Editorial Commentary

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

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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 αvβ3 or the folate receptor α (FRα) 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 FRα-targeting strategy has been explored most for cancer imaging and reached a significant clinical stage for lung and ovarian cancer imaging (3). More specifically, FRα 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 Perdue 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 (FRβ) (13) and could be a source of false positives. Histologically, FRβ 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 FRβ+ 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, FRβ 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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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Tanyi JL, Randall LM, Chambers SK, et al. A Phase III Study of Pafolacianine Injection (OTL38) for Intraoperative Imaging of Folate Receptor-Positive Ovarian Cancer (Study 006). J Clin Oncol 2023;41:276-84.
2. Lee JY, Kim S, Kim YT, et al. Changes in ovarian cancer survival during the 20 years before the era of targeted therapy. BMC Cancer 2018;18:601.
3. Mahalingam SM, Kularatne SA, Myers CH, et al. Evaluation of Novel Tumor-Targeted Near-Infrared Probe for Fluorescence-Guided Surgery of Cancer. J Med Chem 2018;61:9637-46.
4. Ji Y, Jones C, Baek Y, et al. Near-infrared fluorescence imaging in immunotherapy. Adv Drug Deliv Rev 2020;167:121-34.
5. Fukuda T, Yokomizo S, Casa S, et al. Fast and Durable Intraoperative Near-infrared Imaging of Ovarian Cancer Using Ultrabright Squaraine Fluorophores. Angew Chem Int Ed Engl 2022;61:e202117330.
6. Veys I, Pop FC, Vankerckhove S, et al. ICG-fluorescence imaging for detection of peritoneal metastases and residual tumoral scars in locally advanced ovarian cancer: A pilot study. J Surg Oncol 2018;117:228-35.
7. de Valk KS, Deken MM, Handgraaf HJM, et al. First-in-Human Assessment of cRGD-ZW800-1, a Zwitterionic, Integrin-Targeted, Near-Infrared Fluorescent Peptide in Colon Carcinoma. Clin Cancer Res 2020;26:3990-8.
8. van Dam GM, Themelis G, Crane LM, et al. Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor-α targeting: first in-human results. Nat Med 2011;17:1315-9.
9. Randall LM, Wenham RM, Low PS, et al. A phase II, multicenter, open-label trial of OTL38 injection for the intra-operative imaging of folate receptor-alpha positive ovarian cancer. Gynecol Oncol 2019;155:63-8.
10. O'Shannessy DJ, Somers EB, Wang LC, et al. Expression of folate receptors alpha and beta in normal and cancerous gynecologic tissues: correlation of expression of the beta isoform with macrophage markers. J Ovarian Res 2015;8:29.
11. 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 2016;22:2929-38.
12. Hoogstins CES, Boogerd LSF, Gaarenstroom KN, et al. Feasibility of folate receptor-targeted intraoperative fluorescence imaging during staging procedures for early ovarian cancer. Eur J Gynaecol Oncol 2019;40:203-8.
13. Varghese B, Vlashi E, Xia W, et al. Folate receptor-β in activated macrophages: ligand binding and receptor recycling kinetics. Mol Pharm 2014;11:3609-16.
14. 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 2018;25:3350-7.
15. Kleppe M, van der Aa MA, Van Gorp T, et al. The impact of lymph node dissection and adjuvant chemotherapy on survival: A nationwide cohort study of patients with clinical early-stage ovarian cancer. Eur J Cancer 2016;66:83-90.
16. Harter P, Heitz F, Ataseven B, et al. How to manage lymph nodes in ovarian cancer. Cancer 2019;125 Suppl 24:4573-7.
17. Samaniego R, Domínguez-Soto Á, Ratnam M, et al. Folate Receptor β (FRβ) Expression in Tissue-Resident and Tumor-Associated Macrophages Associates with and Depends on the Expression of PU.1. Cells 2020;9:1445.
18. Hyun H, Park MH, Owens EA, et al. Structure-inherent targeting of near-infrared fluorophores for parathyroid and thyroid gland imaging. Nat Med 2015;21:192-7.
19. Hyun H, Wada H, Bao K, et al. Phosphonated near-infrared fluorophores for biomedical imaging of bone. Angew Chem Int Ed Engl 2014;53:10668-72.
20. Hyun H, Owens EA, Wada H, et al. Cartilage-Specific Near-Infrared Fluorophores for Biomedical Imaging. Angew Chem Int Ed Engl 2015;54:8648-52.
21. Park MH, Hyun H, Ashitate Y, et al. Prototype nerve-specific near-infrared fluorophores. Theranostics 2014;4:823-33.
22. Yang C, Wang H, Yokomizo S, et al. ZW800-PEG: A Renal Clearable Zwitterionic Near-Infrared Fluorophore for Potential Clinical Translation. Angew Chem Int Ed Engl 2021;60:13847-52.
23. Choi HS, Kim HK. Multispectral image-guided surgery in patients. Nat Biomed Eng 2020;4:245-6.

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