CHAPTER 10

Summary and General discussion

SUMMARY AND GENERAL DISCUSSION

Over the past decades, major advances have been made in the treatment of CRC patients. The introduction of new surgical techniques and (neo) adjuvant therapies has greatly improved clinical outcome in CRC patients. A great example is the introduction of the total mesorectal excision (TME) technique and pre-operative radiotherapy in rectal cancer, which decreased the local recurrence rate from 11 to 6% [1]. In colon cancer, the introduction of adjuvant chemotherapy with fluorouracil and levamisole greatly reduced the mortality rate by 33% among stage III patients [2]. The addition of oxaliplatin to this regimen further improved clinical outcome in stage II and III colon cancer patients with a three years disease-free survival of 78% in the MOSAIC trial [3]. Final results of this trial reporting on 5-year disease-free survival and 6-year overall survival also proved that adding oxaliplatin to fluorouracil and levamisole was associated with survival benefits. However, significant difference in survival between these two regimens was lost in stage II colon cancer patients [4]. Therefore, the role and benefit of adjuvant chemotherapy in stage II colon cancer patients still remains controversial [4;5]. Altogether, this has led to current recommendations in the Netherlands where patients with stage III and high-risk stage II colon cancer, e.g. those with T4 tumor extent or vascular invasion, are offered adjuvant chemotherapy with the FOLFOX regimen, consisting of oxaliplatin, fluorouracil and leucovorin [3].

In addition to stage II colon cancer patients, the role of adjuvant chemotherapy in rectal cancer remains debatable as well. Up till now, studies have failed to show significant survival benefits for adjuvant chemotherapy in rectal cancer patients, who are, according to current guidelines, treated with preoperative radiotherapy [3;6-8]. Adjuvant chemotherapy in rectal cancer is therefore not implemented in daily clinical practice in the Netherlands.

Even though major advances in treatment of CRC have been made, mortality still remains high. In the Netherlands, each year approximately 9000 patients are diagnosed with CRC and 4000 deaths occur as a consequence of this disease (www.cijfersoverkanker.nl). Morbidity associated with current treatments should not be underestimated as well. For example, studies in rectal cancer have evaluated the short- and long term morbidity of radiotherapy, where preoperative radiotherapy was associated with faecal incontinence, urgency, anal blood loss and sexual dysfunction [9]. A significant number of (neo)adjuvant treated patients will not show any treatment benefit or not even need treatment to increase prognosis, and approximately 30% of stage II colon cancer patients suffer from recurrent disease within 5 years after surgery [10]. Nowadays, prognostication and treatment allocation are majorly influenced by tumor location and tumor stage (TNM). However, tumor classification has become more complex over the past years since the TNM staging system failed to provide clinicians with the optimal staging

tool it was designed for. Patient survival varies widely within each stage and positive lymph nodes, which determine tumor stage, are easily missed in routine pathological assessment. Under-treatment and over-treatment of some patients exists when using this system for treatment allocation [11-14]. Therefore, the use of TNM stage falls short in daily clinical practice and needs to be supplemented with additional biomarkers that can improve current staging and treatment allocation criteria substantially. Predicting the clinical behavior of a tumor through a combination of clinical, pathological and biological characteristics might lead to a well-targeted treatment in the individual patient, thereby increasing treatment benefit and limiting negative side effects. In this thesis we therefore evaluated prognostic and predictive biomarkers in CRC for improved risk stratification and treatment benefit in the individual patient, with the introduction of precision medicine in the near future as ultimate goal. This thesis is divided in three parts. In ***Part one*** we investigated biomarkers related to important hallmarks of cancer, which were able to adequately assess prognosis in CRC patients. In ***Part two*** we established a survival benefit in colon cancer patients treated with low dose aspirin after diagnosis and investigated predictive biomarkers, which were able to predict which patients would benefit from aspirin treatment after a colon cancer diagnosis. Finally, in ***Part three*** we discussed the use of prognostic and predictive biomarkers in clinical practice, its utility and the road to precision medicine.

PART ONE: PROGNOSTIC BIOMARKERS IN COLORECTAL CANCER

In 2000, Hanahan and Weinberg published an important article about ' the hallmarks of cancer', which are six biological capabilities tumors have to acquire during the multistep development of human cancers. These hallmarks are sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death [15]. In 2011, they added two emerging hallmarks; reprogramming of energy metabolism and evading immune recognition and recognized the importance of the tumor-microenvironment in tumor development. The hallmarks constitute an organizing principle for rationalizing the complexities of neoplastic disease. Recognition of these hallmarks will increasingly affect prognostication and the development of new means to treat human cancer [15]. In this part we investigated biomarkers related to some of these hallmarks, such as sustaining proliferative signaling, resisting cell death and evading immune recognition.

The last decades, research has indicated a substantial influence of the immune system on tumor growth, which showed to be both tumor suppressing and promoting [16]. In **Chapter 2 and 3** we investigated the prognostic value of important immune recognition evading mechanisms in colon cancer and in rectal cancer separately by analyzing

HLA class I tumor expression, tumor expression of non-classical HLA class I molecules (HLA-E and HLA-G) and tumor infiltration with immunosuppressive regulatory T cells (Tregs). The goal of these studies was to establish a tumor profile based on biomarkers that reflect a tumor's immune susceptibility status and to determine its relationship to patient outcome.

In 285 colon cancer patients (**Chapter 2**), loss of HLA class I was significantly associated with a better overall survival and disease-free survival, which could be explained by elimination of tumor cells by natural killer (NK) cells once these tumor cells metastasize to the bloodstream [17-19]. When the immune markers were combined, three distinct survival patterns based on immune surveillance were identified. Patients with tumors showing loss of HLA class I and negative HLA-E and –G expression, irrespective of Treg tumor infiltration, showed the best prognosis. Absence of HLA-E and -G expression possibly made these tumors, who have lost their HLA class I expression, even more susceptible to NK cell elimination, further explaining their favorable prognosis [20;21]. In contrast, patients showing the worst prognosis were patients with tumors with HLA class I downregulation and low Treg infiltration, irrespective of HLA-E and –G expression. Since tumors are thought to be 'immunoedited' through a Darwinian selection process into poorly immunogenic tumor cell variants invisible to the immune system [16], we hypothesized that these poorly immune-recognized tumors are already edited by Cytotoxic T-cells (CTL), because they partly lost their HLA class I expression. Consequently, these tumors will elicit a minimal CTL attack, resulting in tumor progression. The absence of Tregs in the tumor micro-environment of these tumors further strengthens our hypothesis. Because of the opposing actions of Tregs and CTL in tumor immunity, Tregs will not be needed for immune escape when CTL presence is scarce [22]. In summary, this study showed a complex and multifaceted interplay between different immune escape mechanisms, highlighting the need for combined immune marker analysis to better reflect patient outcome. We were able to determine three distinct survival patterns in colon cancer based on immune surveillance (Figure 1), which represented significant independent clinical prognostic value in colon cancer patients.

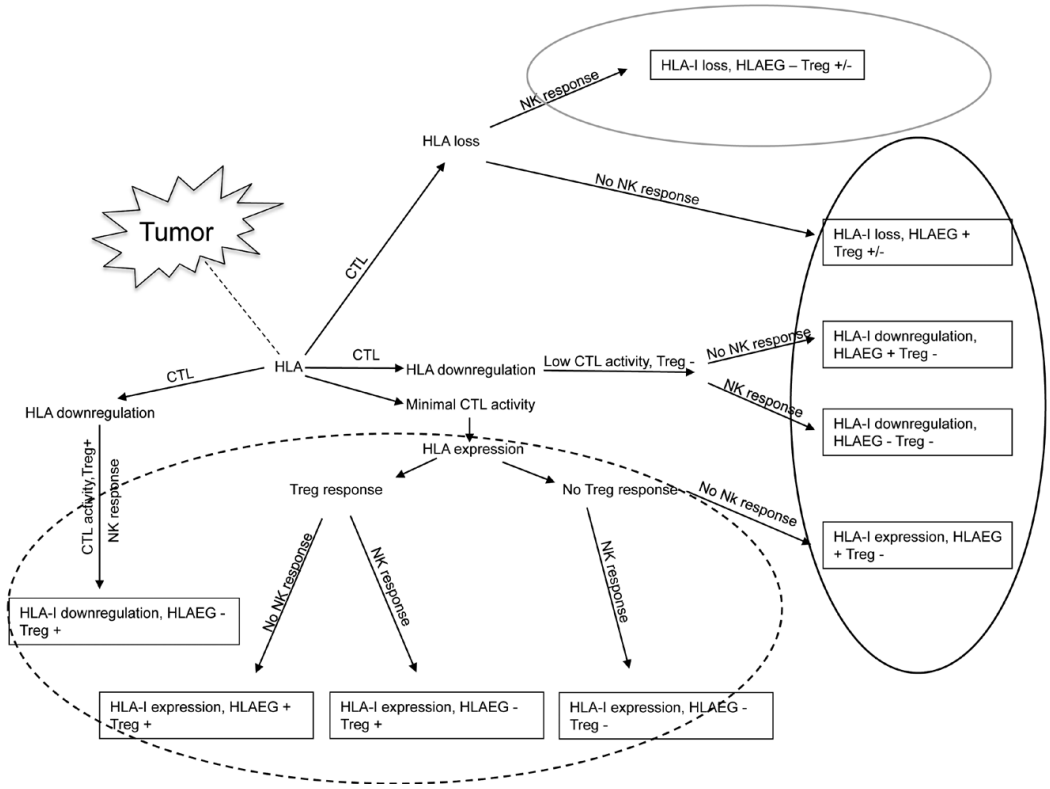

Figure 1: Global overview of immune escape mechanisms based on literature and results we established in a cohort of 285 colon cancers in which HLA class I tumor expression, HLA-E tumor expression, HLA-G tumor expression and Treg infiltration were investigated

The tumors with a certain phenotype in the gray, dashed and black circle indicate tumors that are high, intermediate or low immune susceptible with a good, intermediate and worse prognosis respectively. Treg, immunosuppressive regulatory T cell; CTL, Cytotoxic T cell; NK, natural killer cell.

In **Chapter 3**, we investigated the prognostic relevance of the same immune markers, independently and combined, in 495 rectal cancer patients. In this study, HLA class I tumor expression and a high Treg tumor infiltration were related to a better clinical outcome in these rectal cancer patients. Interestingly, strong HLA-G expression was also significantly related to a better survival. These results are remarkable since HLA-G expression can inhibit NK cells from lysing tumor cells that have lost or downregulated classical HLA class I expression as a secondary immune escape [23;24]. The reason for this seemingly opposing effect of HLA-G expression remains unclear. Immune regulation in cancer still remains complex and multifaceted, and not all immune-related mechanisms are completely clear. Possibly, HLA-G expression does not play an influential role in rectal cancer when HLA class I expression is still present.

When the immune markers were combined, again three distinct patterns in patient survival based on immune surveillance were identified. Prognosis increased with a decrease in negative prognostic markers, thus patients with tumors bearing two or three negative prognostic markers, e.g. loss of HLA class I tumor expression, weak HLA-G

tumor expression and low tumor infiltration with Tregs, showed a worse prognosis and therefore qualified as very low immune susceptible. Furthermore, patients with tumors showing loss of HLA class I expression, low Treg infiltration and strong HLA-G expression showed the worst outcome perspectives. We hypothesized that these patients probably had tumors which were highly 'immunoedited', since these tumors have lost their HLA class I expression, causing a minimal CTL attack and subsequently attracted little to no Tregs. Because of strong HLA-G expression they probably were able to escape further immune recognition through inhibition of NK cell recognition [23;24]. Interestingly, in contrast to what we have reported above, HLA-G expression is in this subset of poorly immune-recognized tumors associated with a worse survival. HLA-G expression might only play an influential role during this phase of 'immuoediting' as second immune escape mechanism, when HLA class I expression has already been lost.

These two chapters have provided us with some confusing and opposing results, as, compared to colon cancer, some different immune escape mechanisms seem to occur in rectal cancer. In colon cancer, loss of HLA class I was significantly related to a better survival. In rectal cancer, best survival outcomes were seen for patients with tumors showing expression of HLA class I. This might suggest biological differences between colon and rectal tumors. One of these biological differences might be the microsatellite status of the tumor. Approximately 50% of all proximal colon tumors show microsatellite instability (MSI), whereas almost all distal colon and rectal cancers are microsatellite stable (MSS) tumors [25;26]. MSI has been associated with loss of HLA class I as well as a better prognosis, possibly influencing prognostic results when analyzing HLA class I in colorectal tumors [27;28]. Unfortunately, in our colon cancer cohort the number of MSI tumors that was successfully determined was too small to perform separate analyses in MSI and MSS tumors.

When all immune markers were combined, differences in immune escape mechanisms became even clearer. In colon cancer, patients with tumors showing loss of HLA class I and negative HLA-E and -G expression, irrespective of Treg infiltration, were related to a better survival. In contrast, tumors with the same characteristics were related to a worse outcome in rectal cancer. Again, microsatellite status might influence these results.

Recently, the Cancer Genome Atlas Network investigated biological differences between colon and rectal cancer, but only established differences in anatomical tumor site with more hypermethylation in right-sided tumors, possibly explained by different embryonic origins of right-and left-sided tumors [29]. Therefore, the question still remains if there are true biological differences between colon and rectal cancer and further studies should focus on separate analyses of these tumors.

In **Chapter 4**, we performed a combined analysis of biomarkers of proliferation and apoptosis in colon cancer, namely Ki67 and cleaved caspase-3. A key factor in tissue

homeostasis, especially of the intestinal mucosa, is a balance between the level of cell death and cell proliferation [30-32]. Disturbance of this balance could contribute to initiation and maintenance of tumor growth and development [15;33]. Previous studies in CRC showed contradicting results with respect to the association between apoptosis and proliferation in tumor resection specimens and patient outcome, especially when comparing tumors originating from the colon and rectum [32;34-39]. Also, the prognostic value of apoptosis and proliferation seems to be influenced by tumor location and microsatellite status [37;40;41].

The contradicting results derived from these studies strengthened our hypothesis that a balance between both these processes determines patient's clinical outcome. Our study showed that a combined analysis of the level of tumor cell proliferation and apoptosis was significantly related to patient outcome in 285 stage I-IV colon cancer patients with respect to disease-free survival and overall survival. Patients with a strong proliferation and presence of apoptosis in their tumors showed the best survival outcomes. Interestingly, the impact of this combined analysis of proliferation and apoptosis on patient outcome varied with tumor location and therefore highly likely with tumor microsatellite status, since significantly more MSI tumors were located on the right side of the colon. Unfortunately, the number of MSI tumors in our cohort was too small to perform stratified survival analysis for microsatellite status.

In the left-sided cohort the patients with a balance between proliferation and apoptosis in their tumors performed better with respect to outcome. As you would expect from high proliferative tumors, patients with left-sided tumors showing high proliferation levels and absence of apoptosis had the worst outcome perspectives. In contrast, right-sided tumors with high proliferation levels and absence of apoptosis performed significantly better. Based on these results we hypothesized that it is either tumor microsatellite status or tumor location, which influences the prognostic value of the balance between tumor cell proliferation and apoptosis. It is not unlikely that the tumor microsatellite status influences the balance between tumor cell proliferation and apoptosis. MSI tumors are known to have high levels of proliferation and tend to accumulate gene mutations leading to increased production of abnormal peptides [40;41]. This might result in an immune reaction leading to higher levels of apoptosis, which possibly explains the favorable prognosis of patients with right-sided tumors showing high proliferation levels [42]. However, further studies investigating these two important hallmarks are necessary and should focus on separate analyses of colon- and rectal cancers, where tumor microsatellite status and location are be taken into account as well.

In **Chapter 5**, we performed a validation of the 12-gene Colon Cancer Recurrence Score® Assay as a predictor of recurrence risk in stage II and III rectal cancer patients treated with surgery alone from the Dutch TME trial [1]. The Onco*type* DX Colon Cancer Recurrence

Score (RS) (Genomic Health, Redwood City, CA, USA) was developed by using tumor gene expression data from 1851 patients with resected colon cancer from four independent clinical trials [43]. This was followed by the design of the 12-gene colon cancer Recurrence Score (RS), which was validated in the QUASAR clinical trial beyond other clinical covariates [44]. Predefined risk groups were categorized as low, intermediate or high risk for tumor recurrence according to patients' RS values, which gave the possibility to specifically allocate cancer patients for (adjuvant) treatment regimens. In this validation study performed in rectal cancer, RS predicted risk of recurrence, risk of distant recurrence, and rectal cancer-specific survival. The effect of RS was most prominent in stage II rectal cancer and attenuated with more advanced stage. RS may be clinically useful in stage II rectal cancer patients, where RS can help identify high-risk patients who could benefit from -- and low-risk patients who may forego -- adjuvant chemotherapy (Figure 2).

Up till now trials failed to show a survival benefit with adjuvant chemotherapy for pre-operatively treated rectal cancer patients [6-8]. However, efforts are underway to study reduced-intensity approaches, including those that spare radiation or even surgery. Incorporation of the Recurrence Score assay into clinical trials, such as the TAILORx and RxPonder trials in breast cancer [45;46], may enable these efforts through improved patient stratification for risk-adapted treatment strategies.

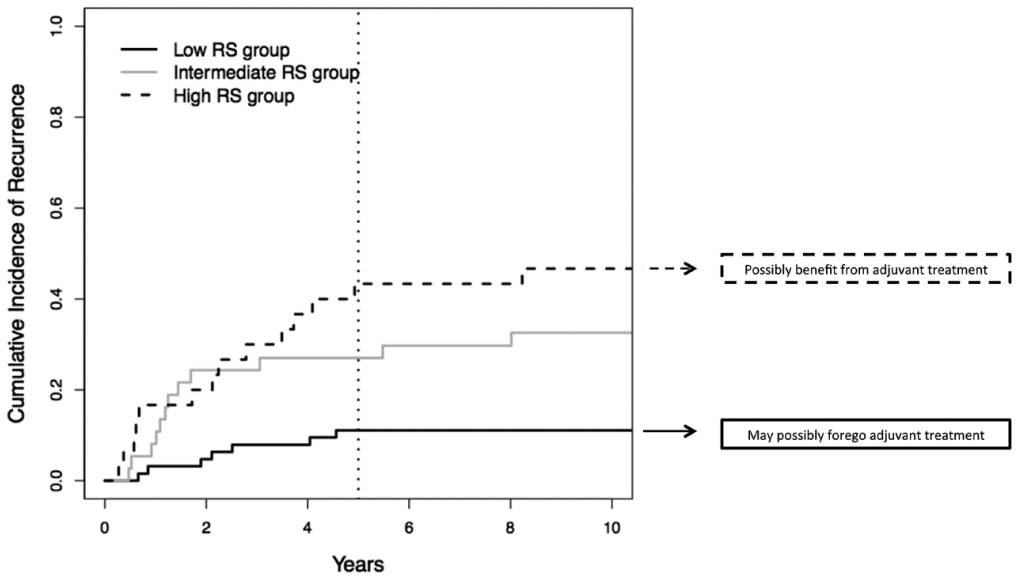

Figure 2: Cumulative incidence of recurrence in 297 rectal cancer patients.
Predefined risk groups were categorized as low, intermediate or high risk for tumor recurrence according to patients' Recurrence Score (RS) values based on the 12-gene Colon Cancer Recurrence Score® Assay, giving the opportunity to specifically allocate adjuvant treatment in the individual patient. This figure is derived from Reimers *et al*., Validation of the 12-gene Colon Cancer Recurrence Score as a predictor of recurrence risk in stage II and III rectal cancer patient, J Natl Cancer Inst 2014 Sep 26:106(11)

PART TWO: TREATMENT OF COLON CANCER AND PREDICTIVE BIOMARKERS

Aspirin and other nonsteroidal anti -inflammatory drugs have shown to be effective in preventing CRC [47-49]. More recently, aspirin has also shown promising results when used after CRC diagnosis [50-52]. In **Chapter 6** we performed a subanalysis in elderly colon cancer patients of the cohort used by Bastiaannet *et al.* [50] to investigate the benefit of low-dose aspirin (80mg) treatment after diagnosis. Patients with rectal cancer were excluded from analysis as these patients did not show any benefit from aspirin treatment. In this study, aspirin use after diagnosis was significantly associated with an improved survival of 40% in older colon cancer patients (≥ 70 years of age) compared to nonusers. This study implicates that aspirin could be an effective adjuvant agent in the treatment of colon cancer, especially in older, chemo-naïve colon cancer patients. Demonstration of a significant therapeutic effect of a well-tolerated, inexpensive drug would be a major clinical advancement.

The exact mechanism by which aspirin exerts its anti-cancer effect still remains largely unknown. It might be that the anti-inflammatory and chemopreventive effects of aspirin are mediated through direct inhibition of COX-1 and COX-2 [53-55]. COX-1 is responsible for platelet aggregation through production of TXA2 in platelets [56]. COX-2 plays an important role in colorectal carcinogenesis, invasion, angiogenesis and metastasis [54] and approximately 70% of colorectal tumors express COX-2 [51;57]. Studies have shown that this COX-2 effect can be reversed by selective COX-2 inhibitors [54]. COX-2 independent pathways, such as suppression of IL-4, NF-κB, insulin-like growth factor 1 (IGF-1), and the inhibition of Wnt-signaling and stem cell growth possibly as the result of enhanced beta-catenin phosphorylation have also been described to contribute to the anti-cancer effects of aspirin [58-62]. Recently, several studies on aspirin benefit in CRC were performed on data from the Nurses' Health Study in the USA. First, Chan *et al*. reported a survival benefit for aspirin use after diagnosis in CRC patients, which seemed to be dependent on COX-2 expression of the tumor. A much lower risk of CRC-specific and overall mortality with tumors that overexpress COX-2 was found [51]. A second study of the same research group showed that the survival benefit from aspirin use after diagnosis was restricted to patients with mutant *PIK3CA* tumors. Patients with wild-type *PIK3CA* tumors did not benefit from aspirin treatment [63]. The phosphatidylinositol 3-kinase (PI3K) signaling pathway plays an important role in carcinogenesis [64]. Mutations in *PIK3CA* are present in approximately 15 to 20% of CRCs [65-67]. Up-regulation of PI3K enhances COX-2 activity and prostaglandin E2 synthesis, resulting in inhibition of apoptosis in colon-cancer cells [68]. Aspirin might suppress tumor development and induce apoptosis by blocking this PI3K pathway [69].

As it is desirable to reduce overtreatment of patients and lower incidental side effects of aspirin treatment, we also tried to find predictive biomarkers for aspirin treatment in

colon cancer. The metastatic potential of cancer cells that are shed into the bloodstream can be modified by environmental conditions, including platelets and bone marrow-derived cells in the vasculature [70]. As soon as cancer cells enter the bloodstream they interact with platelets [71]. Through tumor cell coating, platelets are thought to protect disseminating tumor cells from lysis by immune cells such as NK cells. Tumor cell coating leads to platelet activation and degranulation followed by release of a variety of factors capable of influencing NK reactivity [72]. The interaction between platelets and tumor cells is also thought to transfer HLA class I from the platelet onto the tumor cell surface resulting in a HLA class I-positive phenotype, or 'pseudoself'. This platelet-derived HLA class I blocks NK cell activity. Because platelet-derived HLA class I presents self-peptides, reflecting the normal ligandome of the megakaryocyte lineage, CTLs are not activated as well [72].

Aspirin influences platelet aggregation through COX-1 inhibition [56]. Most likely tumor cell coating and platelet-tumor cell interaction are affected as well. In case of aspirin use, tumor cells are now prone for lysis by immune cells. NK cells preferentially recognize and eliminate cells with low or absent expression of HLA class I [21;23], We therefore hypothesized that the survival benefit associated with low dose aspirin use after a cancer diagnosis would be associated with tumors that have low or absent HLA class I expression. In **Chapter 7** we showed that aspirin use after a colon cancer diagnosis was associated with improved survival if tumors expressed HLA class I on their cell surface, contrary to the original hypothesis. There are two possible explanations for this intriguing observation. First, the disruption of platelet aggregates with aspirin that shield HLA class I expressing, circulating tumor cells might make these cells more susceptible for T-cell mediated immune surveillance. Second, direct contact of platelets and tumor cells results in secretion of TGF-β and activation of the NF-κB pathway, which, in synergistic action, prime circulating tumor cells for subsequent metastases [70]. Aspirin might inhibit platelet-tumor cell signaling and prevents epithelial-mesenchymal transition in circulating tumor cells, thereby reducing the metastatic potential. HLA class I expression might be necessary for this platelet mediated NF-κB signaling in circulating tumor cells resulting in an epithelial-mesenchymal-like phenotype with enhanced metastatic potential (Figure 3).

Our data was not able to confirm the previously published results from the USA group, which demonstrated that the benefits of aspirin after a colorectal cancer diagnosis were associated with strong COX-2 expression in the original tumor and the presence of mutations in *PIK3CA* [51;63]. In our cohort, there was no difference in benefit from aspirin use after a colon cancer diagnosis when the survival analyses were stratified for COX-2 expression and *PIK3CA* mutation status. Interestingly, research performed by an English group recently confirmed the survival benefit of aspirin in *PIK3CA* mutated CRCs, however, the predictive value of COX-2 expression was again not validated in this cohort [73].

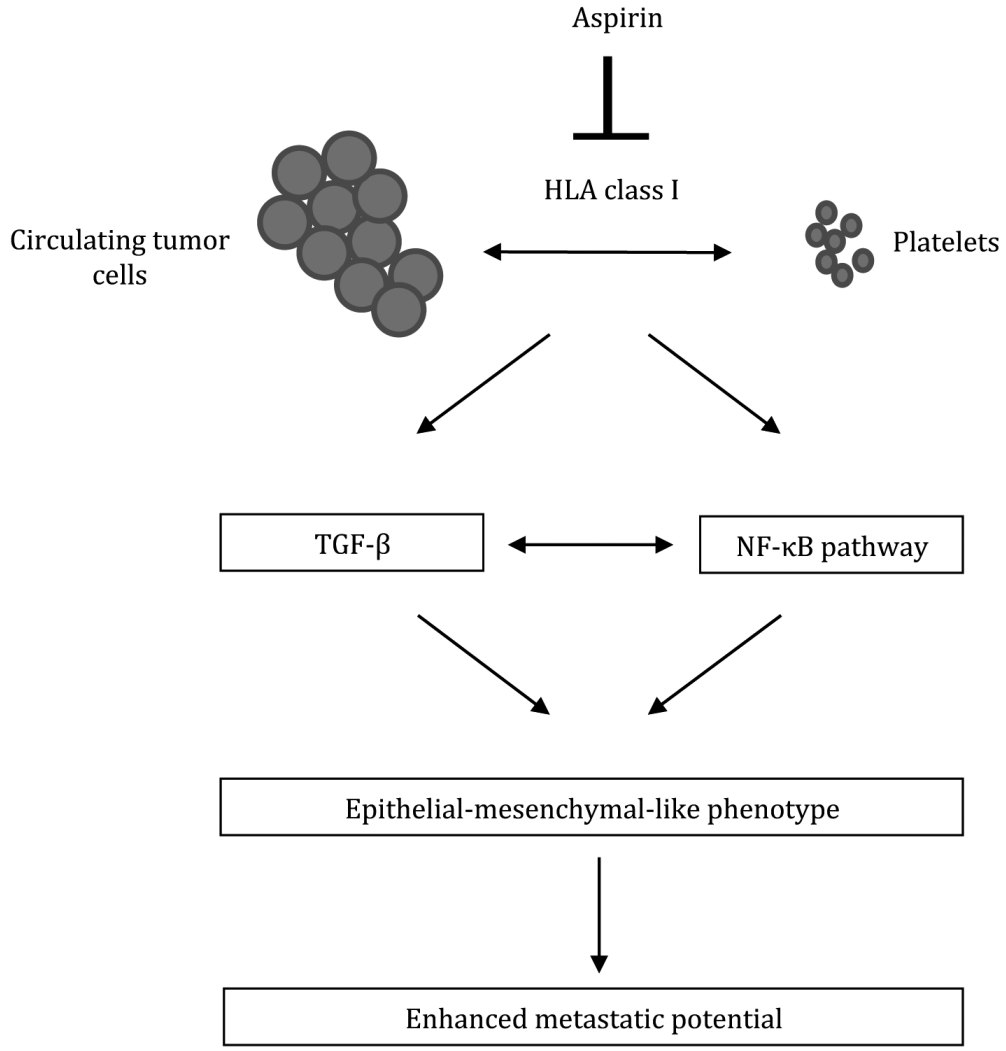

Figure 3:
In this model direct contact of platelets and tumor cells results in secretion of TGF-β and activation of the NF-κB pathway, which, in synergistic action, prime circulating tumor cells for subsequent metastases. Aspirin might inhibit platelet-tumor cell signaling (which is dependent upon intact HLA expression) and prevents epithelial-mesenchymal transition in circulating tumor cells, thereby reducing the metastatic potential.

The contradicting results might be pharmacologically explained, since different dosages of aspirin are investigated in these studies (USA group 325 mg, English group 100 mg, our group 80 mg). Data on aspirin indicate that systemic concentrations of aspirin reached with low-doses are inadequate to permanently acetylate COX-2, but are optimal for platelet inhibition [74]. This might explain why in our cohort, where low-dose aspirin was investigated, strong COX-2 expression and *PIK3CA* mutations were not validated as predictive biomarkers. Furthermore, there may be more than one mechanism of action that accounts for the anti-cancer effects of aspirin; a direct anti-platelet effect due to inhibition of COX-1, that is responsible for the reduction in metastases and only requires

a dose of aspirin that inhibits platelets; and a second mechanism activated with higher or more frequent dosing that inhibits the COX-2 pathway in systemic tissues.

Reflecting on the results derived from this thesis the apoptotic pathway could also be a potential field of interest for studying the anti-cancer effects of aspirin. Aspirin has shown to promote apoptosis, either through suppression of IL-4 gene expression, which is essential for the resistance to DNA damage-induced apoptosis of colon cancer stem cells (CSCs) [58;75], or through inhibition of NF-κB or COX-2 expression [61;68]. Research has shown that MSI confers cell resistance to apoptosis [76]. Consequently, microsatellite status might influence benefit from aspirin treatment. In vitro studies investigating long term aspirin exposure have already shown the selection for MSS and reduction of the MSI phenotype in colorectal and gastric cancer cell lines [77;78]. Goel *et al.* previously showed that aspirin treatment increased mismatch repair protein expression and apoptosis in CRC cells. Interestingly, growth inhibition of all human colon cancer cell lines was independent of microsatellite status, however, different growth regulatory mechanisms were responsible for this inhibition [79]. A recent study also confirmed that aspirin treatment induced NF-κB-driven apoptosis was independent of p53 expression and microsatellite status, suggesting that microsatellite status is not the predominant pathway responsible for aspirin anti-tumor activity [76]. In the preventive setting, for example in Lynch Syndrome families, aspirin could have an important influence on microsatellite status, thereby reducing MSI phenotype and thus cancer progression. However, since the MSI phenotype has been associated with improved survival [80], the survival benefit caused by aspirin will probably not be influenced by the microsatellite status of the primary tumor.

In summary, results from the above mentioned studies still keep us in the dark concerning aspirin's anti-cancer effects. Pooling of data from the different cohorts to improve statistical power in subgroup analyses followed by validation studies and randomized controlled trials are therefore eagerly awaited. In the Netherlands, a randomized placebo-controlled trial investigating low-dose aspirin (80 mg) after surgery in older colon cancer patients will start soon (Aspirin Trial, NTR 3370; EudraCT2011-004686-32). Possibly, more than one mechanism is responsible for the anti-cancer effects of aspirin. Different pathways should therefore be combined, also taken into account that the molecular mechanisms responsible for the anti-cancer effects of aspirin in the adjuvant setting may differ from the ones in the preventive setting.

PART THREE: PRECISION MEDICINE IN COLORECTAL CANCER AND FUTURE PERSPECTIVES

The TNM stage proved to fall short in clinical practice and needs to be supplemented with additional biomarkers to improve current staging and treatment allocation criteria substantially. A lot of research has been dedicated to the discovery and development of clinical prognostic and predictive biomarkers to improve diagnosis and to allocate optimal treatment modalities, introducing precision medicine in the multimodality treatment of cancer. By definition, precision medicine is a multi-faceted approach to medicine that integrates molecular and clinical research with patient data and clinical outcome, and places the patient at the center of all elements. Genomic, epigenomic, patient- and environmental data are studied together to understand individual disease patterns and to design preventive, diagnostic, and therapeutic solutions.

Unfortunately, in spite of a vast amount of available literature on biomarkers in CRC, only a few biomarkers are used on request in clinical practice nowadays, like *KRAS*, *BRAF*, MSI and the Onco*type* DX Colon Cancer Assay for determining whether to treat metastatic CRC patients with cetuximab or panitumumab, for the evaluation of Lynch syndrome and to inform treatment planning in stage II and III colon cancer patients.

In **Chapter 8** we have given an overview of a number of frequently studied biomarkers in CRC and emphasized on the difficulties and controversies that withhold clinical introduction of these biomarkers. In this review we have stated that there is insufficient evidence to introduce other biomarkers in clinical practice. Possible explanations are the use of divergent patient selection criteria, lack of consensus in performing studies and absence of validation studies.

Previously, a stepwise program for the introduction of biomarkers in clinical practice was developed with the first step being biomarker development in a preclinical, exploratory setting, subsequently followed by verification of this biomarker in a large retrospective study, validation and finally confirmation in a prospective randomized controlled trial [81]. Future studies should focus on following this program and standardized methods for performing studies, according to Good Clinical Practice recommendations, have to be developed. Furthermore, since tumor cells may acquire multiple capabilities during tumor development [15], the combination of biomarkers may provide greater prognostic and predictive value than the use of one single marker.

Over the last decade genomic profiling demonstrated its promising prognostic and predictive value in precision medicine and is therefore increasingly used in multidisciplinary consultations for risk-assessment and subsequent treatment planning of the individual cancer patient. The added value of genomic profiling for systemic therapy seems clear. In **Chapter 9** we have focused on the impact of genomic profiling on surgical decision-making. Apart from some single-gene mutations, genomic tumor profiling

in current clinical practice merely impacts surgical decision-making indirectly, as genomic tumor profiling of the biopsy might influence timing, extent and type of surgery by means of optimal tumor shrinkage through targeted neo-adjuvant therapy. Possibly, this may also lead to a wait-and-see approach in case of a pathological complete response (pCR). However, some issues should be resolved before genomic profiling has a clear influence on surgery, such as lack of clarity how to assess a pCR, the ideal timing of clinical, radiological and pathological assessment of response, the uncertainty of the long-term efficacy of this strategy, new follow-up protocols and the question of when to have surgery after neo-adjuvant treatment.

To achieve precision medicine in the future some important steps have to be taken. First, to increase clinical applicability, studies investigating biomarkers should focus on using standardized methods and comparable patient selection criteria in order to validate the results. Second, as current cancer research mainly focuses on the genotypical approach of cancer treatment, which is believed to alter cancer treatment radically in the near future, the phenotype of the cancer patient is ignored. In our greying society, cancer patients often suffer from one or more comorbid conditions, which should be

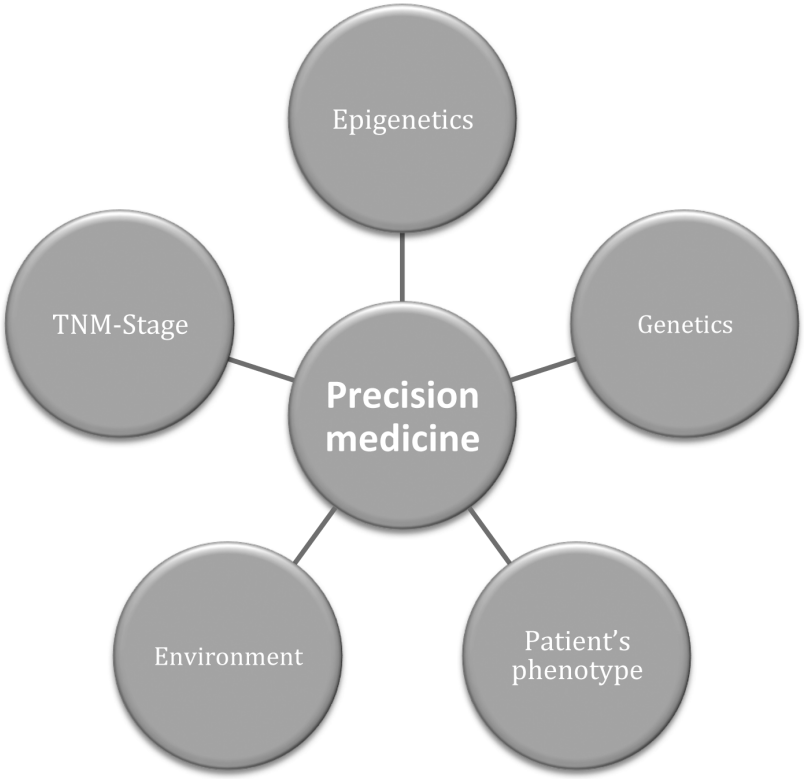

Figure 4: Precision medicine in the multimodality treatment of cancer.
By definition, precision medicine is a multi-faceted approach to medicine that integrates molecular and clinical research with patient data and outcomes and places the patient at the center of all elements.

taken into account when making cancer treatment decisions. Both a direct effect of comorbidity (competing risk of mortality) as well as the interaction with cancer must be weighed in these treatment decisions. Thus, parallel to the existing TNM stage for treatment allocation and the exciting new developments of the epigenetic and genetic fingerprint of the tumor, phenotypic profiling must be incorporated in the treatment approach of an individual patient. Finally, specialists involved in cancer management need to join forces and create a collaborative multidisciplinary approach to provide the most efficient and tolerated treatment in order to achieve precision medicine as ultimate goal (Figure 4).

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