Summarizing discussion

Although heterogeneity in colorectal cancer, both at the tumor and patient level is well recognized, surgical and (neo-) adjuvant treatment, and consequently patient outcome remains basically the same and is mainly based on TNM staging of the primary tumor and age of patients. Classification of colorectal cancer based on current staging systems and histopathological features has so far been insufficient for further substantial improvement. Tumors within the same TNM stage display substantial differences at the molecular level with different clinical phenotypes. Specific profiles of chromosomal aberrations, sequence alterations, microsatellite instability, CpG island promoter hypermethylation, miRNAs and gene expression have been found to be associated with prognosis and response to therapy.[1-4] Therefore, profiling of tumors based on such genetic and epigenetic features has the potential to provide us with more accurate tumor classification and subsequently a personalized approach for therapy selection in patients with colorectal cancer.[5-8]
Beside tumor heterogeneity, patient heterogeneity may also influence surgical outcome, therapeutic decisions and oncological results.[9-11] Different patients characteristics require different treatment strategies. Young, healthy, slim patients for instance need another surgical approach compared to older, acutely submitted patients with colorectal cancer.

This thesis describes several aspects of tumor and patient profiling to predict clinical outcome of patients with colorectal cancer.

Part I of this thesis focused mainly on tumor profiling of stage II colon cancer. Fifty percent of all patients with colon cancer are stage II and thus mostly treated with surgical removal of the tumor, without adjuvant therapy. Approximately 20-30% of these patients still will suffer from a relapse. Histopathology, apart from some specific high risk factors (e.g. T4 tumors, perforation, or poorly differentiated histology) is known to predict recurrent disease insufficiently. Colon cancer is characterized by chromosomal aberrations, which are associated with biological diversity at the DNA level. Distinct chromosomal patterns of adenomas, early stage colon cancers and advanced colonic tumors have been associated with specific clinical outcome.[12-15] Such chromosomal copy numbers can be measured on a genome wide scale using comparative genomic hybridization (CGH).[16] High resolution oligonucleotide array CGH can detect very small aberrations, which harbour only a limited number of genes. Although identification of candidate cancer genes in these small aberrations takes less effort than in large chromosomal aberrations, it still remains a challenge to pinpoint the actual genes that drive the carcinogenetic process. Nevertheless, even in the absence of final prove of such a causative role, these DNA copy number aberrations may serve as new markers for prognosis or as targets for therapy. To further narrow the number of potential cancer genes, mutation analysis or gene expression can integrated with DNA copy number analysis.[17] In **chapter 2** focal chromosomal aberrations (<less 3mB) in patients with stage II colon cancer were identified. Tumor tissue of 38 patients with stage II colon cancer was analysed with a high-resolution oligonucleotide array CGH platform. First, it was demonstrated that small focal aberrations could even be detected with array

CGH in DNA derived from formaldehyde-fixed and paraffin-embedded (FFPE) tissue. Indeed, array CGH with DNA derived from either FFPE tissue or from fresh samples of the same tumor gave identical results. Similar results were obtained when DNA of FFPE tumor tissue of the same patient, was hybridized on two different platforms, namely a 44K Agilent and a 135K Nimblegen array CGH platform. In total 81 focal aberrations (deletions and amplifications) spread throughout the genome were found. These focal aberrations could be validated in publicly available data on copy number changes found in colorectal cancer, breast cancer, pancreatic cancer and glioblastoma. Interestingly, the location of amplifications seemed to be rather colon specific, since they did not show overlap with amplifications previously found in breast cancer, pancreatic cancer and glioblastoma. For 177 candidate driver genes in 81 focal aberrations a correlation between mRNA expression and DNA copy number changes was found, which lends further support to the relevance of focal aberrations. Focal loss of 5q34 and gain of 13q22.1 were independent predictors of survival in patients with stage II colon cancer. Many known and new candidate cancer genes were identified in these focal aberrations, several of which warrant further study.

Next to focal chromosomal aberrations, large scale chromosomal aberrations occur even more often in colorectal cancer. The prognostic value of these large aberrations in colorectal cancer had been studied previously, but not with high resolution array CGH in a homogenous group of patients with stage II colon cancer, like in **chapter 3.** In this study loss of chromosome 4, 5, 15q, 17q and 18q was seen more frequently in patients who had a relapse compared to those without recurrent disease. In MSS patients, loss of chromosome 4q22.1-4q35.2 was associated with poor outcome, but in MSI patients losses on chromosome 4q were not observed. In addition, this study confirmed that in patients with MSI colon cancer DNA copy number changes are not absent, but occur at a much lower frequency, and mainly concern gains. Further validation of the prognostic value of 4q loss in an independent series of patients with colon cancer is needed to establish the potential clinical value of these findings.

ArrayCGH analysis of tumor tissue comes with technical challenges. Wave artefacts in array CGH profiles hamper analysis of breakpoints and biological information may go lost. In **chapter 4** an algorithm is described that was designed to remove wave bias of CGH profiles. Script and instructions are available from http://www.few.vu.nl/~mavdwiel/nowaves.

Tumor biology can be read out at the DNA, RNA and protein level. In **chapter 5** the prognostic value of protein expression of p21, p27, p53, EGFR, Her2/Neu, Ki-67, Cyclin D1, TS, β-catenin and AURKA was determined in a tissue micro-array experiment using immunohistochemistry. Tumor tissue samples of 386 patients with stage II and III colon cancer were studied. Results were analysed separately for patients with MSS and MSI tumors. Low p21, high p53, low cyclin D1 and high AURKA protein expression levels were associated with disease recurrence in patients with stage II and III colon cancer.

In Part II of this thesis characteristics at the patient level in relation to clinical outcome in colorectal cancer were studied. Patients' characteristics, such as co-morbidity and general health at time of operation, may be of crucial importance for short and long term clinical outcome. Objective and reproducible classification and definition of patients' physical condition remains complex. The Charlson co-morbidity index, the ASA classification, or laboratory measurements provide more objective information of patients' health. Scoring systems, including patient features could help to assess a patient's risk of mortality or morbidity in colorectal cancer surgery. Many scoring systems address general surgical practice.[18,19] Scoring systems specific for colon and rectal surgery have also been developed and are reviewed in **chapter 6**. Postoperative mortality is the main outcome studied, but other outcomes of colorectal cancer surgery should also be taken into account when quality of surgical care is assessed. Proper external validation is still needed for most models before reliable comparative audit is possible. In **chapter 7** poor condition at time of operation was classified by the physiologic scores of the POSSUM scoring system, which include cardiac signs, respiratory signs, systolic blood pressure, pulse rate, Glasgow coma scale, serum urea, serum sodium, serum potassium, haemoglobin, white cell count and electrocardiogram signs. The physiologic score was found to be an independent risk factor for long-term overall survival in patients with stage I, II and III colorectal cancer.

Future perspectives

Within the last decade the availability of a complete sequence based map of the Human Genome[20] combined with the wide availability of high throughput profiling technologies has dramatically increased insights in the biology of colorectal cancer and other human solid tumors and provided a basis for potential clinical applications of these findings.[21,22] Recent landmark studies of sequence analysis of all coding exons in breast, colorectal, pancreatic and brain cancer have provided important new information in this context.[23-26] According to these studies, the genomic landscape of many cancers shows a few 'mountains' (genes mutated at high frequency) and many small 'hills' (genes mutated at low frequencies). Many genes mutated at high frequency occur across different tumor types (like PTEN and TP53). On the other hand, tumor specific genes, such as APC in colorectal cancer, also occur. Technological advances now also allow to analyze somatic DNA copy number alterations genome wide at high resolution. One of the largest analyses currently available, containing 3131 high resolution DNA copy number profiles of more than 24 cancer types, gives robust and comprehensive insights in the landscape of somatic chromosomal aberrations in cancer.[27] This study demonstrates that the most prevalent copy number alterations in human cancers are either focal or have the size of a whole chromosome (arm). Furthermore, studies that integrated DNA copy number and sequence alterations discovered many new genes that had not been implicated in tumorgenesis previously. These analyses point at the fact that alterations at the level of pathways rather than individual genes are key for our understanding of tumor biology and consequent clinical implications like prognosis and response to therapy.

These recent studies provide just a first glimpse of the true biological complexity of human cancers. Ongoing initiatives for systematic analysis of cancer genomes will yield many new prognostic, predictive and therapeutic targets. This will eventually lead to a molecular based taxonomy of human cancers. The challenge ahead is to translate these findings into meaningful clinical applications, both in the diagnostic and therapeutic domain, and all of this in a cost effective way. [21] To achieve this goal, a joint effort from all (bio)medical disciplines involved is required, as well as an efficient infrastructure with state of the art clinical trial management, biobanking, data management and data analysis facilities. Only when these conditions are fulfilled, biomedical research will be able to fully benefit from these unprecedented opportunities to innovate and improve treatment of cancer patients.[28]

Simultaneously, medical technologies have improved spectacularly over the last decades. Specialized intensive care units, fast track approaches, image guide radiotherapy, laparoscopic, robot and microscopic surgery have expanded our abilities to cure colorectal cancer patients, with less post-operative morbidity and mortality. Technical innovations in treatment of colorectal cancer patients will further develop, but optimizing patients based features at time of operation is also required. Pre-operative resuscitation and better post-operative monitoring may further improve clinical outcome, especially in high risk patients with colorectal cancer.

At the same time, patients' expectations of medical possibilities and oncological outcomes will increase. Within the next years individual and hospital mortality and morbidity rates will become publicly available. It therefore is important that objective outcome indicators of care will be available, and thorough evaluation of these outcome measures is mandatory. Factors like patient health, mode of presentation and extent of disease should be included. When patient-based variables are included routinely in cancer registries, assessing quality of surgical cancer care may be more accurate.

Ultimately integration of tumor and patient profiling will allow for a more precise prediction of individual prognosis, tailor-made treatment and improved clinical outcome for each patient with colorectal cancer.

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