The role of intestinal microbiota in colorectal and breast cancer treatment

Impact paragraph

Colorectal and breast cancer are common types of cancer. Colorectal cancer is the third most common cancer in the world[1]. The incidence of colorectal cancer almost doubled from 1990 to 2016, with 15,000 new patients yearly in the Netherlands. About 20% of colorectal cancer patients already have metastatic disease at diagnosis[2]. Breast cancer is the most common cancer worldwide[3]. One out of seven women (15%) will develop breast cancer during their lifetime[4]. Approximately 70% of the breast cancer patients have an oestrogen receptor positive tumour and this percentage is increasing[5]. In 2018, almost 80% of the breast cancer patients were postmenopausal at diagnosis[6].

Over recent decades, survival rates of colorectal and breast cancer patients have improved substantially due to improved diagnostic and treatment methods and the implementation of the national screening programme for colorectal cancer in 2014 and breast cancer in 1990. As a result, quality of life has become more and more important. Cancer treatment can, besides curation and its survival impact, also negatively impact quality of life. For instance, chemotherapy, which is administered in half of the patients, can induce side effects like diarrhoea, nausea, vomiting, fatigue, hand foot syndrome, peripheral sensory neuropathy, and alopecia. Furthermore, despite improved treatment strategies, it is still not possible to achieve complete tumour response and cure cancer in all patients. In order to optimize tumour response and reduce side effects in cancer patients, factors need to be identified which potentially impact individual tumour response outcomes and the safety profiles of chemotherapy. Besides the already known factors such as genetic predisposition, environmental influences, other factors, such as intestinal microbiota, are thought to influence tumour response and chemotherapy toxicity.

The human intestinal microbiota is a complex and dynamic ecosystem. Trillions of intestinal microorganisms, consisting of bacteria, archaea, fungi, protozoa, and viruses colonize the human gastro-intestinal tract. Intestinal microbiota are essential for the maintenance of metabolism, immune function, and homeostasis[7]. This results in an ecosystem with dynamic host-microbiota-environment interactions. Every human owns a unique microbiota signature, shaped by personal environmental influences. Due to this exclusive character, knowledge on the influence of the intestinal microbiota in cancer treatment might contribute to the next step in personalised medicine for every cancer patient.

To unravel the first steps of the pathway to new predictive, prognostic, and therapeutic targets of cancer treatment, the overall aim of this thesis was to provide clinical evidence on the role of intestinal microbiota in cancer and systemic cancer therapy, focussing on metastatic colorectal and breast cancer patients treated with chemotherapy.

Scientific impact and relevance

Longitudinal faecal sampling to explore the association between chemotherapy and the intestinal microbiota is highly relevant in studying the dynamic alteration in the intestinal microbiota induced by chemotherapy. However, after completing our review in cancer patient treated with systemic cancer therapy, we concluded that the availability of studies with longitudinal faecal sampling is limited. The new insights resulting from our review, should stimulate future longitudinal microbiota research in cancer patients treated with systemic cancer therapy[8].

In addition, from our own cohort study in metastatic colorectal cancer (mCRC) we concluded that after three cycles of capecitabine, the intestinal microbiota of mCRC patients showed no significant changes. This could be due to the relatively mild impact of capecitabine on the intestinal microbiota, and the low number of treatment cycles that were evaluated. In addition, it could be hypothesized that the intestinal microbiota is already altered due to previous extensive medical treatment of mCRC patients, which includes for example abdominal surgery and/or chemotherapy, earlier administered in 48% of the patients. These new hypotheses will lead to the formulation of additional endpoints and the design of a new research line. In more detail, a follow up period until tumour progression and/or discontinuation of chemotherapy due to chemotherapy toxicity is advised. To further compare microbiota composition of mCRC with primary diagnosed patients, it is necessary to include a cohort with primary diagnosed CRC patients. Therefore, the first steps for a new collaboration between Wageningen University and Maastricht University have already been initiated. The aim is to compare the intestinal microbiota of primary diagnosed non-metastatic CRC patients with our mCRC patient cohort. If the intestinal microbiota of mCRC patients is already depleted or affected by previous antibiotic or chemotherapy treatments and/or operations, early intervention to restore the dysbiotic stage, may be more appropriate. In addition, the type of intervention will depend on the intestinal microbiota composition. Probably, it will not be effective to feed eradicated microbiota with prebiotics. It will be more appropriate to supply eradicated bacteria by probiotic interventions or even provide a faecal microbiota transplantation (FMT). However, at this moment, FMT is a subject of

debate, since the safety in cancer patients has not been fully established[9,10]. As a consequence, primary FMT intervention studies will have to be established in an acceptable ethical situation, for example in patients with metastatic disease without further anti-tumour treatment options. To limit time-consuming faecal donor screenings and minimize the risk of adverse events, a more standardised approach of FMT is attractive. By combining a selection of bacteria from a (single) donor into a capsule[11], a more consistent, safe, and, acceptable approach can be created in this way. This synthetic FMT provides opportunities to manipulate microbial compositions and could be one step towards personalised microbial-based medicine.

To summarize, the scientific impact and relevance of this thesis is mainly based on the design and planning of upcoming clinical microbiota related research. This thesis provides the first steps for the desired longitudinal studies and provides resources for upcoming metabolite analysis and cohort comparisons of colorectal and breast cancer patients.

Implementation and target population

In the first place, the ideas and results of this thesis are and will be published in peer reviewed (inter)national journals. Given the translational character of the data, and lack of primary clinical knowledge, results are submitted to clinical oncologic translation journals. To bridge the knowledge gap between fundamental and clinical research, it is highly relevant to mainly target breast and colorectal cancer physicians and microbiology specialists. By presenting research ideas, oncology specialist should be inspired and informed of the potential role of the intestinal microbiota in systemic cancer therapy. The focus on chemotherapy is of great importance, since chemotherapy remains the backbone of most systemic cancer treatments.

Clinical implementation should be based on the integration of this microbiota research in current and future research. Therefore researchers should invest in long lasting collaborations between departments of microbiology and oncology and join (inter)national collaborations. These collaborations are also of added value for validation studies and exchange of knowledge, materials, and expertise. In more detail, the first steps for a new collaboration with Wageningen University have already been initiated. Furthermore, we are considering the idea of sharing our faecal samples of the breast cancer patients treated with chemotherapy for a validation study of the microbiota research group at the Gustave Roussy Institute in Villejuif, France. By using

metagenomic shotgun sequencing, this collaboration will also provide the opportunity to further reveal associated metabolic microbial functions.

At this stage of research, it is too early to start clinical intervention studies. Further research in the TNO *in vitro* model of the colon (TIM-II), a model that mimics the colon, will bridge the knowledge gap between clinical and fundamental pre-clinical research. During these experiments potential prebiotic compounds will be used to assess the effect of prebiotics on human intestinal microbiota treated with chemotherapy. After completing these pre-clinical experiments, existing multicentre clinical collaborations might be used to set up a clinical intervention study with prebiotics.

In the long term, the ultimate goal will be to integrate the intestinal microbiota as predictive, prognostic, and therapeutic target to improve personalised medicine. Besides the easily accessible and low invasive procedures to obtain non-invasively blood or urine, the faecal microbiota might be an easily accessible tool. Due to the low invasive character, ethical drawbacks of using the faecal microbiota as markers are not expected.

Finally, the results of this thesis are the starting point for future research of the expanding scientific team of Prof. Marjolein Smidt. These data can be used to support upcoming grant applications. In addition, the team will further study and share data with national and international partners. Moreover, it is highly relevant to educate our future scientists at primary schools to make them enthusiastic for microbiota research. Therefore, it is highly encouraged to also participate in for example "de Klokhuis Wetenschapsprijs" contest. In the 2020 edition, our microbiota research ended up in the top three of this contest.