INTRODUCTION

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RATIONALE
INTRODUCTION
Imaging is an essential element in cancer care. Modalities such as X-ray, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT) all have their place in cancer diagnosis, staging, treatment evaluation and follow-up. Preoperative imaging is used for tumor detection, assessment of tumor spread and localization of possible metastases and as such provides a guideline for the surgeon. During surgery however, the surgeon is limited to palpation and visual inspection when assessing the extent of tumor spread and the status of resection margins. Although frozen section analysis provides adequate pathological assessment of dissected tissue during surgery, this technique is time-consuming, not real-time and the amount of tissue that can be examined in this manner is limited.1 Intraoperative fluorescence imaging is a new modality that provides real-time feedback during surgery. Fluorescent agents, when excited by light of a specific wavelength, absorb and subsequently release a photon, thus emitting light of a slightly longer wavelength. Such agents may be administered either locally or systemically. Non-specific contrast agents, which do not bind to cells or structures, are generally used for visualization of anatomical structures like blood vessels (i.e. perfusion), lymphatic vessels and lymph nodes, and bile ducts. Tumor-specific contrast agents are selectively targeted at tumor cells and can be used for tumor delineation and assessment of resection margins. Light in the near-infrared (NIR) spectrum has the best properties for in vivo intraoperative purposes. This light is not visible by the naked eye, but can be detected by an intraoperative camera system.

REQUIREMENTS FOR INTRAOPERATIVE IMAGING
Sensitive intraoperative imaging calls for an unambiguous, bright signal. Colored dyes have been used for more than two decades for detection of the sentinel lymph node; however, these can be difficult to distinguish by the naked eye. The poor penetration of visible light through virtually all human tissue causes the dye to be detected only once the target tissue is fully exposed. Fluorescent agents have a greater contrast with the background and are therefore attractive for in vivo intraoperative imaging. The signal-to-background ratio (SBR) is most optimal in the near-infrared (NIR) range (750-1000 nm).2 Furthermore, NIR light has a larger penetration depth due to decreased scattering and absorption by the tissue. Shorter wavelengths fall in the visible light spectrum (400-750 nm), a range in which the signal is impeded to some degree by autofluorescence and absorption by hemoglobin. Infrared light has longer wavelengths (1000 nm-0.5 cm) and is to a large extent absorbed by water, making it unsuitable for in vivo imaging. (Figure 1)

Currently, only two fluorescent agents have been FDA-approved for clinical use; indocyanine green (ICG) and fluorescein isothiocyanate (FITC). The latter is used as a diagnostic compound in ophthalmology. FITC is suboptimal for intraoperative imaging because of its emission wavelength in the visible light range, causing more autofluorescence and absorption by hemoglobin in the tissue. For superficially spreading lesions, this drawback
is relative. ICG, in contrast, has an emission wavelength of around 795 nm, making it a suitable agent for NIR fluorescence imaging. ICG has been in use since the 1960s, primarily for the evaluation of liver perfusion and ophthalmic angiography, and has very low toxicity.³

Light in the NIR range is not visible by the naked eye; therefore, a specialized camera system is required to register and process the signal for intraoperative real-time imaging purposes. The system used for clinical studies reported in this thesis was developed at the Technical University Munich, Germany.⁴ This system consists of a white light source for illumination of the surgical field, a laser beam for excitation of the fluorescent dye, dichroic mirrors which separate the signal into visible light, intrinsic fluorescence and fluorescence, and sensitive charge-coupled device (CCD) cameras which transfer the signal into a color image and a fluorescence image. (Figure 2)

**This thesis** focuses on the technical aspects and the clinical application of intraoperative fluorescence imaging in surgical and gynecologic oncology.

**NON-SPECIFIC INTRAOPERATIVE FLUORESCENCE IMAGING**

Intraoperative imaging using a non-specific fluorescent agent is suitable for visualization of anatomical structures. **Part I** of this thesis is focused on this subject.

A relatively new application of the fluorescent agent ICG is intraoperative detection of the sentinel lymph node (SLN) in cancer. The SLN is the first draining lymph node from the tumor. (Figure 3) If this lymph node is free from metastases, full lymphadenectomy can be omitted, thus reducing morbidity. The bimodal approach using radiocolloid and a blue dye has proved to be safe in breast cancer, melanoma and vulvar cancer, and is currently regarded the gold standard.⁵-⁷ Disadvantages of this method are the use of radioactivity and the two-step sequence, as radiocolloid is injected several hours to one day prior to surgery.

In the last decade, several studies have been initiated to assess the value of intraoperative fluorescence imaging using ICG for SLN detection. Most data is available from clinical trials in breast cancer, most of which report detection rates comparable to the standard method.⁸⁹ Additionally, first results indicate a possible role for fluorescence imaging in skin cancer and gastric cancer.¹⁰¹¹ A pilot study on fluorescence SLN detection in breast cancer using the above mentioned camera system was performed in the University Medical Center Groningen; the results are described in **chapter 4**.

The applicability of SLN detection in cervical cancer is still matter of debate. The blue dye can be difficult to distinguish deep in the pelvis and the bilateral drainage from the uterine cervix can make lymphatic mapping unpredictable. However, as full pelvic lymphadenectomy is associated with lymphedema in approximately 25% of patients,¹² it is worth exploring new SLN detection methods. We hypothesized that the high signal-to-background ratio of ICG may be advantageous in SLN detection in the pelvic anatomy.
First clinical results are discussed in chapter 2.
As shown in a recent multicenter observational study, the SLN procedure significantly decreased both short-term and long-term morbidity in patients with vulvar cancer; however, the injections in the vulva with radiocolloid several hours to one day prior to surgery pose a painful burden on the patient. The major advantage of SLN detection with ICG is that it provides a one-step procedure during surgery, with the patient under anesthesia, while maintaining high sensitivity through transcutaneous lymphatic mapping and SLN localization. We conducted a pilot study to explore the applicability of fluorescence imaging for SLN detection in vulvar cancer, which is described in chapter 3.
The practical realization of intraoperative fluorescence imaging for SLN detection in cervical and vulvar cancer is illustrated by a video, which is presented in this thesis. The technical background is briefly described in chapter 5; the accompanying video is available on the supplementary CD.

TUMOR-SPECIFIC INTRAOPERATIVE FLUORESCENCE IMAGING
Tumor cells differ significantly from normal cells due to the up- or downregulation of several genes, which may ultimately lead to different expression rates of biomarkers. Targeting a tumor-specific biomarker yields a high tumor-to-normal ratio in diagnostic imaging, whereas a targeted drug that affects only tumor cells and not healthy cells may increase therapeutic efficacy and decrease morbidity. The advantages of tumor-targeting may be obvious; however, target-finding is complex. Every tumor type has its unique biological profile and genomic fingerprint, therefore biomarker expression rates vary greatly between cancers.13 Chances of finding a tumor biomarker that can be used as a ‘one-size-fits-all’ therapeutic or diagnostic marker are small. Exceptions are metabolic markers or markers for more general phenomena in cancer growth like angiogenesis. Consequently, selection of potential tumor-specific targets requires thorough research for every tumor type. Part II of this thesis concentrates on tumor-targeting.
In chapter 6 we present a summary of the several animal models that have been developed in cancer, by using hypoxia in esophageal cancer as an example. Hypoxia plays a role in several solid tumors and is correlated with a worse prognosis. In order to counteract the disadvantageous effects of hypoxia, extensive knowledge of its origin and consequences is indispensable. Furthermore, hypoxia-responsive elements such as VEGF and EGFR are interesting targets for both imaging and therapeutic agents in several cancer types as these factors are frequently upregulated prior to dissemination as is the case in peritoneal carcinomatosis. Animal models are useful for preclinical testing of hypotheses to counteract hypoxia and this review delineates the promises and problems of using such models. The largest value of tumor-targeted intraoperative imaging lies in improved detection of tumor deposits and evaluation of resection margins. In cancers with a peritoneal spreading pattern, such as epithelial ovarian cancer (EOC) and colorectal cancer (CRC), the extent of surgical cytoreduction is a major prognostic factor.14,15 Intraoperative imaging
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using a tumor-specific fluorescent agent may aid the surgeon in staging efforts and in detection and removal of a larger number of tumor spots, thus increasing prognosis. Furthermore, the extent of cytoreduction is of importance in patients eligible for hyperthermic intraperitoneal chemotherapy (HIPEC), as the chemotherapeutics have a limited penetration depth and better exert their function in tumor deposits <2 mm.16,17

Several studies have focused on the expression of biomarkers in EOC or CRC, however, not every marker expressed by a tumor cell is suitable for diagnostic or therapeutic purposes. In chapter 7, we provide an overview of the most promising targets for tumor-specific intraoperative imaging in EOC. This review article focuses on biomarkers that have already been successfully targeted in preclinical studies, and thus are most suitable to find their way into the clinic for tumor-targeted imaging. Similarly, Chapter 8 focuses on targets in CRC, with the emphasis on target selection. We provide a novel scoring system, the TArget Selection Criteria (TASC), which may help in selecting targets that are most suitable for clinical translation.

One of the most promising biomarkers in EOC is the folate receptor alpha (FR-α), as it is overexpressed in 80-90% of cases and virtually absent on healthy cells.18 Furthermore, its substrate folate binds to the receptor with high affinity, is non-toxic and inexpensive, and can be easily conjugated to drugs or imaging agents. Upon endocytosis of the folate conjugate, the FR-α is recycled back to the cell surface within minutes, where it is instantly ready to bind circulating folate.19 In the last decade, several folate-based imaging agents and drugs have been developed.18 However, few data are available on the effect of chemotherapy on expression of FR-α. As (neo-)adjuvant chemotherapy is an important element in the treatment of EOC, targeted agents are only relevant if expression of the target is not affected by chemotherapy. In order to determine the effect of chemotherapy on FR-α expression, and thus the feasibility of folate-based agents in EOC, we evaluated FR-α expression on 361 ovarian cancer tissue samples, including 28 matched samples pre- and postchemotherapy. Additionally, we assessed whether FR-α expression is related to survival. This study is described in chapter 9.

Based on the overexpression of FR-α in EOC, we hypothesized that a folate-based fluorescent agent might be suitable for intraoperative imaging. Currently, the only clinical grade imaging agent available is folate-FITC, which was initially developed for vaccination studies under the code name EC17 (Endocyte Inc.). We conducted a first-in-human pilot study, discussed in chapter 10, to evaluate the technical feasibility of intraoperative fluorescence imaging using folate-FITC in ovarian cancer patients.

In chapter 11 and chapter 12, the results of the studies are summarized, and suggestions for future approaches to intraoperative imaging and tumor-targeting are discussed.
REFERENCE LIST


