Chapter 1

General introduction and outline of this thesis.
1.1 DEFINITION OF COLORECTAL CANCER

The term colorectal cancer (CRC) usually refers to colorectal carcinoma, which is by far the most common cancer type of the large bowel. Other cancer types, such as grade 1 or 2 neuroendocrine tumors, lymphomas and sarcomas are very rare in the large bowel (resp. 1.5%, 0.6% and <0.1%). A carcinoma of the large bowel is a malignant epithelial tumor that has penetrated through the lamina muscularis mucosae into the submucosa or deeper.

1.2 COLON VERSUS RECTUM

Although the colon and rectum are continuous parts of the large bowel without clear boundaries between each other, cancers arising in these two parts of the large bowel are often analyzed separately – as in four out of five main chapters of this thesis (i.e. chapter 2 through 5). The reasons are mainly due to differences in anatomy, therapeutic approach (type of surgery and administration of neoadjuvant therapy), recurrence patterns and biology. Still there are also many similarities between cancers in these two locations, such as histological and etiological factors. Nevertheless, colon and rectal cancers are also frequently analyzed together, as in chapter 6 of this thesis, especially in the stage of metastatic colorectal cancer.

1.3 COLORECTAL CANCER BURDEN IN EUROPE, THE NETHERLANDS AND ICELAND

Colorectal cancer is an important health issue in European countries. In 2012 the incidence in Europe ranged from 12.0 (Albania) to 63.3 (Slovakia) per 100,000 for both genders (using for standardization the European standard age-pyramid). Data from the Netherlands and Iceland were used for this thesis, which is why these countries will be compared in this section. In 2012 the incidence in the Netherlands was the fourth highest in Europe, i.e. 60.1 per 100,000, while the incidence in Iceland was slightly below the European average, 42.8 per 100,000 (see Figure 1). In 2012 CRC was the third most common cancer in the Netherlands after breast and prostate cancer while in Iceland it was the fourth most common cancer after breast, prostate and lung cancer. CRC is a disease of the elderly with a peak incidence around 70 years of age. The 2012 European incidence was higher in men than women.

In 2012 the European mortality was 45% of the incidence. In the Netherlands and Iceland in the year 2012 the estimated mortality was, however, approximately 35% of the incidence in both countries, i.e. 21.2 and 12.1 per 100,000, respectively. Incidence and mortality in European countries is shown in Figure 1. Lower mortality in the Netherlands and Iceland compared to the European average is likely to be partly related to differences in stage distribution at diagnosis. In both countries CRC is the fourth most common cause of cancer death after lung, breast and prostate cancer. Because of the high incidence and mortality and suitability for screening, a nationwide screening
program for CRC was initiated in the Netherlands in January 2014. Nationwide screening has not yet been introduced in Iceland.

1.4 BIOMARKERS IN COLORECTAL CANCER

A cancer biomarker is a term that is defined differently depending by various publications and organizations. Oxford Dictionary defines it as “A naturally occurring molecule, gene, or characteristic by which a particular pathological or physiological process, disease, etc. can be identified.”
In cancer research and medicine, biomarkers are mainly used in five ways; (1) to help diagnose a cancer (diagnostic), (2) to forecast the risk of developing a cancer (prognostic), (3) to forecast the risk of cancer recurrence and death (prognostic), (4) to predict treatment response (predictive), and (5) to monitor the effect of treatment. Usually, the term cancer biomarker refers to a molecule. In theory, histopathological tumor characteristics can also be regarded as biomarkers. Still, they are more typically referred to as just markers or also commonly variables, parameters, or factors.

The Early Research Detection Network (ERDN) proposes that biomarker development can be divided into phases. Phase one is the identification of a promising biomarker. Phase two is the development of a clinical assay. Phase three and four are retrospective and prospective validating studies, respectively. Retrospective studies on prognostic or predictive markers in CRC are common and convenient due to easy access to archival tissue material. Prospective studies are less common. The reasons for that are many. These include contradictory results in retrospective studies, methodological issues pertaining to study designs, lack of robustness of the proposed marker, costs and long time until results from prospective studies are available.

1.5 PROGNOSTIC (BIO)MARKERS AS A BASIS FOR TREATMENT DECISIONS

1.5.1 Colorectal cancer staging

Until we are able to completely prevent colorectal cancer, there is a need to identify prognostic and predictive markers that help us in clinical decision-making. One of the main aims of pathological assessment of colorectal surgical resection specimens is to assess the risk of cancer recurrence and death. This is generally achieved by staging. Cancer staging, as practiced for decades, is the process of determining the extent to which a cancer has developed by spreading locally at the site of the primary tumor and to distant organs. The current staging system for colon cancer stems from 1932 when Dukes proposed a three-tiered staging system for rectal cancer using a combination of two variables, i.e. depth of local invasion and lymph node status. This staging system was based only on the resection specimen and did not take the presence or absence of distant metastases into account. Thus, for both curative resections, which meant that there were no known distant metastases, and palliative resections, when the patient had known distant metastases, the original Dukes staging system used stages A, B and C. In 1967 a fourth category, stage D, was added by Turnbull et al., by that pulling out and categorizing specifically cases with the presence of distant metastases. With that addition Dukes stages A, B, C and D were corresponding to TNM stage I, II and III and IV for colorectal cancer in the 3rd to 7th TNM-staging edition.

The TNM system is currently the most widely used cancer staging systems for colorectal cancer. This simple four-tiered staging system has been useful in identifying patients with similar prognosis and treating them according to the standard protocol per stage. However, the four-tiered staging for colorectal cancer has limitations when it comes to selecting the most appropriate treatment for patients.
1.5.2 Challenges in treatment stratification based on the TNM-staging

In stage I, polypectomy/large bowel resection without any systemic treatment seems sufficient to cure >95% of patients.\(^\text{15}\) At present there is no clear urge to improve that strategy with the addition of adjuvant systemic therapy.

The majority of colorectal cancers are grouped into two intermediate stages, stage II and III, that have wide and overlapping variation in prognosis.\(^\text{16}\) The division between stage II and III is only based on a single variable, i.e. lymph node status. This is based on the idea that the presence of cancer in lymph nodes serves as proof of the acquisition of a metastatic potential.\(^\text{17}\) Patients with positive lymph nodes (stage III), independent of the number of positive nodes, usually receive adjuvant chemotherapy (57% in the Netherlands 2004-2006), but only 20% actually benefit from this.\(^\text{18}\) Most patients with negative lymph nodes (stage II) generally do not receive adjuvant chemotherapy (7% in the Netherlands 2004-2006).\(^\text{18}\) Nevertheless, 15-20% of stage II patients will later develop recurrence and die from CRC.\(^\text{15,18}\) Those patients could potentially benefit from adjuvant chemotherapy. There is ongoing debate about how to identify these high-risk stage II patients and if they would benefit from adjuvant chemotherapy or not.\(^\text{19}\) Furthermore, the administration of adjuvant chemotherapy in certain stage III tumors (pT1–2N1–2M0) has recently been questioned due to relatively good, stage II-like prognosis.\(^\text{16}\) Thus, basing the administration of adjuvant chemotherapy mainly on a single variable, i.e. lymph node status, is obviously too simplistic, and additional variables are needed for more precise prognostication. Furthermore, experience and evidence from various types of studies has shown that the formation of regional lymph node metastases is not a necessary step before metastatic dissemination. To clarify, distant metastases may arise from early disseminated tumor cells from the primary tumor according to the parallel progression model.\(^\text{20}\)

The group of patients in stage IV is heterogeneous with respect to the number and location(s) of the metastases. TNM staging alone is therefore insufficient to decide on the best combination of several treatment modalities, like surgery, systemic treatment (chemotherapy and targeted treatment), local ablation, radiotherapy, trans-arterial embolisation or cytoreduction and hyperthermic intraperitoneal chemotherapy (HIPEC).

1.5.3 Peritoneal metastasis of colorectal cancer and the importance of pT4

The peritoneum is a serous membrane that lines the abdominal cavity. Synchronous peritoneal carcinomatosis (PC) of CRC is detected in 24% of the subgroup of patients with stage IV disease at diagnosis. In other words, 5% of all CRC patients have synchronous PC at diagnosis.\(^\text{21}\) It is the fourth most common site of metachronous metastasis in CRC (11%) after liver (65%), lung (43%) and non-regional lymph nodes (16%).\(^\text{22}\) The prognosis of patients with peritoneal carcinomatosis is very poor.\(^\text{21}\)

Tumors of the colon may spread directly to the peritoneum before lymph node metastases have formed. The T4 category of the TNM system has been identified as an important risk factor of peritoneal carcinomatosis.\(^\text{12,21}\) The histopathological identification of the T4 category has, up to
date, mainly had clinical importance as a high-risk variable in stage II, in that case possibly leading to administration of adjuvant chemotherapy.\textsuperscript{13,19} The clinical importance of the T4 category may increase considerably in the coming years. This is because the presence of T4 can theoretically be used to select patients to receive adjuvant/prophylactic HIPEC. The aim would be to prevent the formation of PC. The COLOPEC trial, which was initiated in the Netherlands in March 2015, aims to shed light on this (Trial registration number: NCT02231086 at Clinicaltrial.gov).\textsuperscript{23}

This brings the histological definition of the pT4 category into discussion. If a marker is supposed to be used for clinical decision-making, it needs to be reproducible and reliable. However, it is known that the identification of pT4 is not uniformly practiced.\textsuperscript{24} The literature on the definition of this important component of the TNM system is surprisingly limited and it has become clear that more research is needed on this understudied problem.

**1.5.4 Other histopathological prognostic markers**

Multiple morphological characteristics have been tested as potential additional prognostic markers through the past decades. These include macroscopic growth pattern, tumor type (eg. mucinous, signet ring cell), grade of differentiation, venous invasion, lymphatic invasion, perineural invasion, tumor border configuration, tumor budding, poorly differentiated clusters, fibrosis/desmoplasia, necrosis, tumor deposits, tumor regression grade, surgical margin, and various forms of inflammation, including lymphocytic infiltrate in different locations. Some of these variables are classified as high-risk variables in stage II CRC and used for therapy decisions. However, the prognostic value of many of these variables still needs clarification.\textsuperscript{25} On that note, Böckelman \textit{et al} wrote the following in a recent review on the risk of recurrence in stage II and III CRC: “The prognostic value of new, additional molecular markers, however, has been explored in hundreds of studies. In this perspective, it is striking that it was difficult to find recent reports on the prognostic value of standard clinicopathological variables, the backbone of all routine care.”\textsuperscript{26}

**1.5.5 Molecular prognostic markers**

Considering the prognostic limitations of the TNM-system, it would be anticipated that additional prognostic markers, that are meant to improve the prognostic stratification of the TNM-system, are probably not difficult to find. The search for such additional prognostic markers has in the past decade mostly been aimed at the molecular biology of cancer. The concept of this translational research is straightforward. By reading out the underlying biology the aim is to forecast the outcome of each patient more accurately. Stratification of patients based on prognosis helps deciding which patients should and shouldn’t be given additional therapy.

There is vastly growing literature on various molecular prognostic markers in CRC. The types of markers range from single molecules to selected panels\textsuperscript{27} and large pools of molecules.\textsuperscript{28} The advances in the ‘omics’ fields, such as genomics and proteomics, have contributed widely to the huge
literature on prognostic molecular variables in CRC. The same time there is also extensive array of different techniques present to perform molecular analyses. Importantly, most of these findings have not been translated to clinical practice as many markers have not or cannot be validated.

In the background of these complexities two markers have emerged as important and commonly used molecular prognostic markers in CRC, microsatellite instability (MSI) and BRAF mutation (15% and 7% of all CRC, respectively; see chapter 5). Microsatellite instability (MSI) is the molecular fingerprint of mismatch repair (MMR) deficiency. MMR deficiency is caused by inactivation of one of the MMR genes and results either from a germline mutation or a sporadic event (MLH1 methylation or biallelic somatic MMR mutation). Microsatellite status can be determined by PCR using a Pentaplex panel of mononucleotide repeat markers. Immunohistochemistry for MMR gene expression can also be used instead of PCR. Generally, MSI is considered to confer favorable prognosis. Interestingly, accumulating data indicates that the prognostic impact MSI is context dependent. To explain, studies have demonstrated that MSI confers better survival than microsatellite stable (MSS) tumors in stage II and paradoxically worse survival than MSS tumors in metastatic CRC.

Various studies have shown that the V600E BRAF mutation carries poor prognosis in MSS cancers. The V600E BRAF mutation, which occurs in about 40% of MSI cancers, is often assumed to carry poor prognosis in MSI cancers as well. However, detailed literature search showed that the prognostic impact of this variable in the subgroup of MSI tumors is still unclear (see Chapter 4).

Many other molecular variables have been extensively analyzed with regard to their prognostic role in CRC. One of these includes KRAS mutation status. KRAS mutation status is generally considered a predictive and not prognostic marker in metastatic CRC. In stage II and III, however, accumulating data indicates that KRAS mutations confer worse survival in CRC. The prognostic impact of KRAS mutations in the MSI subgroup is, however, unknown.

Recently a poor prognosis subgroup of colorectal carcinomas, based on gene expression profiling, has been described. This molecular subgroup is now called CMS4 but names such as stem, serrated and mesenchymal transcriptional subgroup have also been used. This subgroup has abundant stroma that contributes to its transcripts. Furthermore, high levels of copy number aberrations and up-regulation of the TGF-beta pathway characterize it. Future studies are still needed on this intriguing CRC transcriptional subtype.

1.6 PREDICTING RESPONSE TO THERAPY

1.6.1 Adjuvant setting

Adjuvant chemotherapy in colon cancer is mainly based on a one-size-fits-all concept where lymph node status is both used as a prognostic and predictive marker. Currently, the standard adjuvant chemotherapy for stage III in the Netherlands is FOLFOX, i.e. 5-fluorouracil (5-FU), leucovorin and oxaliplatin, or monotherapy with 5-fluorouracil when oxaliplatin is contraindicated. 5-FU and leucovorin can be replaced with oral capecitabine.
MSI-status is currently used as a predictive marker in stage II and III CRC. In stage II, when high-risk variables are present, adjuvant chemotherapy is contraindicated if a cancer is microsatellite instable. This is due to lack of response in that setting and possibly harmful effect.\(^{38,39}\) In stage III, when oxaliplatin is contraindicated and adjuvant 5-FU/capecitabine monotherapy is considered, the presence of microsatellite instability can be used as an argument to skip adjuvant chemotherapy rather than administering 5-FU/capecitabine monotherapy, due to lack of response.\(^{38}\) As an explanation of lack of response of microsatellite instable tumors to 5-FU monotherapy, it has been hypothesized that 5-FU monotherapy has negative impact on the body’s positive immune response towards the cancer, the same time as it has limited effect on the tumor cells themselves.

### 1.6.2 Metastatic setting

In metastatic disease there is more variation in the choice of systemic therapy (chemotherapy and targeted therapy). It depends on the resectability of the metastasis, condition of the patient, \(RAS\) mutation status and if the patient’s cancer progressed under previous systemic therapy. 5-fluorouracil, oxaliplatin and irinotecan are the most commonly used chemotherapeutic drugs in metastatic CRC. EGFR inhibitors/anti-EGFR (cetuximab or panitumumab) and VEGF inhibitors/anti-VEGF (bevacizumab) are targeted drugs, which are commonly used in combination with chemotherapeutic agents or singly.\(^{38}\)

It is a well-known drawback that only a subset of patients will show a clinically relevant response to systemic treatment. The remaining patients are subjected to potentially harmful treatment. Thus, biomarkers that define subgroups of patients that are most likely to benefit or not benefit from specific chemotherapies are needed. Currently, we have one predictive marker in clinical use for metastatic CRC, i.e. the \(RAS\) (\(KRAS\) and \(NRAS\)) mutation status, which has been found to predict response to EGFR inhibitors, i.e. patients that have activating mutations in \(RAS\) are resistant to treatment with EGFR inhibitors.\(^{38}\) There are, however, no predictive markers for chemotherapeutic drugs in metastatic setting or bevacizumab. Thus, there is great need for find predictive markers that could identify those patients who would benefit from systemic therapy and those patients who would not benefit and even suffer from systemic therapy.

Epigenetic alterations are important events in the pathogenesis of colorectal carcinoma. Hypermethylated genes form a particular category of biomarkers since DNA methylation is potentially reversible by DNA methyltransferase inhibitors thus providing a way to restore sensitivity of tumor cells to particular therapy the gene is associated with.\(^{40}\) Some hypermethylated genes have been reported to have predictive role for drug response in CRC patients, although results have been inconsistent.\(^{41-43}\)
1.7 AIMS AND OUTLINES OF THIS THESIS

This thesis has several aims. In the second chapter, we looked at temporal changes observed in incidence, survival, surgery rate, stage distribution, lymph node yield, tumor location and histological type of colon cancer diagnosed in Iceland over a 35-year period (1970-2004). The aim was to find explanations for temporal survival changes and to determine if there were indications for improved colon cancer control in Iceland during this period. Such data can also be used to speculate on which steps should be taken next in order to reduce the burden of colon cancer.

In the third chapter, we looked into the heterogeneity of colon cancer by comparing right-versus left-sided cancers and cancers of young versus old patients. As mentioned in the introduction above, there are differences between colon and rectal carcinoma. Increasing evidence also suggests genetic, biological and demographical difference between right and left colon cancer (see introduction to Chapter 2). Studies have also indicated age differences in the pathology of colon cancer. There is a scarcity of large scale studies that closely examine the pathological differences regarding age and tumor location. The aim of this study was to do an extensive comparison of right- and left-sided colon cancers as well as comparing patients under 50 years of age with older patients.

In the fourth chapter, we aimed to clarify the prognostic importance of several well known but still debated pathological variables related to the survival of colon cancer patients. The study mainly focused on the definition and survival carried by the pT4 category. The study also aimed to cast light on the prognostic value of some of the so-called high-risk stage II variables where their presence may determine whether or not adjuvant chemotherapy is administered.

In chapter five we set out to analyze the prognostic importance of the BRAF and KRAS mutations in stage II and III microsatellite instable colon cancer. The V600E BRAF mutation has been associated with worse survival in MSS CRC. This mutation occurs in 40% of MSI CRC and previous literature is unclear on whether it confers worse survival in this setting. The prognostic value of KRAS mutations in MSI CRC is unknown.

Finally, in chapter six, we identified a novel hypermethylated gene in CRC, DcR1, and evaluated its role in prediction of response to treatment with irinotecan in patients with metastatic colorectal cancer.
1.8 REFERENCES


38. Oncoline [Internet]. *Comprehensive Cancer Centres the Netherlands* [cited 2013 Feb 11]. Available from: http://www.oncoline.nl


