Evaluation of antibody-dependent cell-mediated cytotoxicity activity and cetuximab response in KRAS wild-type metastatic colorectal cancer patients

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Abstract

AIM: To investigate the prognostic role of invariant natural killer T (iNKT) cells and antibody-dependent cell-mediated cytotoxicity (ADCC) in wild type KRAS metastatic colorectal cancer (mCRC) patients treated with cetuximab.

METHODS: Forty-one KRAS wt mCRC patients, treated with cetuximab and irinotecan-based chemotherapy in II and III lines were analyzed. Genotyping of single nucleotide polymorphism (SNP)s in the FCGR2A, FCGR3A and in the 3’ untranslated regions of KRAS and mutational analysis for KRAS, BRAF and NRAS genes was determined either by sequencing or allelic discrimination assays. Enriched NK cells were obtained from lymphoprep-peripheral blood mononuclear cell and iNKT cells were defined by co-expression of CD3, TCRVα24, TCRVβ11. ADCC was evaluated as ex vivo NK-dependent activity, measuring lactate dehydrogenase release.

RESULTS: At basal, mCRC patients performing ADCC activity above the median level (71%) showed an improved overall survival (OS) compared to patients with ADCC
below (median 16 vs 8 mo; \(P = 0.026\)). We did not find any significant correlation of iNKT cells with OS (\(P = 0.19\)), albeit we observed a trend to a longer survival after 10 mo in patients with iNKT above median basal level (0.382 cells/microliter). Correlation of OS and progression-free survival (PFS) with interesting SNPs involved in ADCC ability revealed not to be significant. Patients carrying alleles both with A in FCGR2A and TT in FCGR3A presented a trend of longer PFS (median 9 vs 5 mo; \(P = 0.064\)). Chemotherapy impacted both iNKT cells and ADCC activity. Their prognostic values get lost when we analysed them after 2 and 4 mo of treatment.

CONCLUSION: Our results suggest a link between iNKT cells, basal ADCC activity, genotypes in FCGR2A and FCGR3A, and efficacy of cetuximab in \(KRAS\) wt mCRC patients.

Key words: Metastatic colorectal cancer; Single nucleotide polymorphism in Fc-\(\gamma\) receptors; Cetuximab; RAS family; Antibody-dependent cell-mediated cytotoxicity; Invariant natural killer T cells

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Core tip: A high number of invariant natural killer T (iNKT) cells and a high antibody-dependent cell-mediated cytotoxicity (ADCC) activity, evaluated before therapy, do correlate significantly with a longer overall survival in metastatic colorectal cancer patients treated with immuno-oncology-based chemotherapy and cetuximab in II and III lines. Chemotherapy impacted both iNKT cells and ADCC activity. The prognostic value of ADCC above the median basal level, get lost when we analysed those parameters after 2 and 4 mo of treatment. Correlation of overall survival and progression-free survival with interesting single nucleotide polymorphisms reported as involved in ADCC ability, either in the FCGR2A, FCGR3A or in the 3' untranslated regions of \(KRAS\) gene, revealed not to be significant.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, accounting for 940000 million new cases annually and nearly 500000 deaths each year. Metastatic colorectal cancer (mCRC) previously untreated patients have demonstrated substantial improvements, with a median overall survival time now reaching more than 24 mo, by the development of systemic chemotherapy, including molecular-targeted therapy[1].

The epidermal growth factor receptor (EGFR) signalling pathway is involved in cell differentiation, proliferation, migration, angiogenesis and apoptosis, all processes dysregulated in cancer cells.

Cetuximab is a chimeric immunoglobulin G1 (IgG1) monoclonal antibody (mAb) which binds EGFR with high affinity and inhibits ligand binding[2]. \(KRAS\) activating mutations have been reported in 40% of mCRC showing a negative effect on response to anti-EGFR antibodies[3,4]. Mutations in other downstream effectors of the EGFR signalling pathway, such as \(BRAF\), \(NRAS\) and \(PI3K\) kinase, might also impact the efficacy of monoclonal therapy. Thus, the absence of mutations in \(RAS\) appears to be a reliable marker for predicting the efficacy of cetuximab which was been restricted to mCRC patients with wild-type \(RAS\)[5]. Several studies supported the biological activity of cetuximab in advanced CRC. Cetuximab enhances response rate and progression-free survival (PFS) in first-line therapy in combination with Folfiri and Folfox regimen of chemotherapy[6,7]. However, some clinical studies have failed to show a significant correlation between EGFR expression and the response to cetuximab[8]. The proposed working mechanism of cetuximab is thought to include antibody-dependent cell-mediated cytotoxicity (ADCC)[9].

ADCC utilizes the response of innate immune cells to provide antitumor cytotoxicity triggered by the interaction of the Fc portion of the antibody with the Fc receptor on the immune cell. Immunotherapeutics that target natural killer (NK) cells, \(\gamma\delta\) T cells, macrophages and dendritic cells can, by augmenting the function of the immune response, enhance the antitumor activity of the antibodies[10].

Invariant CD1d-restricted natural killer T (NKT) cells are T lymphocytes characterized by an invariant T-cell antigen receptor-chain rearrangement that co-express NK cell markers[11].

Molling et al[12] in 2007 demonstrated that a severe circulating invariant NKT (iNKT) cell deficiency was related to poor clinical outcome in head and neck squamous cell carcinoma patients, suggesting their critical contribution to antitumor immune responses. Furthermore, screening for iNKT cell levels may be useful for determining which patients can benefit from immunotherapeutic adjuvant therapies aimed at reconstitution of the circulating iNKT cell pool.

Whether ADCC is associated with EGFR expression and/or the mutational status of \(RAS\) and \(BRAF\) in CRC remains unclear. Seo et al[13] 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 \(BRAF\). In this study we aimed to evaluate the prognostic and predictive value of cetuximab-mediated ADCC and circulating iNKT cells levels in mCRC and to analyse their correlation with EGFR level, mutational status of
Lo Nigro C et al. ADCC and cetuximab response in mCRCs

**Table 1** Characteristics of 41 patients in II and III line and tumours

| Gender       | Number of patients | Rates (range) | Median age (range) yr |
|--------------|--------------------|--------------|-----------------------|
| Male (M)     | 23                 | 56%          | 67.5 (51-84)          |
| Female (F)   | 18                 | 44%          | 64.6 (49-83)          |
| Primary tumour |                    |              |                       |
| Right colon  | 7                  | 17%          |                       |
| Left colon   | 21                 | 51%          |                       |
| Rectal       | 13                 | 32%          |                       |
| Grade        |                    |              |                       |
| G1/G2        | 27                 | 65.8%        |                       |
| G3           | 13                 | 31.7%        |                       |
| NA           | 1                  | 2.5%         |                       |
| Metastasis   |                    |              |                       |
| Liver only   | 12                 | 29.3%        |                       |
| Liver plus other sites | 14 | 34.1% | |
| Extra-hepatic sites | 15 | 36.6% | |
| Response     |                    |              |                       |
| Responders   |                    |              |                       |
| CR           | 4                  | 9.8%         |                       |
| PR           | 12                 | 29.3%        |                       |
| SD           | 8                  | 19.5%        |                       |
| Non-responders |                |              |                       |
| PD           | 17                 | 41.4%        |                       |
| Line of treatment |        |              |                       |
| II           | 33                 | 8%           |                       |
| III          | 8                  | 2%           |                       |

NA: Not available; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease.

**KIRAS, NRAS, BRAF, PFS and overall survival (OS) in a prospective cohort of mCRC patients treated with cetuximab-based therapy.**

**MATERIALS AND METHODS**

**Patients and clinical samples**

A total of 41 mCRC patients were enrolled in this study from March 2008 to September 2014. Characteristics of the 41 patients are described in Table 1. An informed consent for tissue collection and use for scientific purpose was obtained from each patient enrolled in this study, approved by the local Ethical Committee and carried out in the respect to Helsinki Declaration. Inclusion criteria for mCRC patients were: Suitability for combination therapy including cetuximab with irinotecan-based chemotherapy in second and third lines and **KIRAS wild type (wt)** status. Patients were evaluated for PFS, OS and response at the end of treatment with CT scan according to RECIST criteria[2]. Median follow-up was 25 mo (range 10-70).

**DNA extraction, genotyping and mutational analyses**

Genotyping of rs1801274 (A > G) in the FCGR2A, rs396991 (T > G) in FCGR3A and rs61764370 in the 3’ untranslated regions (3’ UTR) of KIRAS gene was done on genomic DNA isolated from whole peripheral blood samples using the EZ1 DNA Blood 200 Kit (Qiagen, Germany) according to the manufacturer’s instructions. Analyses were determined using the appropriate

“allelic discrimination assay” from Life Technologies (Foster city, CA, United States); c_9077561_20 for rs1801274; c_25815666_10 for rs396991 and 1350086 for rs61764370 using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems Foster City, CA, United States).

Mutational analyses for KIRAS (codons 12-13-59-61-146), BRAF (codon 600) and NRAS (codons 12-13-59-61-117-146) genes were determined on patients' DNA extracted from Formalin Fixed Paraffin Embedded (FFPE) tumor tissues archived at diagnosis in the Pathology Department of our Institution, by a standard protocol that included proteinase K treatment (EuroClone, Pero, IT).

KIRAS and BRAF gene analyses were performed by pyrosequencing using PyroMark ID System (Biotage, Uppsala, Sweden), while a Real-Time PCR (OncoSreen NRAS; Relab, Jesi, Italy) was employed for NRAS gene using the Rotor-Gene 6000 (Corbett Research, Pty Ltd; Sydney, Australia) according to the manufacturer’s protocol.

**Anti-body-dependent cell-mediated cytotoxicity assay**

Twelve milliliter peripheral blood samples were collected at start of therapy for all the 41 patients and ADCC and NK cells were evaluated at basal level. After 2 and 4 mo of treatment a second collection of blood was done in 30 and 23 patients respectively where ADCC and NK cells were longitudinally studied.

Enriched NK cells were obtained from lymphoprep-peripheral blood mononuclear cell 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 invariant NKT (iNKT) cells by co-expression of CD3, TCR Vα24, TCR Vβ11.

ADCC was evaluated 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[14].

**Statistical analysis**

Statistical analyses were performed using the GraphPad Prism 5 (San Diego, CA, United States) and SPSS version 13 (SPSS, Chicago, IL) programs. The association between ADCC median levels was analyzed using the Fisher’s exact test or the Pearson’s test when appropriate. OS analyses were based on the time from treatment start to death or last contact in which the survivors were censored. PFS analyses were based on the time from treatment start to first event; patients without an event were censored at their last follow-up. OS was calculated using the Kaplan-Meier method with log-rank test for statistical significance. A P-value < 0.05 was considered statistically significant.

**RESULTS**

**Clinical and molecular characteristics of patients**

Clinical characteristics of the 41 mCRC patients are
Survival analysis according to iNKT cells

iNKT cells evaluated before treatment were analysed to seek correlation with OS and PFS either as number of cells/microliter or as % of T cells, since a low level of circulating iNKT cells has been reported to predict poor clinical outcome in patients with head and neck squamous cell carcinoma[12]. iNKT cells median value at basal determination, before treatment, was 0.382 cells/microliter. We did not find any significant correlation of iNKT cells with OS (P = 0.19), albeit we observed a trend to a longer survival after 10 mo in the population of patients (n = 21) with iNKT above median level (Figure 1).

Survival analysis according to ADCC activity

Median ADCC activity before treatment for all the 41 mCRC patients was 71% (range 10%-99%). Comparison between patients with ADCC above and below median value is reported in Table 3. There were no differences in the clinical characteristics between the two groups, although EGFR over-expression was more common in patients with ADCC activity above the median level (P = 0.052; Fisher’s exact test). Correlation with OS and PFS was evaluated. Median OS was 12 mo (range 3-37) and PFS was 6 mo (range 3-37). Patients performing ADCC activity above the median level showed an improved OS compared to patients with ADCC activity below this value (median 16 vs 8 mo; P = 0.026; Long-rank Mantel-Cox Test) (Figure 2). On the contrary, there was no difference in PFS between patients with ADCC below or above the median level (data not shown). When we stratified patients for both iNKT and ADCC activity at basal level, below and above the respective median level, we observed a better OS in patients having both values above the median level compared to all the other combinations (median 23 vs 10 mo; P = 0.0075; Long-rank Mantel-Cox Test) (Figure 3).

Survival analysis according to genotypes of FCGR2A, FCGR3A genes and in KRAS 3’UTR

Correlation in terms of OS and PFS with each genotype, either rs1801274 (A > G) in the FCGR2A, rs396991 (T > G) in FCGR3A or rs61764370 in the 3’ UTR of KRAS gene revealed not to be significant (data not shown).

Patients carrying alleles both with A in FCGR2A (AA/AG genotypes) and T in FCGR3A presented a longer PFS (median 9 vs 5 mo; P = 0.064; Long-rank Mantel-Cox Test) in comparison to all the other subgroups (Figure 4), although the difference was not significant.

Survival analysis according to mutational status in RAS family genes

Due to the limited number of patients we were not able to perform OS and PFS analyses according to all-RAS gene mutations. Nevertheless we observed that of ADCC activity, as median level, was not affected by the presence of a NRAS or a BRAF mutation (data not shown).

How the treatment influenced iNKT cells and ADCC activity

Both iNKT cells and ADCC activity were evaluated over time to seek for dynamic changes during treatment and to investigate the impact of therapy on patients’ ability to perform ADCC and their clinical outcome. iNKT cells median number decreased from 0.382 cells/microliter at basal, before treatment, to 0.193 after 2 mo and to 0.06 after 4 mo of treatment (P = 0.19, Long-rank Mantel-Cox Test). 

Figure 1. Overall survival in 41 metastatic colorectal cancer treated with cetuximab in II and III lines according to median basal level of invariant natural killer T cells (0.382 cells/microliter). iNKT: Invariant natural killer T.

Figure 2. Survival analysis according to ADCC activity. Median OS was 12 mo (range 3-37) and PFS was 6 mo (range 3-37). Patients performing ADCC activity above the median level showed an improved OS compared to patients with ADCC activity below this value (median 16 vs 8 mo; P = 0.026; Long-rank Mantel-Cox Test).
Survival analysis according to variation in ADCC activity during treatment

ADCC determination during treatment lost its prognostic value since there was no difference in OS between patients with ADCC activity above or below the median level after 2 and after 4 mo of treatment (data not shown).

Variation of ADCC values was analyzed by stratifying patients on the basis of to the median values at basal level (71%, 41 patients), after 2 mo (45%, 30 patients) and after 4 mo (45%, 23 patients) of treatment. Combination of longitudinal values generated 4 groups of patients: the first included patients showing both ADCC activities above the median level [ADCCbas above/II (or III) above, where II means on blood drawn after 2 mo and III after 4 mo], the second group a decrease from a basal above median to a II or III determination below median [ADCCbas above/II (or III) below], the third group patients showing instead an increase from below at basal and above at II or III determination [ADCCbas below/II (or III) above] and the fourth group patients with both ADCC activities below the median level [ADCCbas below/II (or III) below].

We then analysed correlation with OS in the 4 groups. Patients performing ADCC activity above the median level both at basal level and either after 2 and/or 4 mo presented a trend in longer OS, albeit not significant (Figure 5).

When we focus on patients presenting ADCC values above the median levels in both determinations (basal and after 2 mo) we found that this 9 out of the 30 patients (30%) showed a higher OS compared to other patients (median 21 vs 13 mo, P = 0.5; Long-rank Mantel-Cox Test). After 4 mo, 8 patients out of 23 (35%) had both values above the median levels of ADCC activity, but their OS was not statistically different from that of the other patients (median 18.5 vs 15 mo; P = 0.42; Long-rank Mantel-Cox Test (data not shown).
Among them, the Fc region of the mAb may also trigger the selective blockade of tumoral membrane receptors. CETUXIMAB, may have mechanisms of action other than exclusive with of 20% of CRCs. Moreover, the have independently been found to give rise to resistance. EGFR only 0.1%-2% of the that tumors without mutations in codon 12 or 13 of the objective response. In fact, it subsequently became clear 20% patients displaying wild-type ADCC, binding via Fv regions the target cell to any of the Fc-γ receptors, i.e., CD16, CD32 and CD64, which are expressed, with different patterns, by cells of the innate immune system, namely monocytes, macrophages, granulocytes and NK. The contribution of the different cell types to the anti-tumor ADCC exerted in vivo by anti-EGFR mAbs is still debated. In general these cell are thought to play a relevant role controlling tumor growth and in preventing metastatic dissemination in humans. In particular, NK cells have been suggested to be the major mediators of the ADCC-dependent therapeutic effect of cetuximab. Moreover, invariant CD1d-restricted NKT cells has been reported to play an allegedly pivotal role in such responses via transactivation of immune effector cells. In particular, a severe circulating NKT cell

**DISCUSSION**

Treatment of mCRC requires a multidisciplinary approach and multiple treatment options are nowadays available. Advances in the understanding of tumor biology have led to the development of EGFR-targeted therapies as mAbs. In fact, the EGFR-signalling pathway regulates important processes involved in cell differentiation, proliferation, migration, angiogenesis and apoptosis, all of which become deregulated in cancer cells. However, the mechanisms that mediate the therapeutic effect of these mAbs are still unclear.

Cetuximab is a chimeric monoclonal antibody that specifically targets EGFR with high affinity and prevents the ligand-mediated activation of the EGFR-dependent pathway. KRAS mutations occur in 35%-45% of mCRC and preclude responsiveness to EGFR-targeted therapy with cetuximab or panitumumab. Initial response rates of about 10% were seen with cetuximab monotherapy in patients with heavily pretreated mCRC. A phase II BOND study demonstrated the ability of cetuximab to circumvent irinotecan-based chemotherapy resistance. Less than 20% patients displaying wild-type KRAS tumors achieve objective response. In fact, it subsequently became clear that tumors without mutations in codon 12 or 13 of the KRAS gene responded in 13%-17% of cases, whereas only 0.1%-2% of the KRAS mutant tumors did.

Alterations in other effectors downstream of the EGFR and deregulation of the PIK3CA/PTEN pathway have independently been found to give rise to resistance. Moreover, the PIK3CA gene is mutated in approximately 20% of CRCs. BRAF is the principal downstream effector of KRAS and its oncogenic V600E mutation is mutually exclusive with KRAS mutations in CRCs.

It has recently become clear that IgG1 mAb, like cetuximab, may have mechanisms of action other than the selective blockade of tumoral membrane receptors. Among them, the Fc region of the mAb may also trigger ADCC, via Fv regions the target cell to any of the Fc-γ receptors, i.e., CD16, CD32 and CD64, which are expressed, with different patterns, by cells of the innate immune system, namely monocytes, macrophages, granulocytes and NK. The contribution of the different cell types to the anti-tumor ADCC exerted in vivo by anti-EGFR mAbs is still debated. In general these cell are thought to play a relevant role controlling tumor growth and in preventing metastatic dissemination in humans.

In particular, NK cells have been suggested to be the major mediators of the ADCC-dependent therapeutic effect of cetuximab. Moreover, invariant CD1d-restricted NKT cells has been reported to play an allegedly pivotal role in such responses via transactivation of immune effector cells. In particular, a severe circulating NKT cell
deficiency was related to poor clinical outcome in head and neck squamous cell carcinoma patients [22].

Thus, the number of iNKT and the level of ADCC activity exerted by NK cells from tumor patients in the presence of cetuximab might be useful prognostic or predictive parameters for response to treatment. With this in mind, we investigated 41 mCRC patients suitable for combination therapy including cetuximab with irinotecan-based chemotherapy in second and third lines and KRAS wild type.

Analyses were carried out at start of therapy for all the 41 patients and ADCC and iNKT cells were evaluated at basal level. After 2 and 4 mo of treatment additional determinations were done in 30 and 23 patients respectively where ADCC and NK cells were longitudinally studied.

Main aim of the project was to study ex-vivo the prognostic and predictive value of the number of iNKT cells and the level of cetuximab-mediated ADCC and to analyse their correlation with EGFR level, mutational status of KRAS, NRAS, BRAF and PFS and OS in our prospective cohort of mCRCs.

We did not find any significant correlation of iNKT cells at basal level with PFS nor with OS, albeit we observed a trend to a longer survival after 10 mo in the population of patients with iNKT above median level. Instead, patients performing, at basal determination, ADCC activity above the median level showed an improved OS compared to patients with ADCC activity below this value.

Moreover, if we combine iNKT number and ADCC basal level and we stratified patients for both determinations, as below or above the respective median level, we observed a better OS in patients having both values above the median level compared to all the other combinations. Of note, when we analysed the same parameters after 2 and 4 mo of treatment, levels of circulating T, NK, iNKT cells were significantly reduced. On the clinical side, we observed that cancer patients exhibited a lower capacity to perform ADCC as compared to the beginning of therapy; this observation has to be replaced in the global context of an immunosuppressed state of cancer patients and the immunosuppressive effect of chemotherapy and it is consistent with earlier reports [20].

Intriguing, during treatment, neither low level of iNKT nor low ADCC activity did correlate to prognosis. In our study this could be in apparent contrast with what observed by us at the beginning of therapy and also with what reported by others, which is patients with a severe numeric iNKT cell deficiency have a strikingly poor clinical outcome in response to chemo and radiotherapy [21].

On the other hand, we’re analysing NK levels and their activity in peripheral blood; we did not have the picture of the functional properties of tumor-infiltrating T and NK cells in patients. A reduced number of NK and iNKT cells in periphery might be “the other side of the coin” and may reflect an increased activity in the tumor infiltrates [21].

The impact of ADCC on the efficacy of cetuximab might also be influenced by the occurrence of polymorphic forms of genes coding receptors for the antibody Fc region. The most relevant polymorphisms regulating Fc:FcR interactions are phenylalanine (F) or valine (V) expression at position 158 of the Fc fragment [22]. In particular, differential response to therapeutic mAbs has been reported to correlate with specific polymorphisms in two of these genes: FCGR2A (H131R) and FCGR3A (V158F) [23]. However, previous studies exploring the relation between the FCGR polymorphisms and cetuximab efficacy in mCRC have demonstrated conflicting and have been mostly low-powered studies with small sample sizes [24].

More recently a variant allele in a let-7 microRNA complementary site within the 3’UTR of KRAS (rs61764370) has been correlated with clinical outcome in mCRC patients receiving cetuximab [25].

In our cohort of mCRCs, correlation in terms of OS and PFS with each genotype, either rs1801274 (A > G) in the FCGR2A, rs396991 (T > G) in FCGR3A or rs61764370 in the 3’UTR of KRAS gene didn’t reveal to be significant. Interestingly enough, patients carrying alleles both with A in FCGR2A (AA/AG genotypes) and TT in FCGR3A presented a longer PFS, although the difference was not significant, probably due to the low number of patients.

For the same reason, we were not able to perform OS and PFS analyses according to all-RAS gene mutations. It is well know, in fact, that activating KRAS mutations are negative predictors of the response to cetuximab therapy in patients with mCRC, since cetuximab is widely considered to be unable to block the signal initiated by oncogenic KRAS [26,27].

Nevertheless we observed that of ADCC activity, as median level, was not affected by the presence of a NRAS or a BRAF mutation.

Seo et al. [12] demonstrated cetuximab-mediated ADCC in human CRC cell lines and observed that ADCC activities for the tumor cells were higher in CRC patients with a high expression level of EGFR. Furthermore, the ADCC activity level was significantly associated with EGFR, but not with the KRAS/BRAF mutational status.

This has to be considered also in the light of the preclinical studies of nakadate and colleagues, who demonstrated that, in an ADCC assay, perforin-dependent target cell lysis was not affected by the KRAS mutation status. On the other hand, perforin-independent ADCC was observed only in CRC cells with wild-type KRAS, but not in cells with mutant KRAS. Their experiments also revealed that the Fas-Fas ligand (FasL) interaction was responsible for the induction of apoptosis and perforin-independent ADCC. Thus, their findings clearly suggested that ADCC is an important mode of action of cetuximab and that KRAS mutation impairs the therapeutic effect exerted by cetuximab-mediated ADCC. In our study, regrettably, the limited number of patients precluded any definitive confirmation of this in our clinical setting of mCRC patients [27]. Therefore, all together, our results seem to suggest a link between iNKT cells, basal ADCC
activity, genotypes in FCGR2A and FCGR3A, and efficacy of cetuximab in KRAS wild-type mCRC patients.

The efficacy of monoclonal anti-EGFR antibodies, like cetuximab, has been proven in mCRC patients. It has been established clearly that response to anti-EGFR antibody treatment is only possible in selected patient groups. However, predictive factors for the efficacy of anti-EGFR therapy have still to be completely elucidated. A factor identified in multiple studies as essential for appropriate assessment of eligibility for cetuximab or panitumumab treatment is the absence of KRAS gene mutations. EGFR expression on the surface of cancer cells does not seem to have a decisive influence on the efficacy of the therapy. There are ongoing studies assessing the predictive value of the number of copies of the EGFR gene, mutations in the NRAS, PI3KCA, P53 and PTEN genes, concentration of EGFR ligands and polymorphisms in the EGFR and EGFR, and the FCGR2A and FCGR3A genes.

In our study, we observed that combining INKT number and ADCC basal level allowed to identify a group of mCRC patients, having both determinations above the respective median level and a longer OS. This combination looks like the best prognosticator in our population of patients. However, it has not as of yet been examined in large randomized prospective studies and hence should still be better elucidated before using as a basis for mCRC patient eligibility for cetuximab treatment.

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Peer-review

This manuscript contributes to shed light to monoclonal therapy response in mCRC patients.
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