Diagnostic and intraoperative targeted molecular imaging for pancreatic cancer

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For years, pancreatic cancer had a dismal prognosis with a long term survival of around 5%. Since the centralization of pancreatic cancer surgery and the introduction of systemic chemotherapy with FOLFIRINOX, the median overall survival increased from 6-13%, after gemcitabine therapy, to around 20%. However, even with the introduction of these novel treatment regimens, the achieved survival rates remain disappointing compared to other cancer types. Consequently, radical tumor-margin free resection provides the patient with the best potential chance for cure. However, due to late onset of symptoms, the majority of patients present with inoperable disease. These patients can benefit from neoadjuvant therapy, or palliative chemotherapy. During clinical practice, this means that decision-making before and during surgery is critical to select the most optimal primary treatment modality for the patient. Currently, conventional imaging modalities lack sensitivity to detect small metastatic lesions, and are unable to visualize treatment response on neoadjuvant therapy.

Tumor-specific molecular imaging in the form of fluorescence and photoacoustic imaging aids the surgeon to accurately recognize and resect malignant tissues in real-time during surgery. The other form of this kind of imaging, PET-CT using radioactive signal, can be used in the perioperative process to assess tumor spread and help in the surgical planning. This thesis focuses on the challenges a surgeon faces during pancreatic cancer treatment, and the potential improvements that could be achieved by the use of tumor-specific imaging. In addition, the regulatory aspects of clinical translation of tumor-specific optical imaging agents are addressed.

Part 1: Development of Targeted Molecular Imaging for Pancreatic Cancer

Chapter 2 describes the current clinical imaging applications for the detection of pancreatic cancer, and the upcoming molecular imaging strategies with emphasize on tumor-specific intra-operative imaging. This technique has shown great potential in order to bridge the gap between diagnostic and intra-operative imaging for pancreatic cancer treatment. In this chapter the need for an optimal biomarker to perform tumor-specific imaging is described together with the advantages and disadvantages of several biomarkers.

To be able to implement a novel technique, such as tumor-specific imaging, in patient care and achieve actual improvement in outcomes, one needs to know

where this technique needs to make a difference. This element is describes in **chapter 3**, where a retrospective study is reported showing that a margin-positive (R1) resection is a major contributor to reduced survival and early recurrence, especially in patients with lymph node negative (N0) disease.

As stressed before, a biomarker is needed to be able to perform tumor-specific imaging. Idealy, this biomarker is able to discriminate between pancreatic cancer and inflammation since this is challenging both at preoperative imaging and during surgery. In **chapter 4** of this thesis an immunohistochemistry study is performed identifying such a biomarker, which is also overexpressed on associated lymph node metastases and on vital tumor cells after neoadjuvant therapy. It was concluded that both integrin αvβ6 and carcinogenic embryonic antigen (CEA) are promising candidates, especially when used in combination. Integrin αvβ6 has the advantage over CEA that this biomarker retains expression after neoadjuvant radiochemotherapy.

Part 2: Validation of Targeted Molecular Imaging for Pancreatic Cancer

In **chapter 5** a novel fluorescent imaging agent is developed targeting integrin αvβ6. This agent consists of an existing cysteine knottin peptide, R01-MG, conjugated to the near-infrared (NIR) fluorescent dye IRDye800. This agent is validated extensively in preclinical mouse models, showing specific targeting to αvβ6 and a significantly higher tumor-to-background ratio (TBR) compared to normal pancreatic tissue in transgenic mouse models. **Chapter 6** focusses on early detection of cancer in preclinical models, using activatable agents. These agents can be used for early detection because the agent is only "turned-on" in the tumor microenvironment, which dramatically improves sensitivity of cancer detection.

Part 3: Clinical Translation of Targeted Molecular Imaging

Targeted molecular imaging is a novel technique which requires regulatory approval before this technique can have wide-spread use in patient. Targeted molecular imaging is unique in its ability to provide real-time feedback to the surgeon in the case of intra-operative imaging, complicating the approval process. In addition, this technique requires the use of both an agent and an imaging device, which are important considerations in this process. **Chapter 7** describes the regulatory hurdles in detail. Important here is that the proposed

"indication for use" will direct all future studies needed for approval, and therefore, should be chosen carefully. Success or failure of optical imaging agents will critically depend on the design of clinical trials and the chosen endpoints. In **chapter 8** insight is given into the process of engaging the FDA in the evaluation of tumor-specific imaging agents, and a guide for designing optimal optical imaging clinical trials.

Part 4: Clinical Application of Targeted Molecular Imaging for Pancreatic Cancer

This part is focused on the clinical use of this novel technique describing early phase clinical trials performed in pancreatic cancer patients. In the diagnostic process, tumor-specific PET-CT imaging could improve the detection of pancreatic cancer and aid the perioperative process to assess tumor spread and help in the surgical planning as shown in chapter 9. Targeting αvβ6 using [18F] FP-R01-MG-F2, provided an elevated imaging signal in the tumor compared to normal pancreas. In chapter 10, the first-in-human use of multimodality molecular imaging for the intra-operative detection of pancreatic cancer is reported using cetuximab-IRDye800 that binds to epidermal growth factor receptor (EGFR). Fluorescent imaging successfully identified tumor with a significantly higher mean fluorescence intensity (MFI) compared to normal pancreatic tissue and inflammation, with a sensitivity of 96.1% and specificity of 67.0%. In addition, the mean photoacoustic signal in the tumor was 3.7-fold higher than surrounding tissue. In chapter 11 a more in-depth analysis is performed on the ability of tumor-specific fluorescent imaging to detect visually occult tumor-positive lymph nodes during surgical resection of pancreatic cancer. With the use of cetuximab-IRDye800 peritumoral lymph nodes were detected with a sensitivity of 100% and specificity of 78% macroscopically. Also, this method was able to detect occult foci of tumor (< 5mm) with a sensitivity of 88%.

Part 5: Future Directions for Targeted Molecular Imaging

In **chapter 12** of this thesis the future of tumor-specific optical imaging is discussed. To eventually achieve wide-spread use of this novel technique patient benefit needs to be shown as discussed in this thesis. Therefore, larger trials are needed with correctly chosen endpoints. To put the results of different studies into a broader perspective, results of different clinical trials should be

comparable. In chapter 12, a format is presented to standardize the way results of tumor-specific imaging trials are reported, which will hopefully lead to a more generalized method in which results can be better appreciated. Eventually, this will ensure that results of different trials can be pooled and hopefully used together in the approval process for novel imaging agents and devices.