Chapter 7

Summary and general discussion.
This thesis has reported on (7.1) epidemiological aspects of colon cancer in Iceland and it has explored both (7.2) prognostic markers in colon cancer and (7.3) predictive markers in metastatic colorectal cancer.

### 7.1 EPIDEMIOLOGICAL ASPECTS OF COLON CANCER IN ICELAND

**Chapters 2 and 3** reported nation-wide epidemiological aspects of colon cancer in Iceland. Histopathological parameters of all tumors were reviewed in a uniform way. Epidemiological studies and especially time trend analyses where all histopathological material from a whole nation has been reviewed has rarely if ever been performed before for colon cancer. This is especially important since reporting of histology has usually neither been uniform through time nor between pathologists. This guaranteed highly accurate and reliable data.

In **chapter 2** we demonstrated improved colon cancer control in Iceland during the 35 year period while at the same time incidence increased for men and stabilized for women. This improvement in survival was related to earlier detection (more stage I), more frequent surgical intervention and reduction in 1-year mortality, mainly observed in stage III and IV. This data provides insights into why we are seeing improved colon cancer survival over time. It would have been interesting to have as well data on the role of chemotherapy, socioeconomic status or comorbidity in this survival improvement. That information was, however, not available.

We also noted that improved lymph node yield coincided with a relative increase in stage III cancers while there was a reduction in stage II cancers, i.e. improved lymph node yield likely lead to stage migration between stage II and III. An increase of median number of examined lymph nodes from 4 to 7 coincided with a 9% increase in the proportion of pN1-2 cases. Further rise in the median number of examined lymph nodes from 7 to 10 coincided, however with lower rise in the proportion of pN1-2 cases, i.e. 3%. This is in accordance with a previous population-based study, which showed that lymph node yield above 5-7 nodes had marginal effects on staging.\(^1\)

There was a slight left-right shift observed in tumor location while the proportions of histological types remained similar. Thus, we could not identify changes in histology that coincided with increased incidence nor possibly influencing improved survival.

It is interesting to use the data in chapter 2 to compare Iceland with other countries and speculate on future steps in Iceland in colon or colorectal cancer control. The proportion of stage I colon cancer was similar to socioeconomically comparable countries in Europe (see discussion in Chapter 2). Previous studies have also shown that CRC survival in Iceland was comparable to other European countries between 1988 and 2002.\(^2\)

These comparisons indicate that colon cancer control is similar to other countries in North and West Europe. Since then, population-based CRC screening has been introduced in many European countries, such as the Netherlands, United Kingdom, Finland and France. CRC screening can reduce...
CRC incidence and death. If Iceland wants to keep up with these countries in CRC control, they will need to invest in CRC screening too. Organized screening for colorectal cancer was actually planned in Iceland but was put on hold after the financial crisis in 2008. Interestingly, there is strong evidence that CRC screening is highly cost effective. Meanwhile, focus has been put on increasing public awareness, especially among men, which is of importance for a high participation rate to a future screening program.

In chapter 3 we demonstrated various differences in pathology profiles between right- and left-sided colon cancers. Most importantly, stage III and IV was more commonly observed in right-sided colon cancer. Factors that have been associated with microsatellite instability in other studies, such as larger tumor size, poor differentiation, mucinous type and expanding tumor border were also more common in right-sided cancers.

In chapter 3, we also demonstrated a linear trend in right-left ratio for women, with high frequency of right-sided cancers in older women while being least common in the youngest age group. A similar linear trend could not be observed for men. Such differences are likely to relate to differences in etiology and carcinogenesis and are reflected in the fact that sporadic microsatellite instable cancers are typically seen in older women with right-sided colon cancers (see also chapter 5). The mechanism underlying tumor location and gender related differences are unknown. Differences in embryologic origin of the right- and left-colon (midgut versus hindgut) and differences in immunology and microbiota composition between the right and left colon are likely to influence molecular events during carcinogenesis.

We analyzed tumor location as a prognostic marker in chapter 4, which will be discussed here, as it was not the main subject of focus in chapter 4.

Tumor location within the colon has been the subject of many survival studies in the past and typically refers to the left or the right side of the colon. This simple dichotomization of the colon has been popular although the assumption of a two-colon model has recently been questioned by Yamauchi et al. who proposed that a multisegmental approach would be more appropriate. We chose to analyze the colon in Chapter 4 in three parts as right (cecum and ascending), middle (transverse and flexures) and distal (descending and sigmoid). We showed that the middle part confers the worst prognosis of the three segments in the overall series (stage I through IV) and that the outcome of patients with right- and left-sided colon cancer does not differ significantly from each other. A similar effect was also seen in stage II only. In stage III the middle part did not retain its relation to survival on multivariable analysis but, surprisingly, right-sided colon tumors were significantly associated with slightly better survival than left-sided tumors. Tumor location was not of prognostic value in stage IV. In addition to a Danish population-based study, worse outcome conferred by the middle part of the colon can also be seen in large datasets from the EUROCARE study and the SEER database but the magnitudes of the 5-year survival differences are small. The underlying causes of these survival differences are not fully understood. Molecular factors, such as dissimilar patterns
of recurrence and various proportions of microsatellite instability, mutations and recently described
gene expression-base consensus molecular subtypes\(^9\) are likely to contribute to these differences.

Taken together, our results from chapter 2, 3 and 4 on the differences in pathology, epidemiology and prognosis depending on tumor location within the colon, support growing evidence that analyzing the colon segmentally instead of the “one colon” approach is informative. Interestingly, recent studies indicate that tumor location (also when corrected for various mutations) may predict response to anti-EGFR therapy.\(^{10,11}\) Also, there is an emerging notion that a significant part of the molecular heterogeneity of CRC may be captured by the location of the tumor.\(^10\) Whether to use a two- or three-colon approach or multisegmental approach remains to be determined. Data on molecular alterations by tumor location will improve our understanding of carcinogenesis of colon cancer with implications for future research.

In chapter 3 we also demonstrated some disparities between younger and older patient population with more frequent occurrence of adverse factors, like lymph node metastases, vessel invasion and infiltrating tumor border configuration, in patients younger than 50 years of age. The differences were however not large, i.e. between 8% and 16%.

We analyzed patients’ age as a prognostic marker in chapter 4, which will be discussed here, as it was not the main subject of focus in chapter 4. In chapter 4 we showed that patients’ age was associated with survival in all multivariable analyses, i.e. younger patients had better prognosis than older patients. This is in agreement with previous studies.\(^8,12\) Interestingly, while patients’ age was prognostic in all multivariable analyses, it was not associated with survival in some of the univariable analyses. In other words, younger patients, who more often present with adverse features (as shown in Chapter 3), can still be expected to live longer than older patients, when corrected for stage-related variables and other prognostic variables. This is likely to be due to factors such as comorbidities/general health and treatment bias.

Of note, in chapter 4 the variable patients’ age demonstrates how important it is to select variables for multivariable analysis not only based on significance in univariable analysis, as has been pointed out in the literature.\(^13\) To explain, the selection of variables for multivariate analysis, based on significance in univariable analysis, has been called the bivariate selection (BVS) method. When the BVS method is used, important confounding variables may be excluded from the multivariable analysis, which may lead to incorrect results. According to Sun et al, “the BVS method in multivariate analysis is one of the most common errors in data analysis in the current literature”\(^13\). Indeed, using the BVS method in the present study, would have led to excluding patients’ age after univariable analysis, while it appeared important in multivariable analysis.
7.2 PROGNOSTIC MARKERS IN COLON CANCER

In chapter 4 we aimed to clarify the prognostic role of various long known histopathological markers in colon cancer, especially focusing on the pT4 variable. In chapter 5 we narrowed our focus down to the MSI subset of colon carcinomas where we analyzed the prognostic impact of BRAF and KRAS mutation status.

In chapter 4 we analyzed histopathological markers in stage I through IV. We showed that the prognostic impact of many variables is stage dependent and only one variable was prognostic in all multivariable analyses, i.e. patient’s age (see Table 2, Chapter 4). The results show that when a variable is prognostic in a model containing cases from different stages (e.g. stage I through IV), the results may not apply to every stage separately. Awareness that prognostic impact in a subgroup may drive the results of a survival analysis, where patients of more than one stage are pooled together, is important when interpreting survival data. In 1989 it was pointed out that most detailed knowledge on prognostic factors would be obtained from survival analyses for each of the individual pTNM stages. This is still often not done, probably mostly due to otherwise too low number of cases. Although this is an important point to consider, especially with regard to analyzing both stage I and IV separately, there still may be valid arguments to analyze stage II and III together. This is due to overlap in prognosis and adjuvant treatment.

In chapter 4 we focused on the definition and prognostic impact of pT4. We showed that this variable is of high prognostic importance in colon cancer, especially in stage II and III where it is of no less importance than lymph node status. Lymph node status has, however, received much more attention in the literature, obviously because of its direct therapeutic consequences regarding adjuvant chemotherapy. If the ongoing COLOPEC trial turns out to show that patients with either clinical or pathological pT4 tumors benefit from adjuvant HIPEC, this variable will receive much more attention in the future.

The identification of pT4 is not always a simple task and the presence of pT4 can easily be missed, similar to a single (and small) lymph node metastasis. Correct macroscopic assessment and both proper and thorough sampling is crucial for the identification of this variable. The other challenge of the pT4 variable refers to the microscopic definition of serosal penetration (called pT4a in the 7th TNM edition). In chapter 4 we showed that the prognosis of the pT4 subcategories (pT4a and pT4b) is dependent on the definition of these categories. By including surrogate markers in the definition of pT4a, such as the LPI2 category of the Shepherd’s LPI classification (see Chapter 4 for details on this variable), the prognostic impact is affected.

In the 2000’s the College of American Pathologists (CAP) decided to include the LPI2 and LPI3 category of the Shepherd’s LPI classification into its definition on identifying T4. What the general practice was, before that guideline was issued, is unclear, but it is likely that mainly cases corresponding to Shepherd’s LPI4 were interpreted as pT4a. In 2008 the CAP removed the LPI2 category
from the definition without any explanation (see discussion in chapter 4). With that, however, the definition of pT4a had become quite variable between pathologists.

It is noteworthy that similar unclarity has also emerged regarding the definition of the so-called tumor deposits in colorectal cancer. That is why the Dutch guidelines still adhere to the 5th edition of the TNM cancer staging system for CRC. There is now call for more rigorous scientific evidence before making changes in the definition of variables within the TNM-system.16

The role of the pT4 category as one of the so-called high-risk stage II variables also needs some discussion. The high-risk stage II variables include, in the Netherlands, poor differentiation, extramural venous invasion, perforation, obstruction and low lymph node count. In some countries or medical centers, lymphatic invasion, intramural venous invasion and perineural invasion are also defined as high-risk variables. Depending on individual oncologists, hospitals, various national guidelines and MSI-status, patients may or may not receive adjuvant chemotherapy in stage II if one or more of these variables are present in a resection specimen. The benefit of adjuvant chemotherapy when having any high-risk variable present has been challenged in a large retrospective study on 24,847 stage II patients.17

We point out in chapter 4 that three of the aforementioned high-risk variables, i.e. lymphatic invasion, low lymph node count and poor differentiation, may not be useful in identifying high-risk stage II patients. Interestingly, there is lack of studies analyzing the predictive value of each of the high-risk stage II variables separately. Thus, it is possible that the inclusion of patients in trials based on the presence of “less useful” high-risk stage II variables, may have obscured the survival benefit in the high-risk subgroup. Interestingly, two studies have recently been published supporting administration of adjuvant chemotherapy in pT4 stage II cancers, specifically. These retrospective studies, published in 2015, showed that patients carrying pT4 stage II cancers survive significantly longer with adjuvant chemotherapy as compared to those not receiving adjuvant chemotherapy.18,19 It can be speculated that adjuvant HIPEC may become the preferred adjuvant treatment for these patients, instead of adjuvant chemotherapy – but clinical trials might be necessary to resolve that.

Regarding the definition of pT4a, the question arises how narrow or open the definition should be. Also, should this variable just be prognostic in a general sense or should it mainly be used as a marker predicting the occurrence of peritoneal carcinomatosis? This is the subject of future research. The author of this thesis is the designated pathologist of the already mentioned COLOPEC trial15 and hopes to be able to shed more light on the pT4 variable with future research.

In chapter 5 we looked into the prognostic impact of the mutational status of two important and well-known genes in colon cancer, BRAF and KRAS. For that, we selected the subgroup of microsatellite instable (MSI) tumors, where the prognostic impact of these variables has been unclear. Firstly, we demonstrated that both BRAF and KRAS mutation in stage II and III MSI colon cancers were associated with poor survival, compared to double wild-type (dWT) cancers, which confer an excellent cancer-specific survival. Secondly, we demonstrated that the concomitant evaluation of both BRAF
and KRAS mutation in MSI colon cancers provides useful prognostic information beyond evaluation of either variable alone.

Guidelines do not recommend adjuvant chemotherapy in stage II MSI CRC demonstrating high-risk features since patients with stage II MSI tumors generally have a good prognosis and randomized controlled trials do not indicate survival benefit from 5-fluorouracil (5-FU). Regarding the favorable survival of MSI versus MSS in stage II; the prognosis may still not be ideal for an individual patient since the chance of experiencing death due to colon cancer in the first 5 years following surgery was 11% in our cohort. In a population-based survival study from Norway (patients diagnosed 1993–2003) the 5-year chance of relapse was 26% for stage II MSI CRC compared to 44% for stage II MSS CRC. These data demonstrate that although the survival carried by stage II MSI cancers is considered more favorable than that of MSS cancers, this patient group is nevertheless heterogeneous and needs prognostic stratification where a subgroup might benefit from adjuvant therapy. In our study, we provide evidence that dWT MSI cancers, especially in stage II, carry excellent prognosis. This means that this patient group should not need any additional treatment, such as adjuvant therapy. Of course the data needs to be confirmed in a separate study.

Unfortunately we did not have detailed enough information on high-risk stage II histopathological parameters to include it in our study on MSI cancers. It would have been interesting to include at least the pT4 variable. Actually, a recent study, that cited our study in Chapter 4, found pT4 to be the most important prognostic variable in stage II and III MSI CRC after adjuvant chemotherapy. They did not, however, have information on BRAF and KRAS mutation status.

7.3 PREDICTIVE MARKERS IN METASTATIC COLON CANCER

In chapter 6 we aimed to identify DNA methylation markers that predict response to treatment with irinotecan in patients with metastatic CRC. The study was conducted using primary CRC tissue samples from the Dutch CAIRO and British FOCUS trials. In these trials, irinotecan was administered as first-line treatment in combination with capecitabine (CAIRO)/5-FU (FOCUS) while patients in the control arms received only capecitabine/5-FU (FOCUS). This provided the opportunity to evaluate predictive markers for irinotecan.

Of a selection of initially 22 candidate genes, using CAIRO samples, Decoy Receptor 1 (DCR1) promoter methylation was identified as a novel hypermethylated gene in CRC and being a promising predictive marker for response to irinotecan chemotherapy. The results were initially validated, using independent samples from the same CAIRO study. In short, patients with methylated tumor DCR1 did not benefit from the addition of irinotecan to capecitabine, in contrast to patients with unmethylated tumor DCR1. However, external validation, using samples from the FOCUS trial, could not validate the findings from the CAIRO trial.

The first question that arises is why? It might relate to differences in methylation between populations and races, as such differences have been demonstrated in various studies and have also
been related to prognosis. Differences in the frequencies of tumor location within the colorectum between the training/internal cohort and the external cohort could also play a role. To explain, the CpG island methylator phenotype (CIMP) in CRC has been shown to carry poor prognosis in MSS CRC and vary considerably in frequency between right- and left-sided colon cancers and decrease gradually along the large bowel. As pointed out earlier in this discussion, tumor location is emerging as an important stratification variable in colon cancer and has been shown to associate with response to cetuximab therapy. Therefore, it would be of interest to look for differences between the CAIRO and FOCUS cohorts with regard to tumor location.

The literature on biomarker development provides a long list of challenges to biomarker development. The drop-out rate in biomarker development is very high and in a recent Nature review paper on biomarker evaluation the authors wrote that “the development and validation of biomarkers is as difficult as the development and approval of a new drug.” In that review the authors divide the challenges in biomarker development into few main categories. Those include biological, logistical and technical challenges, which, when applied to our study, include issues regarding the single molecule approach, intratumoral heterogeneity, differences between primary tumor and metastatic tumors and determining a clinically meaningful cut-off point for dichotomization of methyla-

tion.

Although promoter methylation may have, at least for now, reached its end-point as a predictive biomarker for response to irinotecan-based therapy, other methylation markers, such as the methylation status of WRN, were also identified during the candidate gene selection and are being studied as well.

7.4 FUTURE PERSPECTIVES

The colorectal cancer research field is interesting and lively, with every year exponentially growing literature on very different aspects on colorectal cancer. Although progress is being made in personalized medicine, translating biomedical research into clinical practice is slow and costly. By collaborative, multidisciplinary efforts between researchers, clinicians, institutions, academia and industry, the speed of progress should be able to increase. At the same time it is important to improve the quality of research by adhering to guidelines, such as on the development of prognostic and predictive markers and statistical analysis.

Although colon cancer control in Iceland is quite good compared to similar European countries, there is still space for improvement and progress. Firstly, screening for colorectal cancer should be initiated, as that has been shown to reduce morbidity and mortality and be highly cost-effective. Secondly, assessing the frequency of Lynch syndrome in Iceland (still an uncharted territory) and screening for these patients is an important part of improving colorectal cancer control. A study on the frequency of Lynch syndrome in Iceland is actually underway and the results will be important for colorectal cancer care in Iceland.
Unresolved issues regarding the TNM system, to which new prognostic markers should be compared, also need to be resolved. These issues include the definition of the pT4 category. The author of this thesis is involved in the already mentioned COLOPEC trial and its spin off studies regarding the pathology problems concerning the pT4 component of the TNM-system. Also the list of high-risk stage II histopathology markers and their definition needs to be refined and updated. The assessment of some of these morphological variables is highly dependent on proper grossing and sampling and diligent microscopic assessment. Auditing of grossing, microscopic pathology assessment and guidelines on the minimal number of blocks per tumor (similar to guidelines on the minimal number of analysed lymph nodes) may be needed to increase the quality of pathology assessment. Prognostic histopathology markers with lack of reproducibility should not be used to guide therapy, as treatment decisions need to be based on more solid grounds. It is the hope that molecular analysis, probably using panels of markers, may provide an accurate way to objectively assess prognosis in adjunct to well established histopathological markers.
REFERENCES


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