Molecular imaging of pancreatic and rectal cancer: on a path towards optimized detection and response prediction

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SECTION III

Conclusions

Summary, discussion and future perspectives

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SUMMARY

In this thesis, two large oncological entities are discussed: pancreatic and (colo)rectal cancer. In **Section 1**, research on pancreatic cancer is discussed. As overall survival rates of pancreatic cancer patients are currently very low, there are still major steps to be taken to increase patient outcome. One of the contributing factors to the low survival rate is the delayed detection of the disease, typically occurring in its advanced stages when symptoms become apparent. Additionally, unfavorable biological characteristics such as a high presence of stroma and increased resistance to therapy also play a role. The research in this thesis as described in **Section 1**, has focused on improving detection and therapy response evaluation of pancreatic cancer by investigating novel targets for diagnostic targeted molecular imaging and to help differentiate between therapy induced fibrosis and remaining vital tumor cells after neoadjuvant therapy. Colorectal cancer patients on the other hand have a much better prognosis. Improving quality of life is currently a major focus in this field, for example by improving neoadjuvant treatment regimens for the treatment of rectal cancer. When the number of rectal cancer patients with a complete response of all tumor tissue after neoadjuvant therapy increases, the need for drastic operative treatment with its associated risk of complications can be avoided. The research in this thesis as described in **Section 2**, has focused on exploring novel techniques to predict and monitor response to neoadjuvant therapy to optimize treatment regimens possibly resulting in better patient outcomes.

Pancreatic cancer

Current available imaging techniques are unreliable in assessing response to given therapies. In addition, they are unable to accurately differentiate between (vital) tumor tissue and therapy induced fibrosis and inflammation in pancreatic cancer patients after neoadjuvant therapy. Targeted molecular imaging (e.g. tumor targeted PET/CT) might provide a solution to this problem. **Chapter 2** provides a narrative review of the available scientific evidence on clinically tested tumor targeted PET/CT tracers for the detection of pancreatic cancer. The sensitivity of FDG-PET/CT for detecting pancreatic carcinoma varies, but it is generally reported to be moderate to high (70-90%). However, it is difficult to differentiate pancreatic carcinoma from pancreatitis, which also shows a high [^{18}F]FDG uptake. To overcome this problem, researchers have explored the use of dual-phase PET/CT imaging and various non-FDG imaging tracers to distinguish tumor cells from pancreatitis, therapy-induced fibrosis, necrosis, and inflammation. [^{18}F]FLT, as well as various tracers targeting fibroblast activating protein (FAP), integrin $\alpha_v\beta_6$, and prostate specific membrane antigen (PSMA) showed promise in detecting pancreatic cancer and providing diagnostic aid in distinguishing vital tumor cells from inflammation. The second part of this review describes the current status of targeted radionuclide therapy in pancreatic cancer. These include ^{90}Y, ^{131}I, and ^{177}Lu labeled tracers targeting carcinoembryonic antigen (CEA) and MUC1. Unfortunately, clinical trials have shown conflicting results regarding their effectivity.

As previously mentioned, the treatment of pancreatic cancer is very challenging. This is partly due to the presence of abundant stromal cells, which create physical barriers and prevent systemic treatment from adequately reaching the tumor cells. Stromal cells are part of the tumor microenvironment (TME), which is the environment surrounding tumor cells. The TME includes for example blood vessels, immune cells, stromal cells, and fibroblasts. To illustrate the importance of the TME, malignant cells only account for approximately 30% of tumor mass (depending on cancer type). The rest of the tumor mass consists of e.g. fibroblasts (25%), immune cells (20%), endothelial cells (5%) and macrophages (5%)[1–4]. In recent years, the role and influence of the TME on tumor development and metastases has been studied extensively for both imaging as well as therapeutic purposes. **Chapter 3** provides an extensive overview of the various TME components that can be targeted for imaging purposes, i.e. tumor associated vasculature, immune cells such as macrophages and T-lymphocytes, cancer-associated fibroblasts, and the extracellular matrix. As described in this chapter, a lot of clinical data is available on the use of tumor vasculature targeting agents (e.g. targeting the RGD sequence) for use in various imaging modalities such as PET/CT or NIR fluorescent imaging. In addition, with increasing use of immunotherapy for many indications, there is a lot of interest in immune cell imaging in order to predict response to immunotherapy (e.g. PD-1, PD-L1). Finally, FAP targeted imaging has revealed to be very promising recently, being subject of multiple studies. As FAP shows promise to be a pan-cancer target for imaging and therapeutics, various PET and radionuclide therapy tracers have been developed and are currently tested in clinical trials all over the world. In the future, visualization of the TME might provide extra information about the potential aggressiveness of the tumor or the potential therapeutic efficacy of targeted therapies.

Chapter 4 displays the results from a preclinical study investigating novel targets for molecular imaging of pancreatic cancer, more specifically after neoadjuvant FOLFIRINOX therapy, to aid in the beforementioned diagnostic challenges. Expression of integrin $\alpha_v\beta_6$, carcinoembryonic antigen cell adhesion molecule 5 (CEACAM5), mesothelin, PSMA, urokinase-type plasminogen activator receptor (uPAR), FAP, integrin α5 and epidermal growth factor receptor (EGFR) were evaluated using immunohistochemistry on tissue slides. Integrin $\alpha_v\beta_6$, CEACAM5, mesothelin and PSMA immunohistochemistry stainings showed significantly higher expression in pancreatic cancer compared to tumor associated pancreatitis and pre-existing normal pancreatic parenchyma. No expression of $\alpha_v\beta_6$, CEACAM5 and mesothelin was observed in therapy induced fibrosis. Integrin $\alpha_v\beta_6$ and CEACAM5 allowed for metastatic lymph node detection with a sensitivity and specificity of 83-100% and 100% respectively. In conclusion, targeting integrin $\alpha_v\beta_6$, CEA,

and mesothelin has the potential to distinguish vital pancreatic cancer cells from fibrotic tissue after neoadjuvant FOLFIRINOX treatment. Integrin $\alpha_v\beta_6$ and CEACAM5 detect both primary tumors and tumor positive lymph nodes.

In **Chapter 5**, the next step is taken towards clinical use of a targeted PET/CT tracer in pancreatic cancer. A PSMA targeted tracer, [^{18}F]DCFPyL, which is normally used for the imaging of prostate cancer, was repurposed and its potential to detect primary colon-, gastric- and pancreatic cancer was investigated. A total of 11 patients was included in this clinical pilot study, and all underwent preoperative [^{18}F]DCFPyL and [^{18}F]FDG PET/CT and imaging results were compared. The detection of colon-, gastric- and pancreatic cancers using [^{18}F]DCFPyL PET/CT was feasible, as the primary tumor was detected in 7 out of 10 patients using [^{18}F]DCFPyL. However, relatively low [^{18}F]DCFPyL uptake in the tumor and high physiological uptake in both organs and background hampered clear distinction of the tumor in most patients. As a result, [^{18}F]FDG PET/CT was superior in detecting colon, gastric and pancreatic cancers. Following these results, no further research is warranted into the use of [^{18}F]DCFPyL in these cancer types without prior selection. Such a selection process could for instance consist of PSMA specific immunohistochemistry staining of pre-operative biopsy material, which may possibly be able to detect tumors with high PSMA expression in patients who could benefit from [^{18}F]DCFPyL PET/CT imaging.

(Colo)rectal cancer

Currently, primary staging and restaging of rectal cancer is performed using multiparametric MRI and endoscopy. Unfortunately, previous studies have demonstrated varying and low sensitivity and specificity most evidently seen at restaging after neoadjuvant therapy. In **Chapter 6**, a regional retrospective study in rectal cancer patients demonstrated a low sensitivity of MRI for determining T stage (48.4-58.0%) and N stage (35.5-65.2%). As a result, a significant number of patients received incorrect treatment due to over- or understaging (22.2% in immediate surgery group, 68.8% in short course radiotherapy group). Interestingly, in all cases this was due to incorrect N staging. These results showed a trend towards more overstaging in lower T stages, understaging in higher T stages, and general understaging for N stage. This research adds to the evidence demonstrating low accuracy of MRI for both T and N staging in rectal cancer, and warrants future research to ensure accurate staging, enabling correct treatment decision making.

In **Chapter 7**, an overview is provided on the potential use of [^{18}F]FDG PET/CT for treatment response evaluation in colorectal cancer. This overview was written for educational purposes. Twenty clinical cases with corresponding radiological images are displayed, and teaching points for each case were discussed. Cases discussed in this chapter included response monitoring during and after neoadjuvant chemoradiation, local treatment of liver metastases, neoadjuvant treatment of recurrent rectal cancer and palliative systemic treatment of hepatic and extrahepatic disease.

In **Chapter 8** the feasibility of response prediction using digital [^{18}F]FDG PET/CT and multiparametric MRI before, during and after neoadjuvant chemoradiation therapy in locally advanced rectal cancer patients was investigated. In addition to the anatomical information MRI provides, digital [^{18}F]FDG PET/CT can provide metabolic information on the tumor over time. Moreover, digital PET/CT provides higher resolution over conventional PET/CT scanners, potentially enabling the detection of smaller tumor nodules or metastatic lymph nodes. In this clinical pilot study, 19 rectal cancer patients were included and underwent both digital [^{18}F]FDG PET/CT and multiparametric MRI before, during and after neoadjuvant chemoradiation therapy. From these imaging studies, 57 imaging features were extracted based on their ability to distinguish between good and poor response to neoadjuvant therapy. Twelve features from both imaging modalities were selected to be promising, but should be subject to further investigation in a larger prospective trial.

As we know from colorectal cancer, analysis of specific mutations in tumor cells can guide and predict cancer treatment (e.g. *KRAS* mutation predicts efficacy of EGFR targeted therapies). Likewise, analysis of mutations in preoperative biopsy samples might predict efficacy of (neo)adjuvant therapy in rectal cancer patients. With this purpose in mind, the research described in **Chapter 9** was set up. As a step towards predicting response based on mutational analysis of biopsy samples, the accuracy (i.e. repeatability) of this method had to be established. As we know, tumor heterogeneity results in various clones/populations of tumor cells spread throughout one tumor. With this in mind, analyzing one single biopsy sample taken from only the luminal side of a tumor (as this is the only side accessible by endoscopy) might not be representative for all tumor cell populations in the tumor. This study aimed to investigate the influence of this tumor heterogeneity on the results from mutational analysis from biopsy material. Results from mutational analysis of biopsy material were compared to tissue from 4 other locations within the same tumor using next generation sequencing. Results from this study showed that different mutations were found in various samples from one tumor in 36% of 11 included patients. This resulted in the conclusion that assessment of mutational status on a single pre-operative biopsy sample was inadequate in a substantial proportion of patients, and its use warrants careful interpretation.

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GENERAL DISCUSSION

Although various questions have been answered by the work in this thesis, many more questions and data gaps have been brought to light. More than anything, the work in this thesis once again underlines the complexity of processes involved in cancer treatment. It highlights the fact that using our current "simplistic" approaches (searching for one all-encompassing predicting parameter) is challenging to accurately predict response to therapy.

This challenge in predicting response to therapy is demonstrated by various studies in this thesis. In **Chapter 4**, immunohistochemistry experiments were employed to identify potential targets that could serve as imaging targets (i.e. to predict which imaging targets/ tracers could be successful in the clinic). The degree of expression of a certain biomarker is hypothesized to correlate with tracer uptake during e.g. PET/CT or NIR fluorescent imaging. Although there are certainly many different applications in which this approach has been successful, the immunohistochemical experiments described in this chapter followed by the unsatisfactory results from the clinical implementation in **Chapter 5** demonstrate an example of how difficult it can be to predict clinical imaging results based on immunohistochemistry experiments. Several critical questions regarding the method of using immunohistochemistry to predict clinical imaging results can be posed. First, how accurate and quantifiable is this assessment of the degree of expression? As in this thesis the rating was performed visually by the pathologist, we can at best get an estimate of the percentage of cells staining and the corresponding intensity. As a pathologist is not able to count and assess all cells separately, no exact measurement can be performed using this method. In recent years, (semi)automated software such as QuPath[5] has been developed to more accurately quantify immunohistochemical stainings. After training the software to identify certain cell types (e.g. tumor cells, stromal cells), it can provide detailed information on the percentage and intensity of cells stained. Unfortunately, this software was not yet readily available at the time of the experiments in this thesis.

Next, as only one tissue slide from each tumor was assessed, it could be difficult to make an overall assessment of the 'total available binding sites' that are available in the tumor for imaging agents to bind to. Although one could assume that you can deduct the total biomarker expression in a tumor from a sample tissue slide, various factors can influence this estimation. For example, expression of certain biomarkers can vary throughout the tumor due to e.g. tumor heterogeneity or increased expression in for example the invasive front of the tumor. Such differences in expression pattern can possibly result in an incorrect estimate on overall biomarker expression in a certain tumor. As demonstrated in **Chapter 9**, tumor heterogeneity in for example rectal cancer can significantly influence results from such sample testing approaches. As the assessment of various tissue slides per tumor is very time consuming for the pathologist, the use of software such as QuPath could enable research groups to assess multiple slides per tumor, and improve their estimate of biomarker expression throughout the whole tumor. A third question regarding this approach refers to what degree of biomarker expression is sufficient to enable *in vivo* imaging of this target with satisfactory contrast to physiological uptake in adjacent organs. Intense physiological expression of the target biomarker in adjacent tissue can hamper tumor detection, as experienced in **Chapter 5.** More specific to this study; significant tracer uptake in the gastric wall, pancreas, liver, gallbladder, spleen and small intestines hampered clear identification of pancreatic, colon and gastric tumors. In the search for imaging targets, not only uptake in the target organ itself should be considered, but also uptake in the surrounding organs as this can hamper tumor identification. Depending on the tumor type and location, different background organs should be considered. Finally, in addition to the percentage of cells stained and the intensity of this staining, the cellular location of the found expression should be taken into account. Previously, mostly tumor cells were targeted directly for imaging and treatment purposes. More recently, stromal cells (surrounding the tumor cells) are also being targeted, as these represent a significant part of tumor content as well. To illustrate, stromal cells can account for up to 90% of tumor mass in pancreatic cancer[6]. Neovasculature is part of this stroma, and consists of endothelial cells. As described in **Chapter 4**, moderate PSMA expression was found in the endothelial cells in pancreatic cancer. As endothelial cells only account for a few percent of total tumor mass, this resulted in a relatively low 'total' expression in terms of available bindings sites for imaging tracer (a few percent of the tumor mass x moderate staining = low number of total binding sites). This has possibly contributed to the unsatisfactory results in **Chapter 5**.

A second lesson that can be learned from the work in this thesis, is how difficult it is to find a (combination of) parameter(s) for prediction of clinical results (e.g. response to therapy). Not only the predictive ability of such parameters is important, but also whether they are representative for the whole tumor (in case samples are taken) and whether these samples or measurements are repeatable and result in similar results. A first example of such a challenge regarding representation of the whole tumor and consequent repetition of measurements is found in the work performed in **Chapter 9.** In this study, different mutation profiles were found within various samples from the same tumor in 36% of patients. These results demonstrate how tumor heterogeneity influences the results of mutational analysis when using biopsy material versus using the whole tumor specimen, and thus question the suitability of mutational analysis from biopsy material for response prediction. Unfortunately, only the luminal side of the tumor is accessible for biopsy during endoscopy, thus no data can be acquired on the

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non-luminal tumor parts using this method. Of note, this problem is only relevant for heterogeneic tumors, as in completely homogeneic tumors the results will be identical regardless of the biopsy location. In contrast to using biopsy material and extrapolate results derived from a sample, the use of imaging methods such as PET/CT provide a method to acquire data on the full tumor including all its heterogeneic cell populations. A second example of this challenge to find suitable predicting prarameters is found in the work described in **Chapter 8**, where not only the type of scan (MRI or [^{18}F]FDG PET/CT) but also the timing of the scan in the treatment period and the use of different scanners and scanning protocols is of great importance when trying to predict response. Following this second example, a very strict and consequent study protocol is required to be able to investigate such multimodal approaches. On the other hand, results derived from studies performed in such highly controlled environments might be difficult to translate and apply to the clinical setting as this setting is not as controlled. This results in the following paradox that complex prediction models including data from various different modalities might be able to predict response with sufficient accuracy, but could be difficult to implement in the daily clinical setting. One opportunity to decrease variability in scanning results, could be the use of combined PET-MRI scans, instead of the two separately. One of the many advantages of combined PET-MRI could be increased delineation of the tumor and/or (metastatic) lymph nodes (as you can now reference to MRI instead of CT images).

This paradox should stimulate us as researchers and clinicians to search for ways in which we can use the already available information to support informed clinical decision making. This is more relevant than ever, as there is a vast amount of information gathered in the standard diagnostic work up of every single patient, and tools for analysis and subsequent prediction model development of such large quantities of data improve by the day.

FUTURE PERSPECTIVES

There is a great number of promising developments in the field of molecular imaging. Many new targeted imaging tracers are being developed, scanner technology is constantly improving, and guidelines are being developed to advise on the best (evidence based) way to use these newly available technologies. The use of successfully translated PET/CT tracers such as [^{18}F]FDG have been implemented in national guidelines, as for example to detect recurrent disease (indicated by increase CEA levels) in the follow up after surgical resection of colorectal cancer[7].

Various research groups are currently investigating the further application of [^{18}F]FDG in diagnosis and treatment response monitoring of both rectal and pancreatic cancer. A recent example of this is found in the publication of the PandigiPET study[8]. In this trial, the additional value of digital [^{18}F]FDG PET/CT in primary staging and restaging after neoadjuvant therapy in pancreatic cancer was investigated. Results from this trial demonstrated a strong positive correlation between change in [^{18}F]FDG uptake and change in CT tumor diameter and change in CA19-9. In addition, the application of digital [^{18}F]FDG PET/CT resulted in detection of previously unknown small liver metastases in 5 out of 35 patients. These results warrant further exploration of the additional value of digital [^{18}F]FDG PET/CT in pancreatic cancer, as it could possibly improve both initial staging and response monitoring. Currently, a similar study (IMAGE-PET trial) is being conducted by our colleagues at the Amsterdam UMC to investigate whether a decline in [^{18}F]FDG uptake correlates to surgical resectability, biological tumor marker response and pathological response.

In addition to [^{18}F]FDG, other PET/CT tracers are being developed and tested in clinical trials. In our own research group, we are currently working on the clinical implementation of various novel PET/CT tracers for the detection and response monitoring of pancreatic cancer. These include [^{18}F]Fluciclatide (NL7605), [^{18}F]FP-$R_0$1-MG-F2[9] and [^{68}Ga]Ga-FAPI-46[10], targeting a combination of integrins, integrin $\alpha_v\beta_6$ and the fibroblast activation protein (FAP), respectively. Focusing on the latter one, FAP is expressed by cancer associated fibroblasts in most cancer types. Since its first introduction into human clinical trials in 2019, interest in this tracer has spiked as this may prove to be a novel pan-cancer imaging tracer with great diagnostic and therapeutic potential. As investigated in **Chapter 2** of this thesis in a preclinical setting, and later confirmed by results from various clinical trials, targeting FAP indeed has great potential in diagnosis and treatment of pancreatic cancer[11–13]. A recent systematic review and meta-analysis by our group on the diagnostic test accuracy of FAPI PET/CT in hepato-pancreato-biliary (HPB) tumors (Henrar *et al.*, to be published) concluded that FAPI PET/CT demonstrated higher uptake (mean SUV_{max} 15.6, 95% CI 12.4-18.9) compared to [^{18}F]FDG PET/CT (mean SUV_{max} 6.5, 95% CI 4.4-8.5) in 242 pancreatic cancer patients. In addition, the detection rate of FAPI PET/CT was significantly higher in hepatocellular carcinoma, biliary tract cancers and lymph node-, liver- or distant metastases from all HPB tumors compared to [^{18}F]FDG PET/CT. In addition to its diagnostic potential, it could serve as a theranostic agent, and thus also be used for e.g. radionuclide therapy. A recent review by colleagues from the Radboud Medical Center (Nijmegen, The Netherlands) concluded that FAP targeted radionuclide therapy using tracers such as [^{90}Y]Y-FAPI-46 and [^{177}Lu]-FAPI-46 has already been tested in various limited case series in more than 100 cancer patients[14]. Early results encourage further investigation, with therapy responses observed in difficult to treat end stage cancer patients and manageable adverse events. The first results from prospective clinical basket trials are expected in the upcoming year (e.g. NCT04939610, NCT05723640).

Next to diagnosing cancer, much effort is currently put into developing reliable methods to predict and monitor response to cancer therapy[15–18]. With increasing use of neoadjuvant therapy to enhance both surgical and survival outcomes, many new neoadjuvant treatment regimens are currently under investigation. To illustrate this, both the Dutch Pancreatic Cancer Group (DPCG) and the Dutch Colorectal Cancer Group (DCCG) have conducted various clinical trials over the last years investigating novel combinations for neoadjuvant therapy. These include for example the PREOPANC-1 and -2 trials (and currently ongoing PREOPANC-3 and-4) in pancreatic cancer and the RAPIDO trial in rectal cancer[19–23]. Accurate prediction of response to a certain anti-cancer therapy could be used to choose the most effective treatment regimen at an individual patient level. Inefficient treatment with often serious risk of complications and adverse events could be avoided, and possibly patient outcomes could be improved. An example of this is seen in patients with advanced pancreatic cancer, who according to the local guideline are treated with FOLFIRINOX. Although many patients benefit from this therapy, there is also a subset of patients who are unresponsive to this therapy but do experience severe toxicity (including e.g. neutropenia, thrombocytopenia and diarrhea). The PANCAKE study, another initiative of the DPCG, currently investigates whether certain biomarkers such as ctDNA, microRNA, or SNPs might be able to predict response to FOLFIRINOX therapy[24].

As it is difficult to find one single parameter from clinical, imaging, or pathological data that has enough accuracy to predict response at an individual patient level, much effort is put into the development of prediction models in which multiple of these parameters are combined. As neoadjuvant therapy for rectal cancer patients has been implemented for several years now with great success (pCR rate ~10-30%[25–29]), there is a vast amount of data available to develop such models. To illustrate, a recent publication in the Lancet by Feng *et al*., describes the development and validation of the RAPIDS prediction model[30]. This model was constructed by machine learning based on both MRI radiomics and histological pathomics pre-treatment data. The final model (after training and validating in 3 data sets with over 900 patients) was able to predict pCR with a sensitivity of 89% and specificity of 74%.

In the future, I foresee a significant role for molecular imaging using PET/CT to enable more accurate diagnosis and guide individualized patient treatment. Together with prediction models such as the ones mentioned before in this thesis, I hope this will further increase our ability to select the optimal treatment for the individual patient. By doing so, we might be able to select the most effective anti-cancer treatment and avoid unnecessary adverse events from ineffective treatment regimens.

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