Chapter 10

General discussion

Introduction

The epidermal growth factor receptor (EGFR) antibodies cetuximab and panitumumab are both registered for the treatment of *RAS* wild type metastatic colorectal cancer (CRC). About 40% of the patients have a tumor with a mutation in the *KRAS* gene. In patients with a *KRAS* mutant tumor KRAS is permanently activated which lead to constant cell signaling and proliferation independent of the EGFR. Patients with a *(K)RAS* mutant tumor are considered not to benifit from anti-EGFR therapy. The use is limited to patients with *KRAS* wild type tumors and more recently in *RAS* wild type only[1,2]. After having become chemotherapy refractory, treatment options are limited for this substantial patient group [3]. This means there is an urgent need to optimize anti-EGFR therapy.

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.

Statins and their ability to phenoconvert KRAS mutant CRC

Statins are commonly used to reduce cholesterol levels in patients in order to reduce the risk for cardiovascular events. Statins inhibit HMG-CoA-reductase in the mevalonate pathway and subsequently the formation of cholesterol. Besides cholesterol the formation of farnesylpyrophophate (a C15-group) and geranylgeranylpyrophosphate (a C17-group) are also inhibited. These C15 and C17 groups are used to prenylate the KRAS protein. Prenylation is an essential step in the activation of the KRAS protein. After prenylation, KRAS becomes more lipophilic and associates with the plasma membrane. By preventing prenylation and plasma membrane association the over-activated KRAS protein may be inhibited. We theorised that the inhibitory effect of statins may normalise the KRAS mutant phenotype into a more KRAS wild type phenotype and render *KRAS* mutant colorectal cancers sensitive to EGFR antibodies. Thus, statins may inhibit the expression of the mutant *KRAS* phenotype by preventing prenylation (the addition of C15 or C17 groups) of the KRAS protein.

Besides statins, other KRAS modulators, such as bisphosphonates, farnesyltransferase inhibitors or geranylgeranyltransferase inhibitors, also affect the mevalonate pathway. These KRAS modulators might augment the effect of EGFR antibodies in patients with *KRAS* mutations. In **chapter 2**, clinical studies with these KRAS modulators and their outcomes were reviewed. This review indicates that combinations of EGFR antibodies to target the EGFR with KRAS modulators may be effective in patients with *KRAS* mutant tumors.

To understand the role of statins in CRC and to explore the potential of therapeutic modulation of *KRAS* mutated CRC tumors, an *in vitro* study with *KRAS* wild type and mutant

cell lines was performed. 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 induced a small reduction in growth (**chapter 3**). This effect was not observed in the SW480 cell line harboring a codon 12 *KRAS* mutation. The observation that codon 13 *KRAS* mutated tumors are responsive to EGFR-antibody therapy is in line with other retrospective studies[4-6], which also showed a positive association on outcome for the *KRAS* codon 13 mutations. An *in vitro* study showed that codon 13 *KRAS* mutation may confer weaker transforming capacities on cancer cells, compared to other *KRAS* mutations[7]. Computational analysis revealed that the codon 13 mutated KRAS protein has a similar structure compared to the wild type protein [8]. Our studies revealed that all *KRAS* mutant cells showed a pronounced cytotoxic effect after simvastatin monotherapy. The effect of simvastatin on growth was not observed in the *KRAS* wild type A431 cell line. A possible explanation for the cytotoxic effect may be a strong dependence ("addiction") of the mutant cells on permanently activated KRAS and its corresponding pathways. These pathways are possibly highly activated due to mutant *KRAS*. The observed effect of combination treatment with simvastatin and cetuximab was relatively small; therefore other pathways besides the RAS-RAF-MAPK pathway may play important roles which are not affected by blocking the EGFR receptor.

As the *in vitro* studies (**chapter 3**) showed promising results, we decided to perform a retrospective analysis to evaluate the effect of statin use on outcome in *KRAS* mutant metastatic colorectal cancer patients (mCRC) treated with cetuximab. In the CAIRO2 study 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. **Chapter 4** showed that the use of statins in patients with a *KRAS* mutant tumor was not associated with an improved PFS.

To exclude a possible "generic" effect of statins on survival, patients with *KRAS* wild type tumors and patients in the study-arm without cetuximab were also included in the analysis. Additionally, the study design with an effect modifier helps to identify possible different effects of statins between patients with *KRAS* mutant and wild type tumors.

Possible explanations for the lack of a modulating effect are that the cohort consisted of patients with metastatic disease and hence a relatively short PFS. The detection of a small to moderate effect on PFS may be difficult in this patient group. Another explanation for the absence of an effect was the concomitant use of bevacizumab in the CAIRO2 study. The combination of cetuximab and bevacizumab is not used nowadays, due to an unfavourable outcome. Hypertension, a common side effect of bevacizumab is also a prognostic factor for a better overall survival in CRC patients treated with bevacizumab [9]. A possible negative interaction between bevacizumab and cetuximab may have caused less hypertension in the cetuximab treated group, which contributed to the negative outcome of the CAIRO2 study.

In two prospective studies we investigated the possibility of simvastatin to phenoconvert mutant *KRAS* in CRC patients treated with cetuximab or panitumumab. In the RASTAT C and P studies (**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. In a similar study, Lee et al.[10] tested the addition of 80 mg of simvastatin to cetuximab and irinotecan. The disease controle rate in this study was 65.4%. Their original report indeed showed a low response rate (1 out of 52 patients had a partial remission), however PFS was 7.6 months,

which is even higher than historical results of third-line cetuximab plus irinotecan in KRAS wild type CRC patients. However, in a recent erratum Lee et al[30] reported corrected measurements of PFS in their population. Corrected mean PFS was 3.7 months (range 2.1-5.3), significantly lower than previous reports of cetuximab plus irinotecan as third-line therapy in KRAS wild type [10]. In summary, both our study as well as the study by Lee et al. shows that simvastatin does not render sensitivity to EGFR inhibitor therapy.

Polymorphisms FCGR3A gene and their association with cetuximab efficacy

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. [11]. Previous results from studies investigating the association between *FCGR3A* polymorphisms and cetuximab efficacy are highly variable. A firm conclusion or direction of the effect of *FCGR3A* polymorphisms on cetuximab efficacy cannot be drawn from these studies. These inconsistent findings in studies may have been caused by incorrect genotyping methods[12] or insufficient statistical power[13]. In some studies [14-16], the observed allele frequencies were not in Hardy-Weinberg equilibrium, possibly because genotyping errors may have occurred due to *FCGR3B* co-amplification. The meta-analysis (**chapter 7**) performed on individual patient data shows that the *FCGR3A* polymorphism is not associated with improved survival in cetuximab treated CRC patients and there is no significant difference between patients with *KRAS* wild type and mutant tumors. Lack of effect of the *FCGR3A* polymorphisms may be explained by the fact that most patients were also co-treated with classic chemotherapy or patients received previous lines of chemotherapy, which suppresses macrophages and natural killer cells[17]. Moreover, all included patients had metastatic CRC, which may lead to decreased immune responses and impaired natural killer cell dysfunction in end stage CRC[18] and consequently failure of cetuximab treatment.

Pharmacokinetics of cetuximab and panitumumab in patients with renal or hepatic dysfunction

Both panitumumab and cetuximab are used in patients with metastatic CRC. Some of these patients will present with hepatic impairment, due to liver metastasis. In addition, head and neck cancer patients are heavily pre-treated with chemotherapy and radiotherapy and may have decreased renal function. Cetuximab is used in these head and neck cancer patients because they cannot be treated with cisplatin, due to renal impairment.

Knowledge of dosing in these 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 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 appears to be tolerable and safe (**chapters 8, 9**). However, larger studies in patients with hepatic or renal dysfunction are

needed before firm conclusions can be drawn to guide clinical decision making in daily practice. Successful treatment of solid tumors relies on the ability of EGFR inhibitors to penetrate into the tumor tissue. Clearance of both panitumumab and cetuximab occurs by the EGFR sink and the reticuloendothelial system. Their clearance may also partly depend on the EGFR-positive tumor burden and antigen density in the tumor, i.e. a high tumor burden and/or a high density of EGFR may lead to subsequent higher clearance of EGFR antibodies. In our patients the tumor burden and the antigen density in the tumor were not known. The impact of EGFR binding sites in the liver on serum clearance of EGFR antibodies remains to be fully clarified.

Future research prospectives

In this thesis, the possibility of statins to phenoconvert the KRAS mutant protein to a more wild type protein to overcome EGFR-monoclonal antibody resistance in CRC was studied. The apparently promising results from the preclinical study, however, were not translated to the clinic. Statin use in the CAIRO2 study was not associated with a better progression free survival in cetuximab treated metastatic CRC patients with a *KRAS* mutant tumor. In the RASTAT C en P studies, the combination of cetuximab or panitumumab with simvastatin did not lead to a survival comparable with KRAS wild type patient. In these three studies we were not able to show that statins can phenoconvert the mutant KRAS protein and render these tumors sensitive for cetuximab. In this thesis, the hypothesis that statins can phenoconvert the mutant KRAS to a more favourable phenotype was studied only in patients with advanced colorectal cancer and thus with a short life expectancy. Recent evidence suggest that other *RAS* mutations (in exons 3 and 4 of *KRAS* and exons 2, 3 and 4 of a related gene, *NRAS*) may also be predictive of anti-EGFR resistance. These other RAS proteins also requires post-translational farnesylation to become active. In further studies all *RAS* mutant patients need to be included.

A "perfect world" study where the effect of statins and concomitant chemotherapy and EGFR antibodies is studied in recently diagnosed (*RAS* mutant) patients who did not receive a statin before CRC diagnosis would be of great value. Since these patients have a longer life expectancy, lower tumor burden and possibly a phenotype, which could be modified by statin use.

Besides statins, farnesyl- and geranylgeranyltransferase inhibitors also have a crucial role in the mevalonate pathway and consequently the prenylation of (K)RAS. Inhibiting of (K) RAS prenylation might require combined treatment with these inhibitors. Possibly because of preclinical toxicity geranylgeranyltransferase inhibitors did not proceed to clinical stages. A combination of statin and low doses farnesyl- and geranylgeranyltransferase inhibitors may be an effective treatment in CRC patients with a *(K)RAS* mutant tumor.

In this thesis we studied the effect of the *FCGR3A* gene, which is involved in antibody dependent cellular toxicity (ADCC). Other effector mechanisms and accompanying polymorphisms, such as complement-dependant cytotoxicity, phagocytosis and apoptosis may play a crucial and still unknown role in efficacy. The enormous complexity of cancer makes it debatable whether a single mutation or germ-line polymorphism might have a noteworthy effect on the tumor sensitivity to targeted therapies. Innovative technologies, such as next-generation sequencing, kinase activity profiling of tumors, computational biology and genome-wide association studies, will be useful in achieving a better overview of involved pathways and in further optimizing and personalizing the use of anti-EGFR monoclonal antibodies. By sequencing tumor DNA, targeted treatment can be optimized for the specific characteristics of the tumor.

Many drugs that enter the market are studied in a patient population with adequate organ functions only[19]. No dose recommendations are made for patients with impaired liver or kidney function and formal organ impairment studies are lacking. This is also true for cetuximab and panitumumab. Other than from common sense and some case reports in haemodialysis patients[20-23], liver failure (**chapter 8**) and kidney failure (**chapter 9**), there is no information available to guide our decision on how to use cetuximab or panitumumab in these populations. Larger studies, where the efficacy and safety of EGFR antibodies and chemotherapies is studied in patients with liver and kidney dysfunction, will be of great value. Liver and kidney dysfunction will become more common, since patients are getting older and more treatments become available, which will substantially increase in the incidence of these cases. Development of new drugs should include studies in organ failure patients that reflect clinical dilemmas often encountered in routine patient care. Mandatory additional research with FDA or EMA approval would ensure this process on a timely basis.

Conclusions

The goal of this thesis was to refine EGFR monoclonal antibody treatment in CRC by four different strategies. The first strategy, focused on statins and their ability to phenoconvert KRAS mutant CRC Statin use in metastatic CRC patients with *KRAS* mutant tumors, did not affect progression free or overall survival. The second strategy explored polymorphisms in the gene encoding FCGR3A and their association with cetuximab efficacy. Neither of the *FCGR3A* polymorphisms showed a significant association with improved PFS. Although some studies reported that the effect of the *FCGR3A* polymorphisms on cetuximab efficacy is independent of *KRAS*. The meta-analysis showed that there is no significant difference between patients with *KRAS* wild type or mutant tumors. In conclusion, the result from these two strategies shows that the options for CRC patients with a *KRAS* mutation after failing first line chemotherapy and bevacizumab still remain poor. The outcomes from these strategies demonstrate that the involved pathways are very complex and urgently need further exploration.

The final strategy to optimize anti-EGFR therapy focused on the pharmacokinetics of cetuximab and panitumumab in patients with renal or hepatic dysfunction. The described case reports help clinical decision making in real-life practice. Cetuximab and panitumumab monotherapy seems to be safely applicable in patients with *KRAS* wild type metastatic CRC and hepatic or renal dysfunction, without the need for dose adjustments.

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