



Ovarian cancer-targeted near-infrared fluorophores for fluorescence-guided surgery

Satoshi Kashiwagi, Hak Soo Choi

Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Correspondence to: Satoshi Kashiwagi, MD, PhD. Gordon Center for Medical Imaging, Department of Radiology, Massachusetts General Hospital, 149 13th Street, Charlestown, MA 02129, USA. Email: skashiwagi@mgh.harvard.edu.

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Introduction

Ovarian cancer is typically diagnosed at a late stage with widespread peritoneal dissemination. As a result, it remains one of the deadliest cancers in women worldwide (1). The standard treatment for most frequent epithelial ovarian cancer is debulking surgery followed by chemotherapy. However, surgical removal of small, disseminated nodules in multiple anatomical sites becomes increasingly challenging once ovarian cancer spreads in the peritoneal cavity. Yet, the low 5-year survival rate of patients at advanced stages III and IV (<25%) has not improved significantly over the past decades (2). Since the amount of residual tumor following debulking surgery is the most significant prognostic indicator for patients at advanced stages, complete tumor resection upon debulking surgery is highly desired (3). However, surgeons rely primarily on visual inspection and palpation to identify lesions without real-time guidance, which may lead to oversight of occult tumors and poor outcomes and recurrence. Conventional imaging modalities, including CT, MRI, and ultrasound, are unsuitable for detecting small, disseminated nodules on the peritoneal surface and providing real-time, high-resolution intraoperative imaging guidance for debulking surgery of ovarian cancer.

Fluorescence-guided surgery (FGS) is an optical imaging technology that potentially improves the prognosis of ovarian cancer. FGS typically employs near-infrared (NIR) fluorophores to cancer of interest and/or normal tissue

and provides surgeons with real-time, high-resolution, and sensitive image guidance (4). FGS has been approved for various procedures, including identification of tumor margin, sentinel lymph node (LN) mapping, angiography, and lymphography (4) and has been shown to improve tumor resection rates while minimizing normal tissue damage (5). Therefore, it is a feasible imaging modality for ovarian cancer surgery.

An unmet clinical need in ovarian cancer surgery—a targeted imaging agent

Multiple contrast agents have been used or are in active development for FGS, including multifunctional nanoparticles, antibody-dye conjugates, and small-molecule fluorophores. However, nanoparticles often show undesired biodistribution and pose safety concerns (4), while antibody-dye conjugates are generally too large to penetrate deeply into tumor tissue after targeting specific biomarkers and show nonspecific accumulation in the excretory organs. Due to the slow clearance, antibody-dye conjugates also pose a prolonged washout time after administration. Small molecule fluorophores distribute quickly in their target tissues, and unbound molecules can be excreted rapidly (4). Indocyanine green (ICG; molecular weight = 774.96) is an FDA-approved small NIR fluorophore and takes advantage of tumoral uptake as a result of the enhanced permeability and retention (EPR) effect and has been tested for visualizing ovarian cancer tissues in debulking

surgery (6). However, ICG showed significant limitations with low sensitivity and specificity (62% false positive rate), poor tumor-to-background ratio (TBR), and higher liver and gastrointestinal tract uptake due to its non-targeted nature. A targeted fluorophore conjugate for cancer has been developed to reconcile these issues. This approach includes the use of cyclic arginine-glycine-aspartate motif (cRGD) or folate analog targeting high expression of integrin $\alpha v \beta 3$ or the folate receptor α (FR α) in tumor tissue, respectively. Clinical data showed that these approaches generally exhibit rapid renal clearance and typically require a 2–18 h waiting time prior to surgery (7–9), which is significantly shorter than other approaches.

Folate receptor α -targeted near-infrared fluorophore for ovarian cancer imaging

To date, the FR α -targeting strategy has been explored most for cancer imaging and reached a significant clinical stage for lung and ovarian cancer imaging (3). More specifically, FR α is overexpressed in >90% of epithelial ovarian cancers (10) and, therefore, serves as an excellent surface biomarker to detect ovarian cancer. Dr. Philip Low's group at Purdue University has developed a small molecule ligand–organic fluorophore conjugate to specifically target tumor tissues (3), which is much smaller than nanoparticles and antibody conjugates. Tanyi *et al.* recently completed a Phase III study showing that pafolacianine (a.k.a. Cytalux, OTL38), a folic acid conjugated NIR dye, could intraoperatively identify additional cancers on tissue not detected by visual inspection and palpation in 33% of patients out of 109 FR-positive ovarian cancer patients (1). As expected, rapid and highly sensitive NIR imaging was possible upon a single intravenous infusion of pafolacianine 1 h prior to intraoperative imaging. Although assessment of the long-term clinical benefit of this technology, including a reduction in disease recurrence and improvement in overall survival, has not been completed, these favorable results led to the first FDA approval of an optical imaging agent with tumor targetability. Thus, pafolacianine opened a new era of FGS, which had heavily relied on non-targeted fluorophores during the past 6 decades.

Limitations of FR-targeted NIR fluorophore conjugate

A series of clinical studies demonstrated the high efficacy of pafolacianine but, at the same time, also revealed potential

limitations (1,9,11), suggesting room for improvement and innovation in this area. This review offers a discussion on three critical areas of improvement.

High false-positive rate

In the Tanyi *et al.*'s study (1), a high false positive (fluorescence positive but not confirmed by pathology) rate was consistently noted, while no false negatives were reported. The subject false-positive rate was 24.8% based on 27 FR-positive subjects in whom all lesions detected by fluorescence imaging only were determined to be histologically negative. At the lesion level, the false-positive rate was 32.7% based on 221 false-positive lesions identified out of 616 fluorescence-positive lesions. In previous clinical studies of pafolacianine, these false positives were most commonly detected in the LNs (9,12). It is established that activated macrophages overexpress folate receptor β (FR β) (13) and could be a source of false positives. Histologically, FR β staining was co-localized with CD68+ macrophages in the subcapsular sinuses of LNs, which were free from histologically confirmed micro-metastases (12), supporting the notion that false positive fluorescence is caused by pafolacianine binding to FR β + macrophages in the LNs. A discussion was provided that this false positive signal should not impede the application of pafolacianine because comprehensive lymphadenectomy should be pursued in surgical staging procedures anyway (14). While a positive correlation between the number of resected LNs and overall survival is established for early-stage ovarian cancer patients (15), systematic lymphadenectomy does not benefit patients with advanced ovarian cancers (16). Thus, pafolacianine may need to be used selectively depending on factors such as disease stages and histologic subtypes, which may show differences in LN metastasis rates. In the series of clinical studies, false positives were also seen in the omentum and uterine fibroid other than LNs. In fact, FR β has been shown to be a useful marker not only for macrophages in inflamed tissues and cancer but also for tissue-resident macrophages in normal tissues (10,17). Therefore, benign lesions such as fibrosis and inflammation could be positive for fluorescence signals. These results show that surgeons need to be familiar with all the possible causes of false positive results during FGS to interpret the positive spots correctly.

Complicated chemical synthesis

The chemical synthesis of pafolacianine still relies on

conventional bioconjugation, which requires an extra step of chemistry. ICG is a single small organic molecule and ideal for large-scale and reproducible production at a low cost, which holds an advantage for clinical translation. We have recently reported an ultracompact and ovarian cancer-targeted small molecule NIR fluorophore (5). In this approach, we have used the “structure-inherent targeting (SIT)” concept (18), where all the necessary components for specific tissue targeting and optical imaging are integrated into a small molecule without an additional bioconjugation (5). A squaraine fluorophore (OCTL14) provided durable intraoperative imaging in a preclinical murine model of ovarian cancer between 2- and 24-h post-injection. Contrary to the commonly used ICG, SIT probes are tuned for lipophilicity and noncharged structures, which allows for high uptake in a specific tissue, such as bone (19), cartilage (20), and nerve (21). It is a single small molecule that rapidly distributes in target tissues and is eliminated from the background and eventually from the body to achieve a high TBR and low potential toxicity. Importantly, the SIT strategy allows for large-scale, rapid, and reproducible production with reasonable costs for clinical use. This SIT approach omits a bioconjugation step and can be considered for the next generation of cancer-targeted contrast agents for FGS.

Suboptimal optical property of pafolacianine

Pafolacianine has shorter excitation/emission wavelengths (776/793 nm) (3) compared to ICG (780/820 nm). In order to fully visualize the fluorescence signals of pafolacianine, the current imaging systems optimized for ICG would require further adjustments of optical path and light source. However, it is not always a feasible option in most clinical settings. Therefore, the optical property of current fluorophores under development should be close to ICG than pafolacianine to improve sensitivity and thus facilitate their clinical translation (22). Alternatively, the use of a new fluorophore which enables optical imaging in the second NIR spectral window (NIR-II; wavelengths of 1,000–1,700 nm) could be considered for bioconjugation to a folate analog because NIR-II light allows for deep tissue imaging at a high resolution and sensitivity, owing to reduced light scattering, minimal light absorption, and ultralow levels of autofluorescence (23). However, the current NIR-II fluorophores show poor water solubility and biocompatibility, which is not ideal for this purpose per se. Thus, there is an unmet need for an innovative

strategy to create a water-soluble NIR-II fluorophore for bioconjugation.

Conclusions

Pafolacianine (Cytalux, OTL38), the first-of-its-kind targeted agent for optical imaging of cancer, showed a promising result in Phase III clinical trials to potentially facilitate the complete resection of cancerous tissues and improve the overall survival of ovarian cancer patients. To further improve the sensitivity and specificity of optical imaging agents, the structure inherent targeting strategy and imaging in the NIR-II optical window could be adapted to create the next generation of molecular probes.

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