

Cover Page



Universiteit Leiden



The handle <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



Summary



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;
- and 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.

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 specials 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.