Valorization
Colorectal cancer (CRC) is a major burden on the health care system with over 1.4 million newly diagnosed patients and almost 700,000 deaths annually.\textsuperscript{1} Because of these numbers, the global economic burden is substantial, with an estimated $US14-22 billion each year. Most of the costs occur in advanced stage CRC and are related to hospitalization, chemo- and radiotherapy, treatment of related side-effects and supportive care.\textsuperscript{2} In the Netherlands, CRC is one of the most frequently diagnosed malignancies with over 15,000 new cases and over 5,000 cancer-related deaths per year.\textsuperscript{3} In 2011, €488 million were spent on health care of CRC patients in the Netherlands.\textsuperscript{4,5} In order to decrease CRC rates and thus also the economic as well as the social burden, it is generally accepted that the most effective strategy to manage the disease is early detection.\textsuperscript{6}

In addition to invasive screening methods, i.e. colonoscopy and sigmoidoscopy, several non-invasive methods have been developed such as the fecal occult blood test (FOBT) and fecal immunochemical hemoglobin test (FIT). It has already been studied that population-based screening using FOBT leads to a reduction in CRC mortality rates.\textsuperscript{7-10} Since FIT achieves higher participation and CRC detection rates\textsuperscript{11-13}, population-based screening with this test is expected to improve the mortality rates even more. While FIT is cost-effective and is expected to reduce CRC incidence and mortality, its performance can be improved since the test still misses approximately 20% of CRCs and up to 70% of advanced neoplasia.\textsuperscript{11,14,15} Lowering the cut-off value increases the detection of advanced neoplasia, but results in a decrease of the positive predictive value leading to unnecessary patient anxiety and costs of follow up examinations.

In order to improve CRC screening, we identified several promoter methylation biomarkers in blood and/or feces. In Chapter 2, the potential of promoter methylation detection of GATA4 in fecal DNA is investigated, reaching sensitivities and specificities of 51-71\% and 84-93\%, respectively. Additionally, detection of GATA5 promoter methylation using blood-based assays as described in chapter 6, yielded a methylation frequency of only 18\% with a specificity 99\%. SYNE1 and FOXE1 obtained respectively detection rates of 47\% and 46\% with specificities of 96\% and 93\%. Combining SYNE1 and FOXE1 increased the sensitivity to 58\% with a minimal decrease of the specificity to 91\%. Though these results are promising, the reached sensitivities and specificities are not sufficient for incorporation into a clinical setting. Due to improved fecal and blood DNA isolation and detection techniques, these results might improve in the future. In chapter 4, we showed that detection of NDRG4 promoter methylation in stool samples identified 53-61\% of CRC patients, whereas almost no false positive results were obtained. Before incorporation of a novel biomarker into a screening program can be considered, an optimal sensitivity and specificity should be reached. In addition, extensive validation within the intended target population to confirm the initial results is required.\textsuperscript{16} Combining sensitivity and specificity, NDRG4 is one of the best single early
detection methylation markers published so far. This finding has been validated in independent studies, demonstrating its clinical potential.\textsuperscript{17–20} \textit{NDRG4} methylation as a diagnostic marker for CRC has been patented by our group and biomarker company MDxHealth (Irvine, USA, www.mdxhealth.com) and was licensed to Exact Sciences (Madison, USA, www.exactsciences.com), a molecular diagnostics company developing a molecular maker test for CRC. Exact Sciences incorporated \textit{NDRG4} in their multi-marker molecular diagnostic CRC screening test called Cologuard\textsuperscript{®}, which includes detection of \textit{KRAS} mutations and \textit{NDRG4} and \textit{BMP3} promoter methylation together with a human hemoglobin immunoassay.\textsuperscript{16} The United States Food and Drug administration (FDA) has approved Cologuard\textsuperscript{®} to screen an average-risk adult population of ≥50 years old for CRC.\textsuperscript{21} Additionally, many health insurance companies in the United States of America decided to cover the costs of Cologuard\textsuperscript{®}.

As in many other countries, a population-based CRC screening program has been introduced in the Netherlands since 2014, in which individuals of 55-75 years or older are offered an immunochemical fecal occult blood test (FIT). If positive, a follow-up colonoscopy will be performed. The costs are funded by the national budget and therefore paid with tax revenues. This does not include the costs of the colonoscopy in the case of a positive FIT, which is paid by the health insurance of the identified individual. Comparison of Cologuard\textsuperscript{®} with FIT in a large prospective study, showed a significantly higher detection rate of 85% and a specificity of 95% with the Cologuard\textsuperscript{®} test, while the FIT achieved less false positive results.\textsuperscript{19} Although the sensitivity of the Cologuard\textsuperscript{®} is promising, several adaptations are required before the test can be incorporated in countries such as the Netherlands, in which the logistic system is designed for screeners receiving a FIT which only requires a minimal amount of stool and is feasible for at-home testing. Using Cologuard\textsuperscript{®}, whole stool samples have to be transported to a laboratory which is not only a logistic and economic burden but also requires adequate transportation material and preservation buffers. Improvement of Cologuard\textsuperscript{®} can be obtained by further development of at-home testing on small amounts of stool. Several factors influence the amount of DNA and its quality, such as sample collection, storage buffers and DNA isolation. In order to increase the yield of high quality DNA, these factors should be optimized.

In conclusion, one of the biomarkers identified in this thesis has been incorporated into a commercial biomarker-assay that detects more CRCs when compared to FIT. If this biomarker assay will be introduced in more CRC screening programs, we expect a decrease in CRC morbidity and mortality and consequently a decrease of the social and economic burden.
References