Chapter 1

General introduction

Colorectal cancer and KRAS

Colorectal cancer (CRC) is the second most common tumor type worldwide and accounts for more than 5,000 cancer deaths each year in the Netherlands (www.cijfersoverkanker.nl). The *KRAS* gene has a key role in carcinogenesis, signal transduction and proliferation. Mutations in the *KRAS* gene are found in 40 percent of the CRC tumors. The most frequent mutations in *KRAS* are guanine to adenine transitions and guanine to thymine transversions with 90% of the somatic point mutations occurring in hotspot codon 12 (70%) or 13 (30%) in exon 1. Other, less frequent, mutations are found in codon 61, 62 and 146[1].

KRAS protein prenylation

The activating *KRAS* mutation results in uncontrolled cell growth. To be active, the KRAS protein requires posttranslational prenylation, by binding to a farnesyl- (C-15) or geranylgeranylgroup (C-17). After prenylation KRAS becomes more hydrophobic and associates with the plasma membrane. Membrane association is crucial for the function of the KRAS protein in the RAS-RAF-MAPK pathway. Inactivated KRAS is bound to GDP; activation occurs by the conversion of GDP to GTP by guanine exchange factors. The ratio of GDP and GTP is controlled by guanine exchange factors and GTPase-activating proteins (GAPs). Active KRAS is hydrolyzed by GAPs to return to an inactive state [2].

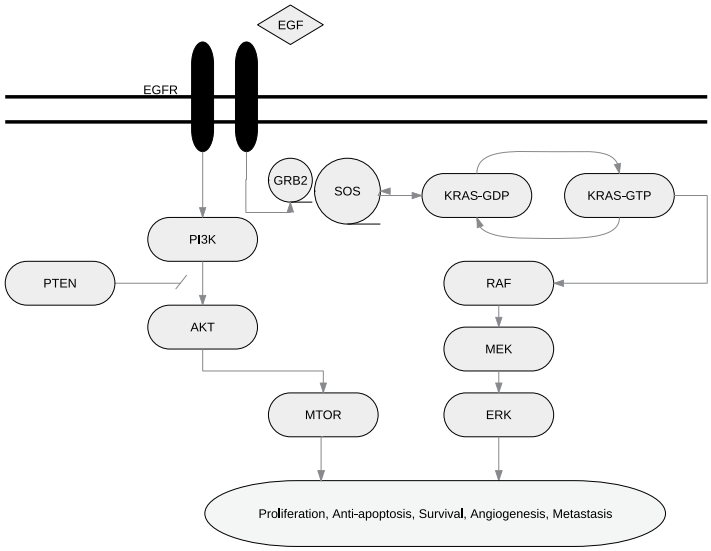

Figure 1: Overview of EGFR-dependent intracellular signaling. Abbreviations: AKT, protein kinase B; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular signal-related kinase; GRB2, growth factor bound protein 2; KRAS-GDP, KRAS bound to guanine diphosphate; KRAS-GTP, KRAS bound to guanine triphosphate; MEK, mitogen-activated protein kinase; MTOR, mammalian target of rapamycin; PI3K, phosphatidyl-inositide-3-kinase; PTEN, phosphatase and tensin homolog; RAF, V-raf murine sarcoma viral oncogene homolog; SOS, son of sevenless.

EGFR antibodies cetuximab and panitumumab

Binding of a ligand to the Epidermal Growth Factor Receptor (EGFR) activates important downstream processes such as the RAS-RAF-MAPK and the PI3 kinase pathway (figure 1). The EGFR is an important target in the treatment of CRC. Blockage of the EGFR leads to inhibition of cancer cell growth. The two registered EGFR antibodies, cetuximab and panitumumab are both indicated for the treatment of metastatic CRC in *RAS* wild type patients only. In *KRAS* mutant patients, KRAS is permanently activated, leading to constant cell signaling and proliferation independent of the EGFR [3].

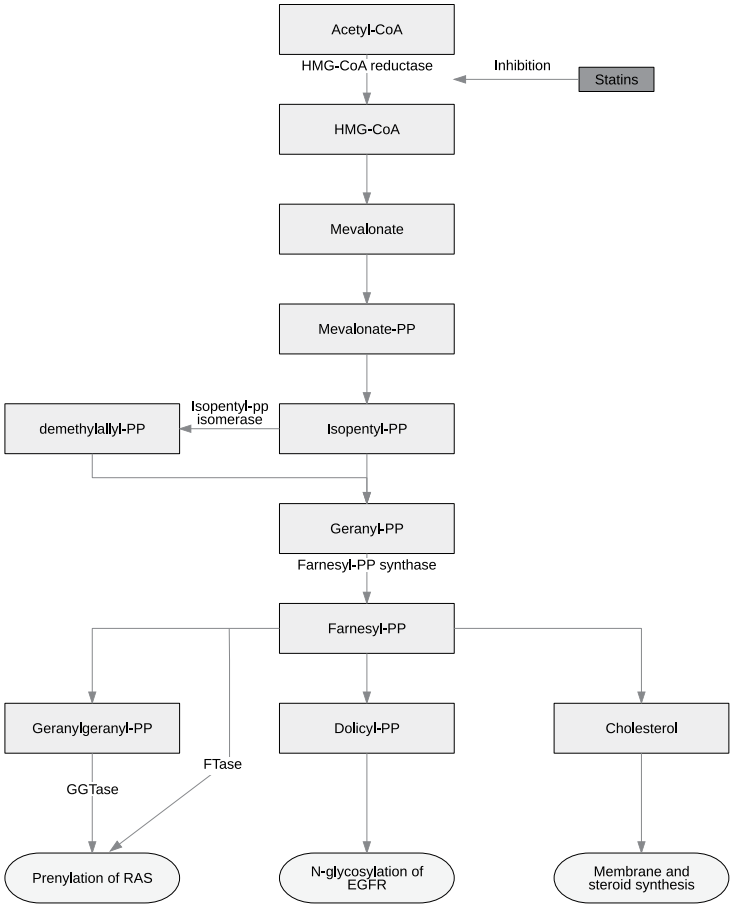

Figure 2: Overview of the mevalonate pathway and the inhibition of HMG-CoA by statins. Abbreviations: Acetyl-CoA (acetyl coenzyme A); EGFR, epidermal growth factor receptor; FTase, farnesyltransferase; GGTase, geranylgeranyltransferase; HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) (reductase); -PP, -pyrophosphate.

Statins and colorectal cancer

Statins inhibit cholesterol synthesis via inhibition of HMG-CoA-reductase in the mevalonate pathway and also prevent protein prenylation (figure 2). We hypothesize that statins may be useful for modulating *KRAS* mutant tumors. It is well known that statins can be used to lower cholesterol and have shown to reduce the number of cardiovascular events and mortality in patients with cardiovascular risks [4]. In addition, the use of statins has been associated with a reduced risk in a variety of malignancies such as colon, rectum, lung and liver cancer [5]. To date, several studies and meta-analysis have investigated statin use and the risk of developing CRC and outcomes but with inconclusive findings[6-8]. Fewer studies focus on effects of statin after diagnosis during treatment [9-14].

Phenoconversion of KRAS to overcome EGFR antibody resistance

Statins may inhibit the expression of the mutant KRAS phenotype by preventing the prenylation of the KRAS protein and as a consequence preventing plasma membrane association and so inhibiting the overactivated KRAS protein. We theorised that the inhibitory effect of statins may normalise the phenotype into a more KRAS wild type phenotype and render *KRAS* mutant colorectal cancers sensitive to EGFR antibodies(5;6).

FCGR3A and cetuximab

An important mechanism of action for some monoclonal antibodies, including cetuximab is antibody-dependent cellular cytotoxicity (ADCC). Monoclonal antibodies are generally molecules of the IgG class and have an antigen-binding fragment (Fab). Fc gamma receptors (FCGR) on effector cells, for example macrophages and natural killer cells, bind to the Fab fragment and this causes lysis of the cancer cell. Germline polymorphisms in the genes encoding the Fc gamma receptor 2A (FCGR2A) c.535A>G, resulting in a change of histidine to arginine at codon 131 and 3A (FCGR3A) c.818A>C resulting in a change of phenylalanine to valine at position 158 have been associated with decreased therapeutic activity of cetuximab[15].

Use of EGFR antibodies in patients with hepatic or renal impairment

Panitumumab and cetuximab are both used for the treatment of metastatic CRC, and a part of the patients will present with liver metastasis and subsequent hepatic impairment. On the other hand, some patients are heavily pre-treated with chemotherapy and radiotherapy and may have decreased renal function. This is especially the case in patients with head and neck cancer where cetuximab is being used in patients that cannot be treated with cisplatin, e.g. due to renal impairment. Knowledge on the dosing in these specials populations is highly relevant; nonetheless the pharmacokinetics and safety of both cetuximab and panitumumab are, to date, not studied in these populations.

Aims and outline of this thesis

The general aims of this thesis with the common denominator 'optimization of EGFR targeted monoclonal antibody therapy in cancer' are to study:

1. *The phenoconversion effects of statins on KRAS mutant colorectal cancer both in vitro and in CRC patient populations and their ability to render KRAS mutant colorectal cancer cells sensitive for the EGFR monoclonal antibodies cetuximab or panitumumab*

2. *The effect of the germline polymorphisms in FCGR3A on cetuximab efficacy*

3. *The pharmacokinetics of the EGFR antibodies cetuximab and panitumumab in patients with renal or hepatic impairment*

In **chapter 2** a systematic review is presented on clinical and preclinical studies with compounds, which interfere with the mevalonate pathway and the prenylation of KRAS, published before April 2009. The novel concept of modulation of the KRAS protein by altering the phenotype and the consequent sensitising for EGFR antibodies is discussed.

In **chapter 3** the effects of the combined treatment with simvastatin and cetuximab are studied *in vitro* in different *KRAS* mutant and wild type cell lines. The sulforhodamine assay is used to study the effects of treatment on survival and proliferation. Upregulated and downregulated tyrosine and serine/threonine kinases and corresponding pathways influenced by concomitant treatment with simvastatin and cetuximab in *KRAS* mutant and *KRAS* wild type cell lines are explored. The aim of this study is to explore the responsible pathways which are affected by simvastatin and cetuximab treatment.

Our hypothesis is that *KRAS* mutant cetuximab treated patients with concurrent statin use may have a favourable outcome from EGFR therapy compared to non-users. **Chapter 4** describes a retrospective evaluation of the effects of statin use in the CAIRO2 study cohort in *KRAS* mutant metastatic CRC patients treated with cetuximab. The primary objective in this study is to determine whether statin use during chemotherapy with CAPOX-bevacizumab and cetuximab is associated with improved progression free survival as compared to non-(statin) users.

In the RASTAT-C and RASTAT-P studies, described in **chapter 5 and 6**, treatment with 80 mg simvastatin daily combined with panitumumab two-weekly or cetuximab weekly is studied in a Simon two stage design single arm clinical trials in patients with *KRAS* mutant CRC. The primary objective is to investigate whether the percentage of patients free from progression and alive 12.5 weeks after the first administration of cetuximab is similar to the results of the *KRAS* wild type population of phase III studies treated with cetuximab or panitumumab.

In the cetuximab arm of the CAIRO2 study the *FCGR3A* 818C (VF plus VV) allele was associated with decreased PFS in the entire group of KRAS mutant and wild type patients. The predictive role of this polymorphism may be independent of *KRAS* status. In **chapter 7** these findings of *FCGR3A* status (in relation to KRAS status) on the progression free survival and overall survival in three cohorts of metastatic colorectal cancer patients treated are combined. In this meta-analysis individual patient data are pooled.

Many metastatic CRC patients will present with liver metastases and some with liver dysfunction. The pharmacokinetics of panitumumab in patients with hepatic impairment has not been investigated, and dosage adjustments are undetermined. **Chapter 8** describes a case

of a patient with progressive metastatic CRC and liver dysfunction treated with panitumumab. Pharmacokinetic data and toxicity of this patient are compared to historical data from a population with adequate liver functions.

In the literature the effect of renal impairment on the pharmacokinetics of anticancer drugs are scarce. **Chapter 9** reports a 68 year old metastatic osteosarcoma patient with impaired renal function due to prior chemotherapy, who was treated on compassionate use basis with 400 mg/m^2 cetuximab. Pharmacokinetic parameters are compared to pharmacokinetic data from a study population with normal kidney function.

This thesis ends with concluding remarks and future perspectives in **chapter 10** and a summary of the results in **chapter 11**.

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