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**Title:** Targeted imaging in oncologic surgery: preclinical studies utilizing near-infrared fluorescence and radioactivity
**Issue Date:** 2017-04-13
Chapter 12

Summary
SUMMARY

Fluorescence-guided surgery (FGS) is an intraoperative imaging technique already introduced and validated in the clinic for sentinel lymph node mapping and biliary imaging. Conjugating a NIR-dye to a specific tumor-targeting vehicle dramatically enhances the specificity of this technique. Hence, a powerful synergy can be achieved when fluorescent imaging is combined with nuclear imaging. The (NIR) fluorescent signal aids the surgeon to accurately recognize and resect malignant tissues and detect (nearby) vital structures in real-time during surgery, while its nuclear counterpart can be used to preoperative assess tumor spread and aid in the surgical planning and guidance. Patients may benefit directly from better tumor detection as the surgical status (R0 or R1) is one of the most import parameters for morbidity and patient survival. This thesis focus on the evaluation of potential targets for image-guided surgery applications (Part I) and describes the preclinical evaluation of novel tracers for (hybrid) image-guided surgery (Part II).

Part I, Chapter 2 describes tumor-specific membrane proteins and their potential to function as target for tumor targeted applications. The majority of tumor-targeting compounds are directed against membrane-bound proteins. Various categories of targetable membrane-bound proteins exist, such as anchoring proteins, receptors, enzymes and transporter proteins. The function and biological characteristics of these proteins determine their location and distribution on the cell membrane and therefore it is important to understand these features. In general, epithelial expressed targets suffer from intratumoral heterogenic expression patterns and are only upregulated in a subset of patients. Tumor-associated stromal targets on the other hand, expressed on for example activated macrophages and/or neoangiogenic cells, are abundantly present, disperse located through and around the whole tumor and generally exists in very high numbers (Chapter 3). Since decades the urokinase plasminogen activator (uPA) system has been associated with the development, progression and invasion of malignant cells. Its receptor, uPAR, is one of the key players in this proteolytic cascade, focusing uPA’s proteolytic activity to the cell surface and functions as a signaling receptor (Chapter 4). uPAR is highly expressed in virtually all human cancers, suggesting possible clinical applications as diagnostic marker, predictive tool of survival or clinical response, and as a target for therapy and imaging. Within the tumor microenvironment of colorectal cancers uPAR is expressed by malignant cells but also on tumor-associated stromal cells like macrophages, (neoangiogenic) endothelial cells and myofibroblasts, where it is independently negatively associated with decreased patient survival (Chapter 5).

Part II focuses on the preclinical development and evaluation of tumor-specific tracers. Two almost solely epithelial expressed and already widely investigated tumor-specific
targets are the Carcinoembryonic Antigen (CEA) and Epithelial Cell Adhesion Molecule (EpCAM). Overexpression of CEA is found in the majority of gastro-intestinal carcinomas. We developed ssSM3E/800CW, a novel CEA-targeted near-infrared fluorescent tracer, based on a disulphide stabilized single-chain antibody fragment (ssScFv) and visualized colorectal and pancreatic tumors in a clinically translatable setting (Chapter 6). Antibody-fragments are favorable since they combine the same affinity as full antibodies with a reduced size, favorable for tumor penetration and biodistribution. Using same methods and models as described above we developed and evaluated F(ab)/800CW, an antibody fragment directed to EpCAM (Chapter 7). EpCAM is known to be a pluripotent target as it is upregulated in many cancer types. NIR fluorescent signals showed high tumor-to-background ratios, which proved suitable for intraoperative detection and delineation of tumor boarders and small (residual) tumor-nodules in mice, between 8h and 96h post-injection.

Chapter 8 describes the clinical translation of the small peptide based NIR fluorescent agent cRGD-ZW800-1 via GMP production, in vitro and in vivo characterization, pharmacokinetics experiments and toxicology studies, to allow administration of cRGD-ZW800-1 in Phase I clinical trials. cRGD-ZW800-1 shows fast and accurate identification of tumors in various clinical relevant human xenograft mouse tumor models and a mean half-life time of approximately 25 min. Single extended dose toxicology study showed no sign of toxicity up-to a dose of 15.04 mg/kg in rats. Being very conservative, a dose of 81µg/kg can therefore be considered as a safe starting dose for first-in-human experiments. Due to the recognition of multiple integrins, cRGD-ZW800-1 has the potential to be a powerful tool for tumor targeting: with high affinity for either the cancer cells, the supporting endothelial cells, or both cell types simultaneously.

Due to the relatively low tissue penetration of NIR fluorescent light (5-10mm) hybrid tracers, integrating pre- and intraoperative diagnostic techniques, must be developed and validated to enhance tumor staging and diagnosis. As previously mentioned, the urokinase receptor (uPAR) plays an important role in the development of cancer, tumor invasion, angiogenesis, and metastasis. Chapter 9 shows the feasibility of an uPAR-recognizing hybrid agent to visualize tumors during image-guided resections using NIR fluorescence, whereas its nuclear component assisted in the preoperative non-invasive recognition of tumors using SPECT imaging. Nuclear and NIR fluorescent signals showed clear tumor delineation between 24h and 72h post-injection and 1-2 mm sized tumors could be clearly recognized by their fluorescent rim. Chapter 10 elaborates on the work described in Chapter 9 and assesses the feasibility of this innovative agent in a clinical relevant xenograft model for locoregional oral cancers. The hybrid agent showed the same optimal imaging window with clear co-expression between BLI, GFP and the NIR fluorescent signals in the tongue, while human cytokeratin staining confirms presence of malignant cells in the positive cervical lymph nodes. Generally, the multimodal strategy
can assist in surgical planning and subsequent precision surgery to reduce the number of incomplete resections. While its fluorescent signals can assist in the recognition of positive resection margins, during surgery and postoperative during the evaluation of the resection specimen at the pathology department.

This thesis describes the successful preclinical evaluation of four novel tumor-specific NIR fluorescent (hybrid) tracers with the potential to be translated for clinical use. If successful translated clinically, these tracers will provide a (solid) real-time identification and demarcation technique for the detection of tumors and will help improve the completeness of surgery and thus patient survival.