45.3) and 46.3 (95% CI interval 39.0 – 54.0) months for *FCGR3A* FF and non FF respectively. For patients with KRAS mutant tumor median OS was equal to 27.7 (95% CI interval 22.6-35.2) and 21.5 (18.9-26.4) months for FCGR3A FF and non FF respectively.

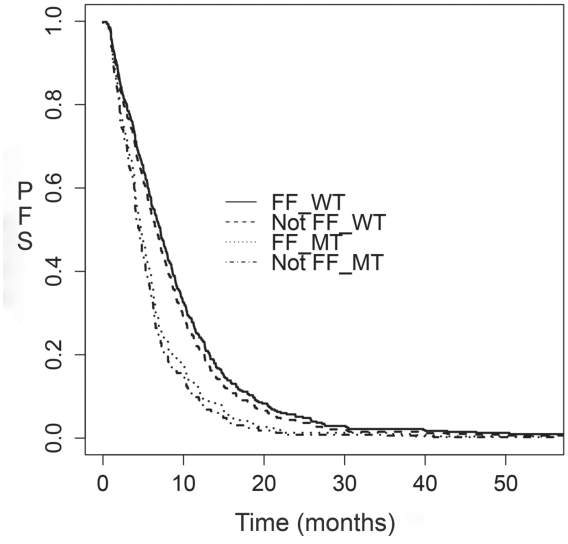

Figure 1: Estimated Kaplan Meier curves for progression free survival in patients treated with cetuximab, stratified by *KRAS* tumor status and FCGR3A status.
Abbreviations: **PFS:** progression free survival, FF_WT: FCGR3A FF and *KRAS* wild type; Not FF_WT, FCGR3A not FF and *KRAS* wild type; FF_MT, FCGR3A FF and *KRAS* mutant; Not FF_MT, FCGR3A not FF and *KRAS* mutant

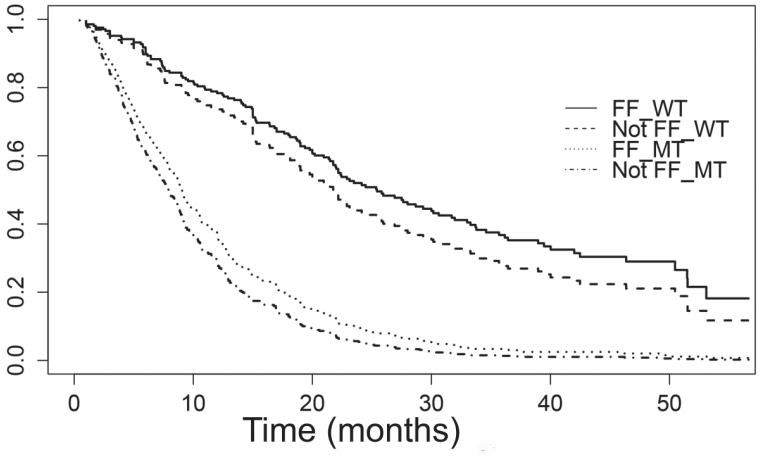

Figure 2: Estimated Kaplan Meier curves for overall survival in patients treated with cetuximab, stratified by *KRAS* tumor status and FCGR3A status.
Abbreviations: **FF_WT:** FCGR3A FF and *KRAS* wild type; Not FF_WT, FCGR3A not FF and *KRAS* wild type; FF_MT, FCGR3A FF and *KRAS* mutant; Not FF_MT, FCGR3A not FF and *KRAS* mutant

Discussion

The meta-analysis performed in this study indicates that the *FCGR3A* (rs396991) polymorphism is not associated with progression free or overall survival in patients with metastatic colorectal cancer treated with cetuximab in patients with either a *KRAS* mutant or *KRAS* wild type tumor. In our meta-analysis we did not find any advantage for the FF genotype in terms of clinical efficacy.

Both *KRAS* wild type and mutant metastatic colorectal cancer patients were included in the analysis, since the interesting results seen in the preclinical[29] and CAIRO 2[24] study. This meta-analysis however shows that there is no difference between the KRAS wild type and mutant populations.

Nowadays cetuximab is used in RAS wild type patients only. In the three included studies only the KRAS status of the tumor was known. Unfortunately, we were not able to extend our research to the effect of FCGR3A in the RAS mutant colorectal cancer patient due to the retrospective design of the studies included in this meta-analysis. Most probably the including RAS mutant colorectal patients in the analysis would not have altered the outcome of this study

Differences between studies due to a specific design and methodology, clinical procedures, different lines of chemotherapy and patients' characteristics can contribute to variability in treatment effect among studies. Heterogeneous studies are a common problem in meta-analysis[36]; to take into account the inter-trial heterogeneity caused by different treatments and lines of therapy used in these studies, we performed a meta-analysis by including studies as random effects, allowing for differences in the treatment effect and different regimens used from study to study and providing a more efficient estimate of the average treatment effect[37].

The inconsistent finding in studies concerning *FCGR3A* polymorphism and cetuximab efficacy shows the importance of genotyping methods, appropriate sample size and proper use of statistical methodology. We pooled data concerning 1,301 patients to improve the statistical power to detect the presence of a treatment effects on survival. Instead of reporting the classical forest plot based on hazard ratio for each individual study, we performed a meta-analysis based on individual patient data, which gave a better estimation of the potential benefit of FCGR3A FF status by using the individual patient data. An individual patient data meta-analysis approach of time to event outcomes, although usually more demanding, allows a deeper investigation.

Advanced and metastasizing CRC and prior lines of chemotherapy may be linked to decreased immune responses and impaired natural killer cell dysfunction and consequently failure of cetuximab treatment[38-40]. This may result in a more limited role of ADCC in cetuximab treated patients with advanced disease. Noteworthy, patients from CAIRO2 received concomitant chemotherapy and in this study, a difference in median PFS between FCGR3A FF and non FF was observed. Nonetheless, no difference was seen for *FCGR3A* on median OS.

In this meta-analysis we have studied the *FCGR3A* polymorphism rs396991 but ADCC is a complex biological process and a more in-depth analysis of alternative crucial steps in the immunological pathway may be of influence. Consideration of other FCGRs, MHC expression, IFN-gamma pathway components and antigen processing machinery genes might provide a broader insight into the role of immunity in cetuximab efficacy.

In conclusion, our results do not support a predictive role for the *FCGR3A* polymorphism (rs396991) in cetuximab efficacy.

References

1 Lievre A, Bachet JB, Le CD, Boige V, Landi B, Emile JF, Cote JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66: 3992-3995.

2 Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359: 1757-1765.

3 Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, McAllister PK, Morton RF, Schilsky RL (2009) 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 27: 2091-2096. J

4 Douillard JY, Oliner KS, Siena S, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocakova I, Ruff P, Blasinska-Morawiec M, Smakal M, Canon JL, Rother M, Williams R, Rong A, Wiezorek J, Sidhu R, Patterson SD (2013) Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 369: 1023-1034.

5 Weng WK, Levy R (2003) Two immunoglobulin G fragment C receptor polymorphisms Iindependently predict response to rituximab in patients with follicular lymphoma. J Clin Oncol 21: 3940-3947.

6 van Sorge NM, van der Pol WL, van de Winkel JG (2003) FcgammaR polymorphisms: Implications for function, disease susceptibility and immunotherapy. Tissue Antigens 61: 189-202.

7 Mellor JD, Brown MP, Irving HR, Zalcberg JR, Dobrovic A (2013) A critical review of the role of Fc gamma receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol 6: 1. 1756-8722-6-1

8 Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de HM (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.

9 de Haas M., Koene HR, Kleijer M, de VE, Simsek S, van Tol MJ, Roos D, von dem Borne AE (1996) A triallelic Fc gamma receptor type IIIA polymorphism influences the binding of human IgG by NK cell Fc gamma RIIIa. J Immunol 156: 2948-2955.

10 Ahlgrimm M, Pfreundschuh M, Kreuz M, Regitz E, Preuss KD, Bittenbring J (2011) The impact of Fc-gamma receptor polymorphisms in elderly patients with diffuse large B-cell lymphoma treated with CHOP with or without rituximab. Blood 118: 4657-4662. blood-2011-04-346411

11 Persky DO, Dornan D, Goldman BH, Braziel RM, Fisher RI, Leblanc M, Maloney DG, Press OW, Miller TP, Rimsza LM (2012) Fc gamma receptor 3a genotype predicts overall survival in follicular lymphoma patients treated on SWOG trials with combined monoclonal antibody plus chemotherapy but not chemotherapy alone. Haematologica 97: 937-942.

12 Kim DH, Jung HD, Kim JG, Lee JJ, Yang DH, Park YH, Do YR, Shin HJ, Kim MK, Hyun MS, Sohn SK (2006) FCGR3A gene polymorphisms may correlate with response to frontline R-CHOP therapy for diffuse large B-cell lymphoma. Blood 108: 2720-2725. blood-2006-01-009480

13 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 (2008) Pharmacogenetic profiling for cetuximab plus irinotecan therapy in patients with refractory advanced colorectal cancer. J Clin Oncol 26: 1427-1434. 26/9/1427

14 Paez D, Pare L, Espinosa I, Salazar J, del RE, Barnadas A, Marcuello E, Baiget M (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.

15 Rodriguez J, Zarate R, Bandres E, Boni V, Hernandez A, Sola JJ, Honorato B, Bitarte N, Garcia-Foncillas J (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.

16 Park SJ, Hong YS, Lee JL, Ryu MH, Chang HM, Kim KP, Ahn YC, Na YS, Jin DH, Yu CS, Kim JC, Kang YK, Kim TW (2012) Genetic polymorphisms of FcgammaRIIa and FcgammaRIIIa are not predictive of clinical outcomes after cetuximab plus irinotecan chemotherapy in patients with metastatic colorectal cancer. Oncology 82: 83-89.

17 Kjersem JB, Skovlund E, Ikdahl T, Guren T, Kersten C, Dalsgaard AM, Yilmaz MK, Fokstuen T, Tveit KM, Kure EH (2014) FCGR2A and FCGR3A polymorphisms and clinical outcome in metastatic colorectal cancer patients treated with first-line 5-fluorouracil/folinic acid and oxaliplatin +/- cetuximab. BMC Cancer 14: 340.

18 Geva R, Vecchione L, Kalogeras KT, Vittrup JB, Lenz HJ, Yoshino T, Paez D, Montagut C, Souglakos J, Cappuzzo F, Cervantes A, Frattini M, Fountzilas G, Johansen JS, Hogdall EV, Zhang W, Yang D, Yamazaki K, Nishina T, Papamichael D, Vincenzi B, Macarulla T, Loupakis F, De SJ, Spindler KL, Pfeiffer P, Ciardiello F, Piessevaux H, Tejpar S (2014) FCGR polymorphisms and cetuximab efficacy in chemorefractory metastatic colorectal cancer: an international consortium study. Gut Epub ahead of print. gutjnl-2014-307234

19 Negri FV, Musolino A, Naldi N, Bortesi B, Missale G, Laccabue D, Zerbini A, Camisa R, Chernyschova N, Bisagni G, Loupakis F, Ruzzo A, Neri TM, Ardizzoni A (2014) Role of immunoglobulin G fragment C receptor polymorphism-mediated antibody-dependant cellular cytotoxicity in colorectal cancer treated with cetuximab therapy. Pharmacogenomics J 14: 14-19. tpj201254 [pii];10.1038/tpj.2012.54 [doi].

20 Bibeau F, Lopez-Crapez E, Di FF, Thezenas S, Ychou M, Blanchard F, Lamy A, Penault-Llorca F, Frebourg T, Michel P, Sabourin JC, Boissiere-Michot F (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.

21 Calemma R, Ottaiano A, Trotta AM, Nasti G, Romano C, Napolitano M, Galati D, Borrelli P, Zanotta S, Cassata A, Castello G, Iaffaioli VR, Scala S (2012) Fc gamma receptor IIIa polymorphisms in advanced colorectal cancer patients correlated with response to anti-EGFR antibodies and clinical outcome. J Transl Med 10: 232. 1479-5876-10-232

22 Zhang W, Azuma M, Lurje G, Gordon MA, Yang D, Pohl A, Ning Y, Bohanes P, Gerger A, Winder T, Hollywood E, Danenberg KD, Saltz L, Lenz HJ (2010) Molecular predictors of combination targeted therapies (cetuximab, bevacizumab) in irinotecan-refractory colorectal cancer (BOND-2 study). Anticancer Res 30: 4209-4217.

23 Dahan L, Norguet E, Etienne-Grimaldi MC, Formento JL, Gasmi M, Nanni I, Gaudart J, Garcia S, Ouafik L, Seitz JF, Milano G (2011) Pharmacogenetic profiling and cetuximab outcome in patients with advanced colorectal cancer. BMC Cancer 11: 496. 1471-2407-11-496

24 Pander J, Gelderblom H, Antonini NF, Tol J, van Krieken JH, van der Straaten T, Punt CJ, Guchelaar HJ (2010) Correlation of FCGR3A and EGFR germline polymorphisms with the efficacy of cetuximab in KRAS wildtype metastatic colorectal cancer. Eur J Cancer 46: 1829-1834. S0959-8049(10)00248-0

25 Mellor JD, Brown MP, Irving HR, Zalcberg JR, Dobrovic A (2013) A critical review of the role of Fc gamma receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol 6: 1. 1756-8722-6-1

26 van der Straaten T, Martijn R, el HT, Baak-Pablo R, Guchelaar HJ (2013) A novel specific pyrosequencing method for genotyping FCGR3A rs396991 without coamplification of homologous gene FCGR3B. Pharmacogenet

Genomics 23: 631-635.

27 Bibeau F, Lopez-Crapez E, Di FF, Thezenas S, Ychou M, Blanchard F, Lamy A, Penault-Llorca F, Frebourg T, Michel P, Sabourin JC, Boissiere-Michot F (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.

28 Park SJ, Hong YS, Lee JL, Ryu MH, Chang HM, Kim KP, Ahn YC, Na YS, Jin DH, Yu CS, Kim JC, Kang YK, Kim TW (2012) Genetic polymorphisms of FcgammaRIIa and FcgammaRIIIa are not predictive of clinical outcomes after cetuximab plus irinotecan chemotherapy in patients with metastatic colorectal cancer. Oncology 82: 83-89.

29 Pander J, Heusinkveld M, van der Straaten T, Jordanova ES, Baak-Pablo R, Gelderblom H, Morreau H, van der Burg SH, Guchelaar HJ, van HT (2011) Activation of tumor-promoting type 2 macrophages by EGFR-targeting antibody cetuximab. Clin Cancer Res 17: 5668-5673.

30 De Roock W., Claes B, Bernasconi D, De SJ, 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 DS, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di FF, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van CE, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S (2010) 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 11: 753-762.

31 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 (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.

32 Tol J, Koopman M, Cats A, Rodenburg CJ, Creemers GJ, Schrama JG, Erdkamp FL, Vos AH, van Groeningen CJ, Sinnige HA, Richel DJ, Voest EE, Dijkstra JR, Vink-Borger ME, Antonini NF, Mol L, van Krieken JH, Dalesio O, Punt CJ (2009) Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. N Engl J Med 360: 563-572. 360/6/563

33 Fiocco M, Putter H, van Houwelingen JC (2009) Meta-analysis of pairs of survival curves under heterogeneity: a Poisson correlated gamma-frailty approach. Stat Med 28: 3782-3797.

34 Fiocco M, Stijnen T, Putter H (2012) Meta-analysis of time-to-event outcomes using a hazard-based approach: Comparison with other models, robustness and meta-regression. Computational Statistics & Data Analysis 56: 1028-1037.

35 Lievre A, Bachet JB, Le CD, Boige V, Landi B, Emile JF, Cote JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66: 3992-3995.

36 Ioannidis JP, Patsopoulos NA, Rothstein HR (2008) Reasons or excuses for avoiding meta-analysis in forest plots. BMJ 336: 1413-1415. 336/7658/1413 [

37 Michiels S, Baujat B, Mahe C, Sargent DJ, Pignon JP (2005) Random effects survival models gave a better understanding of heterogeneity in individual patient data meta-analyses. J Clin Epidemiol 58: 238-245.

38 de Souza AP, Bonorino C (2009) Tumor immunosuppressive environment: effects on tumor-specific and nontumor antigen immune responses. Expert Rev Anticancer Ther 9: 1317-1332.

39 Scartozzi M, Galizia E, Chiorrini S, Giampieri R, Berardi R, Pierantoni C, Cascinu S (2009) Arterial hypertension correlates with clinical outcome in colorectal cancer patients treated with first-line bevacizumab. Ann Oncol 20: 227-230.

40 Kono K, Takahashi A, Ichihara F, Sugai H, Fujii H, Matsumoto Y (2002) Impaired antibody-dependent cellular cytotoxicity mediated by herceptin in patients with gastric cancer. Cancer Res 62: 5813-5817.

41 Negri FV, Musolino A, Naldi N, Bortesi B, Missale G, Laccabue D, Zerbini A, Camisa R, Chernyschova N, Bisagni G, Loupakis F, Ruzzo A, Neri TM, Ardizzoni A (2014) Role of immunoglobulin G fragment C receptor polymorphism-mediated antibody-dependant cellular cytotoxicity in colorectal cancer treated with cetuximab therapy. Pharmacogenomics J 14: 14-19.

Appendix 1: data reconstruction

Starting point for the meta-analysis are the estimated survival curve reported for each study and the minimum and the maximum follow up (min_{FUP} and max_{FUP}) of patients. These quantities may be given directly but most often they will need to be estimated from the manuscript by looking at dates of accrual (if given) and from the date of submission, or perhaps publication of the manuscript. A model for the censoring mechanism based on the minimum and the maximum follow up is assumed here for computing the number at risk and person years for each time. Let C(t) be the function that models the censoring mechanism. Based on the available information we choose the function C(t) as follows

$$C(t) = \begin{cases} 1 & if\ t \leq minFUP \\ 1 - \dfrac{t - minFUP}{maxFUP - minFUP} & if\ minFUP < t < maxFUP \\ 0 & if\ t \geq maxFUP \end{cases} \qquad (1)$$

This function expresses the proportion of patients at time t that have at least t time units of follow-up. Given the number of eligible patients (n), the effective number at risk, the number of revisions at time j and the number of censored are estimated, respectively, as

$$\tilde{r}_j = nS_j C_j \ , \qquad (2)$$

$$d_j = n(S_{j-1} - S_j)\ \frac{C_{j-1} + C_j}{2} \qquad (3)$$

and

$$c_j = n(C_{j-1} - C_j)\ \frac{S_{j-1} + S_j}{2} \qquad (4)$$

This assumes that the censored observations are distributed uniformly over the interval. Under the same assumption, from the number of patients at risk r_j , we can determine the number of person-years over interval I_j , as $r_j = \Delta_j (r_j - c_j / 2)$, where $\Delta_j = t_j - t_{j-1}$ the length of I_j . Following the methodology described the data for each study involved in the meta-analysis have been reconstructed. A model with study as random effects has been fitted to the reconstructed data, to estimate the hazard ratio of progression free and overall survival and its associate confidence interval.