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Part III
Chapter 11

Summary and future perspectives
SUMMARY

The quality of cancer surgery can be improved by assisting the surgeon with better and more objective, real time visual information during the procedure. Fluorescence-guided surgery can play a pivotal role in the intraoperative detection of tumor tissue, lymph nodes and vital structures.

This thesis focuses on a number of important indications in cancer surgery where clinically available near-infrared (NIR) fluorescence contrast agents can be used to improve surgical practice (Part 1) and the clinical translation (first in human study) of newly developed tumor-specific fluorescent contrast agents to improve the real time detection of tumor tissue (Part 2).

Part 1: Exploring clinical available fluorescent contrast agents in cancer surgery

In chapter 2, the detection of sentinel lymph nodes in gastric cancer is demonstrated. The lymph drainage pattern in gastric cancer is difficult to predict. Therefore, a total or partial gastrectomy is often combined with extensive lymphadenectomy. In the present study, indocyanine green (ICG) was adsorbed to nanocolloid to increase its hydrodynamic diameter and to improve retention in first draining lymph nodes. Subsequently, the ICG-Nanocolloid complex was then subserosally injected around the tumor, followed by detection of lymphatic vessels and lymph nodes. In 21 of 22 patients, at least 1 sentinel lymph node was identified using this technique. Interestingly, in 8 of the 21 patients, SLNs outside the standard plane of resection were identified, and tumor involvement was found in 2 of these nodes (in two patients).

Chapter 3 focused on the intraoperative detection of breast cancer using methylene blue (MB). Although the exact mechanism is not exactly clear, we hypothesized that breast cancer could be visualized using MB, based on physicochemical similarities of MB and 99mTc-MIBI. 99mTc-MIBI is a SPECT radiodiagnostic agent with a sensitivity of 83 - 90% to detect breast cancer preoperatively. In 20 out of 24 patients, we successfully identified breast cancer lesions in the resection specimen. And in 2 patients breast cancer tissue was identified in the wound bed using fluorescence imaging. Although MB is a promising agent for breast cancer detection, tumor-ligand specific contrast agents could further improve the accuracy of intra-operative breast cancer imaging.
Chapter 4 described the identification of hepatic metastases from uveal melanoma during laparoscopic liver surgery. ICG was administered 24 hours before surgery. The dye gets excreted by healthy liver into bile, but is trapped in the transition zone between healthy liver and metastatic lesions. At the day of surgery, a clear fluorescent rim around the tumor was seen. Even lesions not detected by pre-operative CT-scan, intraoperative inspection or laparoscopic ultrasound were identified. Therefore, NIR fluorescence imaging during laparoscopic liver surgery is a minimal invasive way to determine whether patients could potentially benefit from a metastasectomy. Moreover, it might help to obtain clear resection margins.

In chapter 5, ICG was administered to patients suffering from a pituitary gland adenoma. We hypothesized that ICG could improve distinction between normal and abnormal pituitary gland tissue during transnasal transsphenoidal selective adenomectomy, based on differences in tissue vascularization. In nine out of 10 patients with a histologically proven pituitary adenoma, the normal pituitary gland showed a stronger fluorescent signal than the adenoma. In two patients, adenoma resection actually took place under direct NIR fluorescence guidance. The available endoscopic imaging system didn't allow simultaneous imaging of color video and fluorescence, which limited the additional value of fluorescence imaging. Introduction of ligand-specific contrast agents and improved imaging systems could further improve fluorescence-guided pituitary gland surgery.

Chapter 6 described a clinical case in which MB was used to identify a paraganglioma, a rare neuro-endocrine tumor. It even allowed the detection of a local metastasis, which was not detected by preoperative imaging and intraoperative inspection.

Based on the principle of comparable biodistribution of MB and the 99mTc-MIBI tracer, chapter 7 elaborated on the intraoperative fluorescence guidance during parathyroid surgery. MB allowed not only identification of parathyroid adenomas, but also identified normal parathyroid glands that were otherwise not detectable. Although promising results with newly developed structure inherent contrast agents are reported, MB can play a vital role in parathyroid surgery until newer probes enter the clinic.

In chapter 8, the enhanced permeability and retention (EPR) effect in ovarian cancer was explored. This effect is based on the fact that macromolecules, such as protein bound ICG, accumulate in tumor tissue due to increased vascular
permeability and reduced drainage. In the current study, several malignant lesions were identified using fluorescence imaging. However, also 13 non-malignant lesions were fluorescent, resulting in a false-positive ratio of 62%. This lack of specificity of the EPR effect shows that it is not suitable for daily clinical practice. A more tumor-specific accumulation profile or ligand-binding mechanism could overcome this limitation.

**Part 2: Clinical translation of innovative tumor-specific fluorescent contrast agents**

Chapter 9 described the use of EC17, a folate analogue coupled to fluorescein, for the identification of folate receptor alpha (FRα) positive ovarian and breast cancer. Intravenous administration of EC17, 2-3 hours before surgery, resulted in clear, bright fluorescent metastatic lesions of ovarian cancer. It resulted in an increased resection of 16% more malignant lesions, and a 70% increase in detection of metastatic lesions on post-operative analyzed images. In breast cancer, the overexpression of FRα is lower than in ovarian cancer. Therefore, preoperative obtained biopsies were stained for receptor expression. This personalized approach guarantees that only patients that can potentially benefit from fluorescence-guided surgery are included in trials using a tumor-specific contrast agent. Also in breast cancer, a clear fluorescent signal was seen in the tumor. Notwithstanding, autofluorescence signal of healthy tissue at 500nm caused false-positive lesions in breast cancer. In addition, this autofluorescence made it difficult to discriminate breast cancer-specific fluorescence from background fluorescence. Imaging in the NIR fluorescence light spectrum could solve this problem.

In chapter 10, we used a newly developed FRα specific contrast agent. This agent, OTL38, consists of a folate analogue coupled to a NIR fluorescent contrast agent (fluorescent at 800nm). First, tolerability and pharmacokinetics in blood and skin were assessed in a randomized, placebo-controlled, dose-ascending trial in healthy subjects. Hereafter three intravenous doses, selected based on the results from the healthy subject study, were administered to 12 patients with epithelial ovarian cancer, and scheduled for cytoreductive surgery. Clear accumulation of OTL38 in FRα positive tumors and metastases was seen, leading to the resection of 29% additional malignant lesions that were not identified by inspection and palpation. Almost no background fluorescence was seen.
GENERAL CONCLUSION

Intraoperative NIR fluorescence imaging using ICG and MB was explored in multiple important indications in cancer surgery. Imaging using ICG resulted in accurate tumor imaging of liver metastases and SLN detection in gastric cancer, with high TBRs and a prolonged fluorescent signal. For these indications, our data suggests that fluorescence imaging should be implemented in the clinic to improve patient care. MB allowed successful imaging of parathyroid adenomas, neuro-endocrine tumors and breast cancer lesions, but accuracy and imaging characteristics can probably be improved by the introduction of more tumor-specific contrast agents. However, in the meantime MB can be used for intraoperative guidance in for instance difficult cases.

Intraoperative imaging of ovarian cancer using the FRα specific EC17 and OTL38 showed highly specific and accurate tumor imaging with a high TBR and prolonged fluorescent signal in malignant lesions. Administration of these low molecular weight contrast agents resulted in rapid accumulation in tumor tissue and fast clearance from the rest of the body. Moreover, with the NIR fluorescent contrast agent OTL38 almost no background signal or autofluorescence was seen. Clinical translation using both healthy subjects and patients allowed a rapid determination of the optimal dose, formulation, and time window for intraoperative imaging, facilitating a fast clinical introduction of newly developed contrast agents in clinical trials.
OVER THE PAST YEARS SEVERAL INDICATIONS ARE IDENTIFIED TO USE CLINICAL AVAILABLE CONTRAST AGENTS FOR IMAGE-GUIDED SURGERY. ICG AND MB HAVE THE POTENTIAL TO IMPROVE CANCER SURGERY BY IDENTIFYING MORE MALIGNANT LESIONS, SPEED UP TIME OF SURGERY, AND AVOID IATROGENIC INJURY DUE TO BETTER IDENTIFICATION OF VITAL STRUCTURES. ALTHOUGH THE NUMBER OF INDICATIONS IS LIMITED DUE TO THE INTRINSIC CHARACTERISTICS OF THESE CONTRAST AGENTS, FLUORESCENCE-GUIDED SURGERY CAN DIRECTLY BE IMPLEMENTED IN DAILY CLINICAL CARE IN FOR EXAMPLE LIVER SURGERY, SLN IMAGING AND EXPECTED DIFFICULT BILE DUCT SURGERIES. UNTIL NEWLY DEVELOPED LIGAND-BINDING, TUMOR-SPECIFIC CONTRAST AGENTS HAVE BEEN DEVELOPED AND INTRODUCED IN THE CLINIC, TUMOR IDENTIFICATION CAN BE IMPROVED IN FOR EXAMPLE BREAST CANCER SURGERY, PARATHYROID SURGERY AND PARAGANGLIOMA SURGERY, AS DISCUSSED IN THIS THESIS.

THE INTRODUCTION OF LIGAND-BINDING, TUMOR-SPECIFIC CONTRAST AGENTS HAVE OPENED NEW POSSIBILITIES FOR MORE ACCURATE INTRAOPERATIVE TUMOR IMAGING. AS LONG AS APPROPRIATE TUMOR OR TISSUE SPECIFIC LIGANDS WILL BE DISCOVERED, IT WILL BE POSSIBLE TO IDENTIFY ALMOST ANY STRUCTURE A SURGEON WANTS TO IDENTIFY DURING SURGERY. AS SHOWN IN OUR STUDIES, INVESTIGATING EC17 AND OTL38, BOTH FOLATE ANALOGUES COUPLED TO FLUORESCENT CONTRAST AGENTS RESULTED IN HIGHLY SPECIFIC TUMOR IMAGING, WITHIN 2-3 HOURS AFTER ADMINISTRATION. MOREOVER, THE FLUORESCENT SIGNAL AND TBR REMAINED EXCELLENT FOR UP TO AT LEAST 6 HOURS.

CONTRAST AGENTS
TO ACHIEVE ACCURATE TUMOR DETECTION, IT IS IMPORTANT TO OPTIMIZE BINDING CAPACITY, BIODISTRIBUTION AND PHARMACOKINETICS OF A CONTRAST AGENT. MULTIPLE COMPOUNDS AS MONOCLONAL ANTIBODIES, ANTIBODY FRAGMENTS, SUCH AS SINGLE-CHAIN ANTIBODY FRAGMENTS AND SMALL PEPTIDES ARE THEREFORE BEING INVESTIGATED1-5. THESE DIFFERENT COMPOUNDS ALL HAVE THEIR OWN PHARMACOKINETIC PROFILES, AND THEREBY DIFFERENT INTERVALS FOR IMAGING BASED ON THE HALF-LIFE OF THE AGENT. ROSENTHAL ET AL.5 REPORTED THE IMAGING OF HEAD AND NECK CANCER USING CETUXIMAB-IRDye800. SURGERY WAS SCHEDULED 3 DAYS AFTER ADMINISTRATION OF THE COMPOUND BECAUSE OF THE PHARMACOKINETIC PROFILE OF THIS MONOCLONAL ANTIBODY BASED CONTRAST AGENT (HALF LIFE 24 – 32 HOURS; MOLECULAR WEIGHT 150 kDa)6. MOREOVER, RELATIVELY HIGH DOSAGES WERE NEEDED TO ACHIEVE SUFFICIENT TBRs (UP TO 25% OF THERAPEUTIC DOSE; 62.5 mg/m²).
Administration of the folate analogue OTL38 (half life 2-3 hours; molecular weight 1.4 kDa) showed a high fluorescent signal in malignant lesions after only 2-3 hours, which prolonged for at least 6 hours (optimal dose 0.0125 mg/kg). Due to the fast clearance of the agent, low background signals were observed. It seems that these properties are ideal for accurate tumor imaging. Future studies will show which compounds are favorable, and this may even differ for different surgical indications.

One would ideally develop one contrast agent that targets almost all solid tumors. It remains however challenging to develop a contrast agent that is highly specific for cancer tissue, while gets excreted from the rest of the body. Receptor expression profiles in tumors can thereby differ. In ovarian cancer for example, over 90% of patients are positive for the FRα, and almost all patients are likely to benefit from treatment with an FRα specific contrast agent. In cancers where expression profiles are more divers, other strategies must be applied. Preoperative obtained biopsies could be used to explore the receptor expression profile of a specific tumor, introducing personalized medicine for precision surgery. This was for example done for the breast cancer patients in our EC17 study. Besides, cocktails with multiple ligand-binding agents can be administered based on biomarker profiles, or so-called multi-headed tumor-specific contrast agents can be developed. These are compounds that have multiple ligands bound to one fluorophore to enlarge the number of receptors that can be bound. A very important hurdle for the design of these agents is that all changes to such molecules can influence binding capacity and fluorescent properties.

One of the main limitations of fluorescence imaging is related to tissue penetration. In the visible light spectrum this is in the order of micrometers, improving to approximately 10 mm in the NIR spectrum. To overcome this limitation, contrast agents that combine fluorescence imaging with other diagnostic imaging modalities are being developed (multimodal imaging, combined optical positron emission tomography (PET) or single-photon emission computed tomography (SPECT) agents). These types of agents allow pre-operative surgical planning, intraoperative guidance towards deeper located targets and real-time visual discrimination of tumors from healthy tissue. In literature, combinations with photoacoustic imaging, magnetic resonance imaging, PET, SPECT and even triple-modal imaging are described. However, most of these agents are still in a pre-clinical phase of development.
Imaging systems
Successful fluorescence-guided surgery depends on the combination of both contrast agents and imaging systems. Therefore, the development of imaging systems is crucial for the wide acceptance of fluorescence-guided surgery. Over the last years, multiple companies and institutes have launched imaging systems, all with their pros and cons. Especially in endoscopic imaging there is much to gain, as suggested in chapter 4 and 5 of this thesis. Moreover, a more standardized approach to compare systems could assist in the development of new improved systems.

Clinical implementation
To provide guidance in the clinical implementation of image-guided surgery, cooperation is necessary, and international society’s, like the International Society of Image Guided Surgery, are formed to discuss regulatory pathways and clinical trial design. Endpoints in phase 1 studies should focus not only on safety, but also on imaging endpoints.

There is a promising role for the development of pharmacokinetic and pharmacodynamic models. These models could give more insight in all the different variables that play a role in the mechanism of action in tumor imaging. Information on compound size, half-life, clearance, receptor density, tumor volume, binding constant, protein binding and so on could be combined in a model. Even imaging characteristics as quantum yield and wavelength can be added. This could potentially predict safety and imaging outcomes of newly designed compounds and could provide information on the optimal dose of a drug. The information obtained from these models could lead to exposure of less healthy subject and patients in first in human studies. It could even predict which imaging system performs best for a specific indication with a specific compound. This would make phase 1 studies more efficient, and lower the hurdle for promising compounds to enter the clinic.

New clinical indications
Cancer treatment requires a multidisciplinary approach where surgery, systemic chemotherapy or immunotherapy and radiotherapy all play a significant role. Recent developments in neoadjuvant chemo-radiation therapy have resulted in an increase in the number of complete pathological response in for example rectal and oesophageal cancer. These patients won't benefit
from a surgical resection, while no vital tumor tissue is present at pathological examination. On the contrary, these surgical resections are associated with morbidity and mortality, and safely avoiding such procedures would have a major impact on quality of life. This has resulted in a paradigm shift toward omitting surgery in patients with a complete clinical response and facilitating organ-preservation. Hereby it’s essential to select patients with a complete clinical response after neoadjuvant therapy and to monitor them. There are several studies reporting on this “Watch and Wait” policy in rectal cancer\textsuperscript{21}, and an international database was recently established to monitor the results of this strategy\textsuperscript{22}. Molecular imaging could have major impact on the selection and monitoring of a patient, because it could give more precise and objective information on the presence of vital tumor tissue in for example the scar tissue after chemo-radiation therapy.

CONCLUSIONS

Fluorescence-guided surgery has shown its potential in visualizing tumor tissue and increasing the number of detected malignant lesions that are otherwise undetectable. Besides, it assists in the identification of lymph nodes and vitals structures. Further studies, preferably randomized controlled trials, should validate whether improved tumor detection results in better patient outcomes by means of improved survival and reduced morbidity. Combined efforts in the rapidly expanding field of fluorescence-guided surgery hopefully leads to clinical implementation and determining its place in standard of care treatment of cancer patients in the near future.
REFERENCES