Targeted imaging in oncologic surgery : preclinical studies utilizing near-infrared fluorescence and radioactivity

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Chapter 1

General introduction and outline of thesis

GENERAL INTRODUCTION AND OUTLINE OF THESIS

Diagnosis, staging, and surgical planning of cancer patients increasingly rely on imaging techniques that provide information about tumor biology and anatomical structures [1-3]. Presently, single-photon emission computed tomography (SPECT) and positron emission tomography (PET) are the only widely implemented (targeted) imaging modalities used to provide insights into tumor location, tumor biology, and the surrounding micro-environment [1]. Both nuclear techniques depend on the pre-operative recognition of tumors and various monoclonal antibodies and peptides, initially developed as therapeutic agents (e.g. cetuximab, bevacizumab, labetuzumab), are labeled with radioactive tracers and evaluated for preoperative imaging purposes or applied for treatment monitoring of neo-adjuvant treatments [4-8]. However, translating information from these two techniques to the operating theatre is difficult due to alteration in body positioning, tissue manipulation by the surgeon and the lack of sensitivity for subcentimeter lesions. Presently, during oncologic surgery intraoperative ultrasound and/or gamma-counters are the only real-time imaging modalities incidentally utilized. To guide cancer resections, surgeons are therefore still mostly dependent on visual inspection and palpation to differentiate malignant disease from healthy surrounding tissues. As a consequence, tumor free resection margins and subclinical and deeper lying tumor foci are difficult to identify. For most solid tumors surgery is the primary treatment, and positive margins (R1, defined as tumor cells located at the edges of the surgical specimen) are associated with increased local recurrences and poor prognoses [9]. Therefore, better tumor detection methods must be developed to improve patient prognosis: Pre-operative to increase the reliability in prediction of resectability, and during the procedure to recognize tumors and resection margins with higher accuracy [10]. For example, surgery offers the sole chance of cure and long-term survival for pancreatic cancer patients, but is one of the most hazardous operations performed with a morbidity rate of 40-50%, positive resection margin rates of more than 50% and a 5-year survival rate of less than 5%. Presumably, cancer patients benefit directly from better tumor detection as the surgical status (R0 or R1) is an import prognostic factor for patient survival.

During the last decade, intraoperative navigation using fluorescence (Fluorescence-guided surgery, FGS) has emerged as a promising strategy to recognize cancer tissue or vital structures during surgery (see Figure 1 for concept). The principle of FGS consist of adding an exogenous fluorophore into the patient that creates a bright spot on a black background on the screen and permits high sensitive detection of any desired target within the surgical field [11]. Utilizing Near-Infrared (NIR) fluorescent light has advantage over visible light due to four key principles [12-16]: Photon absorption in living tissue is minimal between 650-900 nm and photon scatter is much lower in the NIR

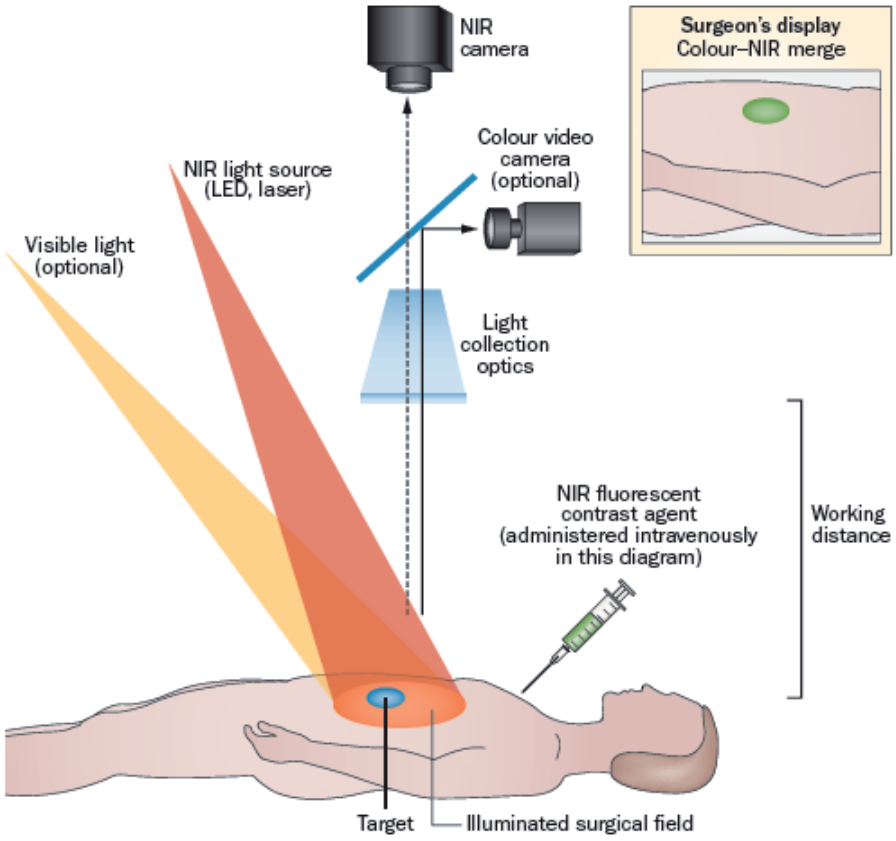

Figure 1: NIR fluorescent contrast agents are administered intravenously. During surgery, the agent is visualized using a NIR fluorescent imaging system of the desired form factor (above the surgical field for open surgery or encased within minimal invasive surgery). All systems must have adequate NIR excitation light, collection optics, filter sets and a camera sensitive to NIR fluorescent emission light. An optimal imaging system includes simultaneous visible (white) light illumination of the surgical field, which can be merged with the generated NIR fluorescence images. The surgeons display can be one of several form factors, including a standard computer monitor, goggles or a wall projector. Abbreviations: LED, light emitting diode; NIR, near-infrared. *Illustration and caption are depicted from Vahrmeijer et al., Nat Rev 2013 [14].*

than in the visible spectrum, both properties permit visualization of tumors and other important structures up-to 5-10 mm below its surface. Further, tissue auto-fluorescence is low in the NIR spectrum – minimizing background signals – and NIR light is invisible for the human eye and therefore does not change the surgical field [17]. Same as for SPECT/PET imaging, linking the (NIR) fluorophores to specific tumor-targeting vehicles, like antibodies or peptides, dramatically enhance the specificity of this technique as it will actively accumulate in the tumor. FGS provides higher spatial resolution than SPECT/PET imaging, provides direct anatomical feedback, and can be used for real-time clinical applications [2]. A powerful synergy can be achieved when these nuclear and fluorescent imaging techniques are combined, leading to improved diagnosis, patient management and surgical planning. Clinically, the advantages of hybrid tracers have

been shown in patients with melanoma and prostate cancer, but these studies used non-specific agents, following the natural lymph drainage pattern of colloidal tracers after peri-tumoral injection to identify the sentinel node [18, 19].

In short, FGS can identify tumor margins and suspicious lesions, allowing surgical guidance, which hopefully translates into more complete tumor resections. Hence, simultaneously, FGS can avoid damage to vital structures such as nerves, arteries or ureters preventing morbidity, and thereby most likely enhancing the quality-of-life significantly. Before routine clinical introduction of targeted (multimodal) FGS can be achieved three major hurdles must be challenged: 1) the choice for a suitable **target**, 2) the choice for an appropriate tumor-specific **tracer,** and 3) the availability of a validated, dedicated (NIR) fluorescence **imaging system.**

1 TARGETS

Not all tumor-associated biomarkers are suitable as oncotarget. If these biomarkers are homogeneously expressed on the cellular membrane in at least 10-times higher densities than on surrounding normal cells and freely available or accessible for the imaging agent then they are considered potential candidates [20]. Biomarkers can be subdivided by their biological function into receptors, anchoring proteins, enzymes, GPI-proteins and transporter proteins [21]. Among the most promising biomarkers for cancer-specific targeting therapies, as recently described by the National Cancer Institute, are the Epithelial Cell Adhesion Molecule (EpCAM) and the Carcinoembryonic Antigen (CEA) [22]. But also integrin $\alpha_V\beta_3$ and the urokinase Plasminogen-type Activator Receptor (uPAR) show promise and are extensively investigated [23]. Unlike CEA and EpCAM, $\alpha_V\beta_3$ and uPAR are not only up regulated on cancer cells but also on tumor-associated stromal cells like angiogenic endothelial cells, myofibroblasts and macrophages. The simultaneous targeting of these tumor and tumor surrounding stromal cells increases the percentage of the total tumor mass that will be targeted. Thereby increasing the absolute number of tumor related cells (e.g. malignant and stromal cells) that can be recognized, potentially increasing the ability to visualize micro-metastasis or tumors that show low biomarker expression.

2 TRACERS

When a biomarker with favorable characteristics is selected, this biomarker can be targeted using various types of (NIR) fluorescent tracers, consisting of a targeting vehicle

and a (NIR) fluorophore (e.g. ZW800-1, IRDye800CW, Cy5). Different types of vehicles can be used, ranging from (therapeutic) monoclonal antibodies, antibody fragments to larger and smaller peptides mimicking the natural receptor ligands such as GE-137 or EMI-137 for c-MET (HGFR), folate for FR-α and the RGD sequence for imaging of various integrins [24-26]. For tumor imaging, important characteristics are efficient tumor penetration and low affinity for surrounding normal tissue and, depending on the application, a reasonable half-life (hours)in the circulation [27]. Monoclonal antibodies are already widely investigated and show specific and sensitive tumor binding characteristics. Because of their large size (150 kDa), injected antibodies possess prolonged blood half-life times (up-to 72h) resulting in early high background signals and subsequently large imaging windows (24-96h). When shorter elimination times are desired, smaller vehicles like F(ab)s (50 kDa), scFv (27 kDa), nanobodies (27kDa) and/or small peptides (1-2 kDa) can be used. In general, the use of smaller tracers increase the tumor penetration capacity of the compound, decrease liver uptake, reduce background signals and shorten the time between injection and imaging. Nevertheless, all these tracers vary considerably in physical characteristics like size, affinity, charge, and possibility to conjugate to (fluorescent) dyes. Therefore, they offer specific advantages and disadvantages for the purpose of achieving appropriate tumor targeting. Various clinical grade (NIR) fluorophores are available that can be conjugated to tumor-specific vehicles like IRDye800CW and ZW800-1 [28, 29].

3 IMAGING SYSTEMS

The development of validated NIR fluorescent imaging systems is dependent on the (clinical) availability of NIR fluorescent tracers and vice versa. Therefore, presently, novel imaging systems are only sparsely developed and clinically introduced. Due to the lack of performance standards for these imaging systems, at present, the combination with a tracer is of major importance for successful functioning and evaluation [30, 31]. For example, the failure of a tracer to delineate tumors could be interpreted as a failure of the tracer, when in fact the unqualified device was simply not sensitive enough for detection. We mainly used a prototype of the FLARE^{TM} imaging system of which an updated version will become clinically available soon and can visualize both 700nm and 800nm signals simultaneously. Furthermore, we validated the novel Artemis
NIR camera system that can be adjusted to visualize 500nm, 700nm and 800nm fluorophores. Both systems already showed clinically feasibility in the surgical theatre for sentinel lymph node mapping, imaging of colorectal liver metastasis and imaging of ovarian cancer [32, 33].

This thesis is divided into two parts: **Part I** focuses on the evaluation of potential tumor targets for FGS and **Part II** describes the preclinical development and evaluation of novel tracers for (multimodal) fluorescent-guided surgery.

Part I, chapter 2 shows an overview of potential membrane-bound proteins for tumor targeting, and **Chapter 3** describes the use of tumor-associated stromal cells as target for fluorescent-guided surgery. **Chapter 4** describes the possible clinical applications of the oncotarget uPAR for imaging in cancer patients. **Chapter 5** explores the expression pattern of uPAR, its association with prognosis in colorectal cancer patients and its expression on various tumor-associated stromal cells.

In **part II**, **chapter 6**, CEA-specific NIR fluorescent imaging is used to visualize and delineate colorectal and pancreatic tumors. **Chapter 7** describes the preclinical development and validation of a novel clinically relevant EpCAM-directed antibody-fragment based NIR fluorescent agent. **Chapter 8** describes the preclinical work to allow administration of cRGD-ZW800-1 in Phase I clinical trials and describes the complete work-up needed for clinical translation. **Chapter 9** and **Chapter 10** show the feasibility of an uPAR-targeting multimodal antibody-based tracer to visualize colorectal and oral cancers during resections using NIR fluorescence, while its nuclear component assisted in the preoperative non-invasive recognition of tumors using SPECT imaging.

In **Part III** the future perspectives are discussed and the chapters are summarized.

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