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# Diagnostic and intraoperative targeted molecular imaging for pancreatic cancer

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**Citation**
Tummers, W. S. F. J. (2018, November 13). *Diagnostic and intraoperative targeted molecular imaging for pancreatic cancer*. Retrieved from https://hdl.handle.net/1887/66717

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**Title**: Diagnostic and intraoperative targeted molecular imaging for pancreatic cancer
**Issue Date**: 2018-11-13

Chapter | 1

# Introduction and thesis outline

# INTRODUCTION AND THESIS OUTLINE

## General

The treatment of disease has changed over the last decennia from population-based treatments to personalized medicine, with no exception to cancer therapy. The focus on personalized treatment in oncology is mainly based on our growing understanding of the pathophysiology of cancer. Molecular imaging has played a major role in this. Molecular imaging can be defined by the noninvasive, real-time visualization of biochemical events at the cellular and molecular level within living cells, tissues, and/or intact subjects.[1] Molecular imaging can be performed in patients during the entire treatment process, with the potential to provide a movie of the patient's disease instead of separate snap-shots. In the diseased state, one can choose to image chemical processes in the body or a target of interest, such as a cell surface receptor. The form of molecular imaging that is currently widely applied, is positron emission tomography using fluor-18-deoxyglucose (FDG-PET), where areas of the body with increased uptake of glucose are highlighted.

The use of molecular imaging could potentially improve the accuracy of cancer detection especially when combined with conventional imaging modalities such as ultrasound and CT, by improving sensitivity and specificity. When performing tissue-specific molecular imaging with a highly specific target and a sensitive imaging system, one should be able to see all involved lesions, even micrometastases. One of the cancer types that is in great need of improved detection and treatment is pancreatic cancer. Pancreatic cancer has a dismal prognosis due to late onset of symptoms and therefore most patients present with advanced stage of disease, resulting in less than 30% of patients to undergo a potentially curative surgical resection. Of those patients, the rate of irradical resections is high, mainly due to invisible tumor boundaries due to the perineural and perivascular growth pattern of the tumor. The most commonly performed surgical procedure for pancreatic cancer is the pylorus-preserving pancreatico-duodenectomy or the classic Whipple procedure. This surgery has a high morbidity with a long recovery period. Since the life expectancy of this disease is low, even after surgery, there is a high need to select only those patients for surgery that will actually benefit from a resection. This highlights the need for a tool able to detect this disease earlier, to properly stratify patients for the optimal primary treatment modality, either surgery

or neoadjuvant chemo- and/or radiotherapy, and in addition, improve results during the actual surgical resection. Tumor-specific molecular imaging has the potential do to this.

## Molecular imaging techniques



Molecular imaging can be done in the diagnostic process in the form of tumor-specific PET imaging, or during surgery using near-infrared fluorescence (NIRF) and photoacoustics. Fluorescence imaging is based on adding an exogenous contrast agent (a fluorophore) into the patient that can create contrast to the dark background, and permits detection of the desired tissue of interest in the surgical field.[2] Fluorescence light in the near-infrared window (NIR) (700-900 nm) has the advantage over visible light in several ways. First, there is an increased depth penetration and decreased autofluorescence compared to light in the visible range since NIR light is impaired by absorbance and scatter. This results in a depth penetration of around 5-8 mm in tissue. Second, since tissue has almost no light excitation in the NIR range, the signal-to-background contrast can be maximized in this range. Lastly, NIR light is invisible for the human eye, and therefore, it will not change the surgical field.[3] This requires specialized cameras that can make the light visible for the surgeon on a screen.[4] The advantage of fluorescence imaging is that it can be used during open and minimally invasive surgery, such as laparoscopy and robotic surgery, depending on the used camera. Currently, there are several cameras that can be used for this purpose. The difficulty of developing a camera is the high dependency on an available imaging agent and vice versa. Choosing the right combination will determine the success of both the agent and the camera, since the failure of an agent to identify tumor in a specific manner could be due to agent failure of because the camera was not sensitive enough to detect the signal.[5]

As described above, NIRF imaging is superior for the detection of superficial lesions < 8 mm of depth. However, to improve the rate of radical resections in pancreatic cancer surgery, a depth of around 5 cm needs to be achieved, since the deep vascular margin is mainly affected. Photoacoustic imaging is based on ultrasound and uses the “light in, sound out”- principle. This technique can image at clinically relevant depths (up to 5 cm). The conversion of light to sound is called the photoacoustic effect, being introduced first by Alexander Graham Bell in 1881.[6] In photoacoustic imaging, tissue absorbs pulsed laser light and emanates ultrasound waves as a result of transient thermoelastic expansion.

Because photoacoustic imaging relies on light only one-way and detection is based on sound waves, deeper tissue penetration is possible. Resulting in two to three orders of magnitude weaker ultrasound scattering than optical scattering in tissue.[7] The key strengths of PAI are its ability to collect functional and molecular information from most tissues, and the high spatial resolution, without the use of non-ionizing radiation.[8]

## Imaging agents

Both for NIRF imaging and photoacoustic imaging, fluorescent dyes are needed to enhance the optical signal. Currently, extensive experience is obtained with the non-specific fluorescent dyes indocyanine green (ICG) and methylene blue (MB). These dyes are approved by the U.S. Food and Drug Administration (FDA) for perfusion imaging, and are proven safe. Therefore, these agents were prefect candidates for off-label use in the first fluorescent imaging studies. When injected intravenously these agents migrate to the tumor using the enhanced permeability and retention (EPR) effect of neovasculature. The dye leaks to the tumor due to low interstitial pressure in the tumor microenvironment and is retained there. This method works well for hypervascular tumors, but it is well-known that pancreatic cancer is hypovascular, has minimal leaky vessels and almost no EPR effect. This is also shown in a study of Hutteman et al. were ICG was used to image pancreatic cancer, without successful tumor demarcation.[9]

Based on the variable results obtained with the non-specific agents, the field changed to developing tumor-specific agents, targeting a specific feature of the tumor or it's microenvironment. These targeted agents consist of a targeting ligand and a signalling moiety. This signalling moiety can either be a fluorescent or photoacoustic dye, or a radionuclide in case of PET imaging. Over the last years, tremendous amounts of literature have been published describing the development and preclinical validation of these novel agents. However, only a few of these agents make it into clinical trials and show successful results. This is mainly due to the extensive approval process to perform clinical trials with new agents, and because preclinical results are hard to translate to the human situation. Currently, a few successful first-in-human trials are conducted for tumor-specific intraoperative imaging with targeted agents in ovarian, and colon cancer.[10-13]

Potentially, molecular imaging in pancreatic cancer could be successful when using a tumor-specific targeted agent, in contrast to the unsuccessful attempt seen with the non-specific ICG. There has been scepsis in the field on whether or not this imaging technique would work for pancreatic cancer. Pancreatic carcinomas have a the dense stroma surrounding the tumor, which could potentially prevent imaging tracers from actually reaching their tumor cell targets. This has been put forward as an explanation for the unpromising results with targeted antibody therapy in pancreatic cancer.[14] In this thesis, we describe the first results and show proof of tumor penetration by tumor-specific imaging agents.

There is a large range of potential targeting ligands, such as small molecules, peptides, aptamers, antibodies, engineered protein fragments, nanoparticles, or micro-sized contrast agent. Each of these types of agents are different in size and thus possesses different pharmacokinetic characteristics. In this thesis, tumor-specific imaging of pancreatic cancer is performed using both an antibody and a peptide, showing the different characteristics of these agents, and their respective advantages.

### Imaging targets for pancreatic cancer imaging

For molecular imaging to be of an additional value in detection and treatment of pancreatic cancer, a highly specific imaging signal is needed. In the case of pancreatic cancer, in order to achieve a high tumor-to-background ratio, one needs to identify a target that is only present on pancreatic cancer, and not or in minimal amount on chronic pancreatitis and/or the surrounding unaffected pancreatic tissue For imaging purposes, the ideal target is a cellular-membrane receptor that is also present in tumor-positive lymph nodes and retains expression in vital tumor cells after neoadjuvant therapy. In this thesis several targets for pancreatic cancer are described, together with the advantages and disadvantages. Important to notice is that, at least, at this point there is no optimal biomarker for pancreatic cancer imaging which also ensures all of the above described features. An agent, or multiple agents, directed against more targets, also known as multiplexing, would probably be ideal. However, at this point the process to receive FDA approval for one novel agent is challenging, since no precedent is available yet. Therefore, a situation where one can inject multiple novel imaging agents into one patient will probably not be realized in the near future.

The experimental parts of this thesis focus on two targets, namely integrin alpha$_v$beta$_6$ (αvβ6) and the epidermal growth factor receptor (EGFR). For now, integrin αvβ6 seems to be the most promising target of the two, due to the low expression in chronic pancreatitis, and the high and homogenous expression in tumor, tumor-positive lymph nodes, and retained expression after neoadjuvant therapy. A disadvantage of this target is that expression in chronic pancreatitis and normal pancreatic tissue is not completely absent, due to very low expression in the normal ductal tissue. Other promising targets that are mentioned in this thesis, but not further studied are CEA,[12,13] and uPAR. The advantage of uPAR is that it is not only present on the tumor tissue, but also on the tumor microenvironment, which is abundantly expressed in pancreatic cancer. A disadvantage is that abundant stroma is also present on chronic pancreatitis, making it harder to differentiate between the two entities.

## Translation from molecule to man

As described above, one of the most challenging aspect of this research is the translation of results generated in the preclinical setting to the clinic. A large amount of the conducted research will not even make it to this stage and will therefore not directly benefit the patient. For this thesis, a unique collaboration was put into place between Stanford University Medical School, the LUMC and the Center for Human Drug Research (CHDR), to ensure an optimal roadmap for promising preclinical agents to make it into the clinic. All of the partners have their unique knowledge and experience, providing the possibility to develop a roadmap from molecule to man. The Gambhir Lab at Stanford University is world-leader in the development and preclinical validation of novel targeted imaging agents. The CHDR on the other hand has the unique position to perform first-in-human studies in a safe and controlled environment, and the Image-Guided Surgery Group at LUMC has the most experience in conducting oncologic trials with fluorescence guided surgery.

## Regulatory aspects

One of the main struggles to get a novel imaging agent into the clinic in the USA are the regulatory aspects set by the FDA. To be able to request for a new drug application (NDA), large clinical trials are needed, and patient benefit needs to be shown. These trials are costly, and clinically relevant and objective endpoints are hard to reach with imaging studies. Especially the costs of these large trials make it impossible for an academic partner to pursue this alone. In addition,

industry partners are less willing to participate since the expected profit of imaging agents is minimal, compared to the development of pharmaceuticals. Another problem is that the FDA sees these agents as diagnostics agents, which means that no adverse events are allowed. However, the field argues that some minor grade I-II adverse events should be allowed, since these agents are used in oncologic patients that generally have a worst prognosis compared to the general population. Another challenge for approval is that at this point no real precedence exists for the products used in tumor-specific optical imaging (e.g. camera's and imaging agents). For optical imaging systems this is challenging, because this makes it hard to get 510(k) approval based on equal performances compared to predicate devices.

## THESIS OUTLINE

The thesis consists of five parts describing the entire process from development of tumor-specific molecular imaging for pancreatic cancer to the clinical application and future directions of this novel method for cancer detection in general and in specific for pancreatic cancer. **Part 1** focuses on the development of targeted molecular imaging for pancreatic cancer. **Part 2** describes the validation of this technique in preclinical setting. **Part 3** focusses on the clinical translation of targeted molecular imaging in general, and **part 4** describes the first-in-human clinical use of tumor-specific imaging agents in pancreatic cancer patients. **Part 5** provides an introduction into the future directions of targeted molecular imaging in general and for pancreatic cancer specific.

**Chapter 2** introduces tumor-specific molecular imaging to improve both diagnostics and intra-operative management of pancreatic cancer. It summarizes the current data available, explains the need to push this field forward, and describes promising novel studies. **Chapter 3** shows the results of a retrospective study on the outcome of pancreatic cancer patients, especially focusing on the effect of tumor margin-positive (R1) resection, and indicates where tumor-specific imaging can make a difference. **Chapter 4** summarizes on optimal molecular targets to detect pancreatic cancer, based on an immunohistochemistry study. In part 2, the achieved results of part 1 are used to translate the use of targeted imaging from *ex vivo* experiments to preclinical setting. **Chapter 5** shows the preclinical validation of a novel fluorescent imaging agent, targeting αvβ6, in

preclinical pancreatic cancer models. **Chapter 6** focusses on different targeted imaging agents and methods to enhance imaging signal for early detection of cancer. In part two the possibility to use targeted imaging in pancreatic cancer in preclinical setting is shown. To be able to translate these promising preclinical results into humans, several crucial steps need to be taken, which are discussed in part 3. **Chapter 7** describes the regulatory aspects to achieve approval for the use of both imaging systems and imaging agents. **Chapter 8** provides an overview for the successful clinical translation of optical imaging agents. In part 4, the clinical use of targeted molecular imaging in pancreatic cancer patients is shown for the first time describing several first-in-human studies. **Chapter 9** describes the results of the clinical translation of targeted PET tracers for pancreatic cancer imaging. **Chapter 10** shows the results of intra-operative identification of pancreatic cancer using fluorescently labelled cetuximab. **Chapter 11** focuses on the detection of visually occult tumor-positive lymph nodes using molecularly targeted fluorescent imaging during surgical resection. Lastly, part 5 provides an insight into the future of this technique and discusses the steps needed to make this a widely used technique in the future. **Chapter 12** describes the need to develop a standardized method in assessing agents for fluorescence-guided surgery. And in **Chapter 13**, a general discussion is provided and the future perspectives are discussed.

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