SUMMARY AND GENERAL DISCUSSION
SUMMARY
Cancer is a leading cause of death worldwide, accounting for 7.9 million cases in 2007 which comprises around 13% of all deaths.\textsuperscript{1} The number of cancer-related deaths is expected to increase with an estimated 9 million in 2015. Overall cancer mortality is mainly caused by solid tumors arising from lung, stomach, liver, colorectum and breast tissue. In this thesis, we will focus on gastric and colorectal cancer with the emphasis on the latter.

Gastric cancer is the second most common cancer worldwide with a varying incidence, being higher in Japan and some parts of South America (> 40 per 100,000) and lower in Western Europe and the United States (< 15 per 100,000).\textsuperscript{2} Environmental exposure rather than genetic factors play a role in the predisposition to gastric cancer.\textsuperscript{3}

Colorectal cancer (CRC) is the third most prevalent cancer worldwide with an estimation of 1.2 million new cases and 630,000 deaths in 2007.\textsuperscript{4} The incidence of CRC is higher in developed countries, making it the second most common cancer in Europe.\textsuperscript{5} The risk for developing CRC increases with age as a result of accumulation of (epi)genetic mutations, with more than 90% of new cases being diagnosed in patients older than 50 years. Most cases of CRC occur in sporadic forms of which about 20% have some component of familial risk.\textsuperscript{6} Approximately 5 to 10% are hereditary.

Surgery is the first choice of treatment in gastric cancer and CRC. After surgery, patients receive adjuvant systemic treatment depending on disease stage. Disease stage is based on the Tumor-Node-Metastasis (TNM) classification system which is one of the most well established prognostic factors in solid tumors.\textsuperscript{7,8} After the introduction in 1987, the TNM system has been revised every few years to allow the incorporation of new evidence. Unfortunately, this staging system does not provide prediction of prognosis for the individual patient leading to both over- and undertreatment.

This thesis assesses whether gastric cancer and CRC patients with minimal metastatic disease at the time of surgery who are at risk for disease recurrence, can be identified by detection of occult tumor cells (OTC) in lymph nodes or bone marrow, or by analyzing the primary tumor for angiogenic and lymphangiogenic markers.

Chapter 1 presents a general introduction and describes the background and outline of this thesis. In short, the TNM staging classification represents the routes of tumor spread namely local invasion (T), lymphatic spread (N) and hematogenous dissemination (M). With the current TNM classification, mostly macroscopical tumor spread is determined by using conventional hematoxylin and eosin stainings and radiological techniques. Therefore, numerous patients who are at risk for disease recurrence after supposedly curative resection are not recognized. By exploring the presence of minimal disease in lymph nodes or
bone marrow or by applying biological markers in the primary tumor related to tumor cell spread, a more individualized portrait of patient’s prognosis might be achieved. The first adjustments have been made in the last revision of this staging system since micrometastases (MM; > 0.2 mm and ≤ 2 mm) and isolated tumor cells (ITC; ≤ 0.2 mm) in lymph nodes were incorporated.

In chapter 2 we investigate whether the presence of OTC in originally considered tumor-negative lymph nodes from curatively resected gastric cancer patients can predict disease recurrence. Also, the value of automated microscopy is evaluated. Forty cases (disease recurrence) and 41 controls (no disease recurrence for at least 5 years) were selected from the previously published multicenter Dutch D1-D2 Gastric Cancer trial. One tissue section was immunohistochemically stained by anticytokeratin antibodies CAM5.2 and examined by conventional and automated microscopy. There was no significant difference in the presence of OTC, MM or ITC between the case and control groups (P = 0.658; P = 0.691; P = 0.887, respectively). However, significantly more cases than controls presented with 20% or more OTC-positive lymph nodes (Chi-square test, 9 (24%) vs one (3%), P = 0.015). A multivariate logistic regression analysis showed that examination of less than five lymph nodes (OR 13.8, 95% CI 1.6-120.6, P = 0.018) was the only significant independent risk factor for disease recurrence, especially for locoregional disease recurrence (OR 20.4, 95% CI 2.2-190.8, P = 0.008). A similar analysis for distant disease recurrence showed a percentage of 20% or more OTC-positive lymph nodes to be the only significant independent risk factor (OR 15.6, 95% CI 1.6-151.4, P = 0.018). The sensitivity of immunohistochemistry evaluated by microscopy to identify cases with 20% or more OTC-positive lymph nodes increased from 8% for conventional microscopy to 22% for automated microscopy (McNemar’s test, P = 0.063). Our results suggest that locoregional disease recurrence might be the result of lymph nodes that were not removed. In addition, when 20% or more of the lymph nodes are OTC-positive, tumor cells may already have travelled to other sites in the body, eventually leading to distant metastases.

Additionally, this chapter presents an overview of previously published immunohistochemistry studies regarding the clinical relevance of OTC in lymph nodes of patients with gastric cancer. There was an extensive variation in the antibodies that were used and in the reported upstaging percentages. Ten of 16 studies reported a negative impact of the presence of OTC in lymph nodes on the clinical outcome. Similar to our study, Siewert et al. also reported clinical significance of the percentage of OTC-positive lymph nodes.

Chapter 3 presents an overview of studies regarding the detection methods and clinical relevance of OTC in lymph nodes in CRC. Three out of four studies using polymerase chain reaction (PCR) for OTC detection,
showed its prognostic relevance. Certain DNA regions that might have contained tumor-specific mutations in the genes p53 or K-ras were amplified by the PCR assay in these studies. Disadvantages such as a non-consistent occurrence of p53 and K-ras mutations in CRC and the large number of codons or even exons in which mutations can be detected, will prevent PCR from becoming a ubiquitously utilized application for OTC detection.

All six studies using reverse-transcriptase polymerase chain reaction (RT-PCR) to detect OTC in lymph nodes showed a negative impact of OTC on patient’s prognosis. However, two realtime RT-PCR studies failed to show any significance of OTC in lymph nodes. We concluded that OTC in lymph nodes detected with RT-PCR show prognostic value provided that fresh or frozen lymph nodes, the markers CEA, CK20 and GCC and the optimal number of PCR cycles are used. Only 9 out of 28 immunohistochemistry studies showed a significantly worse clinical outcome in patients with lymph nodes containing OTC. Particular studies using anticytokeratin antibodies CAM5.2, reported OTC-positive lymph nodes as clinically relevant.

We found upstaging percentages through OTC assessment and the prognostic relevance of OTC in lymph nodes to vary among studies, particularly in the immunohistochemistry studies, which was related to differences in techniques used to detect OTC.

In chapter 4, we assess the value of multiple sectioning of lymph nodes combined with immunohistochemical staining in detecting prognostic relevant OTC in CRC patients. The slides were examined by automated microscopy. Although OTC in lymph nodes detected by RT-PCR, more often are of clinical value, the hospital infrastructure for this technique is more demanding due to the need for fresh frozen tissue. Additionally, morphologic assessment of tissue samples analyzed, is warranted. This study was performed on histologically tumor-negative paraffin-embedded lymph nodes previously studied by Liefers et al. with RT-PCR for CEA for the detection of OTC. They found a five-year survival rate of 50% for the OTC-positive group and 91% for the negative group (P = 0.02).

Lymph node tissue blocks from 20 patients were sectioned (five consecutive sections of 10 levels at 200 μm interval or until no material was left) and immunohistochemically stained using antibodies against cytokeratin, AE1/AE3. The stained slides were analyzed using rapid high capacity flatbed scanning. OTC were detected in all of six (100%) patients with disease recurrence, compared to eight of 14 (57%) patients who did not develop a recurrence (Chi-square test, P = 0.055). Five of six (83%) patients who developed disease recurrence were OTC-positive detected by CEA RT-PCR and three of 14 (21%) who showed no recurrence were OTC-positive.

These findings showed that multilevel sectioning combined with immunohistochemical staining followed by automated microscopy had a higher sensitivity
in predicting disease recurrence than RT-PCR (100% vs 83%) but was lower in specificity (43% vs 79%). In this study, no distinction was made between MM and ITC but groups of cells (≥ 2) were separately noted and these were seen in all six patients with disease recurrence and in five of 14 (36%) patients without disease recurrence. Additionally, all patients with disease recurrence were identified after analysis of four levels at 200 μm distance.

**Chapter 5** addresses whether detailed examination of lymph nodes by multilevel sectioning and immunohistochemical staining can improve prognostication. Thirty-six cases (disease recurrence within 5 years) and 72 controls (no disease recurrence for at least 5 years) were selected. Tissue sections from paraffin-embedded lymph nodes from four levels at 200 μm interval were immunohistochemically stained with anticytokeratin antibodies, AE1/AE3. These slides were analyzed using conventional and automated microscopy for the presence of tumor cells. Altogether, the case group showed more MM (n = 3) than the control group (n = 1). There was no difference in the presence of ITC between the case and control group. Analysis of a second level had led to the additional detection of one case with MM (n = 1), one case with macrometastasis (n = 1) and two cases and seven controls with ITC (n = 9). Examining more than two levels only resulted in detection of additional ITC. All MM and macrometastasis were detected by conventional analysis and automated microscopy only led to detection of more ITC. In a multivariate logistic regression analysis, the presence of macrometastases or MM (OR 34.5, 95% CI 2.7-440, P = 0.006), T4 stage (OR 2.9, 95% CI 0.4-23.5, P = 0.040) and number of lymph nodes (OR 0.9, 95% CI 0.8-1.0, P = 0.025) were independent predictors for disease recurrence. Messerini et al.,22 and Bilchik et al.,23 who also distinguished between MM and ITC reported similar detection rates for MM (9.9% and 3%, respectively). Additionally, the first study also showed prognostic relevance of MM and none for ITC.

Detailed examination of lymph nodes in CRC by multilevel sectioning combined with immunohistochemical staining or RT-PCR in search for OTC is expensive and laborious given the enormous number of lymph nodes that can be harvested. Identification of sentinel lymph nodes (SNs) and selective detailed analysis of only these nodes, is suggested as a possible approach to circumvent this extra workload. Sentinel nodes are the first lymph nodes to drain the primary tumor and therefore have the highest chance of harboring tumor cells (see *introduction, Figure 1*). In **chapter 6**, twenty-five studies on the feasibility and reliability of sentinel node mapping (SNM) in CRC are reviewed with the emphasis on differences in the SNM techniques used. There was a large variation in identification rates and false-negative (FN) rates, ranging from 58% to 100% (average 89%) and 0 to 60% (average 33%), respectively. Factors that might have contributed to failed SNM procedures were incomplete circumferential injection around the tumor,
insufficient volumes of tracer for large tumors, inclusion of advanced tumors and rectal tumors. A major component reported was the learning curve effect, leading to a much higher identification rate of almost 100% in some studies if results from the early phase of the learning curve were not included in the analysis. High FN rates may be explained by extensive nodal replacement and large tumors, which can occlude lymphatic vessels leading to lymph drainage through an alternative route or by a long identification time between injection of the tracer and identification of the SNs, as blue dye travels fast and might have already reached a second echelon lymph nodes. Also, a low FN rate of 20% or less was mostly seen in studies where the number of SNs was limited to the first four blue nodes or the four or five blue nodes closest to the tumor whereas studies reporting a FN rate higher than 20% had a higher number of SNs. The majority of the latter studies considered all blue nodes or radioactive nodes as SNs leading to a wider range of SN. In melanoma and breast cancer lower FN rates are seen. The difference might be in larger tumors found in the colon and rectum and also in the dissimilarity in lymphatic drainage pattern.

Limitations in rectal cancer are the close vicinity of the primary tumor and the pararectal lymph nodes resulting in overlapping radioactivity. Moreover, incising mesorectal tissue during the operation is a contraindication for oncologic adequate removal of rectal cancer. Neoadjuvant radiotherapy and advanced tumors can also hamper the identification of SNs. These factors make SNM in rectal cancer more difficult and of low clinical value.

Two colon cancer studies showed that SNs are significantly more likely to contain tumor cells than nonsentinel nodes\textsuperscript{24,25} whereas conflicting results were seen in a third study published in 2006 in which no difference between SNs and nonsentinel nodes in regard to the frequency of MM or ITC involvement was seen.\textsuperscript{26} One SNM study in colon cancer reported a higher rate of disease recurrence in patients with SNs containing macrometastases or MM detected by hematoxylin and eosin staining or RT-PCR.\textsuperscript{27} Nevertheless, the reliability of SNM in colon cancer has not been clarified yet.

In CRC, disseminated tumor cells (DTC) are found in bone marrow although usually no clinically evident metastases develop in bone.\textsuperscript{28,29} We suspect that the presence of DTC in bone marrow might represent the aggressive nature of the tumor and could therefore be used to predict disease recurrence. This is evaluated in Chapter 7 by assessing whether the presence of DTC in bone marrow from 81 patients with colorectal liver metastases who were scheduled for surgical resection or isolated hepatic perfusion, is associated with a worse outcome of disease. The presence of DTC in bone marrow was analyzed by using quantitative RT-PCR for the expression of cytokeratin 20 (CK20) and or immunocytochemistry (CK-ICC) combined with automated microscopy by use of A45-B/B3, anti-cytokeratin antibodies. Bone marrow samples in 26 of 69 (38%) patients tested positive with the CK20 RT-PCR and samples in 15 of 69 (22%) patients tested positive with the CK-ICC test.
In a multivariate Cox regression analysis a positive CK20 RT-PCR test (HR 2.5, 95% CI 1.2 - 5.2, P = 0.014) and a serum CEA level greater than 200 micrograms per liter (HR 2.4, 95% CI 1.0 - 5.9, P = 0.045) were independent predictors for a reduced disease-related survival. A positive CK-ICC test did not relate to a worse survival but correlated with the presence of extrahepatic disease at the time of surgery (Chi-square test, P = 0.009). No correlation was seen between the CK20 RT-PCR test and the CK-ICC test. Factors that might explain this are the difference in markers used for these tests or RNA detected by RT-PCR may more likely represent active production of cytokeratin whereas CK-ICC may detect dormant or apoptotic cells.\textsuperscript{30}

We found similar results as published by Koch \textit{et al.}\textsuperscript{31} who examined bone marrow samples from 25 patients with colorectal liver metastases who underwent surgical resection. They found a positive CK20 RT-PCR test to be an independent prognostic factor for recurrence-free survival. Previously, a prognostic clinical MSKCC score (Memorial Sloan-Kettering Cancer Center) was proposed by Nordlinger \textit{et al.}\textsuperscript{32} and Fong \textit{et al.}\textsuperscript{33} to select patients who will have optimal chances for cure after hepatic resection. This clinical score consists of the following clinical and pathological risk factors: a positive nodal status of the primary tumor, less than 12 months between the primary tumor and the liver metastases, four or more liver metastases, a maximum diameter of the metastases of more than 5 cm and serum CEA level higher than 200 micrograms per liter. Given our results and others, the CK20 RT-PCR might be an interesting variable to be added to the MSKCC score.

In general, the spread of tumor cells occurs through local invasion and or hematogenous routes and or lymphatic routes. To reach lymph nodes or other tissue compartments in the body, the tumor cells have to overcome many boundaries at the site of the primary tumor. This occurs with the aid of various proteins. In \textit{chapter 8} the markers related to tumor cell spread through blood and lymphatic vessels are studied for their prognostic significance. Specifically, we examine whether disease recurrence in lymph node-negative CRC patients can be predicted by assessing their primary tumors for the expression of the angiogenic and lymphangiogenic factors sialyl Lewis X (sLeX), vascular endothelial growth factor (VEGF)-C, and VEGF-D, and for blood and lymphatic microvessel density (BMVD and LMVD), and for the presence of blood and lymphatic vessel invasion. This is performed in a case-control design studying the same patient group as in \textit{chapter 5}. Thirty-six cases (disease recurrence within 5 years) and 72 controls (no disease recurrence for at least 5 years) were selected. Tumor sections were stained by antibodies CSLEX1 also known as CD15s (sLeX), anti-VEGF-C, anti-VEGF-D, anti-CD31 (BMVD) or D2-40 (LMVD) to determine the parameters as mentioned above.

A multivariate analysis showed sLeX expression and high LMVD (OR 5.1, 95% CI 1.3-20.0 and OR 3.1, 95% CI 1.0-10.0, respectively) to be independent factors predicting disease recurrence. Expression of sLeX correlated with liver metastases (P = 0.015).
A high LMVD was related to regional intra-abdominal or intrapelvic metastases in lymph nodes and distant metastases other than in the liver and lungs such as peritoneum, bones, brain and adrenal glands (P = 0.004). A high BMVD in the invasive front correlated with lung metastases (P = 0.018).

When comparing the results in chapter 5 and 8, the following is noticed. There is a significant correlation between the presence of macrometastases or MM and lymphatic vessel invasion (Fisher’s exact test, P = 0.001 and P = 0.014, respectively) but none with a high number of LMVD (Fisher’s exact test, P = 0.170) (data not published). Also, lymph node metastases are related to distant metastases (Fisher’s exact test, P = 0.001).

This study is one of the first studies that combine a multiple set of tumor markers each correlating with a hematogenous or lymphatic tumor spread as previously described by Pantel and Brakenhoff (see introduction, Figure 3). Additionally, these results imply two types of mechanisms involved in metastasis (passive and active intravasation) as previously described by the group of Jain (see introduction, Figure 2). First, there is the mechanical way of tumor cell dissemination through blood and lymphatic microvessels, resulting in tumor cell arrest in the narrow capillary network in different organs including lymph nodes. There they might proliferate and develop clinically evident metastases. This may explain the correlation between a high BMVD and lung metastases and the correlation between a high LMVD and regional intra-abdominal or intrapelvic metastases in lymph nodes with eventually distant metastases.

Moreover, also biological processes determine outgrow of metastases as reflected in the “seed and soil” theory. This theory suggests that a subpopulation of tumor cells with metastatic potential, recently identified as colon-cancer-initiating cells, disseminate through the whole body. These cells proliferate and differentiate to form clinically evident metastases at preferential sites depending on local molecular interaction among which availability of local growth factors. In our study, the interaction of sLeX on tumor cells and E-selectin on endothelial cells might have facilitated tumor cell invasion in blood microvessels, extravasation and migration into distant tissue. As blood microvessels drain to the portal vein, the tumor cells arrive in the liver where their sLeX expression might prefer the liver to grow out to form clinically evident metastases due to interaction with local E-selectin.

**GENERAL DISCUSSION**

**Chapters 2 to 5** assess the detection of OTC in lymph nodes and its clinical relevance. OTC in lymph nodes from gastric cancer patients (chapter 2) are of clinical value if they are present in at least 20% of the lymph nodes whereas in CRC (chapter 5), MM and not ITC are found to be of clinical relevance. The difference between these studies in OTC relevance might be explained by the higher number of harvested lymph nodes in gastric cancer compared to CRC (median 15 with a range of one to 92 vs median 6 with a range of one to 26).
Besides being a staging tool, lymphadenectomy has also been reported to have therapeutic value.³⁹⁻⁴¹ In CRC and bladder cancer, improved survival was shown in both node-negative and node-positive patients when an increased number of lymph nodes was examined.³⁹⁻⁴⁰ The results of our study in gastric cancer patients (chapter 2) support the therapeutic value of a higher number of dissected lymph nodes as a significantly higher number of harvested lymph nodes was seen in less than 20% OTC-positive group (n = 27) compared to the greater or equal than 20% OTC-positive group (n = 10) (Mann-Whitney test, median 18 (range 6 to 92) vs median 8.5 (range one to 35); P = 0.021). This again corresponds to the survival benefit of patients with N2 disease⁴² who underwent a D2 dissection as previously reported.⁴³

We do not expect the number of levels examined to be a factor for the difference between chapter 2 and 5 as the percentage of OTC-positive lymph nodes after analysis of the first level in CRC also did not differ between cases and controls. Furthermore, the anticytokeratin antibodies CAM5.2 and AE1/AE3 had been compared and no difference in staining pattern was observed (data not published).

In chapter 4, we do not differentiate between ITC and MM. More patients are seen with OTC detected by multilevel sectioning and immunohistochemical staining in patients who have developed disease recurrence than in patients without recurrence, but it does not reach significance (P = 0.055). When disregarding single tumor cells as relevant, the sensitivity of the test remains 100% and the specificity rises from 43% to 64% in predicting disease recurrence. Nevertheless, the specificity of the RT-PCR test is still higher (79%). This may relate to the fact that RT-PCR detects RNA which represents active cells whereas immunohistochemistry detects proteins which might also be in apoptotic tumor cells.

An additional finding in the study presented in chapter 5 are four macrometastases in the first sectioning level which had not been recognized as such on the conventional hematoxylin and eosin stained sections. They were reported as vascular invasion or tumor deposits without lymphoid tissue. These patients had been considered as node-negative and did not receive adjuvant chemotherapy. The combination of serial sectioning and immunohistochemical brown-staining of tumor cells might lead to a more clear differentiation from surrounding lymphoid tissue. A recent review by Nagtegaal et al.⁴⁴ shows that various studies have tried to determine the importance of tumor deposits based on contour, size and origin but all fail to provide an evidence base to substantiate its use in the TNM system. Detailed analysis of lymph nodes may aid in examining tumor deposits in perirectal and pericolic fat.

To further elucidate the role of MM and ITC in lymph nodes, it would be advisable for further studies to follow the guidelines recently provided by Turner et al.⁴⁵ These guidelines regard criteria for clusters of tumor cells, measurements of OTC and a more extensive definition of ITC and MM.
In their study, the variability of pathologists in their distinction between MM and ITC decreased after examining the set of clearly defined histologic criteria. Agreement improved from 76% to 97% leading to a reproducible nodal classification. Given our results and others, MM are predictors of disease recurrence in CRC and therefore correctly used in the current TNM staging classification. There is no clarity on ITC as yet. Stroma analysis, staining for apoptosis, laser capture followed by single cell analysis or other techniques might clarify their relevance. Also, automated microscopy might be helpful in detection of OTC.

Both in chapter 2 and 5, the number of examined lymph nodes are of clinical relevance. In chapter 5 that regards archival material and includes rectal cancer patients, only 20% of the patients had undergone the recommended examination of at least 12 lymph nodes by the American Joint Committee on Cancer (AJCC). Similar findings are seen in other studies investigating large archival material. Higher numbers of lymph nodes have been found in prospective studies investigating OTC detection implying a more dedicated search for lymph nodes. Fat clearance techniques increases the number of harvested lymph nodes, especially small lymph nodes less than 5 mm. However, these methods are time consuming, expensive and impractical as they involve noxious volatile agents. Recently, the use of modified Davidson’s fixative instead of conventional formalin fixation, showed an increase in lymph node yield, especially of smaller lymph nodes. An alternative might be injection of blue dye which also leads to detection of small lymph nodes. Nevertheless, at best 50% of patients in the United States undergo adequate lymph node evaluation for colon cancer. Risk factors reported for <12 lymph nodes examined are male sex, older age, earlier T stage tumor, low-volume hospitals and preoperative (chemo)radiotherapy. Studies have suggested a wide range for adequate nodal evaluation but guidelines from the National Comprehensive Cancer Network, the College of American Pathologists, the National Cancer Institute and the AJCC all recommend the examination of 12 or more lymph nodes because this is a feasible and effective threshold. To improve the number of harvested lymph nodes, guidelines from the Santa Monica Conference in 2007, are recommended. If less than 12 lymph nodes are found on initial examination, the pathologist should reexamine the specimen, possibly including microscopic examination of extramural soft tissue. A harvest of less than 12 lymph nodes, should be documented in the pathology report including the type and extent of reexamination undertaken. Also, the surgeon bears responsibility for submitting both sufficient tissue for examination and a meticulous description of the surgical resection. As Jass et al claim “there is no unacceptably low number of lymph nodes for an individual dissection, but the mean number of lymph nodes in a series of colon cancer dissections should approximately be between 12 and 15. In the case of rectal cancer with the increasing use of neoadjuvant radiotherapy, the mean number may be lower than 12 despite intensive search.
Summarizing, the number of examined lymph nodes is a prognostic factor proven in numerous studies. Accordingly, it might also be a factor to include in a revised TNM system to aid in the decision-making regarding adjuvant systemic treatment.

**CLINICAL PERSPECTIVES**

Although surgical resection is the cornerstone in the treatment of both gastric cancer and CRC, surgery alone is not able to cure patients with minimal metastatic disease. These patients at risk for disease recurrence might benefit from additional systemic treatment and therefore their identification is a major issue. Detection and treatment of minimal metastatic disease is of increasing importance given the expected rise in the incidence of early-stage gastric and colorectal cancer patients through the use of gastroscopy, fecal occult blood tests and coloscopy. Additionally, curative surgical resection of isolated colorectal liver or lung metastases is more often becoming an option for treatment. Also in these patients, determination of minimal metastatic disease may direct further treatment. This thesis shows prognostic significance of occult tumor cells in lymph nodes or bone marrow and of primary tumor markers related to lymphatic and hematogenous spreading in patients who had undergone a supposedly curative resection, and, therefore, may indicate the presence of minimal metastatic disease.

For years, chemotherapy failed to show survival benefit in gastric cancer. In 2001, the Intergroup 0116 trial showed adjuvant chemoradiotherapy (5-fluorouracil plus leucovorin and radiation) to improve overall survival in gastric cancer. Also, a Japanese trial confirmed an increase in overall survival after adjuvant chemotherapy with an oral fluoropyrimidine S-1. The MAGIC trial (Medical Research Council Adjuvant Gastric Infusional Chemotherapy) showed perioperative chemotherapy (pre- and postoperative epirubicin, cisplatin and 5-fluorouracil) to result in improved progression-free and overall survival. Therefore, the gastric cancer patient group with minimal metastatic disease at risk for disease recurrence might optimally benefit from adjuvant chemotherapy. Currently, patient’s prognosis after neo-adjuvant chemotherapy and curative surgery followed by postoperative chemoradiotherapy or chemotherapy is investigated in the CRITICS (ChemoRadiotherapy after Induction chemoTherapy In Cancer of the Stomach) study which is being performed in the Netherlands.

Until recently, adjuvant systemic therapy in colon cancer had consisted of 5-fluorouracil and leucovorin. Now, results with capecitabine, an oral fluoropyrimidine, suggests that 5-fluorouracil can be replaced by capecitabine. Also, the MOSAIC (Multi-center International Study of Oxaliplatin/5-fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer) trial showed that addition of oxaliplatin improved disease-free survival of patients with stage III disease. The value of adjuvant chemotherapy for patients with stage II disease remains controversial because evidence is inconsistent that adjuvant 5-fluorouracil-based chemotherapy is associated with a better overall survival.
compared to surgery alone. However, subgroups of patients with stage II CRC at risk for disease recurrence including T4 stage, perforation, complete obstruction and given the results of this thesis, the presence of minimal metastatic disease, might benefit from adjuvant chemotherapy to a similar extent as shown for stage III CRC patients. Nevertheless, future studies have to prove this.

Results of other studies investigating the survival benefit in gastric cancer and CRC patients treated with biological agents attacking several pathways such as bevacizumab and cetuximab have to be awaited. Concluding, considerable numbers of patients who underwent curative surgical resection of their gastric or colorectal tumor are at risk for disease recurrence. In this thesis, we have shown that patients treated for gastric or colorectal cancer who are at risk for disease recurrence can be identified through occult tumor cell detection in lymph nodes, disseminated tumor cell detection in bone marrow and analysis of angiogenic and lymphangiogenic features of the primary tumor. These findings can be implemented in new strategies for identification of high risk patients, leading to individualized therapy preventing over- and undertreatment.

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