Received: 25 July 2024 | Revised: 13 August 2024 | Accepted: 14 August 2024

DOI: 10.1002/pbc.31293

Pediatric Blood & Cancer

BRIEF REPORT

Let it glow: Intraoperative visualization of pulmonary metastases using pafolacianine, a next-generation fluorescent agent, for young adults undergoing pulmonary metastasectomy

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Level of Evidence: IV

Abstract

A new generation of disease-specific molecular imaging agents is poised to revolutionize fluorescence-guided surgery. Pafolacianine has been approved for adult lung and ovarian cancers. We demonstrate a proof of concept for pediatric surgeons treating young adults with pulmonary metastatic sarcomas. Five successful fluorescence-guided pulmonary metastasectomy operations were performed in young adult patients with metastatic osteosarcoma or Ewing sarcoma following administration of pafolacianine. All osteosarcoma lesions identified using standard techniques were also markedly fluorescent in patients. Novel fluorescent molecular agents targeted to tumor-specific receptors have promise of increased sensitivity and specificity for detecting metastatic nodules and enhancing surgical clearance of disease.

KEYWORDS
fluorescence, pulmonary metastases, thoracoscopic, thoracotomy

1 | INTRODUCTION

Pulmonary metastasectomy stands as a critical intervention in the multimodal treatment of pediatric patients with metastatic solid tumors, including osteosarcoma, Ewing sarcoma, Wilms tumor, and soft tissue sarcoma.[1] Among these pediatric cancers, osteosarcoma and non-rhabdomyosarcoma soft tissue sarcoma particularly rely on surgery as a key management strategy, as they are less responsive to adjuvant therapy.[2] Despite strides in multimodal therapy, the current management of metastatic disease remains suboptimal, necessitating innovative approaches to augment surgical accuracy and therapeutic efficacy. Fluorescence-guided surgery (FGS) has emerged as a promising modality to assist surgeons in real-time visualization of cancerous lesions, offering the potential for precise tumor resection.[3–9] While current FGS techniques often rely on non-specific fluorescent agents such as indocyanine green (ICG), recent endeavors have focused on developing molecular agents targeted to tumor-specific receptors, aiming to enhance sensitivity and specificity in detecting metastatic nodules.

Among these emerging agents, pafolacianine (CYTALUX) has garnered attention for its ability to target the folate receptor, which is frequently expressed in tumors metastasizing to the lung, including osteosarcoma.[10] Notably, pafolacianine clinical trials in adults have demonstrated its efficacy in improving surgical outcomes in lung and ovarian cancers by aiding in the identification of occult tumors and close surgical margins,[11–13] leading to FDA approval in 2022. However, very little is known about its potential utility for pediatric as well as adolescent and young adult (AYA) tumor histologies.

2 | METHODS

Four young adult patients at two separate institutions, with pulmonary metastases secondary to osteosarcoma plus one patient with pulmonary metastases secondary to Ewing's sarcoma, underwent FGS for

Abbreviations: FGS, fluorescence-guided surgery; FRα, folate receptor-alpha; FRβ, folate receptor-beta; ICG, indocyanine green; LLL, left lower lobe; NIR, near-infrared.

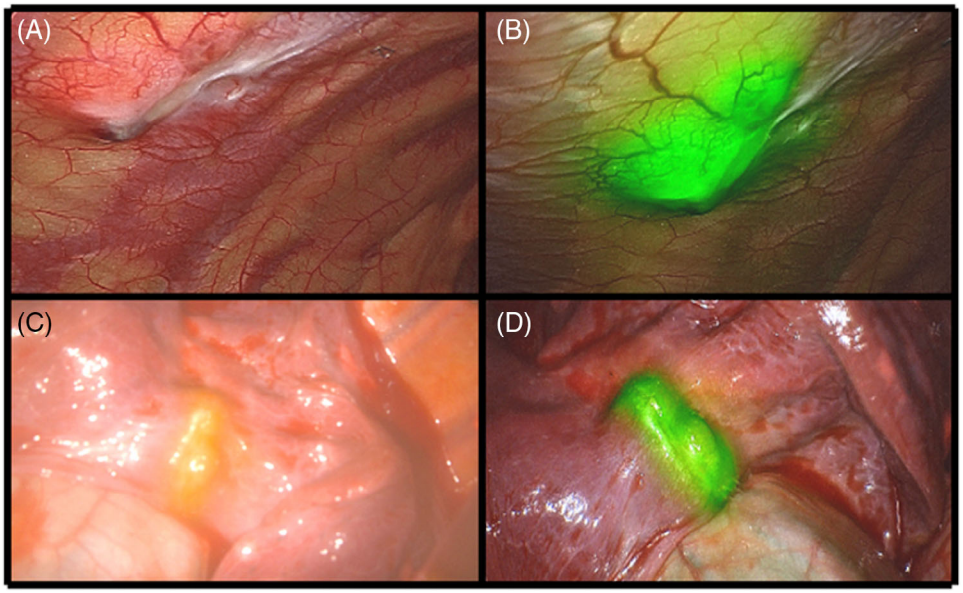

FIGURE 1 White light and near-infrared (NIR) visualization of lesions. (A) Pleural-based nodule seen by standard thoracoscopy. (B) Same nodule under NIR illumination after administration of pafolacianine. (C) Metastatic lung lesion in white light and (D) NIR.

pulmonary nodule resection after preoperative intravenous administration of 0.025 mg/kg of pafolacianine between 3 and 8 hours prior to surgical visualization. One patient with osteosarcoma underwent staged procedures, with left pulmonary metastatectomy followed by a subsequent operation for right pulmonary nodules; pafolacianine was used in each case. Both thoracoscopic techniques and thoracotomy were utilized.

For thoracoscopy, a 5-mm endoscope was used in combination with an endoscopy tower with fluorescence capabilities to excite at 760–785 nm and detect emission at 790–815 nm (Stryker Endoscopy 1788 platform). Generally, the operations began with standard white light visualization, with frequent toggled use of the overlay, contrast, and color segmented modes per surgeon discretion. For open thoracotomy, following exposure and retraction a similar thoracoscopic setup was introduced into the pleura through the wound and manually navigated by the surgeon. This combination of white light and near-infrared (NIR) imaging to observe lesions that fluoresced was used to identify lesions for resection.

3 | RESULTS

Five fluorescence-guided pulmonary metastasectomy operations were performed in four young adult patients with known malignancy and pulmonary nodules. Patients' age ranged from 18 to 25 years. Weights ranged from 67 to 90 kg, and the cohort was 100% male. Four operations were in patients with osteosarcoma, and one in a patient with Ewing sarcoma. Pafolacianine was infused the same day in all five operations. Three operations were performed thoracoscopically, and two via thoracotomy. In one of the thoracoscopic cases, a trocar incision was enlarged to allow digital palpation also. Resection was successful in all patients, and there were no significant surgical complications or serious adverse events related to pafolacianine infusion.

For osteosarcoma, fluorescence identified a pleural-based lesion in one case that had not been previously noted under white light, which was found to contain viable malignancy upon pathology evaluation (Figure 1). In two cases in patients with osteosarcoma, nodules visible under both white light and fluorescence were removed, and subsequent pathologic examination revealed benign intraparenchymal lymph nodes. In contrast, for Ewing sarcoma, a 4-mm nodule was not distinguishable by fluorescence in situ despite histologic confirmation of disease. The lesion was noted to be faintly avid following resection.

4 | DISCUSSION

These results demonstrate the promising utility of FGS with pafolacianine, a novel molecular imaging agent targeting the folate receptor, in aiding the detection and resection of metastatic osteosarcoma lesions during pulmonary metastasectomy procedures in AYA patients. This aligns with previous research demonstrating the superior sensitivity of fluorescence imaging with this agent in detecting lesions that may otherwise go unnoticed under conventional visual inspection.[11,12,14,15]

Only a single prior case report has shown evidence for use of pafolacianine for osteosarcoma.[14] In a 24-year-old man with osteosarcoma, who was noted to have suspicious lesions in his left lower lobe (LLL), pafolacianine was administered same day of planned thoracoscopic resection. Intense fluorescence in the LLL lesion as well as an additional nodule within the major fissure were confirmed as osteosarcoma metastases upon histopathological analysis.[14] Recent evidence indicates that osteosarcomas exhibit unique molecular mark-

ers, with folate receptor-alpha (FRα) being one of them. Yang et al. conducted a study involving osteosarcoma samples.[10] The results showed detectable FRα mRNA in 78.5% of samples, highlighting FRα as a viable target for therapeutic intervention with pafolacianine.

Pafolacianine binds to FRα receptors and enters cells via endocytosis. It also binds to beta receptors, potentially exerting its effects through a synergistic mechanism. This involves binding to FRα on tumor cells and/or folate receptor-beta (FRβ) on tumor-associated macrophages. When the area is excited under NIR light within the appropriate range, the lesions fluoresce, aiding surgeons in identifying osteosarcoma cancer. This facilitates more comprehensive resection, detection, and removal of synchronous lesions, and ensures margins free of disease. Pafolacianine has undergone evaluation in ovarian cancer, and in phase 2 and phase 3 studies for intraoperative imaging during lung cancer surgery.[11–13]

The identification of fluorescence in an intraparenchymal node without histological evidence of tumor cells raises questions regarding the specificity of FGS. Most patients in prior phase 1 studies showed brightly fluorescent lymph nodes, yet only a minority of these actually contained cancer metastases.[16,17] This disparity may stem from pafolacianine's affinity for FRβ, found on activated macrophages within the non-cancerous lymph nodes.[16–18] This apparent limitation of false-positive fluorescence can, in reality, offer utility, as activated macrophages may include tumor-associated macrophages, contributing to tumor progression.[19,20]

Pafolacianine's utility may be limited by factors such as pretreatment and tumor necrosis. For instance, we observed that Ewing's sarcoma did not exhibit fluorescence distinguishable from surrounding tissue in situ following administration of pafolacianine, although this was only a single case and fluorescence was noted after the specimen was removed and examined ex vivo. Additional limitations include the unique excitation/emission parameters required for the operating room equipment that vary from typically used ICG (Excite 700–800 nm, Emit 830–880 nm). As experience with pafolacianine accumulates, insights into the optimal preoperative timing of administration may emerge, potentially influencing the agent's effectiveness. Furthermore, achieving ideal visualization will necessitate a delicate balance between the retention of pafolacianine in abnormal tissues of interest and its clearance rate from surrounding parenchyma and lymph nodes.

5 | CONCLUSION

These findings support the clinical utility, safety, and versatility of FGS in aiding the precise localization and resection of metastatic lesions during pulmonary metastasectomy procedures in young adult, osteosarcoma patients. These novel agents targeted to tumor-specific receptors have promise of increased sensitivity and specificity for detecting metastatic nodules and enhancing surgical clearance of disease. There is significant opportunity for use in children with osteosarcoma lung metastases, warranting further exploration and investigation in a younger population. Continued research and refinement of FGS techniques are essential to optimize its effectiveness and further improve outcomes for pediatric cancer patients undergoing surgical treatment.

AUTHOR CONTRIBUTIONS

Alison Lehane: Methodology; writing—original draft preparation; visualization. **Ashley Dodd**: Writing—reviewing and editing. **Stephanie Polites**: Conceptualization; writing—reviewing and editing. **Seth Goldstein** and **Timothy Lautz**: Conceptualization; methodology; writing—reviewing and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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How to cite this article: Lehane A, Polites SF, Dodd A, Goldstein SD, Lautz TB. Let it glow: Intraoperative visualization of pulmonary metastases using pafolacianine, a next-generation fluorescent agent, for young adults undergoing pulmonary metastasectomy. *Pediatr Blood Cancer*. 2024;71:e31293. https://doi.org/10.1002/pbc.31293