Near-infrared fluorescence imaging in colorectal cancer and its metastases

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SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES

The rapidly evolving field of near-infrared (NIR) fluorescence-guided surgery is gradually finding its way into the clinic. Before becoming standard of care patient benefit needs to be unequivocally demonstrated. This thesis primarily focuses on clinical applications for NIR fluorescence-guided surgery in colorectal surgery. Both targeted and non-targeted fluorescent agents are addressed.

PART I: NIR FLUORESCENCE IMAGING IN COLORECTAL CANCER SURGERY

The first part of this thesis focuses on the use of NIR fluorescence imaging in colorectal cancer patients. It includes an extensive review followed by three chapters that detail studies involving non-targeted fluorescent agents.

Chapter 2 comprises a comprehensive overview of fluorescence-guided surgery in colorectal cancer. It displays current clinically available applications, and focuses on promising future modalities. The discussed applications encompass the imaging of primary and recurrent colorectal tumors, sentinel lymph node imaging, visualization of peritoneal and liver metastases, nerve imaging, urinary tract imaging, as well as perfusion assessment. The first studies show additional value of this technique regarding change in surgical management. Future trials should focus on patient related outcomes such as quality of life, complication rates, disease free survival, and overall survival.

In **chapter 3** of this thesis, the literature on the application of NIR fluorescence imaging for sentinel lymph node (SLN) mapping in colorectal surgery is examined. SLN mapping shows promise as a valuable tool in the treatment of colorectal cancer patients. Nevertheless, conventional lymph node mapping methods using a combination of a radiocolloid tracer and patent blue, face limitations because of poor depth penetration of patent blue, while the logistical challenges associated with radiocolloid tracer pose additional hurdles. With NIR fluorescence imaging, the SLN can be accurately identified in most patients resulting in more accurate lymph node staging. Despite its potential benefits, current technical challenges (like endoscopic dye injection and the low negative predictive value of the SLN) withhold surgeons from incorporating this application into routine practice.

Despite the promising results on indocyanine green (ICG) perfusion analysis there is still debate about the interpretation of the observed fluorescence signal. The current visual interpretation is subjective and therefore hampers standardisation. In **Chapter 4**, a case study, four patients are described with possible compromised bowel perfusion after mesenteric resection, revealing challenges in interpreting uncorrected fluorescence signals. This study emphasizes the importance of quantifying NIR fluorescence signals for assessing tissue perfusion during surgery. Bowel perfusion, as assessed clinically by independent surgeons based on NIR fluorescence imaging, resulted in different treatment strategies, three with excellent clinical outcome, but one with a perfusion related complication, highlighting the complexities of interpreting uncorrected fluorescence signals. Post- surgery quantitative analysis of fluorescence dynamics showed different patterns in the affected bowel segment compared to the unaffected reference segments for the four patients. The study suggests that real-time standardized quantification of NIR fluorescence imaging could improve surgical decision-making in the future. However, few studies explore the use of quantification software beyond feasibility due to method limitations.[1]

Chapter 5 introduces a protocol for a multicenter, randomized controlled clinical trial evaluating the effectiveness of NIR fluorescence imaging with ICG in reducing anastomotic leakage (AL) rate in colorectal surgery. In the final study, 931 patients were randomized between the Fluorescence Guided Bowel Anastomosis group and the Conventional Bowel Anastomosis group.[2] There was no significant differences in overall AL rate. However, subgroup analysis showed a significantly lower AL rate in patients undergoing left-sided colorectal surgery, especially for patients who underwent rectosigmoid resection. It underlines the value of NIR fluorescence imaging with ICG in reducing anastomotic leakage rates in colorectal surgery.

PART II: INTRAOPERATIVE IMAGING USING SGM-101; A TUMOR TARGETED NIR FLUOROPHORE

The second part of this thesis focusses on the clinical results of the carcinoembryonic antigen (CEA) specific antibody SGM-101. This anti-CEA fluorescent antibody is primarily used in colorectal cancer, but other malignancies have been studied as well.

The process of clinical translation of novel fluorescent agents is an essential part in the evolution of NIR fluorescence guided surgery. **Chapter 6** gives an overview of the clinical translation of SGM-101, a novel fluorescent anti-CEA monoclonal antibody.

SGM-101 can aid in improving detection and complete resection for CEA-positive tumors. In **chapter 7**, the performance of SGM-101 for the detection of colorectal and pancreatic liver metastases was investigated. In this pilot study,

all clinically suspected malignant lesions were detected with NIR fluorescence-guided surgery. These findings show that SGM-101 can facilitate real-time detection of liver metastases, potentially aiding surgeons in achieving more precise and complete tumor removal.

On the contrary, only 31% of the colorectal lung metastases were intraoperatively detected (**chapter 8**). In this chapter we have explored the feasibility of NIR fluorescence-guided surgery, using SGM-101, for the detection of colorectal lung metastases. Metastasectomy is a common treatment option for patients with colorectal lung metastases (CLM). Challenges exist with margin assessment and identification of small nodules, especially during minimally invasive surgery. Intraoperative fluorescence imaging has the potential to overcome these challenges. The study focuses on *in vivo*, back table, and closed-field bread loaf imaging. It demonstrated the potential of fluorescence imaging of CLM with SGM-101. CEA expression was observed in all tumors and closed-field imaging showed excellent CEA specific targeting of the tracer to the tumor nodules. The full potential of SGM-101 for *in vivo* detection of the tracer can be achieved with improved minimal invasive imaging systems and optimal patient selection.

The difference between colorectal liver and lung metastases may be a consequence of the use of minimal invasive NIR fluorescence imaging systems, as this was used in most lung metastases cases (85%), but in only 1 (9,1%) of the liver procedures. Moreover, closed-field imaging showed excellent results for colorectal lung metastases. This confirms the hypothesis that improving minimal invasive NIR systems should lead to better intraoperative imaging results. The promising results from those phase I/II studies served as the basis for a phase III multicentre trial (NCT03659448), including patients in Europe and the US. This trial focuses on primary, recurrent and abdominal metastatic colorectal cancer, aiming to demonstrate added value of fluorescence guided surgery regarding resection margins and the detection of additional lesions.

Chapter 9 reports on the first in-human surgical trial using SGM-101 to evaluate the suitability for molecular imaging-guided lung cancer resections, along with its correlation with the expression of carcinoembryonic antigen-related cell adhesion molecule type 5 (CEACAM5) glycoprotein. Among the 4 patients undergoing surgery, lesions with 2+ and 3+ tissue CEACAM5 expression had outstanding tumor fluorescence, displaying a mean tumor-to-background ratio of 3.1. Conversely, the absence of SGM-101 fluorescence was linked to benign lesions and a lack of CEACAM5 staining. This study demonstrated SGM-101 localization to CEACAM5-positive tumors with the detection of real-time near-infrared fluorescence in situ, *ex vivo*, and by immunofluorescence microscopy. These findings suggest that SGM-101 is a receptor-specific, and feasible intraoperative molecular imaging fluorochrome that should be further evaluated in randomized clinical trials.

PART III: NEW WAYS FOR IDENTIFICATION OF NOVEL TARGETS FOR NIR FLUORESCENCE IMAGING

The final section of this thesis focuses on the future: finding new targets for NIR fluorescence imaging.

Selection of novel cell surface biomarkers for fluorescence-guided surgery is conventionally done by reviewing key publications from recent literature. Unfortunately, this is time consuming. Nowadays a data driven selection method is available that combines multiple databases and interlinks literature (**chapter 10**). This chapter encompasses a study assessing cell surface biomarkers for non-small cell lung cancer that are potentially suitable for fluorescence-guided surgery. The selection of these biomarkers involves a data driven selection using >275 multi-omics databases, literature, and experimental evidence. Ten biomarkers were selected, and tumor expression was assessed by immunohistochemical staining. Epithelial cell adhesion molecule (EPCAM), carbonic anhydrase 9 (CAIX), and collagen type XVII alpha 1 chain (COLLAGEN XVII) were identified as promising targets for intraoperative fluorescence imaging for both major subtypes of non-small cell lung carcinomas and should be investigated further in future studies. This approach offers researchers unique insights and has the potential to significantly reduce the time required compared to a conventional literature search. On the other hand, data driven selection can lead to new targets, for which no clinical fluorescent target ligands are yet available. These completely new targets will therefore require more research, probably including immunohistochemistry (IHC). Although IHC serves as an indicator for potential clinically useful fluorescent agents, its correlation with NIR fluorescence-guided surgery endpoints, like the tumor-to-background ratio, is unknown.

In conclusion, this thesis underscores the burgeoning potential of NIR fluorescence-guided surgery, especially in colorectal cancer. Through comprehensive analyses of both targeted and non-targeted fluorescent agents, significant advancements have been identified that could transform surgical precision and patient outcomes. Despite promising preliminary results,

challenges such as standardization of fluorescence signal interpretation and technical limitations of current imaging systems persist. Future research should focus on large-scale clinical trials to unequivocally demonstrate patient benefits, refine imaging techniques, and explore new biomarkers for a broader range of malignancies. Ultimately, the integration of NIR fluorescence-guided surgery into routine practice hinges on continuous innovation and robust clinical validation to enhance surgical accuracy and patient care.

REFERENCES

1 Van Den Hoven P, Osterkamp J, Nerup N, Svendsen MBS, Vahrmeijer A, Van Der Vorst JR, *et al.* Quantitative perfusion assessment using indocyanine green during surgery-current applications and recommendations for future use. Langenbecks Arch Surg. 2023;408(1):67.

2 Faber RA, Meijer RPJ, Droogh DHM, Jongbloed JJ, Bijlstra OD, Boersma F, *et al.* Indocyanine green near-infrared fluorescence bowel perfusion assessment to prevent anastomotic leakage in minimally invasive colorectal surgery (AVOID): a multicentre, randomised, controlled, phase 3 trial. Lancet Gastroenterol Hepatol. 2024.