The handle [http://hdl.handle.net/1887/33832](http://hdl.handle.net/1887/33832) holds various files of this Leiden University dissertation

**Author:** Krens, Lisanne  
**Title:** Refining EGFR-monoclonal antibody treatment in colorectal cancer  
**Issue Date:** 2015-07-02
Chapter 11
The use of the epidermal growth factor receptor (EGFR) antibodies cetuximab and panitumumab is limited to colorectal cancer (CRC) patients with KRAS wild type tumors and more recently in RAS wild type only. After having become chemotherapy refractory, treatment options are limited for this substantial patient group. This means that there is an urgent need to optimize anti-EGFR therapy. The work presented in this thesis aimed at optimising EGFR targeted monoclonal antibody therapy in metastatic CRC.

This thesis investigates several strategies to refine EGFR targeted monoclonal antibody therapy in CRC by:

- statins and their ability to phenoconvert KRAS mutant CRC;
- exploration of polymorphisms in the gene encoding FCGR3A and their association with cetuximab efficacy;
- investigating the pharmacokinetics of cetuximab and panitumumab in patients with renal or hepatic dysfunction.

In patients with KRAS mutant tumors, the KRAS protein is highly active and these patients’ tumors do not respond to anti-EGFR therapy. Before the KRAS protein exerts its important function in the cell signaling cascade, prenylation of the KRAS protein is required. Prenylation is the addition of C15 and C17 fatty acid chains to the KRAS protein. Prenylated KRAS is more lipophilic and can easily associate with the membrane. Membrane association of KRAS is crucial for its function in the RAS-RAF-MAPK signaling pathway. Statins and other KRAS modulators, such as bisphosphonates, farnesyltransferase inhibitors or geranylgeranyltransferase inhibitors affect the prenylation of the KRAS protein. Inhibition of the prenylation may lead to a more wild type KRAS phenotype. The modification of the KRAS mutant phenotype to a more KRAS wild type phenotype may augment the effect of EGFR antibodies in patients with KRAS mutations.

In chapter 2, clinical studies with statins and other KRAS modulators and their use in cancer treatment are reviewed. This review indicates that combinations of EGFR antibodies to target the EGFR with KRAS modulators may be an effective approach in patients with KRAS mutant tumors.

Chapter 3 describes an in vitro study using KRAS wild type and mutant cell lines. The aim of this study was to understand the role of statins in CRC cells and to explore the potential of therapeutic modulation of KRAS mutated CRC tumor cell lines. Western blot analysis showed that simvastatin inhibited the prenylation of the KRAS protein. The inhibition by simvastatin resulted in less membrane association of KRAS. A survival assay was used to study the effects of simvastatin and cetuximab on proliferation in colorectal cancer cell lines. In KRAS G13D mutated HCT116 and LoVo cell lines a combination of simvastatin pre-treatment and cetuximab resulted in less proliferation. This effect was not observed in the SW480 cell line harbouring a codon 12 KRAS mutation.

Since the in vitro studies showed promising results, we decided to perform a retrospective analysis to evaluate the effect of statin use on outcome in KRAS mutant metastatic CRC patients treated with cetuximab. In the CAIRO2 study by the Dutch Colorectal Study Group Metastatic CRC patients were treated with capecitabine, oxaliplatin, bevacizumab with or without cetuximab. We retrospectively analysed the effect of statin use at time of diagnosis on progression free survival (PFS) in CRC patients with KRAS mutant tumors treated with cetuximab and described the results in chapter 4. In our study we showed that the use of statins in patients with a KRAS mutant tumor did not lead to an improved progression free survival.
Summary

In two prospective studies we investigated the potential of simvastatin to phenoconvert mutant KRAS in CRC patients treated with cetuximab or panitumumab. In the RASTAT C and P studies described in chapter 5 and 6, metastatic CRC patients who failed on first- and second-line therapy, were treated with 80 mg of simvastatin daily and cetuximab (RASTAT C) or panitumumab (RASTAT P). Both studies were terminated after a planned interim analysis of the Simon two-stage design, because similar survival as seen in KRAS wild type patients was not observed.

An important mechanism of cetuximab induced cell-killing is antibody-dependent cellular cytotoxicity (ADCC). Fc gamma receptors (FCGR) on effector cells, for example macrophages and natural killer cells, bind to the Fc fragment of the cetuximab molecule and this causes lysis of the cancer cell. The germline polymorphism (rs396991) in the Fc gamma receptor 3A (FCG3A) c.818A>C results in a change of phenylalanine to valine at codon 158. Previous results from studies investigating the association between F158V FCGR3A polymorphisms and cetuximab efficacy are highly variable and firm conclusions cannot be drawn. To clarify the effect of the FCGR3A F158V polymorphism on efficacy a meta-analysis was performed. The individual patient data meta-analysis (chapter 7) shows that FCGR3A polymorphism is not associated with improved survival in cetuximab treated CRC patients. Some earlier studies showed that patients with specific FCGR3A polymorphisms might benefit from cetuximab treatment regardless of their KRAS mutational status. In this study, there is no significant difference in cetuximab efficacy between patients with KRAS wild type and mutant tumors.

Both cetuximab and panitumumab are used in patients with advanced or metastatic disease. Due to previous treatment or metastatic disease these patient are likely to have renal or hepatic insufficiency. Knowledge of dosing in special populations with impaired renal or hepatic function is highly relevant. The pharmacokinetics and safety of both cetuximab and panitumumab have not been studied in these special populations. In two case reports, with panitumumab and cetuximab in cancer patients with liver and kidney dysfunction respectively, we showed that dose adjustments in patients with liver or kidney failure are not necessary and that treatment seems to be tolerable and safe (chapters 8 and 9).

In chapter 10 the results from the performed research are discussed and future perspective are presented. Despite the promising results from the preclinical study, KRAS modulation with simvastatin is not applicable in the clinic and other strategies are needed for colorectal cancer patients with tumors harbouring a KRAS mutation who failed standard therapy. Besides statins, farnesyl- and geranylgeranyltransferase inhibitors also have a crucial role in the mevalonate pathway and consequently the prenylation of KRAS. A combination of statin and low doses farnesyl- and geranylgeranyltransferase inhibitors may be an effective treatment in CRC patients with a KRAS mutant tumor. Some studies reported that the effect of the FCGR3A polymorphisms on cetuximab efficacy is independent of KRAS status. The FCGR3A polymorphisms did not show a significant association with PFS. Moreover, no differences in cetuximab efficacy were found between patients with a KRAS mutant and KRAS wild type tumor. The results from these two approaches show that treatment options for CRC patients with a (K)RAS mutant tumor after failing chemotherapy and bevacizumab still remain poor.

The described case reports in this thesis help clinical decision making in real-life practice. Cetuximab and panitumumab monotherapy seems to be safely applicable in patients with RAS wild type metastatic CRC and hepatic or renal dysfunction, without the need for dose adjustments.