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A phase II, multicenter, open-label trial of OTL38 injection for the intra-operative imaging of folate receptor-alpha positive ovarian cancer☆,☆☆

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HIGHLIGHTS

- OTL-38 binds preferentially to ovarian cancer tumors in this phase 2 study.
- OTL-38-guided surgery was safe and detected additional ovarian cancer implants in half of subjects.
- This study led to FDA support of a phase III trial NCT03180307.

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ABSTRACT

Purpose. OTL38 is a folate-indole-cyanine green-like conjugate to folate receptor alpha (FRa). The objectives of this prospective trial were to assess the safety and efficacy (sensitivity and positive predictive value (PPV)) of OTL38 for intraoperative imaging during epithelial ovarian cancer surgery.

Methods. Patients with suspected ovarian cancer planned for cytoreductive surgery were eligible to receive OTL38. Near-infrared (NIR) imaging was used to visualize target lesions that were evaluated by two blinded pathologists. A modified intent to treat (mITT) population of lesions from all patients who received OTL38-NIR imaging, underwent surgery, and had at least one FRa + target lesion was used to determine sensitivity and PPV. Two generalized linear models, with and without random effects, were employed to estimate sensitivity and PPV.

Results. Forty-four patients were evaluated for safety, and 225 lesions from 29 patients (the mITT population) were evaluated for efficacy. When assuming no correlation of interlesional results within a patient, sensitivity was estimated at 85.93% (95% lower boundary CI = 81.19) and PPV at 88.14% (95% lower boundary CI = 83.59). When controlling for actual correlation of detection among multiple lesions within a single patient (a random effect), sensitivity was estimated at 97.97% (95% lower boundary CI = 87.75) and PPV at 94.93% (95% lower boundary CI = 86.13). A total of 48.3% [14/29, (95% CI 0.29–0.67)] of patients had at least one additional lesion detected by OTL38 alone. Eight patients had mild drug-related adverse events including infusion reaction, nausea, vomiting, and abdominal pain.

Conclusions. OTL38-NIR was safe and efficacious in this phase II study regardless of folate expression levels and merits phase III evaluation.

1. Introduction

Ovarian cancer remains the most lethal gynecologic cancer with an overall 5-year survival of 45% [1]. Multiple targeted therapies and chemotherapeutic agents have been developed over the last 20 years with some degree of benefit. However, complete surgical cytoreduction is the single most important prognostic indicator for survival. A meta-analysis of 6885 patients with stage III or IV ovarian cancer treated with maximal cytoreduction demonstrated that each 10% increase in optimal cytoreduction was associated with a 5.5% increase in median

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survival [2]. Vergote et al. showed that complete resection (no gross residual disease) of all macroscopic disease (both at primary and interval surgery) is the strongest independent variable in predicting overall survival [3]. The importance of complete cytoreduction was further emphasized by du Bois et al. in a retrospective review of 3126 patients with stages IIB–IV epithelial ovarian cancer from three prospective randomized trials (AGO-OVAR 3, 5, and 7) [4]. The patients were selected in three groups, group A (1046 patients) patients with complete resection, group B (975 patients) patients with optimal cytoreduction (1–10 mm), and group C (1105 patients) patients with suboptimal cytoreduction, (>10 mm). Multivariate analysis showed improved progression-free (PFS) and overall survival (OS) for group A compared to groups B and C ($p < 0.0001$) [4]. Although bulky disease can be easily recognized, sub-centimeter implants are often difficult to discriminate from adjacent normal tissue and may not be recognized and resected. Intraoperatively, a surgeon has only two tools to improve the outcome of the tumor resections: visual inspection and palpation.

Intraoperative molecular imaging requires a fluorescent contrast agent that can be injected systemically and selectively accumulates in tumor tissue. In order to target ovarian adenocarcinomas with a fluorophore, we selected the folate receptor alpha (FRa). FRa is overexpressed 10- to 100-fold in non-mucinous epithelial ovarian carcinomas compared to normal cells [5–8]. Furthermore, the FRa expression is not altered after chemotherapy so it is a strong, reliable target for molecular imaging [8], even in the interval and secondary cytoreductive setting. OTL38 is comprised of the vitamin folic acid conjugated to an indocyanine green-like near infrared dye termed S0456. OTL38 was developed in order to detect folate receptor positive lesions in ovarian cancer patients using an investigational camera imaging system.

We hypothesized that the intravenous injection of OTL38 was safe and had adequate sensitivity and positive predictive value (PPV) to warrant further investigation as an adjunct to surgery for epithelial ovarian cancer.

2. Materials and methods

2.1. Study design

This was a single-arm, open label, prospective phase II study that was conducted at 4 tertiary ovarian cancer centers with gynecologic oncologists experienced in radical surgical cytoreductive procedures. The two primary objectives were to determine the efficacy of OTL38 according to sensitivity and positive predictive value (PPV) for FRa positive ovarian cancer by immunohistochemistry (IHC) and to report the safety and tolerability of single-dose OTL38. Sensitivity and PPV of OTL38 were determined by comparing OTL38 results with the "gold standard" of FRa status (positive or negative). Safety was investigator-assessed from the time of study drug administration and at follow-up visits on days 7 ($\pm$ 4) and 28 ($\pm$ 4) after surgery. Incident adverse events (AEs) were recorded based on Medical Dictionary for Regulatory Activities (MedDRA) definitions. AEs were classified as mild, moderate, or severe in nature and the attribution to study drug was classified as definitely related, probably related, possibly related, or not related by the investigator. The secondary objective of the study was to assess the safety of three NIR imaging systems: Quest Artemis, Novadaq PINPOINT LI, and Visionsense VS3 fluorescence imaging systems. An exploratory objective was to determine the number of additional lesions identified by OTL38 that were undetected by usual visual and tactile techniques.

2.2. Patient population

Women over age 18 with known or suspected ovarian cancer planned for cytoreductive surgery by laparotomy were eligible to participate. Surgical cytoreduction was permitted in the settings of primary (no prior surgery or chemotherapy), interval (prior neoadjuvant chemotherapy), or secondary (regional, oligometastatic recurrence) surgery. All surgery was performed as standard of care. Exclusion criteria were pregnancy, impaired renal function (eGFR <50 mL/min/1.73 m2), impaired liver function (ALT, AST, or total bilirubin > 3× the upper limit of normal), abnormal ECG at baseline, known brain metastases, receipt of another investigational agent up to 30 days prior to surgery, and previous anaphylactic reaction to any drug. The study was IRB approved at each of the participating site and was conducted in accordance with ICH-GCP guidelines and US laws and regulations. All subjects provided informed consent prior to study enrollment. The trial was registered with ClinicalTrials.gov with identifier: NCT02317705.

2.3. Study drug

An open-label single dose of OTL38 was administered intravenously. The chemical structure, preparation, and dose selection of OTL38 were elucidated in the phase I trial informing this study [9]. In the phase I trial, the initial dose of 0.025 mg/kg yielded acceptable side effects and optimal tumor to background ratio of the OTL38 signal, but a higher dose could shorten the image acquisition time. Therefore, the dose was escalated to 0.05 mg/kg. Nausea, abdominal pain and pruritus increased at this higher dose, but not to a dose-limiting level. Therefore, the initial plan for this study was to perform a safety lead-in of 5 subjects at the 0.025 mg/kg dose and subsequently escalate the dose to 0.05 mg/kg for the remainder of the study if the safety profile was acceptable. However, after an interim safety review of the lead-in cohort, the 0.025 mg/kg dose was chosen as the phase II dose and was the only dose used in this study. The injection was administered in the preoperative area approximately 2 to 3 h prior to surgery. Vitals signs (blood pressure, pulse, peripheral oxygen saturation, respiratory rate, temperature, skin examination, and ECG) and adverse events were assessed every 15 min for 1 h and then every 30 min until in the OR where they were monitored continuously.

2.4. Surgical procedure and pathology

The abdomen was entered through a vertical incision per standard practice. The operating surgeon performed an assessment of the disease distribution by standard white light (SWL) and palpation techniques, which was recorded on an anatomic schematic diagram. The surgical field was then reassessed under NIR with the respective fluorescent imaging system. Lesions visible by NIR alone were recorded on the schematic. All disease visible by SWL and/or NIR that was safe and clinically meaningful to remove was resected. No resection occurred prior to imaging. Start and stop times for NIR image acquisition were recorded to determine total imaging time for device safety purposes.

Malignant cells were confirmed by histology and FRa IHC, both performed in a CAP Biorepository Accredited laboratory by a single central pathologist who was blinded to the surgical assessment. The 26B3.F2 antibody clone against FRa was internally optimized and used for IHC (Biocare Medical, Pacheco, CA). Positive FRa IHC staining was defined as >10%. Up to 10 excised lesions identified under both visible light and fluorescence (⊗), were sent for pathology and Immunohistochemistry evaluations. Additional excised lesions identified under both visible light and fluorescence (⊗), were sent for pathology per the site practice. All excised lesions identified under visible light only (O) and fluorescence only (X) were sent for pathology and immunohistochemistry evaluations. If an organ/large section of tissue (e.g. omentum) with multiple lesions was excised *en bloc*, each lesion was considered unique for the purposes of the study. If miliary disease was identified, "MIL" was recorded.

Following the initial cytoreduction under normal light, the field was illuminated with the imaging system again to detect any remaining fluorescence-positive lesions, and any such lesions were noted with a "P" on a schematic sheet, excised as determined by the Investigator, and sent for pathology and immunohistochemistry evaluations. The reason for not excising any remaining lesion(s) was noted.

All excised lesions were sent for on-site pathology and to a central laboratory to confirm presence and type of ovarian cancer. The expression of FRa on specimens was independently evaluated at the central laboratory by two blinded pathologists. Discrepancies between the on-site and central laboratory were resolved by the central pathology laboratory for inclusion in the efficacy data.

2.5. Statistical methods

The safety population consisted of all patients who received OTL38 and was used to assess adverse events. The intent to treat (ITT) population consisted of all patients who signed the Informed Consent at Visit 1 and reflects the number and demographics of patients enrolled. A modified intent to treat (mITT) population consisted of all patients who received OTL38, underwent cytoreductive surgery for efficacy analysis, were exposed to fluorescent light using the imaging system, and had at least one FRa + target ovarian cancer lesion. All efficacy analyses were conducted using the mITT population.

Lesions with positive results for both ovarian cancer and FRa, or negative result for both tests were only included in the primary analysis. A separate exploratory analysis was performed to include all types of lesions. Sensitivity for the detection of FRa + ovarian cancer lesions was defined as the ratio (multiplied by 100) of the number of FRa + ovarian cancer lesions confirmed by both fluorescent light and by pathology and/or immunohistochemistry (True Positive = TP) over the number of FRa + ovarian cancer lesions confirmed by the pathology and/or IHC (TP + FN, where FN=False Negative). PPV for the detection of FRa + ovarian cancer lesions was defined as the ratio (multiplied by 100) of the number of FRa + ovarian cancer lesions confirmed by both fluorescent light and the pathology and/or IHC (TP) over the number of FRa + ovarian cancer lesions confirmed by fluorescent light (TP + FP, where FP=False Positive). Healthy (non-cancerous) tissue was not removed as part of this study, and therefore, a true negative (TN) sample was not available.

Since the correlation was unknown for the ovarian tumor lesions from the same subject, 2 different statistical models were used, separately for sensitivity and PPV [10]:

- Proc Glimmix in SAS for a binomial distribution with logit link function, without a random effect for patient and assuming no correlation among multiple lesions in a single patient, a Generalized Linear Model (GLM).
- Proc Glimmix in SAS for a binomial distribution with logit link function, with a random effect for the patient allowing for the possibility of some correlation among lesions within a single patient, Generalized Linear Mixed Model (GLMM).

At least 135 FRα + lesions were required to have an 80% chance that the lower boundary of the boundary 95% confidence interval for sensitivity would be above 85% assuming the "true" sensitivity is 92% or more. Assuming 7 individual tumor lesions per patient, it was estimated that 20 patients with FRa + ovarian cancer would provide 140 FRa + lesions for excision and testing. The sample size calculations assumed lesions within each patient are uncorrelated. All statistical analyses were performed using Statistical Analysis Software SAS® (SAS Institute Inc., Cary, NC, version 9.4.)

3. Results

A total of 48 patients provided informed consent and were enrolled in the study (ITT population). The majority (44/48, 91.7%) of these patients received OTL38 and were included in the Safety population. Data from 29/48 (60.4%) patients were included in the mITT population and were used in the efficacy analysis. Of the 19 patients that were excluded from the mITT population, 7 did not have at least one FRa + ovarian cancer lesion, 1 did not undergo fluorescence imaging, 4 did not receive study drug, and 7 were enrolled only in the safety population after the efficacy endpoint had been met.

Table 1 shows the demographic and oncologic characteristics of the study cohort. The mean age of the women who participated was 64 years, ranging from 37 years to 82 years. Patients were primarily white (79.5%), and the median BMI was 25.3 g/m^2. Most patients had International Federation of Gynecology and Obstetrics (FIGO) stage ≥ III ovarian cancer (70.3%) of serous histology (61.4%) with a typical peritoneal disease distribution.

The total length of time patients were exposed to fluorescence imaging pre-resection ranged from 2 to 23 min. The maximum duration of exposure to fluorescence imaging, which included any post-resection imaging, was 46 min. No patient discontinued from the study due to an adverse event related to the imaging system.

3.1. Treatment efficacy in the mITT population

A total 225 lesions were obtained from the 29 patients, comprising the mITT efficacy population. 171 lesions were positive by both OTL38 NIR and FRa and were counted as true positives (Table 2). There were 23 lesions that fluoresced but did not test positive for both FRa and ovarian cancer, which were counted as false positives. Lesions that did not fluoresce but were FRa and ovarian cancer positive equaled 28 false negatives, and there were 3 true negative lesions that did not fluoresce and were negative for both FRa and ovarian cancer. The GLM analysis, assuming no correlation of lesions within patient (excludes a random effect for patient), resulted in an estimated sensitivity of 85.93%, with a 95% lower boundary CI = 81.19. The estimate for PPV was 88.14% with a 95% lower boundary CI = 83.59. The GLMM analysis that accounts for possible correlation of lesions within the patient estimated a sensitivity of 97.97%, with a 95% lower boundaryCI = 87.75. The

Table 1
Demographic and tumor characteristics of the safety population (n = 44).

Characteristic	Intent to treat (n = 44)
Age (years)	
Mean (range, SD)	63.8 (37–82, 0.19)
Ethnicity	
Hispanic or Latino	0 (0%)
Not Hispanic or Latino	37 (84.1%)
Unknown	7 (15.9%)
Race	
American Indian or Alaska Native	0 (0%)
Asian	3 (6.8%)
Black or African American	1 (2.3%)
Native Hawaiian or other Pacific Islander	0 (0%)
White	35 (79.5%)
Other	4 (9.1%)
Multiple races checked	1 (2.3%)
BMI (kg/m^2)	
Mean (SD)	24.94 (3.699)
Tumor stage	
IC	1 (2.3%)
IIC	1 (2.3%)
III NOS	1 (2.3%)
IIIA	0 (0%)
IIIB	2 (4.5%)
IIIC	17 (38.6%)
IVA	10 (22.7%)
Unknown	10 (22.7%)
Tumor histology	
High grade serous	27 (61.4%)
Clear cell	1 (2.3%)
Endometrioid	2 (4.5%)
Adenocarcinoma NOS	5 (11.4%)
Other	8 (18.2%)

Table 2
Sensitivity and PPV of OTL38 in detecting FRa + ovarian cancer lesions.

	OTL38 mITT population (N = 29)		
	Number of lesions	Estimate (lower one-sided 95% CI)[a] (with patient as random effect)	Estimate (lower one-sided 95% CI)[b] (without patient as a random effect)
TP	171		
FP	23		
FN	28		
TN	3		
Sensitivity[c]		97.97 (87.75)	85.93 (81.19)
PPV[d]		94.93 (86.13)	88.14 (83.59)

Note: Includes lesions with positive result for both ovarian cancer and FRa + or negative for both tests.
TP: Lesions that fluoresced and tested positive for FRa and ovarian cancer.
FP: Lesions that fluoresced but did not test positive for both FRa and ovarian cancer.
FN: Lesions that did not fluoresce but tested positive for both FRa and ovarian cancer.
TN: Lesions that did not fluoresce and did not test positive for both FRa and ovarian cancer.
[a] Estimated using Proc Glimmix in SAS® for binomial distribution with patient as a random effect.
[b] Estimated using Proc Glimmix in SAS® for binomial distribution.
[c] Sensitivity of OTL38 for the detection of FRa + ovarian cancer lesions.
[d] Positive Predictive Value (PPV) of OTL38 for the detection of FRa + ovarian cancer lesions.

estimate for PPV was 94.93% with a 95% lower boundaryCI = 86.13. The "goodness of fit" test indicated that the GLMM model was the better fitting model due to a non-zero correlation (confirmation of intrapatient correlation of image-guided detection of lesions).

When considering all histologically-confirmed ovarian cancer lesions, whether FRa positive or negative, as the "gold standard", OTL38 demonstrated a sensitivity of 96.82 (lower boundary 95% CI = 86.09) and a PPV of 92.62 (lower boundary 95% CI = 83.35) determined by the GLM model and 83.9 (lower boundary 95% CI = 79.4) and 85.34 (lower boundary 95% CI = 80.93), respectively, by GLMM modeling (Table 3).

Additional patient level analyses revealed that 14 of the 29 (48.3%, 95% CI 0.29–0.67) mITT patients had at least one additional pathology-confirmed FRa + ovarian cancer lesion that was detected by OTL38 alone. Miliary disease was identified in 20 FRa + ovarian cancer lesions. In 17 of these 20 cases, 2 or more lesions were identified by OTL38 alone as compared to normal light. In 8 of these 20 (40%) cases, 4 or more lesions were identified by OTL38 alone, and were not detected by normal white light. In addition, 29 falsely positive lesions were detected in 11 patients by each of the 4 investigators. False positives were detected in various anatomic sites, but lymph nodes were the most common location (Table 4).

Table 3
Sensitivity and PPV of OTL38 in the detection of any ovarian cancer lesion (FRa positive or negative).

	OTL38 mITT Population (N = 29)		
	Number of lesions	Estimate (lower one-sided 95% CI)[a] (with patient as random effect)	Estimate (lower one-sided 95% CI)[b] (without patient as a random effect)
TP	198		
FP	34		
FN	38		
TN	7		
Sensitivity[c]		96.82 (86.09)	83.90 (79.40)
PPV[d]		92.62 (83.35)	85.34 (80.93)

Note: Includes all lesions with pathology results.
TP: Lesions that fluoresced and tested positive for ovarian cancer.
FP: Lesions that fluoresced but tested negative for ovarian cancer.
FN: Lesions that did not fluoresce but tested positive for ovarian cancer.
TN: Lesions that did not fluoresce and tested negative for ovarian cancer.
[a] Estimated using Proc Glimmix in SAS® for binomial distribution with patient as a random effect.
[b] Estimated using Proc Glimmix in SAS® for binomial distribution.
[c] Sensitivity of OTL38 for the detection of ovarian cancer lesions.
[d] Positive Predictive Value (PPV) of OTL38 for the detection of ovarian cancer lesions.

3.2. Safety population

All patients in the Safety population had at least 1 treatment-emergent adverse event (TEAE) reported during the study. The most common TEAE was procedural pain. Procedural pain was reported in 40 of 44 (90.9%) patients within 1 to 2 days following surgery; none of which were considered related to study drug or imaging device. The majority of TEAEs were of moderate severity (26/44 patients; 59.1%). There were 6 (13.6%) patients who had mild TEAEs and 12 (27.3%) patients who had severe TEAEs. Of the severe TEAEs the most common were infections and infestations (6/44; 13.6%) and blood and lymphatic system disorders (5/44; 11.4%). The most common SAEs were infections (7/44 patients; 15.9%) and included postoperative wound infection (2/44 patients; 4.5%) and sepsis (2/44 patients; 4.5%), which occurred on Days 9/10 and Day 7, respectively. None of the SAEs were considered related to study drug or to imaging device. All of the SAEs resolved during the study.

Study drug-related TEAEs were reported for 8/44 (18.2%) patients (Table 5 and 6). The most common study-drug related TEAEs were gastrointestinal disorders (5/44; 11.4%), specifically nausea (4/44 patients; 9.1%), vomiting (3/44 patients; 6.8%) and abdominal pain (1/44 patients; 2.3%). Infusion-related reactions were reported in 5/44 (11.4%) patients, and sneezing and increased lacrimation were reported in 1/44 (2.3%) patient. All study drug-related TEAEs in the 8 patients resolved within 1 day of onset without sequelae.

4. Discussion

Here we report the results of the second phase of drug development for OTL38 NIR for the intraoperative imaging of ovarian cancer. In this prospective study, NIR imaging with OTL38 was feasible among various

Table 4
False-positive lesions detected by OTL38 and investigator.

Subject	Number of false positive lesions	Location, pathology, FRa and FRb status
1	1	Right anterior abdominal wall (2 true positives adjacent; lesion was both FRa and FRb negative)
2	5	Uterus; histopathology was abnormal; FRb positive Cervix; abnormal histopathology; FRb positive Left fallopian tube; suspicious for malignancy; Fra and FRb positive Right fallopian tube; abnormal histopathology; FRb positive Appendix; normal histopathology; FRb positive
3	2	Cervix; normal histopathology Ileum lymph node (LN)
4	1	Ileum LN; FRb positive
5	1	Lesser omentum; normal histopathology
6	1	Rectum LN
7	9	2 right paracolic gutter LN; abnormal histopathology; both FRb positive 3 Ileum LN; 2 abnormal histopathology; 1 confirmed cancer but was FRa negative
8	1	Ileum LN abnormal histopathology
9	6	Greater omentum; suspicious cells; FRb positive Greater omentum; cancer histopathology; FRa negative but FRb positive Greater omentum LN FRb positive Sigmoid colon Ileum Descending colon
10	1	Greater omentum; normal histopathology; FRa and FRb negative
11	1	Leiomyoma in the uterus; FRb positive

Table 5
Investigator-assessed drug-related (possible, probable, or definite) and non-related treatment-emergent adverse events (TEAEs) by organ class (n = 44).

MedDRA system organ class/preferred term	Related n (%)	Non-related n (%)
Patients reporting ≥ TEAE	8 (18.2%)	36 (81.8%)
Gastrointestinal disorders	5 (11.4%)	21 (47.7%)
Nausea	4 (9.1%)	8 (18.2%)
Vomiting	3 (6.8%)	11 (25.0%)
Abdominal pain	1 (2.3%)	1 (2.3%)
Injury, poisoning and procedural complications	5 (11.4%)	36 (81.8%)
Infusion related reaction	5 (11.4%)	0
Respiratory, thoracic and mediastinal disorders	1 (2.3%)	7 (15.9%)
Sneezing	1 (2.3%)	0
Eye disorders	1 (2.3%)	0
Lacrimation increased	1 (2.3%)	0

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities; TEAE = treatment-emergent adverse event.

surgeons at 4 separate institutions and across 3 imaging platforms. In addition, the study met its primary endpoint of showing at least 85% sensitivity with acceptable toxicity.

The proof-of-concept study for folate-based intraoperative imaging in ovarian cancer was reported by van Dam et al. whose group showed that imaging could improve disease detection over usual visual and tactile methods [12]. Their study, which employed a folate-fluorescein isothiocyanate (FITC) conjugate (EC17), showed the preliminary safety and feasibility of incorporating this technique into real-time surgery with a median imaging duration of 10 min (range: 4–36 min). The FITC fluorochrome, however, has a spectral wavelength of 490–530 nm, which overlaps with visible light more than NIR indocyanine component of OTL38 (wavelength 774–794 nm). Thus, NIR imaging has the benefit of less auto-fluorescence, which improves specificity, in addition to deeper tissue penetration, which is particularly important for the detection of metastases in the lymph nodes or in the parenchyma of the spleen or liver. De Jesus et al. performed preclinical testing of this hypothesis in an in vivo mouse model, reporting a 3.3 fold (range 1.48–5.43) increase in signal to background ratio with OTL38 compared to EC17 [13]. Another disadvantage of FITC is that it overlaps with the absorption spectrum of hemoglobin, weakening the signal in a surgical field obscured by blood.

Table 6
Treatment-emergent serious adverse events (SAE) by system organ class (n = 44).

System organ class SAE	
Any SAE (n = patients, not events)	13 (29.5%)
Infections	7 (15.9%)
Postoperative wound infection	2 (4.5%)
Sepsis	2 (4.5%)
Clostridium dificile infection	1 (2.3%)
Hematoma infection	1 (2.3%)
Pneumonia	1 (2.3%)
Septic shock	1 (2.3%)
Wound abscess	1 (2.3%)
Gastrointestinal disorders	3 (6.8%)
Constipation	1 (2.3%)
Nausea	1 (2.3%)
Small intestinal obstruction	1 (2.3%)
Vomiting	1 (2.3%)
Respiratory, thoracic and mediastinal disorders	2 (4.5%)
Atelectasis	1 (2.3%)
Hypoxia	1 (2.3%)
Pleural effusion	1 (2.3%)
Vascular disorders	3 (6.8%)
Deep vein thrombosis	1 (2.3%)
Haematoma	1 (2.3%)
Haemorrhage	1 (2.3%)
Blood and lymphatic system disorders	1 (2.3%)
Anaemia	1 (2.3%)
General disorders and administration site conditions	1 (2.3%)
Fever	1 (2.3%)
Renal and urinary disorders	1 (2.3%)
Acute kidney injury	1 (2.3%)

The first prospective study of OTL38 in 30 healthy volunteers yielded an optimal dosage range and time window for intraoperative imaging [9]. In 12 patients with ovarian cancer, OTL38 accumulated in FRa + tumors and metastases, enabling the surgeon to resect an additional 29% of malignant lesions that were not identified previously using inspection and/or palpation. Though this study did not have a sufficient sample size to calculate a true specificity or PPV for the agent, it provided the preliminary data necessary to justify proceeding to this phase II clinical trial.

Our study demonstrated the sensitivity of 85.93–97.97% for FRa + ovarian cancer with lower 95% confidence interval boundaries of 81.19–87.75 depending on the statistical model used to analyze the data. The GLMM analysis that accounts for possible correlation of lesions within the patient estimated the higher sensitivity of 97.97%. Even in the unselected (FRa+ and FRa− combined) population, OTL38 achieved sensitivity and PPV comparable to that shown in the FRa+ specific patients. This is relevant because FRa status is not typically known pre-operatively, and this suggests that neither unknown FRa status nor FRa− status would contraindicate the use of OTL38 in surgery. Also, in contrast to the phase I study, this expanded cohort revealed an additional 48% of patients who had at least one lesion identified by imaging alone, and 40% of the patients with miliary disease having 4 or more that would have been left behind without the use of the investigational agent.

Because removal of normal tissues was not built into the study design, a true specificity of OTL38 could not be calculated. However, 29 false positive lesions were detected in 11 patients by each of the 4 investigators (Table 4). Most of the lesions classified as false positives expressed FR-beta and were located primarily in the lymph nodes, though 2 true positive lymph nodes were misclassified by the on-site pathologist. Falsely positive lymph nodes were also observed in the preceding phase I study of OTL38 in ovarian cancer [9]. These findings should be considered in the context of the prospective, randomized Lymphadenectomy in Ovarian Neoplasms (LION) trial, which reported excess morbidity and mortality from the removal of clinically negative lymph nodes, 56% of which were microscopically positive, with no gain in survival time [14]. Therefore this technology has the potential to increase surgical risk if all NIR+ nodes are removed. Further studies are needed to confirm and further characterize falsely positive lymph nodes. When analyzed in the phase 1 trial, falsely positive nodes with FRb expression were a marker for the presence of tumor-activated macrophages that have potential immunotherapeutic relevance [15,16].

Toxicity in this study was consistent with that observed in the preceding phase I study [9] and was not dose-limiting. When considering all toxicity irrespective of study drug attribution, all patients experienced at least one adverse event. The most common AE was procedural pain and occurred independent of the study drug. The most common drug-related AEs/TEAEs were gastrointestinal related nausea, vomiting, and abdominal pain. While these AEs are considered probably related to the study drug, it is possible that concurrent medications given in the pre-operative setting such as intravenous narcotics could also cause these effects. However, a dose-response increase in these AEs was noted in the phase I study, increasing the likelihood that these are truly drug related. Given the typical disease distribution of ovarian cancer, miliary disease deposits involving the gastrointestinal tract, there might be a biologic rationale for this consistently observed effect. Regardless, drug-attributable toxicity with this agent is mild and resolves within 24 h of study drug receipt.

The development of an imaging adjunct to ovarian cancer surgery that can be applied “real-time” during surgery has many potential applications. First, such a technique could improve the accuracy of cancer staging that is performed in the setting of an isolated ovarian mass. The standard staging operation relies on systematic removal of lymph nodes and omentum with peritoneal biopsies of tissues that appear normal and cancer free. In this situation, targeted imaging could allow detection of metastatic lesions that are not visible under white light and, thus, could be missed by standard staging techniques. Second, intraoperative imaging could identify more disease during cytoreductive surgery and contribute to the strongly prognostic outcome of complete tumor resection. In a study by Eskander et al., women deemed to have completely resected disease were found to have measurable disease on CT scan performed one month postoperatively for clinical trial enrollment [17]. This may be due to underestimation of residual disease volume, aggressive disease biology, or progression of occult surgical lesions. Third, the proportion of women receiving neoadjuvant chemotherapy (NACT) prior to surgery has significantly increased from 8.6% to 22.6% between the years of 2004 and 2013 ($p < 0.001$), and adoption of this treatment modality occurred primarily after 2007 (95%CI 2006–2009; $p = 0.001$) [11], following the publication of EORTC 55971 [3]. Despite improved rates of complete disease resection post-NACT, complete resection in the interval debulking setting has not translated into a similar magnitude of benefit as that observed with primary debulking. Since NACT reduces tumor burden to a microscopic level in many cases, the risk of leaving behind disease not recognized by usual means of visual inspection and palpation is increased. Moreover, pathologic complete response (pCR) following NACT is highly prognostic in the breast cancer population, but there is a need to better define this for multi-focal diseases like ovarian cancer. Therefore, image-guided surgery might be particularly beneficial in this increasingly common treatment group. Finally, the controversial benefit of secondary cytoreductive surgery for women with ovarian cancer recurrence is the objective of two prospective clinical trials, GOG 213 (NCT00565851) and DESKTOP III (NCT01166737). Should the data be positive in this setting, removal of all existing disease will be important in order to achieve the maximum benefit of surgery.

In conclusion, with the application of OTL38 and NIR imaging at the time of cytoreductive surgery, we propose a more accurate method to increase the rate of complete tumor resection and moving this hypothesis in a phase III clinical trial.

Declaration of Competing Interest

The institutions of LMR, RMW, SCD, and JLT all received funding to conduct the clinical trial. None of the investigators received funding outside of this mechanism. PSL is the Founder and Chief Scientific Officer of OnTarget Laboratories. He was involved in the scientific concept and study design from the perspective of optimization of the drug reconstitution and timing of administration, but he did not participate in the clinical trial. He only reviewed the manuscript from a scientific perspective, and did not author or edit any of the clinical findings.

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Author contribution

LMR, RMW, SCD, and JLT all enrolled and treated patients per study protocol and wrote and edited the manuscript. PSL advised the study on scientific aspects of OTL38 drug preparation. The manuscript was written and edited independent from the study sponsor.

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