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ARTICLE

Folate-targeted intraoperative fluorescence, OTL38, in robotic-assisted laparoscopic partial nephrectomy

Jay E. Sulek[a], James E. Steward[a], Clinton D. Bahler[a], Max H. Jacobsen[b], Amitha Sundaram[a], Cheuk Fan Shum[c], George E. Sandusky[b], Philip S. Low[d] and Chandru P. Sundaram[a]

[a]Department of Urology, Indiana University School of Medicine, Indianapolis, IN, USA; [b]Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, IN, USA; [c]Department of Urology, Khoo Teck Puat Hospital, Singapore; [d]Department of Chemistry, Institute for Drug Discovery Purdue University, West Lafayette, IN, USA

ABSTRACT

Objective: To investigate the safety and efficacy of OTL38, a folate-targeted, intraoperative fluorescence agent, in patients undergoing robotic-assisted laparoscopic partial nephrectomy.

Methods: Patients with proven or suspected localized renal cell carcinoma at a single academic institution were selected from 2016 to 2018. Patients received one dose of OTL38 at 0.025 mg/kg prior to robotic-assisted laparoscopic partial nephrectomy. The da Vinci Fluorescence Imaging Vision System was used to identify the tumor and inspect for residual disease after resection. Immunohistochemistry was performed to quantify folate receptor alpha in both the tumor and surrounding normal parenchyma. Patient follow-up was 1 month. Outcome data included descriptive statistics of the patient cohort and surgeon and pathologist surveys.

Results: Ten cases were performed. Mean patient age was 62.9 years (range = 50–70). Mean tumor size was 2.45 cm. Pathologic tumor stages ranged from T1a–T3a. Histologic tumor types included clear cell, chromophobe, type 1 papillary renal cell carcinoma and oncocytoma. The tumors did not fluoresce, while the surrounding normal parenchyma did show fluorescence. No adverse reactions were seen. Staining for folate receptor alpha was localized to the proximal renal tubules. Average staining in normal surrounding renal parenchyma was significantly greater than staining observed in tumor tissue (0.2086 vs 0.0467; $p = 0.002$). The mean difference in staining between tumor tissue and surrounding normal renal parenchyma was 0.1619 (95% CI = 0.0796–0.2442).

Conclusions: Based on our initial experience, OTL38 shows potential as a safe, effective and easy to use tool to improve visualization and resection of renal tumors.

Abbreviations: BMI: body mass index; CI: confidence interval; cm: centimeters; ECOG: Eastern Cooperative Oncology Group; FRα: folate receptor alpha; ICG: indocyanine green; IRB: institutional review board; kg: kilogram; LLC: limited liability corporation; mg: milligram; mL: milliliters; RCC: renal cell carcinoma; RPN: robotic-assisted laparoscopic partial nephrectomy; USA: United States of America

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Renal cancer; folate; fluorescence; robotics; partial nephrectomy

Introduction

Robotic-assisted laparoscopic partial nephrectomy (RPN) is an increasingly common procedure among urologists [1,2]. With growing adoption of this technique comes new challenges. Blood loss and complication rates are higher when partial nephrectomy is performed in larger tumors, and the incidence of a positive margin can be as high as 18% in certain cases, such as a solitary kidney or bilateral renal masses [3]. For difficult cases, intraoperative imaging of tumors has the potential to improve clinical outcomes [4,5].

OTL38 is a fluorescent tag which targets folate receptor alpha (FRα) [6]. In clinical trials, OTL38 has been used to identify tumor tissues with upregulated expression of folate receptors [7–9]. OTL38 has shown fluorescence in lung, ovary, pituitary, bladder, stomach and kidney tissues [10]. In the kidney, FRα is involved in folate reabsorption. Expression of this receptor reaches 100% at the apical surface of the proximal tubules of the kidney [11]. The use of OTL38 for intraoperative tumor imaging requires differential expression of folate receptors between normal and malignant tissues [12]. In previously published work, we found that expression of FRα was significantly less in kidney tumors compared to surrounding kidney parenchyma. This difference in expression was reflected intraoperatively and allowed for differentiation of the tumor from its surrounding parenchyma [13].

OTL38 is fundamentally different than currently used intraoperative fluorescence agents. Indocyanine green (ICG), for example, is used to identify areas of the renal parenchyma that continue to receive blood flow. Conversely, OTL38 targets normal renal parenchyma independent of blood flow and can aid in identification of benign vs malignant tissue [13]. Another way OTL38 differs from ICG is the time it remains in the tissue. ICG binds to plasma proteins and is removed from the tissue as it travels through the

CONTACT Chandru P. Sundaram sundaram@iupui.edu Department of Urology, Indiana University School of Medicine, 535 Barnhill Drive, RT 420, Indianapolis, IN 46202, USA

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vasculature [14]. In contrast, OTL38 binds to the folate receptors on nephrons of normal kidney parenchyma [6]. This difference allows for OTL38 to maintain its fluorescence for hours, as opposed to minutes in the case of ICG [6,14]. Herein we present our experience with OTL38 as a tool for intraoperative fluorescence for robotic partial nephrectomy.

Materials and methods

After Institutional Review Board approval (IRB#: 1511879268), patients with localized renal cell carcinoma (RCC) were selected to undergo RPN after administration of OTL38 (On Target Laboratories LLC, West Lafayette, IN) folate-targeted intraoperative fluorescence from May 2016 to February 2018. Eligible patients were at least 18 years old, had proven or suspected diagnosis of clinical stage T1–2 RCC, good performance status and were scheduled for RPN. Exclusion criteria included participation in an alternate clinical trial, known adverse reaction to folate or analog, renal or hepatic impairment or any medical conditions that could potentially jeopardize patient safety or limit the ability to complete the study.

Patients received a single dose of OTL38 at 0.025 mg/kg and a single dose of 25 mg diphenhydramine within 2 hours of skin incision. The da Vinci Fluorescence Vision Imaging System (Intuitive Surgical, Sunnyvale, CA). Fluorescence imaging was used to visualize the OTL38 signal to help identify the tumor prior to resection and then to inspect the resection bed and tumor for evidence of a positive margin. All tumors were excised with scissors after clamping the main renal vasculature. The surgeon alternated between visible light and near-infrared imaging during tumor resection. A frozen section was obtained based on the surgeon's discretion. A two-layered renorrhaphy was performed in each case. Images were taken in visible and fluorescent light immediately before and after tumor excision. Once resected, the tumor was sent to the pathologist for processing and sectioning. Further details of the study protocol may be found in previously published work [13].

Immediately after the procedure, the surgeon completed a questionnaire describing the usefulness of folate-targeted intraoperative fluorescence for tumor identification and excision. A coinvestigator, a blinded surgeon uninvolved in the case, viewed the visible and fluorescent light images and completed the questionnaire to assess interobserver agreement.

Post-operatively, patients underwent serum laboratory collections, including complete blood counts, serum electrolytes and renal function. A review visit at 1 month after surgery provided assessment of clinical outcomes and identified any delayed adverse events from the study. The final histopathology of the tumor specimen was noted.

Tissues were collected and processed with the aid of a surgical pathologist according to documented standard operating procedures. Immunostaining steps were performed using an immunostainer (Dako/Agilent, Santa Clara, CA). Slides were blocked with protein blocking solution (Dako/Agilent) and FRα (Biocare Medical, LLC, Concord, CA) was added to slides which were then incubated for 30–45 min at room temperature. Following washing, visual detection was performed using Envision Flex Link and 3,3′-diaminobenzidine chromogen (Dako/Agilent). A whole slide digital imaging system, ScanScope CS, (Aperio/Leica, Wetzlar, Germany), was used for imaging. Computer-assisted morphometric analysis of the digital images was performed using ImageScope software (Aperio/Leica) which uses an algorithm approved by the US Food and Drug Administration for clinical trials. The Positive Pixel Count Algorithm (Aperio/Leica) was used to quantify the amount of stain present. Only regions with positive or strong positive staining were used to calculate positivity. It was observed that weak positive staining was mostly background staining and was therefore not counted. Slides were evaluated by two pathologists. Positive and negative controls were included. Positivity was calculated for tumor tissue and normal surrounding renal parenchyma for each patient. Data was analyzed using SPSS, Version 24 (IBM, Armonk, NY).

Results

Ten patients underwent RPN with assistance of OTL38 imaging and follow-up as per study protocol (Table 1). Patients ranged from 50–70 years old. Nine patients were male and one female. All patients identified as Caucasian. BMI ranged from 23.2–34.4 (mean = 29.9). All patients had an ECOG score of 0. All patients presented with an incidental small renal mass ranging from 1.0–3.7 cm. The mean time from administration of study drug to surgery was 101.5 min (range = 57–152). Mean operative time was 211 min (range = 164–252). Clamp time ranged from 10–17 min (mean = 14.7). There were no intraoperative complications and no allergic reactions to OTL38.

During the procedure, near-infrared imaging revealed strong fluorescence in the normal parenchyma but no fluorescence in the tumor. Fluorescence in the tumor was not identified in any patient, including the patients with oncocytoma and non-clear cell RCC. Figure 1 illustrates clear cell RCC and papillary RCC in visible vs near-infrared light. Normal renal parenchyma surrounding the tumor was categorized as 'very bright fluorescence' in two cases, 'bright' in seven cases, and 'mild' in one case. In each of the 10 cases, the surgeon characterized the difference between the fluorescence in the tumor and the kidney as 'tumor much less than kidney'. In all cases, fluorescence aided in identifying the tumor 'a lot' or 'somewhat'. In terms of resection, fluorescence was described as 'a lot' or 'somewhat' in all cases except one in which the surgeon was 'not sure'. Histologically, each tumor showed 'no fluorescence' when examined in near infrared light (Figure 2).

The mean estimated blood loss was 117.5 mL and ranged from 25–200 mL. No patient required transfusion. There were no complications intraoperatively or at 1-month follow-up. The mean length of stay was 2 days and ranged from 1–3 days.

On final pathology, seven of the patients had clear cell RCC. Each of these cases were characterized as Fuhrman grade 2 and pathologic stage T1a. There was one case of papillary RCC which was pathologic stage T3a due to microscopic invasion of surrounding fibroadipose tissue, and one

Table 1. Demographics and clinical characteristics.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10
Age	67	70	50	65	62	58	70	53	70	64
BMI	31.2	23.2	32.8	23.2	32.0	31.2	30.7	30.5	34.4	24.6
Pathology	ccRCC	ccRCC	pRCC	ccRCC	ccRCC	chromophobe	oncocytoma	ccRCC	ccRCC	ccRCC
Stage	pT1a	pT1a	pT3a	pT1a	pT1a	pT1a	N/A	pT1a	pT1a	pT1a
Grade	2	2	2	2	3	3	N/A	2	2	2
Margin	negative	negative	negative	negative	negative	negative	N/A	negative	negative	negative
EBL (mL)	200	200	50	25	100	100	50	100	250	100
Tumor fluorescence[a]	5	5	5	5	5	5	5	5	5	5
Normal parenchyma fluorescence[a]	3	2	2	1	2	2	2	1	2	2
Helpful in locating tumor[b]	2	2	2	1	2	2	2	1	1	1
Helpful in tumor resection[b]	3	2	2	1	2	2	2	1	1	1

ccRCC: clear cell renal cell carcinoma; pRCC: papillary renal cell carcinoma (type 1); chromophobe, chromophobe renal cell carcinoma; N/A: not applicable; BMI: body mass index; EBL: estimated blood loss.
[a]1 = very bright fluorescence; 2 = bright fluorescence; 3 = mild fluorescence; 4 = minimal fluorescence; 5 = no fluorescence.
[b]1 = helps a lot; 2 = helps somewhat; 3 = not sure.

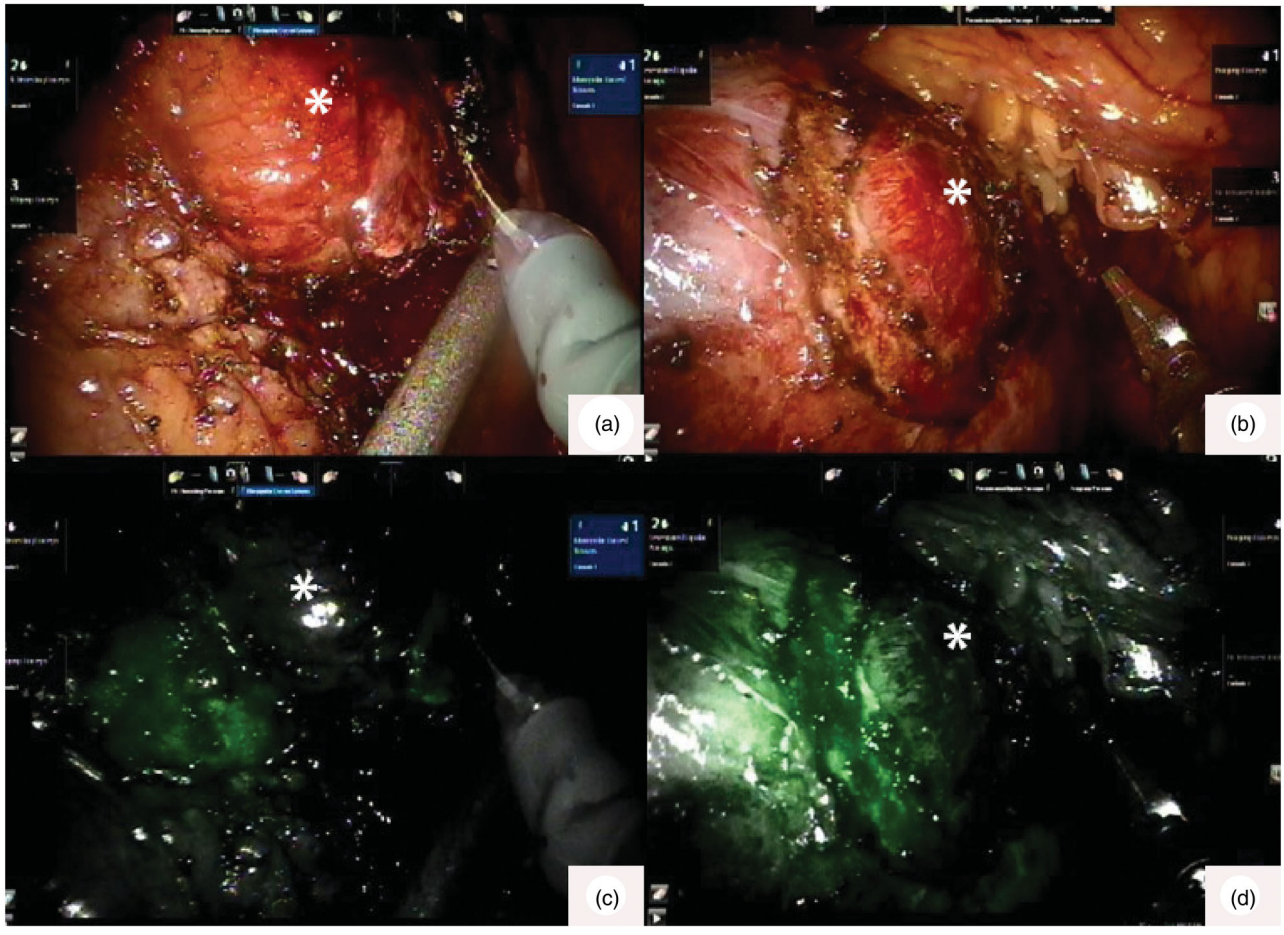

Figure 1. (a and b) Visible light intraoperative images of a clear cell renal cell carcinoma and papillary renal cell carcinoma, respectively. (c and d) The corresponding infrared intraoperative images. The asterisks identify the tumors. The areas of fluorescence correspond to normal renal parenchyma.

case of a chromophobe tumor which was pathologic stage T1a. There was also one benign case of a 3.3 cm oncocytoma. A frozen section was obtained intraoperatively in five of 10 cases and was negative in each of these cases. Final tumor margin was negative in all cases.

Staining for FRα was localized to the proximal renal tubules. Average staining in normal surrounding renal parenchyma was significantly greater than staining observed in tumor tissue (0.2086 versus 0.0467; $p = 0.002$). The mean difference in staining between tumor tissue and surrounding normal renal parenchyma was 0.1619 (95% CI = 0.0796–0.2442). Expression of FRα in the case of papillary RCC (0.0320) and in the case of a chromophobe RCC (0.0197) was less than the average expression seen in clear cell RCC tumors (0.0479). Expression in the oncocytoma case was somewhat greater when compared to the RCC tumors (0.0842), though still much less than the surrounding renal parenchyma.

Discussion

An ideal protocol for imaging renal tumors during RPN would be safe, easy to administer, reproducible, and effective

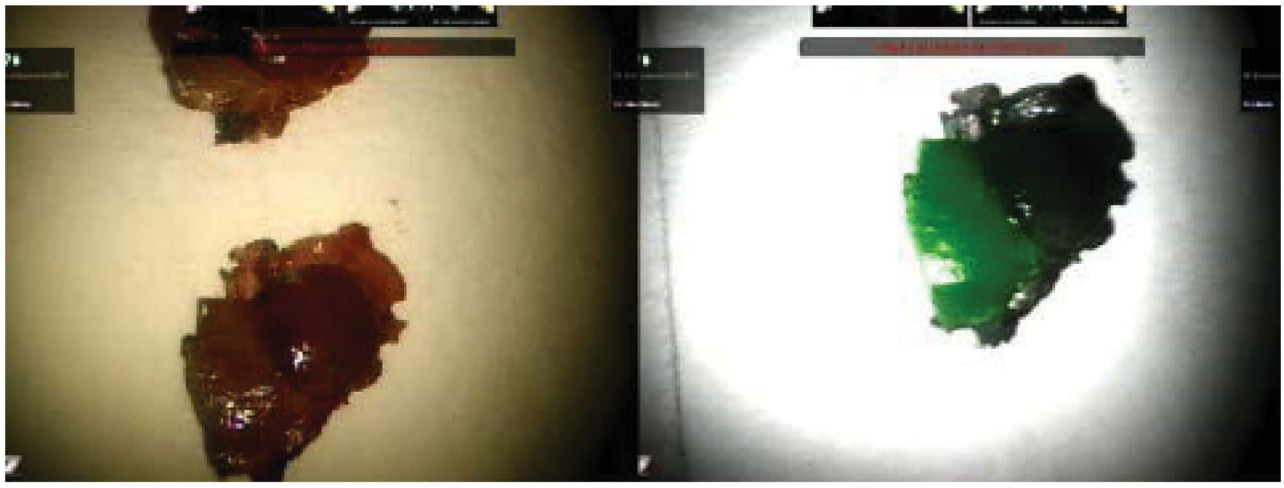

Figure 2. Visible light (left) and infrared (right) images of histologic sections. On the right, the fluorescence is seen in normal renal parenchyma, helping to identify the tumor margin.

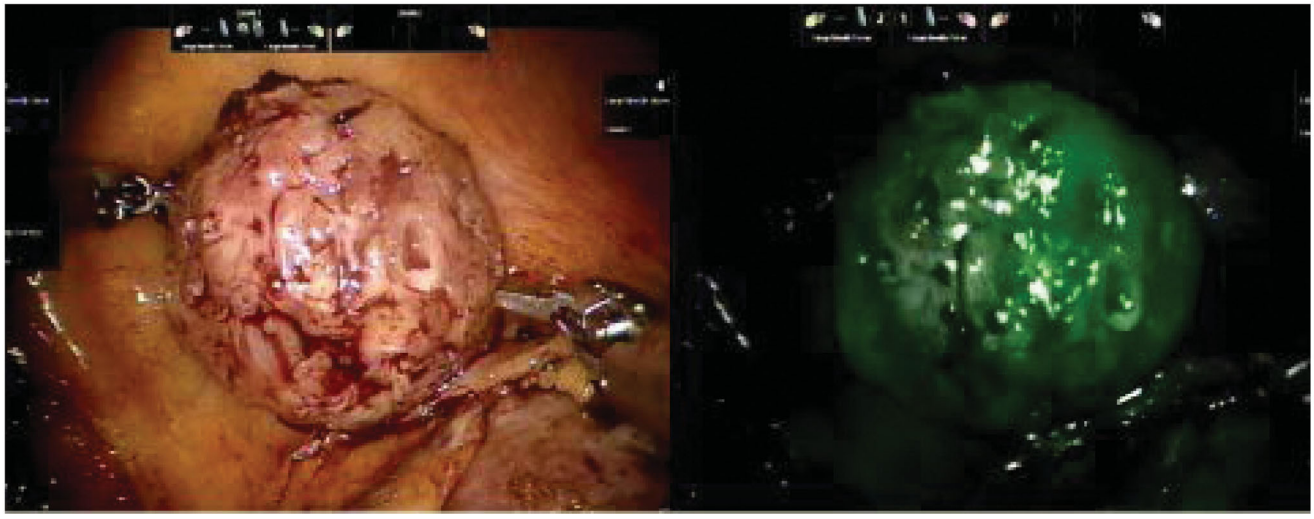

Figure 3. Visible light image (left) and near-infrared imaging (right) of the normal parenchyma at the base of a tumor after resection. The presence of fluorescence confirms normal renal parenchyma surrounding the resected tumor, as the tumor itself does not fluoresce.

in all tumor types. Additionally, it would aid in tumor localization, identification of margins and identification of any residual tumor tissue after resection, without increasing operative or clamp time. Based on our initial experience, OTL38 shows promise as a safe, easy to administer and reproducible method to visualize renal tumors intraoperatively during RPN.

No intraoperative or postoperative complications were seen. While the time from administration of OTL38 to surgery start time ranged from 57–152 min, there was no fluorescence seen in the tumor regardless of time to surgery. A distinct difference was seen between the fluorescence of the surrounding kidney parenchyma and the tumor in each case.

OTL38 targeting of FRα was shown to be useful for several tumor types. Expression of FRα in the cases of papillary RCC and chromophobe RCC was observed to be less on immunostaining than the average expression seen in clear cell RCC tumors. The expression of FRα in the oncocytoma case was greater than that seen in clear cell RCC cases (0.0842 vs 0.0473), although still far less than staining in surrounding normal parenchyma. Intraoperatively, the oncocytoma tumor showed a distinct contrast in amount of fluorescence compared to the surrounding parenchyma. FRα may make for a useful target for intraoperative imaging in a wide variety of renal tumors. This is in contrast to another target of interest for intraoperative imaging, carbonic anhydrase IX, which is only expressed in clear cell RCC tumors [15].

Improved intraoperative imaging has the potential to improve RPN outcomes in a variety of ways. A frozen section is occasionally obtained by the operative surgeon once the tumor is resected to ensure that the margin is negative. While this practice can be beneficial and, in fact, was performed in five of ten cases in this study, procuring a frozen section can potentially increase warm ischemic time and has not been shown to improve positive margin rate in most trials [16]. Figure 3 shows normal renal parenchyma surrounding the tumor immediately following tumor resection.

Infrared light image shows the fluorescent normal renal parenchyma which is present at the entire cut edge. OTL38 imaging may help the surgeon to identify whether the margin is positive or negative on inspection of the resected tumor, thus eliminating the need for a frozen section.

While partial nephrectomy has become feasible in larger and more complex T1b and T2 tumors, the likelihood of complications is increased in these cases [17]. Moreover, while the overall rate of positive margin is low, risk of positive margin increases with increasing tumor complexity [18]. In these more complex cases, improved intraoperative imaging may lead to better patient outcomes. Enhanced intraoperative tumor imaging may be particularly helpful to less experienced surgeons [19]. The learning curve for RPN ranges from 44–300 cases [20,21]. Improvements in console time, trifecta achievement, ischemia time, blood loss, transfusion rate, and hospital stay may occur well into the learning curve [21]. While it is difficult to determine the exact reasons experienced surgeons excel, an ability to quickly localize the tumor and develop a spatial understanding of its borders have been proposed as important factors [22]. OTL38 intraoperative fluorescence imaging has the potential to assist both experienced and inexperienced surgeons in locating and resecting the tumor during partial nephrectomy.

OTL38 offers potential benefits compared to other fluorescence agents such as ICG. OTL38 targets folate receptors, which are significantly greater in number in normal renal parenchyma [13]. This can help determine benign versus malignant tissue. ICG on the other hand is more suitable to identify areas that continue to receive vascular flow before tumor resection [14]. In addition, since OTL38 binds directly to folate receptors, the fluorescence remains for a much longer period of time compared to ICG [13]. The unique properties of targeting a specific tissue type and increased fluorescence time make OTL38 a potentially valuable tool in partial nephrectomy.

Limitations of this study include the nonrandomized nature, small study population, and relatively small size of tumors resected. In addition, tumor fluorescence is subjective, and clinical benefit may vary among providers. While the short-term outcomes for our series of patients were similar to published outcomes for T1a masses [23], the small number of cases and retrospective nature make comparison difficult. Moreover, improved intraoperative imaging of tumors may not provide a clinically significant benefit in this population. In the future, a prospective, randomized trial, including larger, more complex tumors, would help to better determine whether this technique is truly beneficial. All surgeries in this series were performed at a referral center by a highly experienced surgeon. Further studies should include surgeons of varying experience levels, as improved tumor imaging may provide a greater benefit to the inexperienced surgeon.

Conclusions

Folate-targeted fluorescence using OTL38 shows potential as a safe, easy to administer, and reproducible method to visualize renal tumors intraoperatively during RPN. Further studies are required to determine the clinical situations in which OTL38 imaging might be of greatest benefit.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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