Retrospective evaluation of ADCC activity and cetuximab response in KRAS wild-type metastatic colorectal cancer patients (mCRC)

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, accounting for 940,000 million new cases annually and nearly 500,000 deaths each year.
Metastatic colorectal cancer (mCRC) previously untreated patients have demonstrated substantial improvements [1], and one of the responsible factors may be the development of systemic chemotherapy, as molecular-targeted therapy with cetuximab.
Cetuximab is a chimeric immunoglobulin G 1 (IgG1) monoclonal antibody (mAb) which binds the epidermal growth factor receptor (EGFR) with high affinity and inhibits ligand binding [2].
Cetuximab has been restricted to mCRC patients with wild-type RAS genes, since KRAS activating mutations have been reported in 40% of mCRC showing a negative effect on response to anti-EGFR antibodies [3-4]. Moreover, additional mutations in the downstream effectors of the EGFR signalling pathway, such as BRAF, NRAS, and PI3 kinase, might also cause loss in monoclonal therapy effectiveness. Therefore, the absence of mutations in RAS appears to be a reliable marker for predicting the efficacy of cetuximab [5].
The proposed working mechanism of cetuximab is thought to include antibody-dependent cell-mediated cytotoxicity (ADCC). However ADCC association with EGFR expression and/or mutational status of RAS and BRAF in mCRC remains unclear.
Indeed some clinical studies have failed to show a significant correlation between EGFR expression and the response to cetuximab [6].
Yuki Seo et al demonstrated that the ADCC activities were significantly associated with the cell surface expression levels of EGFR but not with the mutational status of KRAS and BRAF [7].
Moreover single nucleotide polymorphisms (SNPs) of genes encoding the Fcγ receptors [Fc fragment of IgG receptor 2A (FCGR2A) and 3A (FCGR3A)], which influence their affinity for the Fc fragment, have been linked to the pharmacodynamics of monoclonal antibodies.

AIM

In this study we aim to evaluate the prognostic and predictive value of cetuximab-mediated ADCC and to analyse its correlation with progression free survival (PFS) and overall survival (OS) in a cohort of 41 mCRC patients treated with cetuximab-based therapy.
Moreover we aim to investigate the association between the SNPs inside FCGR2A and FCGR3A genes and the effect of cetuximab treatment in this mCRC patients' cohort.

PATIENTS AND METHODS

Patients and clinical samples

We retrospectively identified, from March 2008 to September 2014, 41 mCRC patients who received chemotherapy with cetuximab (9 received cetuximab in first, 26 in second and 6 in third line).
An informed consent, approved by our local ethical committee, for tissue collection and use for scientific purpose was obtained from each patient enrolled.
Patients were evaluated for PFS, OS and response at the end of treatment with CT scan according to RECIST criteria.

DNA extraction, genotyping and mutational analyses

Genotyping of rs1801274 (A>G) in the FCGR2A, rs396991 (T>G) in FCGR3A and rs61764370 in the 3' UTR of KRAS genes was done on genomic DNA isolated from peripheral blood samples using a commercial kit (Qiagen, Germany) according to the manufacturer's instructions.
Analyses were determined using appropriate the allelic discrimination assays from Life Technologies (Foster city, CA, US): c_9077561_20 for rs1801274; c_25815666_10 for rs396991 and 1350086 for rs61764370 using the ABIPRISM 7000 Sequence Detection System.
Mutational analysis for KRAS (codons 12-13-59-61) gene was determined on patients' Formalin Fixed Paraffin Embedded (FFPE) tumor tissues archived at diagnosis in the Pathology Department of our Institution. KRAS gene analysis was performed by pyrosequencing using PyroMark ID System (Biotage, Uppsala, Sweden).

Antibody-dependent cell-mediated cytotoxicity (ADCC) assay

Peripheral blood samples were collected at start of therapy and every 2 months, during treatment and follow-up.
Enriched natural-killer (NK) cells were obtained from PBMC pellets using the human NK Cell Isolation Kit (Miltenyi Biotec, Cologne, Germany). NK cells were defined as CD56+/CD3-; T cells as CD3+/CD56- and invNKT cells by co-expression of CD3, TCR V24, TCR V11.
ADCC was evaluated over time as *ex vivo* NK-dependent activity with a standard lactate dehydrogenase (LDH) assay (Cytotox 96® non radioactive cytotoxicity assay, Promega, Madison, WI) as set up in our Laboratory [8].

Statistical analysis

Statistical analysis was performed using Prism software (version 5.0, GraphPad Software, La Jolla, CA).

RESULTS

Mutational analysis

KRAS mutational analysis in the 41 mCRC patients (**Table 1**) showed 2 mutated samples (G12V and Q61H) in patients treated with cetuximab in first line (N=9), while the remaining patients treated in second (N=26) and third (N=6) line resulted wild-type.

Genotyping analyses

The genotype distributions obtained for rs1801274 of FCGR2A, rs366991 of FCGR3A and rs61764370, located in the let-7 complementary site 6 (LCS6) of the KRAS 3' UTR, in the 41 mCRC patients of this study are presented in **Table 2**.

Survival Analysis according to ADCC activity

Median ADCC activity at treatment start for all the 41 mCRC patients was 68.5% (range 10-99%).
Correlation with OS and PFS was evaluated only in the sub-group of 26 patients treated with cetuximab in second line as it was the major group.
Characteristics of the 26 patients treated in second line are described in **Table 3**.

For this latter group of 26 patients treated with cetuximab in II line, median follow-up was 13 months (range 3-37) for OS and 5.5 months (range 2-37) for PFS, while the median value of ADCC was 71%.
Patients performing ADCC activity above the median value showed an improved OS compared to patients with ADCC activity below this value (median 21 vs 12 months; P=0.045; Long-rank Mantel-Cox Test) (**Figure 1**).

Survival Analysis according to genotypes of FCGR2A, FCGR3A genes and Let7 in KRAS 3'UTR.

Correlation in terms of OS and PFS with genotypes of rs1801274 (A>G) in the FCGR2A, rs396991 (T>G) in FCGR3A and rs61764370 in the 3' UTR of KRAS genes resulted significant in PFS for SNP in FCGR2A (P=0.04).
Patients carrying alleles with A presented a longer PFS in comparison with GG genotype (median 8 vs 3 months; p=0.04; Long-rank Mantel-Cox Test) (**Figure 2**).

Moreover patients showing the TT genotype of SNP rs396991 in FCGR3A gene presented also a longer PFS in comparison with patients carrying allele G (GT or GG), although this difference was not significant (median 11 vs 5 months; p=0.053; Long-rank Mantel-Cox Test) (**Figure 3**).
This effect resulted even amplified when PFS was evaluated in FCGR2A favourable alleles stratified for FCGR3A.

Patients carring both the A allele (AA or AG) for SNP rs1801274 of FCGR2A gene and the TT genotype of FCGR3A gene performed better than all the other subgroups (median 11 vs 5 months; p=0.024; Long-rank Mantel-Cox Test) (**Figure 4**).

CONCLUSION

These results indicate a link between ADCC activity, genotypes for FCGR2A and FCGR3A genes, coding FcRII and FcRIII, and efficacy of cetuximab in KRAS wild-type mCRC patients. This is in particular evident for patients treated with cetuximab in II line. Our results should be confirmed by further large prospective studies.

Table 1. Mutational analysis for *KRAS* gene in the 41 mCRC patients.

Gene		Patients treated in first line (N=9)	Patients treated in second line (N=26)	Patients treated in third line (N=6)
KRAS	WT	7	26	6
	Mutated	2	0	0

Table 2. *FCGR2A* (rs1801274; A>G), *FCGR3A* (rs366991; T>G) and LCS6 of *KRAS* 3' UTR (rs61764370) genotypes with the correspondent aminoacid in the 41 mCRC patients included in this study.

Gene	SNP	Genotype	Aminoacid	Number of subjects (%)
FCGR2A	rs1801274	A/A	H131H	14 (34%)
		A/G	H131R	20 (49%)
		G/G	R131R	7 (17%)
FCGR3A	rs396991	T/T	F158F	11 (27%)
		T/G	F158V	23 (56%)
		G/G	V158V	7 (17%)
KRAS 3' UTR	rs61764370	T/T	---	33 (80%)
		T/G	---	8 (20%)
		G/G	---	0 (--)

Table 3. Characteristics of 26 patients in II line and tumours.

	Number of patients	Rates	
Gender			**Median age (range) yrs**
Male (M)	17	65.4%	66.6 (51 – 77)
Female (F)	9	34.6%	71.7 (49 – 82)
Primary tumour			
Right Colon	2	7.7%	
Left Colon	14	53.8%	
Rectal	10	38.5%	
Grade			
G1/G2	19	73%	
G3	7	27%	
Metastasis			
Liver only	9	35%	
Liver plus other	8	30%	
Extra-hepatic	9	35%	
Response			
Responders			
RC	3	11.5%	
RP	8	30%	
SD	5	20%	
Non-Responders			
PD	10	38.5%	

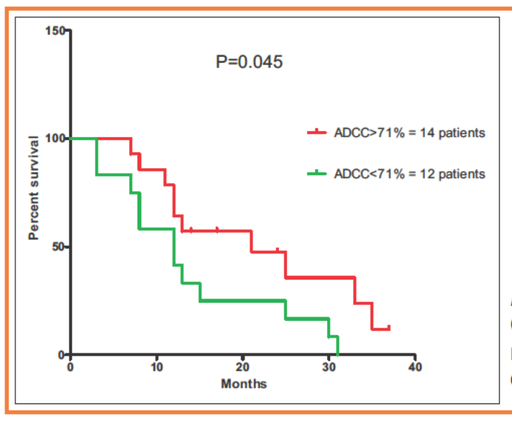

Figure 1. Overall survival in 26 mCRC treated with cetuximab in II line.

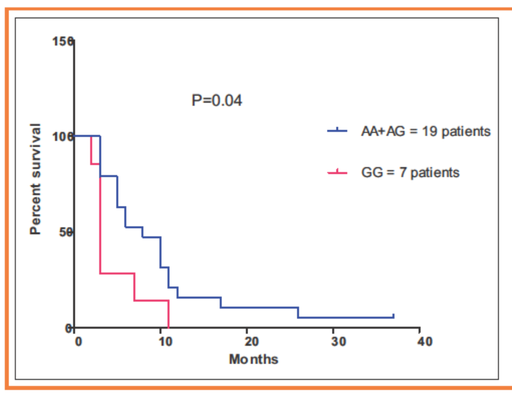

Figure 2. PFS for SNP rs1801274 in FCGR2A gene inside 26 mCRC patients' group.

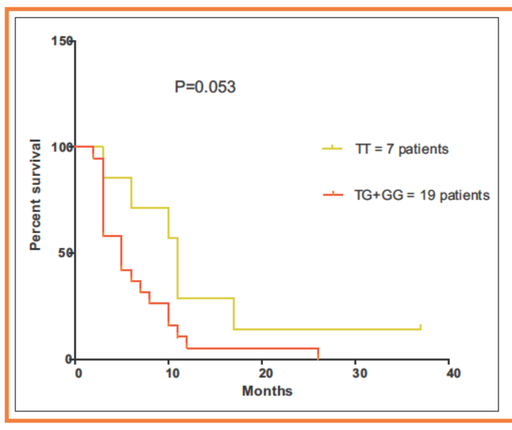

Figure 3. PFS for SNP rs396991 in FCGR3A gene inside 26 mCRC patients' group.

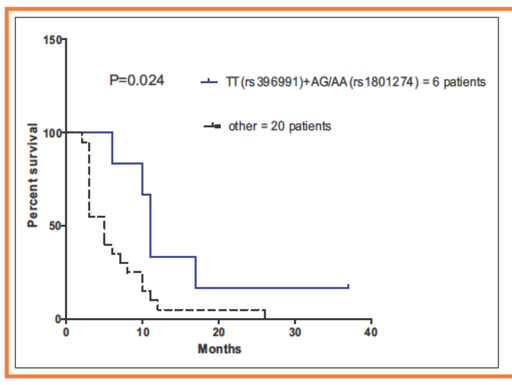

Figure 4. PFS for compound heterozygote patients for SNPs rs1801274 in FCGR2A and rs396991 in FCGR3A genes in the 26 mCRC patients' group.

REFERENCES

1. Kopetz S, Chang GJ, Overman MJ, et al: Improved survival in metastatic colorectal cancer is associated with adoption of hepatic resection and improved chemotherapy. *J Clin Oncol* 27: 3677-3683, 2009.
2. Cunningham D, Humblet Y, Siena S, et al.: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastaticcolorectal cancer. *N Engl J Med* 2004, 351:337-345.
3. Allegra CJ, Jessup JM, Somerfield MR et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009; 27:2091-2096.
4. Sartore-Bianchi A, Di Nicolantonio F, Nichelatti M, et al. Multi-determinants analysis of molecular alterations for predicting clinical benefi t to EGFR-targeted monoclonal antibodies in colorectal cancer. *PLoS One* 2009; 4: e7287.
5. De Roock W, Claes B, Bernasconi D et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; 11:753–62.
6. Marechal R, De Schutter J, Nagy N et al. Putative contribution of CD56 positive cells in cetuximab treatment efficacy in first-line metastatic colorectal cancer patients. *BMC Cancer 2010*; 10: 340-350.
7. Yuki S, Yoshiyuki I, Hiroki O et al. Cetuximab-mediated ADCC activity is correlated with the cell surface expression level of EGFR but not with the KRAS/BRAF mutational status in colorectal cancer. *Oncology Reports* 2014, 31: 2115-2122, 2014.
8. Monteverde M, Milano G, Strola G, Maffi M, Lattanzio L, Vivenza D, Tonissi F, Merlano M, Lo Nigro C. The relevance of ADCC for EGFR targeting: A review of the literature and a clinically-applicable method of assessment in patients. *Crit Rev Oncol Hematol.* 2015 Mar 12. pii: S1040-8428(15)00048-7.