Fluorescence imaging during abdominal surgery: real-time imaging of ureters and malignancies

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PART 1

CLINICAL TRANSLATION OF NOVEL ZWITTERIONIC AGENTS

Chapter II

THE CLINICAL TRANSLATION OF NOVEL NEAR-INFRARED FLUOROPHORES FOR FLUORESCENCE GUIDED SURGERY

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ABSTRACT

Near-infrared fluorescence imaging is a promising intraoperative technique for real-time visualization of vital structures and tumor tissue during surgery. This manuscript describes the applications and limitations of NIR fluorescence imaging, and provides a general overview of two novel fluorescent agents and the process of clinical translation. The process of clinical translation of novel fluorescent agents is an essential part in the evolution of NIR fluorescence guided surgery. Treatments and surgeries are constantly advancing, which can occasionally cause challenges or difficulties for the surgeons. Poor visualization of tumors during surgery is one of the major challenges surgeons often face in oncologic patients, mainly due to the improved neo-adjuvant treatment patients receive. In these cases, NIR fluorescence imaging with the use of tumor-targeted fluorescent agents can play an essential role and help provide better results or outcomes. However, before this technique can be implemented in standard of care, optimal tumor-targeted fluorescent agents need to be developed and novel fluorescent agents need to undergo a successful process of clinical translation.

INTRODUCTION

Intraoperative near-infrared (NIR) fluorescence imaging is a novel technique that combines the use of a fluorescent agent with a dedicated NIR camera system, to allow real-time visualization of lymph nodes, tumor tissue and/or vital anatomic structures for surgical guidance.[1] NIR light ranges in the wavelength of 700-900 nanometers, is invisible to the naked eye and can only be detected with the use of a dedicated NIR imaging system, which are currently available from various commercial companies. Fluorescent agents are mainly administered intravenously at a given time prior or during surgery, in order to enable real-time imaging during surgery. The time of injection is dependent on the distribution factors of the specific agent. After injection the fluorescent agent is cleared by either the liver and/or kidneys and accumulates in the target (i.e. lymph node, tumor or vital structure) by either physiological processes (enhanced permeability and retention effect), specific targeting or through their clearance route.

Enhancing tumors or vital structures using NIR (tumor-targeted) fluorescent agents is an innovative technique that is currently under extensive development. Visual inspection and palpation have always been the principle methods in surgery to differentiate between different types of tissue or uncover vital structures. However, due to the technical advancements, such as the introduction of laparoscopes and robots, the palpation aspect in surgery has disappeared in most cases and surgeons are limited to visual inspection. With the use of NIR fluorescence imaging, tumor tissue and vital structures can be enhanced in real-time during surgery, to assist surgeons and conceivably improve surgical (i.e. improved radical resections and less iatrogenic damage of vital structures) and patient outcomes.

A known challenge in the field of fluorescence-guided surgery is the development of ideal NIR fluorophores. Currently, the only clinically available NIR fluorophores are methylene blue (MB) and indocyanine green (ICG), which have shown successful imaging results in different clinical studies.[2–4] However, both MB and ICG are non-targeted fluorophores and cannot easily be conjugated to other molecules, which is conversely an essential part for the development of tumor-targeted fluorescent agents. For advancement in the field of NIR fluorescence guided surgery, the following steps need to embrace the development and clinical translation of novel conjugatable fluorophores.

NOVEL FLUORESCENT AGENTS

In the past years, several translational studies have been conducted with different kind of tumor-targeted fluorescent agents.[5–10] However the most optimal agent still needs to be found. Recently, a promising novel fluorophore has been introduced that could potentially cause a paradigm shift in fluorescent guided surgery.

zw800-1

zw800-1 is a novel fluorophore, geometrically neutral, with unique and improved optical and biodistribution properties. This fluorophore emits light at a wavelength of approximately 788 nm, produces a high fluorescent signal with low non-specific binding and uptake in normal tissue and most importantly, it has a renal-exclusive clearance.[11,12] This extraordinary clearance route is a major step forward for reliable intraoperative imaging of gastrointestinal tumors. ICG, for example, is a well-known fluorophore and has been used in several different imaging indications, but has an exclusive hepatic clearance, which is often disadvantageous. This clearance route results in high uptake in the liver, often resulting in compromised imaging of the gastrointestinal tract (i.e. gastrointestinal tumors).[3]

zw800-1, however, was extensively investigated in small and large animal studies and showed that the renal-exclusive clearance route is particularly suitable for NIR fluorescence imaging of the ureters.[12,13] As iatrogenic ureteral injury is a feared complication of lower abdominal surgery, the clinical need

for improved visualization of these vital structures is sought.[14–17] Most of the ureteral injuries occur in patients during oncologic surgeries, as these patients frequently have increased risk factors, such as previous multiple surgeries and/or radiotherapy in the pelvic area. An urethral injury can often be restored when detected during the surgical procedure, however most injuries remain undetected and come to light a few days later resulting in long-term complications and morbidity. As avoiding damage to vital structures is of clinical importance during surgery, NIR fluorescence imaging with ZW800-1 may be a novelty for adequate and safe ureter mapping during surgery.

Regardless of the fact that ZW800-1 can be used to create a major step forward in the clinic with ureter imaging, the true promise of this novel fluorophore lies in the conjugation to targeting moieties. ZW800-1 can easily be conjugated to other molecules, creating endless possibilities for developing novel targeted ligands. These targets can either be tumor-specific molecules, known to be upregulated on malignant cells or tumor-associated tissue, or mechanisms involved in tumor survival such as neoangiogenic vessel formation or stroma. This concurrent advantage of ZW800-1 can potentially solve the longstanding problem of optimal targeted-fluorophore development.

cRGD-ZW800-1

Tumor visualization with NIR fluorescence imaging is an innovative and powerful surgical tool that can aid surgeons during oncologic surgery. Discriminating between malignant and benign tissue during surgery can occasionally be challenging as oncologic patients frequently receive neo-adjuvant chemo-radiotherapy, which often results in diminished tumor visibility during surgery. Different strategies, such as the use of tumor-targeting agents, are being explored to overcome the problem of poor tumor visibility.

The different hallmarks of cancer describe the underlying principles for the existence of cancer cells and can be used as a guideline for the development and identification of tumor-specific targets for fluorescence surgery. An effective target for NIR tumor imaging is integrins associated with neoangiogenesis, such as $\alpha v\beta_3$. The integrin $\alpha v\beta_3$ is a receptor for RGD-containing proteins, and has shown to be upregulated in the process of angiogenesis.[18,19] Tumors larger than 1-2 cm depend on neoangiogenesis to acquire sufficient oxygen and nutrients to grow.[20] Therefore, integrin $\alpha v\beta_3$ is highly and widely expressed on angiogenic endothelium, in both tumor stroma and tumor cells, and not on normal human tissue.[21,22]

cRGD is a peptide that recognizes and binds to the integrins associated with neoangiogenesis, therefore being a functional target to enable NIR imaging of tumors. This resulted in a development of a novel tumor-targeted agent, cRGD-ZW800-1, which subsequently has already preclinically been validated *in vitro* on glioblastoma and colorectal cell-lines, and also in orthotopic mouse models with colorectal, breast, pancreatic and head-and-neck tumors.[23] According to the preclinical data this novel tumor-targeted fluorescent agent has the potential to become a useful agent for real-time intraoperative imaging of different cancer types.

CLINICAL TRANSLATION

The pathway from invention to proof of efficacy, i.e. clinical adaption, of novel fluorescent agents is a costly and time-consuming path. For successful implementation in the clinic, several important steps need to be completed.

Development of a novel fluorescent agent

The number one step is the development of a promising fluorescent agent. A clinical need to improve the intraoperative detection of either a vital structure or specific kind of malignancy during surgery is needed to initiate the development of a novel agent. Once this has been determined, the following vital step is identifying the specific target that will enable the NIR imaging. As previously mentioned the different hallmarks of cancer are often used as a guideline for the identification of tumor-specific targets. Subsequently, this target will need to be conjugated to a fluorescent dye to form a fluorescent agent suitable for NIR imaging. This novel agent will then undergo pilot testing in animal models to determine whether further translation is useful.

Preclinical studies

Preclinical studies are of great importance, as it acts as a pilot study to determine whether further studies are consequential, and most importantly they are crucial in the step towards the clinic (i.e. humans) as the preclinical data is needed to validate and determine the toxicity of a novel agent before it gets exposed to humans. The studies provide useful information regarding the toxicology as well as the initial diagnostic value of a new agent. Once the novel agent has passed the validation process of whether it is useful for further employment, it will undergo the Good Manufacturing Practice (GMP) production so that it can be used in humans. The GMP batch will yet again undergo, more extensive, toxicity testing in animals.

For any first-in-human study, the toxicological profile is needed to determine a safe starting dose for humans. The guideline of the Food and Drug

Administration (FDA) is usually recommended, where the approach is based on the no-observed adverse event level (NOAEL) in animals, specifically in the most sensitive species in the preclinical toxicology study.[24] This dose is then calculated to the human equivalent dose using an algorithm and safety factors.

In most preclinical studies, especially for new therapeutic drugs for example, the maximum tolerated dose is also recognized. However, a major difference between therapeutic study drugs and fluorescent agents is that for fluorescent agents the maximal tolerated dose is not necessarily required to obtain the best result. The effect of a fluorescent agent is based on the ratio of fluorescence seen in the target (i.e. tumor) and the surrounding normal tissue, which is usually achieved with well below therapeutic dosage. The ratio between the target and the surrounding tissue is also known as the tumor/target-to-background ratio (TBR) and the higher the ratio, the more distinct the fluorescence of the target will be. Experience has shown that low doses of a fluorescent agent is often enough to achieve an optimal effect, as high doses frequently lead to unwanted excessive background fluorescence, resulting in low TBRs. Therefore, in studies with fluorescent agents a more informative concept can be applied; the concept of the 'pharmacological active dose' where lower conservative doses can be employed to obtain the effective result.[25] With this concept possible irrelevant and unsafe high dosages can also be avoided.

Phase I: First-in-human studies

After completion of the preclinical studies and approval by the medical ethics committee, the first major step in the clinic can be performed, which is the exposure of the novel agent in humans. First-in-human studies with novel fluorescent agents can be performed in either healthy volunteers or on a selected group of the target patients. However, a first-in-human study in healthy volunteers is superior to patients. The safety, tolerability, pharmacokinetics and pharmacodynamics of the fluorescent agent can adequately be studied in a controlled setting with a homogenous healthy subject population, based on medical screening, minimalizing contamination of safety data. Additionally, it prevents that vulnerable (oncologic) patient groups are being exposed to potentially harmful adverse events. Furthermore, easy escalation and de-escalation procedures can take place where different doses can be evaluated in a short period of time. Pharmacokinetics can also be assessed in more detail without having to interrupt standard of care procedures. Blood and urine samples can be obtained and fluorescence imaging of the skin, for example, can be performed at specified time points which provides valuable information regarding the time window of infusion to imaging (in the surgical setting) in a time and cost effective manner.

Phase II: Patient studies

Once the novel agent has proven to be tolerable and safe in healthy volunteers, it can be implemented in patients to study the feasibility, optimal dose and timing of injection for suitable and optimum fluorescence imaging. The pharmacokinetic data obtained in healthy volunteers can be effective in designing an efficient patient study, as the pharmacokinetics in healthy volunteers can fairly predict the dose range and injection time range of the fluorescent agent for further assessment. This is crucial as the guiding principle in these studies is to avoid exposing too many (vulnerable) patients to suboptimal doses. Due to the frequently adequate dose and timing predications, patient studies can be completed in a timely manner while preserving safety of the patients. Blood and urine samples are often also collected in patients, which can substantiate the pharmacokinetic results in healthy volunteers. Furthermore, determining the efficacy of the fluorescent agent is also accomplished in patients, where the assessment and concordance of the fluorescence with the pathology results (histological evidence of tumor for example) is performed.

Phase III/IV trials

Once the feasibility, efficacy, optimal dose and injection time has been established in the phase I/II studies, the translation of the agent is proceeded in a phase III trial. In this phase the goal is to acquire more proof for the benefit of the imaging technique using the novel fluorescent agent in a large patient population group and compare it to the standard of care procedures. These studies are therefore often blinded and randomized for adequate validation. Phase IV studies are often also known as post-marketing studies and are usually done after clinical approval for optimization of the product.

CONCLUSION

The use of NIR imaging and novel fluorescent agents are currently under wide development and exploration. NIR imaging itself is an emerging field with great potential to improve and change the surgical practice. However, major steps still need to be taken before this technique can be used in standard of care for oncologic procedures. The clinical translation of ZW800-1 and CRGD-ZW800-1 are rapidly on its way, where phase I/II studies have been performed. The package of preclinical, healthy volunteer (phase I) and patient (phase II) data have proven to be important and indispensable in the design of the ensuing phase III/IV studies, for cost-effective and timely adaption in standard-of-care. The unique collaboration within the Image Guided Surgery group in Leiden, the Netherlands,

between the Centre for Human Drug Research (CHDR) and Leiden University Medical Center (LUMC), has helped develop an optimal roadmap for the clinical translation of promising fluorescent agents.

This collaboration has also permitted the clinical translation of other tumor-targeted agents such as OTL38 (a folate receptor alpha (FRα) targeting moiety), SGM-101 (a carcinoembryonic agentic (CEA) targeting agent), and VB5-845D-800CW (an Epithelial Adhesion Molecule (EPCAM) specific agent). NIR fluorescence imaging has emerged rapidly with significant potential for clinical efficacy, especially in the field of surgical oncology. Despite its progress, there are still many opportunities for growth in the field, such as advancements in NIR clinical imaging systems and further refinement of imaging agents to provide better precision and clarity necessary for clinical translation.

REFERENCES

1 Vahrmeijer AL, Hutteman M, van der Vorst JR et al. Image-guided cancer surgery using near-infrared fluorescence. Nat Rev Clin Oncol **9**, 507-518 (2013).

2 Boogerd LS, Handgraaf HJ, Lam HD, et al. Laparoscopic detection and resection of occult liver tumors of multiple cancer types using real-time near-infrared fluorescence guidance. Surgical endoscopy **31**, 952-961 (2016).

3 Schaafsma BE, Mieog JS, Hutteman M, et al. The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery. J Surg Oncol **104**, 323-332 (2011).

4 Verbeek FP, van der Vorst JR., Schaafsma BE, et al. Intraoperative near infrared fluorescence guided identification of the ureters using low dose methylene blue: a first in human experience. J Urol **2**, 574-579 (2013).

5 Hoogstins CES, Boogerd LSF, Sibinga Mulder BG, et al. Image-Guided Surgery in Patients with Pancreatic Cancer: First Results of a Clinical Trial Using SGM-101, a Novel Carcinoembryonic Antigen-Targeting, Near Infrared Fluorescent Agent. Ann Surg Oncol **11**, 3350-3357 (2018).

6 Boogerd LSF, Hoogstins CES, Schaap DP, et al. Safety and effectiveness of SGM-101, a fluorescent antibody targeting carcinoembryonic antigen, for intraoperative detection of colorectal cancer: a dose-escalation pilot study.Lancet Gastroenterol Hepatol **3**, 181-191 (2018).

7 Hoogstins CE, Tummers QR, Gaarenstroom KN, et al. A Novel Tumor-Specific Agent for Intraoperative Near Infrared Fluorescence Imaging: A Translational Study in Healthy Volunteers and Patients with Ovarian Cancer. Clin Cancer Res **12**, 2929-38 (2016).

8 Boogerd LSF, Hoogstins CES, Gaarenstroom KN, et al. Folate receptor-α targeted near-infrared fluorescence imaging in high-risk endometrial cancer patients: a tissue microarray and clinical feasibility study. Oncotarget **1**, 791-801 (2017).

9 Tummers QR, Hoogstins CE, Gaarenstroom KN, et al. Intraoperative imaging of folate receptor alpha positive ovarian and breast cancer using the tumor specific agent EC17. Oncotarget **22**, 32144-55 (2016).

10 Handgraaf HJM, Sibinga Mulder BG, Shahbazi Feshtali S, et al. Staging laparoscopy with ultrasound and near infrared fluorescence imaging to detect occult metastases of pancreatic and periampullary cancer. PLoS One **11** (2018).

11 Choi HS, Gibbs SL, Lee JH, et al. Targeted zwitterionic near-infrared fluorophores for improved optical imaging. Nat Biotechnol **2**, 148-153 (2013).

12 Choi HS, Nasr K, Alyabyev S, et al. Synthesis and *in vivo* fate of zwitterionic near-infrared fluorophores. Angew Chem Int Ed Engl **28**, 6258-6263 (2011).

13 Hyun H, Bordo MW, Nasr K, et al. CGMP-Compatible preparative scale synthesis of near-infrared fluorophores. Contrast Media Mol Imaging **6**, 516-524 (2012).

14 Delacroix Jr SE, Winters JC. Urinary tract injures: recognition and management. Clin Colon Rectal Surg **2**,104-112 (2010)

15 Andersen P, Andersen LM, Iversen LH. Iatrogenic ureteral injury in colorectal cancer surgery: a nationwide study comparing laparoscopic and open approaches. Surg Endosc **6**, 1406-1412 (2015).

16 Engel O, Rink M, Fisch M. Management of iatrogenic uretral injury and techniques for uretreral reconstruction. Curr Opin Urol **4**, 331-335 (2015)

17 Minas V, Gul N, Aust W, et al. Urinary tract injuries in laparoscopic gynaecological surgery; prevention, recognition and management. TOG **1**, 19-28 (2014).

18 Cheresh DA. Human endothelial cells synthesize and express an Arg-Gly-Asp-directed adhesion receptor involved in attachment to fibrinogen and von Willebrand factor. Proc. Natl. Acad. Sci **18**, 6471-6475 (1987).

19 Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alphav beta3 for angiogenesis. Science **5158,** 569-571 (1994).

20 Naumov GN, Akslen LA, Folkman J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch. Cell Cycle **16**, 1779-87 (2006).

21 Beer AJ, Niemeyer M, Carlsen J, et al. Patterns of alphavbeta3 expression in primary and metastatic human breast cancer as shown by 18F-Galacto-RGD PET. J Nucl Med **2**, 255-9 (2008).

22 Avraamides CJ, Garmy-Susini B, and Varner VA. Integrins in angiogenesis and lymphangiogenesis. Nat Rev Cancer **8**, 604-617 (2008).

23 Handgraaf JMH, Boonstra MC, Prevoo HAJM et al. Real-time near-infrared fluorescence imaging using cRGD-ZW800-1 for intraoperative visualization of multiple cancer types. Oncotarget **13**, 21054-12066 (2017).

24 FDA. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. 2340-2341

25 Cohen A. Should we tolerate tolerability as an objective in early drug development? Br J Clin Pharmacol **64**, 249-252 (2007).