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Review Article

Cancer detection during surgery: FDA-approved use of pafolacianine

Mihaela Elisabeta Dindere[1,2], *Octavian Bucur*[1,3,]*

[1]Victor Babes National Institute of Pathology, Bucharest, Romania
[2]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
[3]Viron Molecular Medicine Institute, Boston, MA, USA

* *Corresponding author*: Octavian Bucur, MD, PhD, Next Generation Pathology Group, Victor Babes National Institute of Pathology, Bucharest, Romania and Viron Molecular Medicine Institute, Boston, MA 02108, USA; octavian.bucur@ivb.ro; octavian.bucur@gmail.com

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ABSTRACT

While cancer is on the rise, surgeons have limited reliable tools for intraoperative tumor detection and real-time margin assessment. The incomplete resection of the malignant lesions often leads to increased recurrence rate and ulterior interventions, as the surgeon must identify the cancer tissues mostly by inspection and palpation. Currently, the progress in finding novel tumor-specific tracers used for intraoperative molecular imaging came just in time with the advances of surgery towards minimally invasive operative approaches. Therefore, the FDA approval of pafolacianine, a FRα binding fluorescent agent used for near-infrared imaging in ovarian cancer patients, represents a step closer to achieving radical tumor excision and improved overall outcomes in oncologic patients. To date, numerous studies tested the safety and efficacy of intraoperative molecular imaging with pafolaciane in several types of cancer, but rigorous results are expected to ascertain these optimistic findings in the near future. This review highlights the beneficial use of intraoperative near-infrared imaging with pafolacianine in a wide variety of malignancies, this new developed technique succeeding in improving long-term outcomes in cancer patients by providing enriched tumor detection during surgery, and ultimately, by reducing cancer relapse rate, morbidity and mortality, costs and most importantly, the negative impact on patient survival.

SUMMARY

Abbreviations

U.S. Food and Drug Administration (FDA); folate receptor alpha (FRα); tumor-to-background ratio (TBR); folate receptor β (FRβ); non-small cell lung cancer (NSCLC); computed tomography (CT); endometrial cancer (EC); magnetic resonance imaging (MRI).

Keywords

Pafolacianine, intraoperative, tumor detection, folate receptor, molecular imaging, near- infrared spectrum, ovarian cancer, lung cancer.

1. Introduction

Despite the progress of modern medicine in finding new ways of improving diagnosis and treatment methods, cancer remains a major public health problem, one of the leading causes of death worldwide and the second leading cause of death in the United States, after cardiovascular diseases. According to the American Cancer Society, 1.918.030 new cancer cases will be diagnosed, and 609.360 cancer deaths are estimated to occur by the end of 2022 in the United States[1].

For the vast majority of cancer patients, surgery is the most prevalent and successful option for completely removing malignant lesions, while the rate of effectiveness is dependent on the surgeon's ability to detect the cancer tissues relying mostly on inspection and palpation[2,3]. These conventional intraoperative cancer detecting tools often lead to high recurrence rate and ulterior interventions, revealing the need of advanced techniques that may allow precise resection of the tumors during surgery[3,4].

In these circumstances, the oncological scientific community focused its interests towards finding proper technologies that could enable the surgeons with enhanced intraoperative detection of the cancer tissues, for additional guidance besides the standard visual and tactile feedback. Therefore, the intraoperative molecular imaging (including fluorescent imaging), used for accurate visualization of the malignant lesions, appeared just in time with the evolution of surgery towards minimally invasive operative approaches and robotic-assisted surgery[4].

After numerous studies stated positive results of intraoperative molecular imaging using pafolacianine, a fluorescent drug also known as OTL38, the U.S. Food and Drug Administration (FDA) approved pafolacianine (Cytalux, On Target Laboratories, LLC) on November 21, 2021, as a tumor-targeting drug used for intraoperative near-infrared imaging, intended to serve the surgeons for precise tumor detection and rigorous margin assessment in adult patients with ovarian cancer[5,6].

Pafolacianine ($C_{61}H_{67}N_9O_{17}S_4$) is an optical imaging agent, a small molecule which consists of folic acid linked by its γ-carboxyl and a short spacer to a fluorescent dye called SO456. The drug absorbs light in the near-infrared spectrum within a range of 760 nm to 785 nm and, upon excitation, it exhibits fluorescence within a range of 790 nm to 815 nm[7,8].

The near-infrared ligands show significant advantages over the visible spectrum ones, in terms of contrast, light scattering, background autofluorescence and depth of penetration[9–11].

Pafolacianine specifically binds folate receptor alpha (FRα), a membrane glycoprotein physiologically overexpressed on the surface of a restricted group of polarized epithelia, particularly in kidney, choroid plexus or placenta[12,13]. This receptor is also upregulated in various types of cancer, including ovarian cancer, lung cancer, triple negative breast cancer or gastric cancer[14–25].

Pafolacianine targets FRα-positive cancer cells with an affinity of ~1 nM[26] and is internalized via receptor-mediated endocytosis (Figure 1), thus, being accumulated in the FRα-expressing malignant

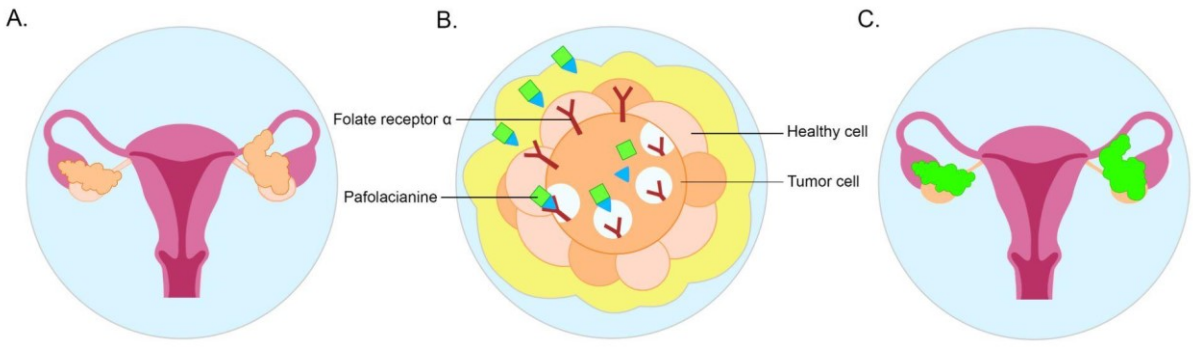

Figure 1. Mechanism of near-infrared imaging using pafolacianine.
A. Ovarian cancer under visible light. **B.** FRα-mediated endocytosis of pafolacianine into the tumor cells. **C.** Ovarian cancer in near-infrared imaging using pafolacianine
(created using the information provided by drugbank.com[8]).

lesions[8].

This review highlights the beneficial use of intraoperative molecular imaging with pafolacianine in a wide variety of malignancies, this new developed technique succeeding in improving outcomes in cancer patients by providing enriched tumor detection during surgery, and ultimately, by reducing cancer relapse rate.

2. Pafolacianine for ovarian cancer detection during surgery

Ovarian cancer represents 2.5% of all malignancies among female patients and 5% of all cancer deaths among women due to low survival rates, especially because of late-stage diagnosis and incomplete resection of the tumors during surgery[27]. Ovarian cancer comprises a heterogeneous array of malignancies individualized by cell/site of origin, pathologic grade, risk factors, prognosis and treatment[28–31]. Epithelial ovarian cancer is the most frequent one among females of all ethnic/racial groups, accounting for 90% of all cases and implying a real diagnostic challenge due to obscure clinical signs in the early stages and poor cancer visualization using preoperative radiologic approaches, which are not tumor specific[27,32].

To date, several studies proved that the adequate treatment of advanced-stage ovarian cancer generally consists of cytoreduction, succeeded by combination chemotherapy[3]. Thus, the recurrence rate and the overall outcomes are directly dependent on the degree of cytoreduction and the minimization of the residual malignant deposits that persist post-surgery[33–37].

In this scenario, tumor-targeting intraoperative fluorescence imaging could be the perfect opportunity for achieving a meticulous resection of the malignant lesions and more tailored therapeutic interventions by facilitating visualization of the hard-to-detect lesions and providing real-time feedback on margin assessment, with higher resolution and sensitivity[3].

FRα is upregulated in 80-96% of ovarian cancer cases, with characteristic overexpression in the fallopian tube cancer and primary peritoneal cancer[12,19,20]. This great overexpression level in the malignant tissues compared to the minor one in the normal cells grants excellent tumor-to-background ratios (TBRs) and makes the FRα-targeting ligands, such as pafolacianine, remarkable candidates for intraoperative molecular imaging in ovarian cancer patients[32].

According to Vahrmeijer et al. in a phase I clinical trial conducted in 12 patients with ovarian cancer, the optimal dose of pafolacianine (0.0125mg/kg) used for near-infrared imaging made possible the detection of the malignant lesions with high specificity and sensibility, enabling the surgeons to visualize 29% of all resected tumors that would have been omitted with the naked eye. The lesions were clearly visible during surgery which led to obtaining real-time images even with a short camera exposure time. However, the ideal exposure time was notably dependent on the drug dose, the longer exposure time at higher doses inducing saturated images. Moreover, the average TBR was 4.4, constant throughout the intervention, and usually decreasing with the increasing dosage, probably because of high background autofluorescence. The study reported a false positive rate of 23% mostly due to pafolacianing accumulating in the non-malignant regional lymph nodes (52% of all false positive lesions)[3] which were harboring activated macrophages with folate receptor β (FRβ) upregulated on their surface, also a binding target for pafolacianine[38–41]. This may be, however, only a seeming disadvantage, as the macrophages accumulated in the sinuses of the lymph nodes are thought to be activated macrophages which play a substantial role in cancer metastasizing[42–44]. The remaining false positive results emerged because of the noncancerous epithelial cells within the uterus and the fallopian tubes which express FRα, being generally resected during this kind of surgical procedures[3].

In terms of efficacy, near-infrared imaging using pafolacianine proved a more than 2-fold improvement in the surgeons' ability to identify malignant lesions, the technique did not interfere with the proper performance of the cytoreductive surgery, the depth of penetration was up to 1 cm below the tissue surface[3], a sizable advantage over the visible range ligands[45,46] and the surrounding healthy tissues exhibited low autofluorescence, providing great contrast[47,48]. Moreover, the pharmacokinetic behavior of pafolacianine allowed the surgeons to detect the lesions for at least 6 hours after infusion, the drug being delivered shortly before surgery (unlike fluorescent antibodies)[49], having long residence time in the tumor and quick plasma clearance. Safety wise, the toxicity was

minor and the mild side effects could have been easily reduced by adjusting the drug's formula[3].

Later on, a phase II clinical trial evaluated the safety and effectiveness of intraoperative near-infrared imaging using pafolacianine in 29 adult patients with diagnosed or suspected ovarian cancer. The enrolled patients underwent cytoreductive surgery, enhanced by intraoperative molecular imaging. All women had at least 1 adverse reaction post-surgery, the most common being the procedural pain which was reported in 90.9% of patients within 1 to 2 days following the surgery. The study-drug adverse effects were described for 18.2% of patients, being mostly gastrointestinal disorders, such as vomiting, nausea or abdominal pain. Regarding the efficacy, data showed that 48.3% of the patients (95% CI, 0.29-0.67) had at least one cancer lesion detected by intraoperative molecular imaging with pafolacianine that would have been missed if the surgery was performed relying only on tactile and visual feedback[50].

The studies on intraoperative near-infrared imaging using pafolaciane culminated in a phase III clinical trial, which provided the necessary data for the FDA approval. It included 178 females with diagnosed or suspected ovarian cancer, who were scheduled for cytoreduction, recurrent ovarian tumor surgery or interval debulking. Pafolacianine was administered to all patients, but only 134 women have benefited from intraoperative fluorescent imaging. The technique proved significant advantages, as 36 patients (26.9%) had at least one cancer lesion detected by fluorescent imaging during surgery and not identified by palpation or inspection in visible light. However, the pathologic evaluation confirmed a false positive rate of 20.2% (95% CI 13.7, 28.0%), the causes of these false positive results being similar with the ones highlighted in the previous studies[6]. Moreover, no toxicity was associated and the drug implied moderate adverse reactions (nausea, abdominal pain, vomiting, hypersensitivity, chest discomfort, dyspepsia, pruritus or flushing)[4], being retained for a couple of hours in the FRα-positive tissues, but with a half-time of <30 minutes in the non-expressing ones[26].

Currently, FDA recommends an optimal pafolacianine dose of 0.025mg/kg intravenously administered 1 to 9 hours before the surgical intervention, after prior avoidance of folic acid or folate-based drugs intake for 48h before pafolacianine administration[6].

Therefore, the FDA approval of pafolacianine for intraoperative near-infrared imaging in ovarian cancer patients means a step closer to upgraded staging of ovarian malignancies, enhanced tumor detection during surgery, decreased relapse rate and improved overall outcomes in females suffering from ovarian cancer.

3. Pafolacianine for lung cancer detection during surgery

Lung cancer is the most common type of cancer in male population and the second most common in females, after breast cancer. It is estimated that by the end of 2022 there will be 236,740 new diagnosed cases of lung cancer in the U.S.[51], recent statistics mentioning that 1 in 16 people will be diagnosed with pulmonary cancer in their lifetime[52].

The most frequent type of invasive lung cancer (85%) is the non-small cell lung cancer (NSCLC), the most lethal cancer in the U.S.[53] which represents a real challenge in terms of early-stage diagnosis and surgical treatment, as the data report that 10%-20% of NSCLC patients develop primary metastases, usually not identified during surgery because of poor intraoperative cancer detecting tools[54].

Even though the implementation of lung cancer screening protocols and the increasing use of high-resolution computed tomography (CT) led to a growing incidence of indistinct pulmonary nodules and ill-defined ground-glass opacities (GGOs-radiologic findings of abnormal lung parenchyma), resection remains the only feasible option for reliable histologic assessment. Unfortunately, in these circumstances, the architectural changes in the GGOs' parenchyma can represent a supplementary complication for the standard visual and tactile detection methods[55].

Thus, multiple preoperative and intraoperative tumor marking procedures have been tested to improve GGOs' localization, including cancer lesions staining with methylene blue, indocyanine green, technetium 99 or ethiodized oil, intraoperative CT or fluoroscopy, microcoils, fiducials or hook wires placements[56].

According to several studies, the most universal procedure involves CT-guided hook wire needle detection, an approach which proved successful localization in 95% of patients[57]. However, the intervention presents various drawbacks and limitations: it requires rigorous planning,

coordination and monitoring, it implies different types of complications such as pneumothorax (35% of patients) and pulmonary hemorrhage (15% of patients)[58], it harbors morbidity and the displacement of the hook wire may occur in ~5% of patients, reducing the success rate by 50%[57,59,60].

Since these complementary procedures fail in providing real-time margin assessment or detection of small nodules and synchronous disease, the miniaturization of surgical devices and the progress in finding novel tumor specific contrast agents made intraoperative fluorescent imaging a promising alternative for reaching complete resection of the tumors in pulmonary cancer patients[61,62].

Therefore, the FRα overexpression in 62% of pulmonary adenocarcinomas[18] and 20%-40% of pulmonary squamous cells carcinomas represents an excellent support for intraoperative near-infrared imaging with pafolacianine in most pulmonary adenocarcinoma patients and almost a third of those patients with other types of NSCLC[14,15].

Additionally, studies evaluated the FRα expression in different NSCLC lines, reporting strong signal in A549 (bronchoalveolar carcinoma), L55 (adenocarcinoma), and ChaGo-K-1(adenocarcinoma) models and a moderate one in H2170[18].

To date, numerous studies proved the favorable use of intraoperative fluorescent imaging with pafolacianine in lung cancer patients, succeeding in identifying malignant pulmonary nodules with high affinity[63], yielding accurate real-time margin assessment and precise localization of small or peripheral lesions, providing rapid intraoperative interpretation and no toxicity associated[55].

4. Pafolacianine for the detection of other types of cancer intraoperatively

4.1 Endometrial cancer

In endometrial cancer (EC) patients, the most meaningful prognostic factor for survival is the presence of lymph node metastases. Therefore, in patients with a high probability of metastatic lesions (such as non-endometrioid endometrial cancer patients) the pelvic and para-aortic lymph node sampling is proposed as a fundamental part in the surgical staging procedure[64]. Because of the limitations in the surgeon's ability to identify metastatic lesions relying only on visualization and palpation, the need for adjuvant intraoperative approaches became of great interest. Thus, a tracer that could selectively highlight cancer cells, such as pafolacianine, would be an essential help for gynecologists, enabling real-time tumor detection, localization of peripheral metastases and proper removal of metastatic lymph nodes[3].

In endometrial cancer patients, 82% of serous and clear cell carcinomas express FRα, while the healthy uterine epithelium presents a constitutive FRα expression, leading to an uncertain intraoperative distinction between the cancer lesions and the normal background tissues, yet irrelevant in staging or cytoreductive surgery, because all patients undergo a total hysterectomy[39,65]. Moreover, a histopathological analysis of uterine tissues exposed a high FRα expression in adenomyosis cells, previously reported in 17/18 endometriosis samples[66].

In the first study of intraoperative cancer detection with pafolacianine during staging and cytoreductive surgery in patients with either serous or clear cell EC, the technique helped surgeons identify all the omental and lymph node metastases, including one metastatic lesion that would have been missed by inspection and finger palpation alone. Additionally, all the metastatic lymph nodes were clearly identified, even the ones located ~1 cm below the tissue surface such as para-aortic lymph modes. The false positive results were observed in 17/50 non-malignant lymph nodes due to pafolacianine targeting FRβ, overexpressed on the surface of tumor-associated activated macrophages. The study also reported 100% sensitivity, 70% specificity and 48% positive predictive value when detecting metastatic lymph nodes with a mean TBR of 6.3[67].

4.2 Renal Cancer

The high level of FRα expression in the normal healthy kidney parenchyma (almost 100% expression at the apical surface of the proximal tubules providing physiologic folate reabsorption)[21] and the low expression level in the renal tumors[22,68] present a completely opposite pattern from the one previously described, as the healthy tissues are brightly fluorescent while the cancer lesions appear dark (10%-30% staining) when using intraoperative molecular imaging[23].

Since the goal of the partial nephrectomy in renal cancer patients is the integral removal of the tumors, while preserving as much as possible the function of

the healthy tissues and providing a proper margin surrounding the lesion, without generating a long ischemic time[69], near-infrared imaging with pafolacianine allows a smooth and precise cancer detection[70,71], especially when using laparoscopic visualization and robotic assistance[72–75].

To date, optimistic findings state positive results of intraoperative molecular imaging with pafolociane in renal cancer patients, the technique proving great contrast against the background tissues, enhanced cancer detection and accurate real-time margin evaluation[69].

4.3 Gastric cancer

In gastric cancer patients, the diagnostic and staging techniques include endoscopic ultrasound, preoperative radiologic approaches (computed tomography, magnetic resonance imaging, positron emission tomography etc.) or diagnostic laparoscopy with peritoneal washings[76]. Regarding the curative options, gastric resection is the suitable approach for localized tumors (T1-2N0) and locally spread cancer, following neoadjuvant chemotherapy in patients with primary metastases[77].

Because of the limitations in terms of endoscopic ultrasound performance, diagnostic laparoscopy with peritoneal washings, surgical lymph node staging, imaging evaluation of neoadjuvant therapy response and intraoperative pathologic assessment on frozen sections, novel technologies are extremely needed for improving cancer diagnosis and staging, tumor demarcation, localization of metastatic lymph nodes and margin assessment during surgery[78–87].

Several studies reported FRα upregulation in more than 30% of gastric adenocarcinomas, highlighting the possible application of near-infrared imaging using pafolacianine during surgery. Thus, the technique showed encouraging results such as visible fluorescence even at poor FRα expression[10,88], excellent depth of visualization (enabling detection of T3 cancer lesions which did not penetrate the gastric wall), great contrast with minimal background autofluorescence and favorable patient safety properties[17].

4.4 Pituitary cancer

Multiple studies demonstrated FRα overexpression in most nonfunctional pituitary adenomas while the functional adenomas have insignificant levels of FRα expression[89–92]. This impressive FRα upregulation in the nonfunctional pituitary adenomas together with the need for a precise visualization of the malignant lesions that could enable the neurosurgeons to reach total resection, made near-infrared imaging with pafolacianine a promising approach to achieve a proper tumor detection and decreased relapse rate in pituitary cancer patients[9].

For the nonfunctional adenomas, intraoperative molecular imaging using pafolaciane provided 75% sensitivity, 100% specificity, 100% positive predictive value, 62% negative predictive value and estimated margins with 100% accuracy, a complete resection being suggested by the absence of the fluorescence, according to the postoperative MRI evaluation[93–95].

To date, various studies tested the beneficial use of intraoperative near-infrared imaging using pafolaciane in the presented types of malignancies, but further research should confirm these encouraging results and also explore the advantages of this technique in the remaining types of cancers that overexpress FRα.

5. Conclusion

For the most cancer patients, surgery is the most effective curative option in the early-stage cancers, and it may also be used to treat advanced-stage malignancies along with the help of adjunct oncologic approaches. Since the surgeons must detect the malignant lesions mostly relying on tactile and visual feedback, novel intraoperative tumor-targeting techniques are in high demand. Thus, the FDA approval of pafolacinanie, a tumor specific fluorescent ligand used for near-infrared imaging during surgery in ovarian cancer patients, could pave the way to improved cancer diagnosis and staging, enhanced intraoperative tumor detection and positive long-term outcomes in oncologic patients. Currently, there are multiple studies which investigated the feasibility of this technique in a wide range of other malignancies, providing an encouraging platform for further research on this topic. Therefore, comprehensive studies should address the safety and efficacy properties of intraoperative molecular imaging with pafolaciane in the remaining eligible malignancies for this technology, in order to broaden the spectrum of cancer detecting tools during surgery.

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MD and OB designed and organized the review. MD analyzed, summarized the information, and wrote the manuscript. OB supervised the work and contributed to the writing and improvement of the manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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