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Introduction to this thesis

Colorectal cancer

Colorectal cancer is one of the most prevalent forms of cancer and the third leading cause of cancer related death worldwide.[1;2] Disease stage is the most important prognostic factor, with a 5-year survival of only ten percent for patients with stage IV metastatic disease.[3] At present, approximately 20 percent of patients present with distant metastases[4;5], and another 26 percent will develop metastases in the first 5 years after surgery.[6]

Survival for metastasized colorectal cancer (mCRC) has increased substantially over the last four decades. This increase in survival is due to the improvement of surgical techniques, including the introduction of hepatic resection for liver-only metastases, but it is also largely the result of the implementation of new multidrug systemic therapies.[5;7]

Whereas 5-fluorouracil (5-FU) was the only systemic treatment available until the turn of the century, the treatment arsenal has since then been expanded and now also includes other fluoropyrimidine analogues (such as capecitabine), oxaliplatin, irinotecan, and drugs targeted against angiogenesis (bevacizumab) or the endothelial growth factor receptor (EGFR; cetuximab and panitimumab). During this time, overall survival for mCRC patients has risen from 5.9 months on 5-FU monotherapy, to 23 months for patients treated with modern-day combination treatment.[4] At the same time, a larger proportion of mCRC patients are now prescribed chemotherapy, increasing from 23 to 64 percent between 1989 and 2006 in the Netherlands.[5]

Despite encouraging results, not all patients benefit equally from these developments. There is great inter-patient variation in efficacy of treatment, and adverse events do not affect all patients to the same extent. Pretreatment predictors of efficacy and toxicity could safeguard patients from unnecessary adverse events, as well as reduce health care costs by preventing pointless treatments in patients who will not respond.

Genetic variation between patients and between their tumors is responsible for at least some of the inter-patient difference in treatment response. Tumor DNA harbors somatic mutations, on top of germline genetic characteristics, which can interfere with treatment efficacy. As an example, mutations in *Ras* oncogenes restrict activity of EGFR-inhibiting treatment, reducing its efficacy to almost zero percent.[8-11]

In contrast, pharmacogenetics focus on variation in germline DNA, present in all nucleated cells. Genetic alterations in a myriad of genes are thought to induce phenotypic changes in pharmacokinetics and dynamics for cytostatic drugs. The information obtained from genotyping in healthy DNA can therefore be of aid in the choice and dosage of anti-cancer treatment. Whereas treatment efficacy is mostly affected by tumor characteristics, toxicity associated with anti-cancer treatment is determined by its effect on healthy tissues. Therefore, it is likely that adverse events are far better predicted by germline genetic variation than by somatic changes in tumor DNA.

Scope of this thesis

The aim of this thesis is the search for germline genetic markers to pre-emptively predict treatment efficacy and adverse events of capecitabine and oxaliplatin, prescribed to patients with advanced colorectal cancer. For this thesis, patient data and DNA were obtained from patients included in the CAIRO and the CAIRO2 study by the Dutch Colorectal Cancer Group (DCCG). The CAIRO trial was a multicenter open label randomized phase III trial, comparing sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in a total of 803 patients with mCRC.[12] The phase III CAIRO2 trial was conducted in 755 mCRC patients, and randomized between first-line treatment with capecitabine, oxaliplatin and bevacizumab (CAPOX-B) versus CAPOX-B plus cetuximab.[13]

The studies described in this thesis address different aspects of pharmacogenetic research in colorectal cancer. **Chapter 2** gives an overview of the current knowledge on the effect of genetic variation on chemosensitivity for most common anti-cancer drugs prescribed in colorectal cancer treatment. New studies are reported every week and knowledge is constantly increasing. As a result, at the moment this thesis is published, more recent and more elaborate information will certainly be available.

Germline DNA for pharmacogenetic research is derived from peripheral blood leukocytes or other healthy tissue in most studies. However, all early pharmacogenetic studies in colorectal cancer treatment, as well as several more recent publications, have used archived paraffin-embedded surgical resection samples as the primary source of DNA for their analyses. For reliable comparison between studies, it is essential to know if the source of DNA affects genotype for the markers under investigation. **Chapter 3** addresses a basic element of pharmacogenetics research, whether there is sufficient concordance between tumor and germline DNA, to exclude the source of DNA as a confounding factor in the interpretation of data from different studies.

In the following section of this thesis, focus is on pharmacogenetics of capecitabine. Fluoropyrimidines have been the mainstay of systemic treatment for metastatic colorectal cancer, since their first development in 1957. The oral pro-drug capecitabine is equal to 5-FU in terms of efficacy, although pharmacokinetics differ.[14] The cytotoxic effect of fluoropyrimidines is thought to derive from interference with the thymidylate synthase (TS), an essential enzyme for the formation of DNA precursors, as well as direct incorporation into DNA and RNA. Methylenetetrahydrofolate, which is under the direct influence of methylene tetrahydrofolate reductase (MTHFR), is an essential component for binding of 5-FU to TS. *MTHFR* polymorphisms could therefore influence 5-FU chemosensitivity. **Chapter 4** focuses on the effect of *MTHFR* polymorphisms on adverse effects of capecitabine-based chemotherapy. In **chapter 5**, additional single nucleotide polymorphisms (SNPs) are investigated for their association with capecitabine efficacy. Some of these SNPs were selected based on their genome wide significance in an *in vitro* study using lymphoblastoid cell lines. Additionally, SNPs were selected based on their location in the gene encoding for methylene synthase reductase (MTRR), which, like

MTHFR, is involved in the folate pathway.

Next, focus is shifted to oxaliplatin. Organic cation transporters (OCTs) play an important role in the uptake of oxaliplatin into the cells.[15] Several types of OCTs have been associated with adverse effects of oxaliplatin, specifically platinum-induced neurotoxicity.[16] **Chapter 6** focusses on genetic variation in the genes encoding for three OCTs and the correlation with oxaliplatin-induced neurotoxicity.

The cytotoxic effect of oxaliplatin depends on the formation of DNA interstrand crosslinks. Repair of this DNA damage by the nucleotide excision repair (NER) system is likely to affect cellular chemosensitivity to oxaliplatin. Excision Repair Cross-Complementation group 1 (ERCC1) is a major component of the NER system. In **chapter 7**, the effect of a common variation in this gene, *ERCC1* C118T, on oxaliplatin response is addressed, combining *in vitro* studies and clinical association analysis.

In addition to the candidate-gene approach that was adopted in the previous chapters, in **chapter 8** a genome wide search for predictors of efficacy of combination chemotherapy is presented. Progression free survival of patients treated with first-line chemotherapy consisting of capecitabine-oxaliplatin-bevacizumab (CAPOX-B), either with or without cetuximab, is explored.

Finally, **chapter 9** gives a summary and general discussion on the results presented in this thesis.

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