CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214907Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NME, 505 (b)(1)
Application Number	NDA 214907
Priority or Standard	Priority
Submit Date	December 29, 2020
Received Date	December 29, 2020
PDUFA Goal Date	November 29, 2021
Division/Office	Division of Imaging and Radiation Medicine/ Office of Specialty Medicine
Review Completion Date	November 23, 2021
Established/Proper Name	Pafolacianine
Trade Name	Cytalux
Pharmacologic Class	Optical Imaging Drug
Applicant	On Target Laboratories
Dosage Form and Strength	Injection: 3.2 mg/1.6 mL (2 mg/mL) of pafolacianine in a single-dose vial.
Applicant Proposed Indication/Population	For adult patients with ovarian cancer as an adjunct for intraoperative identification of malignant (b) (4) lesions (b) (4)
Applicant Proposed SNOMED CT Indication Disease Term	363443007: Malignant tumor of ovary
Recommendation on Regulatory Action	Approval
Recommended Indication/Population	Cytalux is an optical imaging agent indicated in adult patients with ovarian cancer as an adjunct for intraoperative identification of malignant lesions
Recommended SNOMED CT Indication Disease Term for each Indication	363443007: Malignant tumor of ovary

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DIRM=Division of Imaging and Radiation Medicine
DO1=Division of Oncology 1
OPQ=Office of Pharmaceutical Quality
OPDP=Office of Prescription Drug Promotion
OSI=Office of Scientific Investigations
OSE= Office of Surveillance and Epidemiology
DMEPA=Division of Medication Error Prevention and Analysis
CDRH=Center for Devices and Radiological Health
DMGP=Division of Molecular Genetics and Pathology
DHTIVA= Division of Health Technology IVA

Glossary

ADME	absorption, distribution, metabolism, excretion
AE	adverse event
BIMO	Office of Bioresearch Monitoring
BLA	biologics license application
BMI	body mass index
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
CI	confidence interval
CMC	chemistry, manufacturing, and controls
CNS	central nervous system
CRF	case report form
CRO	contract research organization
CSR	clinical study report
CT	computerized tomography
ECG	electrocardiogram
EFD	embryo-fetal development
FAS	full analysis set
FDA	Food and Drug Administration
FGS	fluorescence guided surgery
FPR	false positive rate
FR	folate receptors
GCP	good clinical practice
GD	gestation day
GI	gastrointestinal
GLMM	generalized linear mixed model
GLP	good laboratory practice
HD	high dose
hERG	human ether-a-go-go-related gene
ICH	International Conference on Harmonization
IHC	immunohistochemistry
IND	investigational new drug
IV	intravenous
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
LD	low dose
MD	mid dose

MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NDA	new drug application
NIR	near-infrared
NME	new molecular entity
NOAEL	no-observed-adverse-effect-level
NZW	New Zealand White
OPQ	Office of Pharmaceutical Quality
OSI	Office of Scientific Investigation
PBS	phosphate buffered saline
PD	pharmacodynamics
PFS	progression-free survival
PI	prescribing information
PK	pharmacokinetics
PPAS	per protocol analysis set
PPV	positive predictive value
SAS	safety analysis set
SD	study day
SOC	standard of care
SPA	special protocol assessment
TPR	true positive rate

1. Executive Summary

1.1. Product Introduction

Pafolacianine sodium injection (referred to as Cytalux and also known as OTL-38), is a folate analog ligand conjugated with an indocyanine green-like dye. Pafolacianine binds to Folate Receptors (FRs) and enables the intraoperative imaging of ovarian tumors that overexpress FRs. Following intravenous injection, pafolacianine distributes throughout the body and is retained in areas with a high concentration of folate receptors. Through this new drug application (NDA) the Applicant seeks approval for the adjunctive use of 0.025 mg/kg intravenous (IV) dose of Cytalux for intraoperative identification of malignant lesions in patients with ovarian cancer. Cytalux, excited by near-infrared (NIR) light between the wavelengths of 760 (b) (4) nm (with maximum excitation at (b) (4) 776 nm), emits light at wavelengths in the NIR spectrum (maximum emission (b) (4) 796 nm). This fluorescence of malignant tissue can be used adjunctively to guide surgical resection of ovarian tumors.

Cytalux contains pafolacianine active ingredient as a tetrasodium salt referred to as pafolacianine sodium. Pafolacianine sodium is a water soluble, amorphous, hygroscopic dark green to black solution that is light- and heat-sensitive and the drug product is stored frozen, in dark until preparation for administration. Cytalux injection will be supplied as a single dose vial for IV administration. Cytalux is a (b) (4), single use, sterile, solution provided in an amber glass (to protect from degradation in light) vial with (b) (4) (b) (4) rubber closure and crimp seal. Each vial contains 3.2 mg (2 mg/mL) pafolacianine (equivalent to 3.4 mg pafolacianine sodium),14.4 mg sodium chloride, 0.23 mg potassium phosphate monobasic, 1.27 mg sodium phosphate dibasic heptahydrate in 1.6 mL volume. The pH is adjusted with sodium hydroxide and/or hydrochloric acid and is between 7.1 to 7.8.

Cytalux is stored frozen and in the original carton to protect it from light. Prior to use, the vials are thawed at room temperature for 90 minutes. Cytalux is not intended to be administered directly, as supplied. The required dose must be diluted in a 250 mL 5% Dextrose Injection, USP under aseptic conditions prior to administration. Other diluents should not be used with Cytalux. The diluted solution may be stored for up to 24 hours in a refrigerator and protected from light. Once removed from refrigeration, the infusion should be completed within 3-hours. The product should be shielded during infusion to protect it from light.

The Applicant submitted NDA 214907 on December 29, 2021 under the 505(b)(1) pathway . On 08/11/2021, a major amendment regarding the product quality was received and the PDUFA date was extended. The drug was initially reviewed under IND 118215.

Conclusions on the substantial evidence of effectiveness.

Cytalux is classified as an optical imaging drug. Evidence submitted with this application demonstrates the efficacy of Cytalux as an adjunct for intraoperative identification of malignant lesions in adult women with ovarian cancer. The main support for efficacy was derived from one adequate and well-controlled Phase 3 Study OTL-2016-OTL38-006 (Study 006), along with supporting data from a Phase 2 Study OTL-2014-OTL38-003 (Study 003). Both studies were conducted prospectively and are similar in study design, patient selection criteria, and dosage.

Study 006 was conducted in 150 patients under a Special Protocol Assessment. The primary efficacy end point for the protocol was the proportion of patients with identification of at least one ovarian cancer lesion confirmed by central pathology that was detected using Cytalux plus NIR fluorescent light but not under normal light and/or palpation. The pre-defined clinically significant threshold deemed necessary to establish success of this trial was set at 10%. The selection of this threshold is to be viewed in the context of the need for improving outcomes of surgery in patients with ovarian cancer – a serious disease with largely unmet medical needs. The primary efficacy endpoint was achieved with 36 of the 134 subjects (26.9%, 95% confidence interval (CI) [19.6, 35.2], $p < 0.001$)in the Intent-to Image set.

The phase 2 study (Study 003) also met its primary efficacy endpoint with the reported estimate for sensitivity at 97.97%, with a lower 1-sided 95% CI of 87.75%. In summary, the Applicant has submitted adequate data providing substantial evidence of effectiveness for Cytalux as an adjunct for the intraoperative identification of malignant lesions in patients with ovarian cancer.

1.2. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

Cytalux is an optical imaging agent indicated as an adjunct for intraoperative identification of malignant lesions in adult patients with ovarian cancer. Cytalux is administered intravenously at a dose of 0.025 mg/kg body weight (BW) prior to surgery. In this new drug application (NDA), data supporting the efficacy and safety of use of Cytalux in this patient population are derived primarily from the phase 3 study OTL-2016-OTL38-006 (Study 006) with supporting data from OTL-2014-OTL38-003 (Study 003) . Additional data for safety were derived from OTL-2019-OTL38-005 (Study 005), a lung cancer study. For the efficacy analysis (Study 006), data from 134 patients of the 178 patients who completed the study were analyzed. For the Safety Analysis Set (SAS), there were 194 patients in the ovarian cancer group and 100 in the lung cancer group making up a total of 294 patients. The primary efficacy endpoint for the Phase 3 study is the proportion of patients with at least one evaluable ovarian cancer lesion confirmed by central pathology (truth standard) that was detected with Cytalux and fluorescent light but not under normal light or palpation, with the pre-defined win criterion threshold set at 10%. This pre-defined threshold was achieved in the overall population (26.9 %; 95% CI 19.6, 35.2) and in sub-groups of patients undergoing different surgical approaches including primary cytoreductive and interval debulking surgery. The lesion-level false positive rate was 32.7% . No major safety concerns were identified. The most common adverse reactions were nausea, vomiting, abdominal pain, flushing, dyspepsia, chest discomfort, and pruritus. In the clinical studies, 2.4% of patients experienced reactions during the period of administration of Cytalux with a typical onset within 15 minutes of the start of infusion. To address these infusion reactions, mitigation steps were included in Section 2 (Dosage and Administration) and Section 5 (Warnings and Precautions) of the Prescribing Information (PI). These studies showed that Cytalux administered at a dose of 0.025 mg/kg achieved the primary efficacy endpoint with a favorable benefit-risk profile for its use as an adjunct for intraoperative identification of malignant lesions in patients with ovarian cancer, a serious disease with largely unmet medical needs. Therefore, the approval of this application is warranted.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Advanced epithelial ovarian cancer (EOC) is one of the common cancers in women. It presents with extensive locoregional metastatic disease, has poor response to therapy and high risk of recurrence despite advances in surgery and chemotherapy.	• Epithelial ovarian cancer is a serious condition that is typically detected in late stages and causes substantial morbidity and mortality (Jochum et al. 2020). • Incomplete tumor removal at surgery and greater residual tumor burden results in

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Cytoreduction – i.e. reducing the overall tumor burden in the patient plays an important role in optimizing the tumoricidal effects of systemic chemotherapy and improving its success. • Optimal debulking surgery involves identifying most, if not all, of the tumor nodules in the abdomen and pelvis and maximizing their safe removal with minimal surgical morbidity.	poorer survival (Sioulas et al. 2017; Gadducci et al. 2019). • Intraoperative fluorescence guided surgery has been proposed for optimizing cytoreduction due to better visualization of tumor nodules (Charlotte E. S. Hoogstins M.D. et al. 2017; Mahalingam et al. 2018).
Current Treatment Options	• Cytoreduction of locoregional disease with pre- or post-surgery chemotherapy, guided by standard imaging techniques such as computerized tomography (CT), positron emission tomography (PET)/CT, ultrasonography (US) and magnetic resonance imaging (MRI) is the standard of care in patients with EOC. • While various imaging methods are used pre-surgery to develop the surgical excision plan, they cannot guide the surgeon in real time. • Intraoperatively, the surgeons use white light and palpation to guide resection during debulking surgery to maximize the safe removal of cancerous tissue. • In addition, CA-125, a serum ovarian cancer tumor marker, may be used to assess the degree and persistence of cytoreduction and for follow up of patients after treatment.	• Despite the availability of different techniques for imaging ovarian cancer, there is an unmet need for better intraoperative guidance to maximize optimal debulking. • While standard-of-care imaging with ultrasound, CT, MRI and PET/CT can generally help identify large lesions in the pre- or post- surgical settings, they cannot be used in real-time for intraoperative guidance during debulking surgery. • Some of the tumor nodules in the abdomen and pelvis are not clearly visualized during standard surgery under white light and may thus be unresected , increasing the chances of recurrence and treatment failure (Ibeanu and Bristow 2010).

Dimension	Evidence and Uncertainties	Conclusions and Reasons
		• Using intraoperative tumor-targeted fluorescent agents and near infrared imaging could help identify and delineate tumor nodules that can be removed during debulking surgery to maximize cytoreduction.
Benefit	• Results of one Phase 3, Study 006, study with supporting results from Phase 2, Study 003, were submitted by the Applicant with this NDA to support the efficacy of Cytalux as an adjunct for intraoperative identification of malignant lesions in patients with ovarian cancer. • Study 006 enrolled a total of 178 patients. Of these, twenty eight did not receive Cytalux. From the 150 patients who received Cytalux, 134 patients were randomized to a group that received normal light followed by fluorescent light, 6 patients were randomized to a group that received normal light only and 10 patients were not randomized to any imaging group. Twenty five patients did not have central pathology confirmation for at least one ovarian cancer lesion detected under normal light or near-infrared (NIR) fluorescent light resulting in 109 patients in the full analysis set (FAS). (b) (4) The primary efficacy endpoint for study 006 was the proportion of patients with at least one confirmed ovarian cancer lesion not already intended for removal that was detected by Cytalux plus fluorescent light but not under normal light and or palpation; the threshold for study success was set at 10%. • Study 006 resulted in identifying at least one FR+ positive tumor lesion	• Considering the generally poor outcome in patients with Stage III/IV epithelial ovarian cancer with standard of care debulking surgery, the identification of additional cancerous lesions during debulking surgery can help improve the overall surgical outcome for these patients. • Results from Study 006 indicate that the primary endpoint was achieved in all and in sub-groups of patients in the trial. • These results show the beneficial role of Cytalux as an intraoperative adjunct to debulking surgery in patients with ovarian cancer, a disease with generally poor outcome and largely unmet medical needs.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	in 26.9% of the study population with a positive predictive value of 67.3%. The primary endpoint was also achieved in the various sub-group analyses. • Study 003 enrolled 48 patients; 29 patients were included in the main population for efficacy analysis, modified intent to treat (mITT) population and 44 were included in the safety population. Forty three patients completed the study. Results showed that the primary endpoints were met with an estimated sensitivity of 97.97% with a lower 1-sided 95% CI of 87.75% and a positive predictive value (PPV) of 94.93%.	
Risk and Risk Management	• The safety population consisted of 294 patients from the two ovarian cancer studies (study 006 and study 003) and study 005 in patients with lung cancer. • No deaths, serious adverse events (AEs), or study withdrawals related to the drug were reported. • The reported AEs were rare and mild and included nausea, vomiting, abdominal pain, flushing, dyspepsia, chest discomfort, pruritus and hypersensitivity. • The false positive rate (FPR) for lesion identification in study 006 was calculated to be 32.7% .	• No major safety concerns were identified for the study drug. • To address potential adverse reactions such as nausea, vomiting, abdominal pain, flushing, dyspepsia, chest discomfort, pruritus and hypersensitivity that were reported during Cytalux infusion, the following recommendations were included the Prescribing Information (PI) - Consider pretreatment with antihistamines and/or anti-nausea medications - Interrupt the infusion if an infusion reaction occurs , treat as necessary, and complete the infusion within 3 hours of the start of the initial administration

Dimension	Evidence and Uncertainties	Conclusions and Reasons
		• The possibility of false positive (FP) and false negative (FN) errors exists when using Cytalux and hence the labeling cites the risk of misinterpretation.

1.3. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

☐	**The patient experience data that were submitted as part of the application include:**			Section of review where discussed, if applicable
	☐	Clinical outcome assessment (COA) data, such as		
		☐	Patient reported outcome (PRO)	
		☐	Observer reported outcome (ObsRO)	
		☐	Clinician reported outcome (Clinton)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify):		
☐	**Patient experience data that were not submitted in the application, but were considered in this review:**			
	☐	Input informed from participation in meetings with patient stakeholders		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Other: (Please specify):		
X	**Patient experience data were not submitted as part of this application.**			

2. Therapeutic Context

2.1. Analysis of Condition

The American Cancer Society estimated that in 2018, approximately 22,240 new cases of ovarian cancer were diagnosed and there were 14,070 deaths in the US (Torre et al. 2018). Even with a better understanding of its pathogenesis and clinical presentation, ovarian cancer is the leading cause of death from gynecologic malignancies, ranking fifth in cancer-related deaths among women. The 5-year survival rate is 30% to 40% with many of the patients presenting in advanced stages, largely due to a lack of effective early detection measures, and with limited therapeutic options (Gourley and Bookman 2019).

Ovarian cancers are a heterogenous group of malignancies differentiated by cell or site of origin, pathological grade, risk factors, prognosis, as well as treatment. Epithelial cancers are the most common, accounting for 90% of all cases across various races. Epithelial ovarian cancers (EOC) are classified by tumor cell histology as serous (52%), endometrioid (10%), mucinous (6%), or clear cell (6%), with one-quarter being more rare subtypes or unspecified. Ovarian cancers are also classified into two groups - Type I, that are generally considered low grade, and Type II, that are high grade involving both ovaries, late stage disease with low survival (Kurman and Shih Ie 2016).

Surgery plays a primary role in the management of ovarian cancer even in advanced disease (Jelovac and Armstrong 2011; Al Rawahi et al. 2013). Currently, the standard treatment for patients with ovarian cancer is primary debulking cytoreductive surgery followed by chemotherapy. The goal of a debulking surgery is to maximize removal of cancer, while at the same time minimizing removal of benign tissue, to achieve optimal cytoreduction. Prognosis depends on the effectiveness of cytoreductive surgery in removing cancerous tissue. Debulking surgery followed by chemotherapy has a higher incidence of recurrent chemoresistant disease (Kim et al. 2018) compared to interval debulking surgery after neo-adjuvant chemotherapy. In one study, patients with ovarian cancer and residual disease of 1 to 10 mm had better progression-free survival (PFS) and overall survival (OS) than patients with residual disease greater than 10 mm (Shih and Chi 2010; Sioulas et al. 2017). Despite tumor debulking surgery – primary or interval (following neo-adjuvant chemotherapy), remaining the mainstay of current treatment, the ovarian cancer lesions can be diffuse, numerous, of varying sizes, and often not readily visible in the surgical field, leading to a wide variation in achieving cytoreduction across surgeons (Ibeanu and Bristow 2010; Boogerd et al. 2018). Neo adjuvant chemotherapy and interval debulking was introduced to improve the outcome, especially in patients with stage IIIC/IV disease. Reports in the scientific literature generally support the role of debulking surgery in ovarian cancer patient management. Complete or near complete resection of all

macroscopic gynecologic disease was reported to be an independent variable in predicting OS (Vergote et al. 2010). Though primary debulking surgery and interval debulking surgery may have similar OS and PFS, surgical complexity and postoperative complications are reduced in the interval debulking surgery (IDS) group and the extent of pathological response and cytoreductive status are associated with improved PFS (Bristow et al. 2002; Kumar et al. 2016; Brand et al. 2017; Chiofalo et al. 2019; Liang et al. 2019). Each 10% increase in cytoreduction was reported to correlate with a 5.5% increase in median survival (Wakabayashi et al. 2008). Selecting patients for neoadjuvant chemotherapy and interval debulking surgery, based on laparoscopic evaluation of resectability, appears to prolong the PFS without worsening the OS compared to patients who were not completely debulked with primary debulking surgery (Kobal et al. 2018; Gunakan et al. 2020; Nishio and Ushijima 2020). Real-time fluorescence imaging with tumor-targeted fluorophores is proposed to improve the process of debulking surgery through more accurate intraoperative differentiation of cancer from adjacent normal tissue.

Fluorescence imaging-guided surgery:
Conventional surgery is largely guided by normal white light visualization and palpation by the surgeon to identify, feel, and resect the tumor lesions in a patient. While preoperative imaging with computerized tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) provides staging information with the size and extent of ovarian cancer, these modalities do not provide full real-time assistance in complete removal and debulking of tumor. Intraoperative optical imaging guidance with exogenously administered fluorescent contrast agents has been proposed for additional real-time assistance by identifying occult tumor nodules (Lee et al. 2019). Real-time optical imaging (OI)-guided surgical removal of cancer has been studied in a number of cancers and Gleolan was approved in May of 2017 for debulking of high grade Glioma. Non-specific and targeted OI agents are being studied in cancers (Lee et al. 2019). Fluorophores that operate in the near-infrared (NIR) region are typically used in fluorescence guided surgery (FGS). A number of tumor targets, including ovarian cancer, have been identified for FGS. In this NDA , the Applicant has proposed the use of pafolacianine that localizes in Folate Receptors (FR) that are overexpressed in ovarian cancer.

Folate receptor expression:
FR is a folate-binding protein located on the cell surface that takes up folate via receptor-mediated endocytosis and is a target for optical imaging in ovarian cancer (Parker et al. 2005; Scaranti et al. 2020); The FRα isoform is the most widely expressed FR isoform and is overexpressed in very high copy numbers in EOC, particularly in high-grade and advanced stage disease that has a higher rate of recurrence after treatment (Kalli et al. 2008). Pafolacianine has been studied in other tumors to evaluate identification and removal of tumor during surgery (Mahalingam et al. 2018).

Because of folate's role in the biosynthesis of nucleotide bases, it is consumed in elevated quantities by proliferating cells, including cancer cells. The degree of FRα over-expression

correlates with a higher histological grade and more advanced stage of the cancer and is seen in over 90% of cases (Kalli et al. 2008). Chemotherapy does not seem to affect FR expression in ovarian cancer (Crane et al. 2011) indicating that prior chemotherapy is unlikely to diminish binding of FRα targeting pafolacianine in patients who received neo-adjuvant chemotherapy before debulking surgery. Non-neoplastic ovarian tissue and about 5-10% of ovarian carcinomas will be FR-receptor negative (Markert et al. 2008; van Dam et al. 2011). Pafolacianine has been known to be taken up by both FRα and FRβ with sufficient affinity and has 93.7% plasma protein binding with minimal partitioning into red blood cells. Pafolacianine absorbs light in the NIR region (760-785 nm) and emits fluorescence with a peak emission of 796 nm.

Pafolacianine is a FRα-targeting fluorescent agent, a folate-fluorescein isothiocyanate with a high affinity for FRs that are 90-95% overexpressed in epithelial ovarian cancers (van Dam et al. 2011; De Jesus et al. 2015).

2.2. Analysis of Current Treatment Options

The use the diagnostic modalities in the management of advanced ovarian cancer is summarized in Table 1 below. While the referenced modalities are diagnostic tools in the broader sense, pafolacianine is proposed as an intraoperative adjunct to debulking surgery in patients with ovarian cancer to identify malignant lesions.

Table 1. Current Diagnostic Options for Detecting Ovarian Cancer

Product(s) Name	Clinical Use	Clinical Context	Important Safety and Tolerability Issues	Other Comments
Ultrasound	Diagnosis and staging	Identifying primary and metastatic tumor lesions prior to initial surgery or during follow up. May also play a role in early detection and intra-operative guidance.	Intraoperative availability. However, it is non-specific and has low resolution limited by tumor size.	Non-specific anatomic imaging, therefore, not optimal for accurate staging and follow up (Shetty 2019; Rizzo et al. 2020).
CT	Initial staging and restaging	Identifying primary and metastatic tumor lesions prior to initial surgery or during follow up	Challenges to intraoperative availability, radiation exposure issues	Non-specific anatomic imaging, and therefore not optimal for accurate staging and re-staging (Shetty 2019; Rizzo et al. 2020).
MRI	Initial staging and restaging	Identifying primary and metastatic tumor lesions prior to initial surgery or during follow up	Challenges to intraoperative availability, magnetic safety issues.	Non-specific anatomic imaging and therefore not optimal for accurate staging and restaging (Shetty 2019; Rizzo et al. 2020).
F-18 FDG PET/CT/MR	Initial staging and restaging	Identifying primary and metastatic tumor lesions prior to initial surgery or during follow up	Uses short lived PET radiopharmaceutical, radiation exposure Challenges to intraoperative availability.	Non-specific imaging of glucose metabolic activity in tumors and inflammation for staging, restaging, assess treatment response (Sharma et al. 2016; Khiewvan et al. 2017; Rizzo et al. 2020).
CA-125 tumor marker	Serum marker for monitoring disease burden, recurrence etc.		None and no intraoperative application.	Non-specific tumor marker that provides information on the persistence and or recurrence of tumor (Bottoni and Scatena 2015).

Source: Generated by the reviewer
Abbreviations: CA=cancer antigen, CT=computerized tomography, F-18=fluorine 18, FDG=fluorodeoxyglucose, MR=magnetic resonance imaging, PET=positron emission tomography

The role of pafolacianine in aiding surgery depends on the likelihood of unknown disease present in the imaged location that very likely might be missed by the standard of care surgery performed under normal light and palpation. Intraoperative optical imaging is primarily performed to identify additional disease to optimize debulking in patients with advanced ovarian cancer, where the intent is to maximize tumor removal while minimizing removal of non-cancerous tissue.

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Pafolacianine sodium (Cytalux) is a new molecular entity.

3.2. Summary of Presubmission/Submission Regulatory Activity

A new drug application for pafolacianine was received by the US Food and Drug Administration (FDA) on December 29, 2020, NDA 214907 from On Target Laboratories Inc.

The original Investigational New Drug (IND) 118215 application was received on April 26, 2013, followed by other submissions as detailed below in Table 2.

Table 2. Summary of Key Regulatory Interactions With FDA for the Clinical Development of Pafolacianine

Date	Interaction	Type of Meeting	Reference
05/28/2013	PIND, Clinical Development plan, nonclinical testing, CMC information	Type C	ID 3530499
04/03/2014	Follow up	Type C	
06/23/2014	Addendum to PIND meeting		ID 3530499
12/23/2014	Orphan Drug Designation granted		12/23/2014
11/10/2015	Reproductive Toxicity Waiver granted (Fertility and embryonic development to implantation; pre- and postnatal development, including maternal function)		Email 11/10/2015
05/12/2016	CMC, nonclinical, clinical data design Phase 3 development	Type B, EOP2	
08/11/2016	Fast Track designation granted		Letter 08/11/2016
11/04/2016	Proposed Phase 3 clinical protocol for SPA submission	Type B EOP2	ID 4095943
06/02/2017	SPA agreement letter		ID 4106198
01/22/2019	SPA modification, use of another imaging system, recalculation of sample size and increased number of study sites	Letter	ID 4378474
07/06/2020	CMC program support NDA and Division would accept rolling NDA	Written response	ID 4635243
11/09/2020	QTc waiver	Email	Email 11/09/2020
11/13/2020	Pre NDA meeting	Type B Pre-NDA	ID 4699807

Source: Applicant supplied Table 2, 2.5.1.7 Regulatory guidance
Abbreviations: CMC=chemistry, manufacturing, and controls, EOP2=end of phase 2, NDA=new drug application; NIR=near infrared, QTc=corrected, PIND=pre investigational new drug, QT=interval, SPA=special protocol assessment

4. Significant Issues From Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

The Office of Scientific Investigation evaluated the following aspects of the clinical trial: protocol compliance and overall number of evaluable lesions at site #2 and site #5, training, Standard Operating Procedures (SOPs), and the role of (b) (4), a Contract Research Organization (CRO), for training, monitoring, reporting and remediation. Handling of pathology reports at the Central Pathology (b) (4) in accordance with Good Clinical Practice (GCP) was also evaluated by the OSI. The final OSI's Clinical Inspection Summary (Summary 2021) dated July 14, 2021 was filed in FDA's Document Archiving, Reporting and Regulatory Tracking System (DARRTS) (4825762 July 14, 2021).

The final Clinical Inspection Summary document (FDA Reference 4825762) from the Office of Scientific Inspections, takes into account the responses from the Applicant to several IRs . Table 3 below is a compilation of the salient points from the Clinical Inspection Summary (CIS):

Inspections of the clinical investigators' site #2 and site #5, and the CRO raised questions related to the documentation of the investigational product (IP), start and stop time of the infusion, concomitant medication usage, and a number of other protocol violations.

Inspection of the central pathology laboratory identified the lack of an audit trail needed for validation of the primary endpoint truth standard data. A blinded re-read of histopathology slides was needed to verify the concordance of the re-read with the initial read.

Table 3. Salient Points From the Final OSI Clinical Inspection Summary

Investigator/ Site	Findings	Resolution	OSI Explanation/ Comments
Site #2	• Start and end time of infusion • Concomitant medication usage • Other protocol violations • 40% under reporting of AEs • IP documentation issues e.g. "vortex time"	• Applicant updated NDA to include all unreported AEs	• No other concerns • Data were verifiable • Documentation issues - Due to use of forms which did not specify vortex time and not clear if the procedure was performed or not. - Lack of documentation of start and stop times in 2/11 subjects is a regulatory violation, OSI concluded that *"this finding is sporadic and does not appear to be clinically significant."*

Investigator/ Site	Findings	Resolution	OSI Explanation/ Comments
Site #5	Records reviewed by OSI: • ICFs • Regulatory binders for IRB communication/approvals • Form 1572s • Financial disclosure forms • Drug accountability logs • Monitoring reports • Subjects source documents • Lab reports • Study endpoint data and questionnaires • AE logs • Concomitant meds • Logs and deviation logs		• There was no underreporting of drug related AEs. Provided material for review (Subjects (b) (6) • Minor protocol deviations related to missed assessments and out of window vial sign assessments (Subjects (b) (6) *Reviewer comments: " Based on the information available for review, the missed assessments are sporadic."*
(b) (4)			
Site #5	• Discrepancies between reported adverse events to FDA and those in patients/source records.	• Applicant updated NDA to include all unreported AEs	• List included previously reported AEs • All unreported events were non-serious and deemed unrelated to the investigational product (IP).

Investigator/ Site	Findings	Resolution	OSI Explanation/ Comments
(b) (4)			

Source: OSI Clinical Inspection Summary for NDA214907 dated July 14, 2021.
Abbreviations: AE=adverse event, CRO=contract research organization, DIRM=Division of Imaging and Radiation Medicine, HPE=histopathological examination, ICF=informed consent form, IRB=institutional review board, IP=investigational product, (b) (4) NDA=new drug application, OSI=office of scientific investigations, SOP=standard operating procedure

Summary and Conclusion:
From a review of the above deficiencies identified by OSI and Applicant's responses to the Information Requests, we deem the deficiencies to be relatively minor and unlikely to impact the efficacy and safety findings of Cytalux. The sponsor report of the results of the re-reads of histopathology slides from a sample patient group as described in Table 3 above showed adequate concordance with the initial read.

4.2. Product Quality

Reference is made to the Chemistry, manufacturing, and controls (CMC) Review. Integrated Quality Review by OPQ added on October 19, 2021 in DARRTS, (874961 October 19, 2021).

The applicant has provided sufficient information to assure the identity, strength, purity, quality, including sterility of the proposed drug product. The proposed drug product is a single use, sterile, liquid injectable solution provided in an amber glass vial. The drug is temperature sensitive and photosensitive, so it is packaged in an amber glass vial with outer carton and is to be stored in freezer at -20°C (± 5°C). At the time of use, the product is thawed and diluted with 250 mL of 5% dextrose solution (D5W) for intravenous administration to yield a light blue green clear solution. All the issues around freezer storage, thaw period, dilution, storage and use of diluted infusion solution have been adequately resolved and are reflected in the product labeling.

The Applicant evaluated saline as the diluent for the proposed drug product and found that this resulted in hypersensitive reactions in study subjects. An investigation was conducted and concluded that the hypersensitivity was most likely due to the formation of Cytalux aggregates.

Hence following warning has been included in the section 5 of PI: Use of the incorrect diluent to prepare the Cytalux infusion solution can cause the aggregation of pafolacianine and can induce infusion reactions. Use only 5% Dextrose Injection USP to prepare the Cytalux infusion solution. Do not use other diluents.

During the course of review, the applicant changed the drug product assay method (submission dated 06/20/2021). This method was also used to determine the assay of incoming OTL0038 drug substance batches received by the drug product manufacturer. The revised UHPLC based assay method (same method for drug substance and drug product) along with supporting validation data were submitted to the NDA in the major amendment dated 08/13/2021). The applicant performed an equivalency assessment between GRAM-TM-0060 (assay method used during the clinical trials) and GRAM-ATM-1074 (the assay method proposed for the commercial drug product). The UHPLC method gives results that are consistent with historical values generated with the UV method, and hence the safety and efficacy assessment is not impacted. Additionally, it was recommended that the drug substance be granted a (b) (4) retest date, as opposed to the (b) (4) originally requested by the applicant, due to inadequacy of the drug substance stability data. The company agreed to (b) (4) retest date for the drug substance when stored (b) (4) in the designated container closure system (11/16/2021). All the key review issues (assurance of sterility, container closure integrity, assay, chiral purity, impurities, storage and use conditions, expiration and use periods and the cGMP status of a listed facilities) have been adequately resolved. The labeling include adequate quality information, as required. All associated manufacturing, testing, packaging facilities were deemed acceptable. Based on the OPQ review team's evaluation of the information provided in the submission, Cytalux (pafolacianine) injection possesses the necessary attributes to ensure indicated safety and efficacy.

The Applicant has provided adequate drug product stability data to support the proposed storage and shipping condition (frozen; -20ºC ± 5°C) and the proposed shelf life of 36 months under the recommended storage conditions.

Based on data, the drug product can tolerate three freeze thaw cycles. Further, the drug product has been shown to tolerate up to 24 hours of thawed time during processing and handling. For use, the drug is thawed, removed from the vial, and is diluted into D5W, protected from light, and can be stored for up to 24 hours at 2-8°C prior to administration to the patient.

4.3. Clinical Microbiology

This section is not applicable to this NDA.

4.4. Devices (b) (4)

Pafolacianine is not part of a combination product. The Applicant used two devices in Study 006 – 1. (b) (4) (FDA reference 510K (b) (4) and 2. (b) (4) (b) (4) (FDA reference 510K (b) (4) (b) (4) has satisfied the 501 (k) requirements for clearance by FDA's Center for Devices and Radiation Health (CDRH) (4893385 November 23, 2021).

CDRH was also consulted to assess the regulatory status and the validation data for the commercially available immunohistochemistry (IHC) kit for assay of folate receptor expression used by the Applicant.

Per the Applicant –

- The kit was from Biocare Medical (Intellipath FLX™), catalogue number - IP4006K G10
- Antibody 26B3.F2 in the kit was licensed from Morphotek® (4868034 October 5, 2021)
- The kit contains reagents required to complete an IHC staining procedure for formalin-fixed, paraffin-embedded specimens
- Folate Receptor alpha IHC Assay Kit (IPI4006K) was formulated for use with Biocare's intelliPATH™ Automated Slide Stainer
- The staining characteristics were scored using the following grades 0, 1+, 2+ and 3+ based on staining intensities

CDRH assessment following the review of the information provided by the Applicant was completed on June 3, 2021, (ICCR00084169 and ICC2100438 2021), and submitted in DARRTS on October 5, 2021 (4868034 October 5, 2021). Highlights from the response are listed below: (b) (4)

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

This NDA is approvable from a nonclinical perspective.

The Applicant conducted a comprehensive nonclinical program that supports the marketing authorization of Cytalux (pafolacianine or Pteroyl-L-tyrosine-S0456; OTL-0038) at the

recommended dose of 0.025 mg/kg in adult patients with known or suspected ovarian cancer as an adjunct for intraoperative identification of malignant lesions . This decision to approve is based on nonclinical findings that established specificity, efficacy, adequate margins of safety (300-fold safety margin for extended, single dose and repeat dose toxicity studies in rats and dogs) and absence of significant toxicologically-relevant findings. The pivotal studies include clearly defined *in vitro* / *in vivo* proof-of-concept and safety pharmacology studies, pharmacokinetic, single and repeat dose general toxicity studies, in vitro and in vivo genotoxicity, and embryofetal-developmental toxicity studies.

All nonclinical safety pharmacology, pharmacokinetic, and toxicology studies were conducted by or for the Sponsor in accordance with Good Laboratory Practice (GLP) regulations.

In vitro and *in vivo* Pharmacology Studies:
Pharmacodynamic proof-of-concept studies were conducted in mice primarily to demonstrate receptor binding, tumor visualization, biodistribution and to investigate image results using varying drug doses.

Safety Pharmacology:

There were no significant safety findings identified for pafolacianine by a battery of safety pharmacology studies which included GLP conducted *in vitro* human ether-a-go-go-related gene (hERG) assays (IC_{50} > 300µM), central nervous system (CNS) safety pharmacology by functional observational battery in Sprague Dawley rats (up to 46.3 mg/kg), a cardiovascular and respiratory safety pharmacology study in Beagle dogs (up to 13.9 mg/kg), and CNS safety pharmacology in Sprague Dawley rats.

PK/ADME:
Absorption, distribution, metabolism and elimination (ADME) of radiolabeled pafolacianine in plasma was similar between sexes. Using radiolabeled [^{14}C]-pafolacianine, a similar pattern of excretion was observed in males and females with approximately equal fraction of the administered dose being excreted in urine and feces over 168 hours, post-dose. Tissue-to-plasma ratios for radioactivity were >1.0 for all tissues in male and females with the exception of bone (femur), brain, eye and fat, indicating a binding of [^{14}C]-pafolacianine and/or its metabolites to tissues. Overall, [^{14}C]-pafolacianine was widely distributed throughout the body with the greatest calculated dose exposure being received by the fat, liver, kidneys, muscle, and skin. Lastly, the findings of protein binding studies showed that pafolacianine was bound to plasma protein from 93.7% to 99.1% in human, rat, and dog plasma.

Single and Repeat-Dose Toxicity:
Extended single-dose and repeated-dose toxicity and toxicokinetic studies were conducted in Sprague Dawley rats and Beagle dogs. Taken together, the results of toxicokinetic studies were generally benign except for the prominent finding of green discoloration observed throughout

multiple tissues (injection site, skin, extremities, facial area, urogenital area, kidneys, and urinary bladder); green extremities and green discoloration of the urine. There were no histopathological correlates to the tissue discoloration occurring at the mid and high dose levels of pafolacianine and the discoloration was considered non-adverse in both the single and repeat-dose toxicity studies in both species. Based on the findings of the rodent and non-rodent single dose toxicity studies, the administered high dose of 46.3 mg/kg in rats and 13.9 mg/kg in dogs, were determined as the no-observed-adverse-effect-level (NOAEL) and a 300-fold safety margin based on the proposed clinical dose of 0.025 mg/kg in the label. Similar findings of broad tissue discoloration were observed in repeat dose toxicity studies in rats and dogs at the mid and high dose. Safety margins of 300-fold were determined based on NOAELs of 46.3 mg/kg and 13.9 mg/kg for repeat dose toxicity studies in rat and dogs, respectively based on non-adverse findings of green tissue discoloration which may have been due to folate receptor binding.

Genotoxicity, Reproductive Toxicity, Carcinogenicity, and Other Nonclinical Studies:
Pafolacianine was negative for genotoxic potential by a battery of *in vitro* and *in vivo assays* that included an *in vitro* bacterial reverse mutation assay, *in vitro* mammalian cell micronucleus assay in Chinese hamster ovary (CHO) cells and *in vivo* micronucleus assay in rats. Carcinogenicity studies were not conducted and are not required for single-use optical imaging agents.

The Sponsor requested and was granted a waiver from conducting fertility and embryonic development (FEED) and pre- and postnatal development (PPND) studies. Reproductive and developmental toxicity studies conducted with pafolacianine were limited to exploratory and definitive embryo-fetal toxicity studies in rats and rabbits that resulted in embryo-fetal toxicity. In an exploratory follow-up embryo-fetal development study conducted subsequently in rats with the unconjugated fluorescent dye component of pafolacianine (b) (4), no evidence of embryo-fetal toxicity was demonstrated. These data suggested that embryo-fetal toxicity noted in both rats and rabbits was associated with the folate moiety component of pafolacianine. In definitive studies, the NOAELs for maternal (F0) and developmental (F1) toxicity were each 1.5 mg/kg/day in rats and 3 mg/kg/day in rabbits. Steady-state maternal systemic exposure (AUC) to pafolacianine at the NOAEL was 9,630 ng·h/mL in rats and 34,800 ng·h/mL in rabbits.

In a local tolerance study conducted in rabbits, administration of a single perivascular, intramuscular, or subcutaneous injection of pafolacianine resulted in slight, reversible irritation at the site of injection. The findings also included the presence of non-adverse greenish discoloration within the perivascular and subcutaneous injection sites at 24- and 96-h post dose. Evidence of perivascular hemorrhage and tissue inflammation with minimal to moderation infiltration of lymphocytes and neutrophils was also observed in the rat and dog repeat-dose toxicity studies but was reversible following recovery.

Lastly, in a photoactivation study conducted in rats, there was no evidence of phototoxicity as demonstrated by the absence of adverse effects on clinical observations, body weights, clinical pathology endpoints, selected organ weights and histopathology of selected tissues and organs following acute IV administration of pafolacianine (at up to 46.3 mg/kg) and a single 15-minute exposure of the surgical incision site, liver, kidney and jejunum to near infrared light (760 nm) under surgical conditions.

5.2. Referenced NDAs, BLAs, DMFs

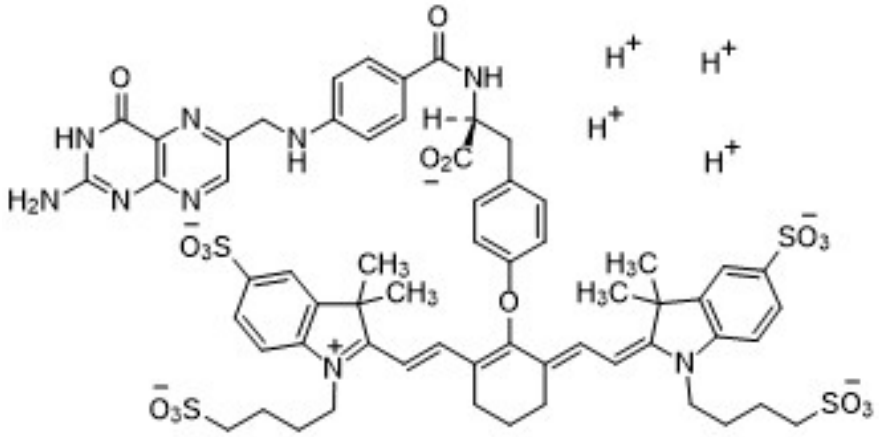
None.

5.3. Pharmacology

5.3.1. Introduction

Pafolacianine (Pteroyl-L-tyrosine-S0456 or OTL-0038), is a folate receptor-targeted tumor imaging agent intended for use in patients who overexpress folate receptor alpha (FRα). Pafolacianine is a small molecule fluorescent marker that consists of a pteroic acid moiety conjugated to a near-infrared (NIR) cyanine dye. The pharmacology of pafolacianine as described in this review is comprised of in vitro and in vivo primary pharmacodynamic studies, and safety pharmacology studies. The structure of pafolacianine is indicated in Figure 1.

Figure 1. Structure of Pafolacianine



Source: CAS#: 1628423-76-6 (pafolacianine sodium)

5.3.2. Mechanism of action and Proof-of-Principle Studies

Mechanism of Action:
Pafolacianine is a fluorescent marker that consists of a NIR dye and a folate-derived ligand that allows it to bind to alpha-type folate receptors (FRα) overexpressed in ovarian cancer cells and absorbs light in the NIR region (760 – 785 nm) with peak at 776 nm and emits fluorescence within a range of 790 – 815 nm with peak emission at 796 nm. Pafolacianine is retained in FRα-expressing tissues and can be excited with light from a camera system thus enabling

visualization of the fluorescence of the dye. Under such visualization, FR-positive cells appear as fluorescent masses in contrast to dark or possibly auto-fluorescent normal cells. Binding affinity is described under *In vitro Pharmacodynamic (PD) study.*

Proof-of-Principle:
The initial scientific rationale for developing pafolacianine as an intraoperative imaging agent in patients with folate receptor positive (FRα) ovarian cancer was provided by *in vitro* and *in vivo* primary pharmacodynamic studies.

Folic acid (folate or vitamin B9) is an essential vitamin that acts as a precursor for cofactors that regulate a variety of biochemical processes that support critical cellular functions e.g., cell proliferation, mitochondrial respiration, and epigenetic regulation. These processes require binding to folate receptors (FR) which consists of FRα, FRβ and FRγ. FRs are cysteine-rich cell surface glycoproteins that bind folate with high affinity to mediate the uptake and function of folic acid. Pafolacianine was evaluated *in vitro* for affinity and specificity of FRα binding in tumor cells, and *in vivo* to assess potential as a tumor imaging agent in nude mice bearing human tumor xenografts. For this purpose, *in vitro* and *in vivo* proof-of-concept studies were conducted in mice to demonstrate receptor binding, tumor visualization, biodistribution and to investigate image results with different doses. Intense fluorescence was observed in tumors of nude mice bearing FR-positive tumor xenografts after IV administration. Pafolacianine, as a folate-seeking imaging agent, is intended for use in the detection of folate alpha receptor positive (FRα+) ovarian cancer and specifically targets and illuminates cancer cells overexpressing folate receptors.

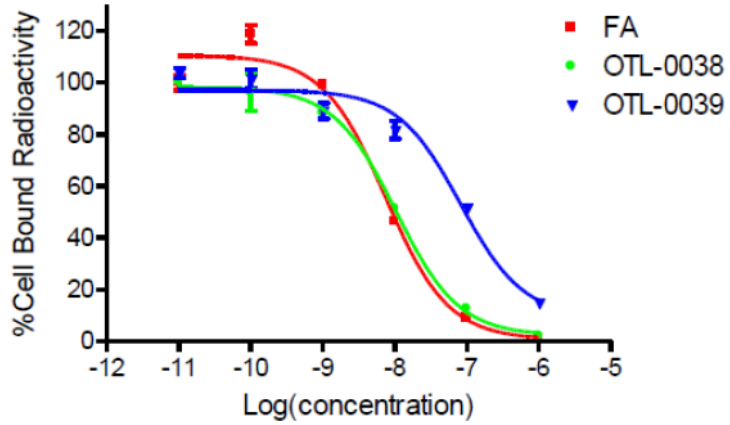
In vitro Pharmacodynamic (PD) Study:

Study title: Binding of OTL-0038 and OTL-0039 (D-isomer of OTL-0038) to folate receptors in vitro (Study number: OTL13-001)

Based on *in vitro* binding studies, pafolacianine showed FRα binding that compared well with the binding affinity of folic acid (K_D of 10.4nM versus 7.4nM, respectively). Results of whole body and tissue distribution studies showed that pafolacianine accumulated mainly in folate receptor positive (FR+) tumors, with no substantial fluorescence activity in the other tissues except for the kidneys, which also express high levels of folate receptors.

In vitro FR binding of pafolacianine (L-isomer), OTL-0039 (D-isomer of pafolacianine) were compared to folic acid (the cognate ligand for folate receptor) was evaluated in a human cervical cancer cell line (KB cell line) known to overexpress FRα. The relative binding of pafolacianine, OTL-0039 and [^{3}H]folic acid (FA) are shown in Figure 2 below.

Figure 2. Relative Binding Affinity of Folic Acid (FA), OTL-0038 and OTL-0039



Source: Figure 1; 2-6-2 Pharmacology Written Summary, p.8 of 25

The relative binding affinities of the three compounds is shown in Table 4. Binding affinities of OTL-0038, OTL-0039 and folic acid to folate receptor indicate the binding specificity of OTL-0038 and folic acid in contrast to OTL0039. The dissociation constants (K_D) were 10.4 nM, 81.8 nM, and 7.4 nM for OTL-0038, OTL-0039 and folic acid, respectively are shown in Table 4 below.

Table 4. Binding Affinities of Folic Acid, Pafolacianine (OTL-0038) and OTL-0039

Test Article	Dissociation Constant (K_D), nM	Relative Binding Affinity
Folic acid	7.4	1
OTL-0038	10.4	0.7
OTL-0039	81.8	0.09

Source: Table 2; 2-6-2 Pharmacology Written Summary (p.8 of 25)
Relative binding affinity is defined as the molar ratio of the compound required to displace 50% of [^{3}H]-folic acid bound to FR on cells; relative affinity of folic acid=1; relative affinity <1 indicates weaker affinity for FR; relative affinity >1 indicates stronger binding to FR.

Key Findings:

- Pafolacianine showed a high affinity for folate receptors (FR) and compared well with the binding affinity of folic acid (K_D of 10.4nM vs. 7.4nM, respectively).
- OTL-0039 (D-isomer of pafolacianine) has a lower affinity for FR when compared to folic acid and pafolacianine.

Conclusions:

Pafolacianine demonstrated high affinity for FRα and it compared well with the binding affinity of folic acid (K_D of 10.4nM versus 7.4nM, respectively). OTL-0039 (D-isomer of OTL-0038) had a lower affinity for FR when compared to folic acid and OTL-0038. Based on the results, OTL-0038 competed well with [^{3}H]-folic acid for binding to FR, indicating that OTL-0038 is highly specific for FRα.

***In vivo* Pharmacodynamic (PD) Studies:**

Whole body and tissue distribution studies demonstrated accumulation of pafolacianine in folate receptor alpha-positive (FR+α) tumors (Study Number: OTL-13-002). The potential of pafolacianine binding to non-FR positive tissue was not evaluated in this review).

An *in vivo* whole-body imaging study in female *nu/nu* mice (5 weeks old, 18-20 g) bearing FR-positive (KB cell) or FR-negative (A549 cell) xenografts to demonstrate tissue distribution. The *nu/nu* mouse are immunodeficient nude mice because they lack a thymus and are unable to produce T-cells.. Mice were administered pafolacianine in varying doses (0.3-30 nmol/mouse). The primary objective was to determine folate receptor (FR)-positive tumor uptake of pafolacianine and OTL-0039 and tissue distribution after an intravenous administration to tumor-bearing athymic mice.

Key Findings:

- Substantial fluorescence was observed in tumors at 2.5h after administration of 10 nmol pafolacianine in mice bearing FR+ KB tumor xenograft. A weaker signal was observed in kidneys, which are known to express FR in proximal tubules with little or no substantial signal detected in normal tissues.
- OTL-0039 tissue distribution pattern was similar to that of pafolacianine but showed weaker fluorescence intensity consistent with FR binding data. The optimal pafolacianine dose range for tumor-to-background ratio was 1-30 nmol/mouse with fluorescence intensity increasing with dose. Pafolacianine did not target FR-negative (A549 cell) tumor xenografts *in vivo*.

Conclusions:
Pafolacianine demonstrated high specificity for FR-expressing tumors. Pafolacianine accumulated primarily in FR+ tumors, with no substantial fluorescence activity in the other tissues based on the results of whole-body and tissue distribution studies. The highest fluorescence intensity was observed in FR+ tumors without any accumulation in the other tissues except for the kidneys, which also highly express folate alpha receptors in the apical membrane of the proximal convoluted tubules (PCT)..

5.3.3. Safety Pharmacology

Summary of Safety Pharmacology Studies:

A core battery of safety pharmacology studies was conducted to investigate the effects of pafolacianine on vital physiological functions. Based on the results, it was concluded that pafolacianine had no significant effects on cardiovascular and respiratory systems. The core battery of safety pharmacology studies conducted with pafolacianine indicated no undesirable pharmacodynamic effects of pafolacianine on physiological functions in relation to exposure in the diagnostic range and above. No functional changes in vital organs or systems which are

likely to be of importance in clinical testing of pafolacianine were identified based on these studies.

Human Ether-à-go-go-related gene (hERG) Assay (ChanTest)
Study Title/number: Effect of OTL-0038 on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney (HEK) Cells / 130219.PUM
GLP Compliance: Yes
QA Statement: Yes

Study Objective:
The objective of the study was to examine the *in vitro* effects of pafolacianine at up to 300µM on the hERG (human ether-à-go-go-related gene) channel current (a surrogate for I_{Kr} - the rapidly activating delayed rectifier cardiac potassium current) at near-physiological temperatures.

Key Findings:

- Pafolacianine inhibited the hERG current by (Mean ± SEM; n=3), 0.9±0.5% at 10 µM and 1.0±0.4% at 300 µM versus 1.0±0.3% (n=3) in control. hERG inhibition at 10 and 300 µM was not statistically significant ($P < 0.05$) when compared to vehicle control values. The IC_{50} for the inhibitory effect of pafolacianine on hERG potassium current was > 300 µM (estimated).
- Under similar conditions, the positive control (60nM terfenadine, n=2 cells) inhibited hERG potassium current by 82.2 ± 4.8% (Mean ± SD), confirming the sensitivity of the assay.

Conclusion:
There were no drug-related effects on the hERG current observed in OTL0038-treated cells.

Cardiovascular and Respiratory Assessment Following Intravenous Administration to Conscious, Radiotelemetry-Instrumented Beagle Dogs

Study Title/number: Cardio-Respiratory Assessment of OTL-0038 in the Radio-telemetered Beagle Dog / (b) (4)963003
GLP Compliance: Yes
QA Statement: Yes

Study Objective:
The purpose of this study was to evaluate the potential acute effects of intravenous administration of pafolacianine on cardiovascular and respiratory parameters, and body temperature in conscious, radiotelemetry-instrumented male Beagle dogs.

Key Findings:

- A single intravenous injection (slow bolus, administered over a 1-2 min period) of pafolacianine at 0.9, 9.3, or 13.9 mg/kg to male Beagle dogs did not affect the heart rate, systolic, diastolic, mean arterial, and pulse pressure, body temperature, electrocardiogram (ECG) intervals (PR, QRS, RR, QT, and QTcV), or respiratory system (respiratory frequency, tidal volume, and minute volume).
- Pafolacianine administration resulted in a bluish discoloration of the forelimbs, ears, and gums; a blue discoloration of the sclera and urine (9.3 and 13.9 mg/kg) and green discoloration of the urine at the same dose levels.
- Findings were not considered adverse as the tissue and urine discoloration was attributed to test article color.

Conclusion:
A single intravenous injection of pafolacianine at 0.9, 9.3, or 13.9 mg/kg to male Beagle dogs did not affect the cardiovascular system (heart rate, systolic, diastolic, mean arterial, and pulse pressure), body temperature, ECG intervals (PR, QRS, RR, QT, and QTcV), or respiratory system (respiratory frequency, tidal volume, and minute volume).

5.4. ADME/PK

Table 5. Summary of ADME/PK Data

Type of Study	Major Findings
Absorption	
Please see PK data reported following Excretion.	
Distribution	
Pharmacokinetics, Tissue Distribution and Excretion Balance of [^{14}C]-OTL-0038 following Intravenous (IV) administration to Rats **((b) (4)963010)**	Based on the AUC_{last}, radioactivity was rapidly distributed to most tissues. In both sexes, the tissue: plasma ratios were greater than 1.0 for all tissues except in bone (femur), brain, eye, and fat, an indication of notable partitioning of [^{14}C]-tyrosine-pafolacianine at a dose of 2 mg/kg (approx. ~2.5 µCi/kg).
Distribution and Metabolism in the Rat Following a Single IV administration of [^{14}C]-OTL-0038 **(OTG/01)**	Distribution of the radioactivity was widespread at the first sampling time point, 8 hours, post-dose. Maximal concentrations were not observed until 120 and 168 hours, post-dose in most tissues evaluated. The greatest radioactive uptake was observed in the kidney cortex at all time points, with substantial uptake in the lymph nodes, choroid plexus, and some exocrine glands.
Plasma Protein Binding of OTL-0038 **(IAS-13-MS-044-DM)**	Plasma protein binding of pafolacianine was evaluated *in vitro* in fresh plasma from human, dog, and rat donors by equilibrium dialysis. Mean percentage of plasma protein bound pafolacianine was 99.1%, 98.6%, and 93.7% for rat, dog, and human, respectively.
Blood to Plasma Partitioning of [^{14}C]-OTL-0038 in Rat, Dog, and Human **(14693)**	Blood to plasma partitioning ratios of 0.70 (rat), 0.69 (dog), and 0.60 (human) were determined indicating there was no notable partitioning of pafolacianine into blood cells.
In Vitro Evaluation of OTL-0038 as an Inhibitor and Substrate of	Study evaluated pafolacianine as an inhibitor and substrate of human transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2,

Type of Study	Major Findings
OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K and a Substrate of P-gp and BCRP **(XT168061)**	MATE1, and MATE2-K) and as a substrate of the human transporters P-gp and BCRP. 1 µM pafolacianine was shown to be a substrate of OATP1B1, OATP1B3 and OAT1. Studies with pafolacianine and MATE1 or MATE-2K transporters appeared inconclusive due to low test article accumulation in control and transporter-expressing cells. OTL-0038 was not a substrate for P-gp, BCRP, OAT3, or OCT2.
Metabolism	
In vitro studies: Studies were performed with pooled plasma, urine and feces from rats, administered a single IV bolus injection of [^{14}C]-tyrosine-pafolacianine or [^{14}C]-dye-pafolacianine at 2 mg/kg (25 -100 µCi/kg), and in patients who received a single IV infusion of pafolacianine at 0.025 mg/kg in Phase 3.	
Stability Assessment and Metabolite Profiling of OTL-0038 in the Presence of Human, Monkey, Dog, Rat, and Mouse Hepatocytes **(IAS-13-MS-033-DM)**	Study was conducted to determine the hepatic stability of pafolacianine and to identify metabolites formed in the presence of human, monkey, dog, rat, and mouse hepatocytes. Percentage of pafolacianine remaining in hepatocyte incubations ranged from 32-84% at 4 hr. while the percentage remaining in the negative control reaction (i.e., fraction excluding hepatocytes) was 52%.
Stability Analysis of OTL-0038 in Rat, Dog, and Human Whole Blood **(14200)**	Study assessed the stability of pafolacianine in rat, dog and human whole blood (WB). The repeated blood stability experiment conducted in the absence of any acid, supported the conclusion that pafolacianine was stable in blood for all species tested through at least 8 hr.
Stability of [^{14}C]-OTL-0038 in Rat Whole Blood and Plasma During Sample Processing **((b) (4) 963025)**	This study determined the stability of [^{14}C]-tyrosine-pafolacianine-derived radioactivity in male SD rats (whole blood/plasma) during sample processing and storage under conditions. Blood was collected from 5 SD rats in tubes containing K_2EDTA. [^{14}C]-tyrosine-pafolacianine was spiked into blood and incubated at room temperature for 0, 0.5 or 1 hr. Following centrifugation, plasma samples were stored at -80°C for 2 weeks, followed by extraction and analysis. Data showed that despite the absence of acid and the blood and freezer stability, the predominant radio-chromatographic peaks included [^{14}C]-tyrosine-pafolacianine.
In vivo studies: (Metabolite profiling) Studies were performed with pooled plasma, urine and feces from rats, administered a single IV bolus injection of [^{14}C]-tyrosine -pafolacianine or [^{14}C]-dye pafolacianine at 2 mg/kg (25-100 µCi/kg), and in patients who received a single IV infusion of pafolacianine at 0.025 mg/kg in Phase 3.	
Excretion	
Pharmacokinetics, Tissue Distribution and Excretion Balance of [^{14}C]-OTL-0038 Following IV Administration to Rats **((b) (4) 963010)**	In the excretion mass balance phase, 3 rats/sex administered a single IV bolus dose of [^{14}C]-tyrosine- pafolacianine (2 mg/kg; 25 µCi/kg) were placed in individual metabolism cages for periodic urine and fecal collection through 168 hr. post-dose. After final excreta collection, animals were euthanized and excreta samples analyzed for total radioactivity. Following a single IV dose of [^{14}C]-pafolacianine, radioactivity was nearly equally excreted in the urine vs. the feces through 168 hr. post dose, indicating liver and kidney involvement in the excretion of [^{14}C]-pafolacianine-related radioactivity. After carcass processing, additional 35-39% of total [^{14}C]-pafolacianine-derived radioactivity was recovered, resulting in an overall total recovery of 97.4-99.8%. Profiling of urine/fecal samples showed that unchanged [^{14}C]-tyrosine-pafolacianine was the main radioactive

Type of Study	Major Findings
	entity suggesting that [^{14}C]-tyrosine-pafolacianine was primarily excreted unchanged.
TK Data from General Toxicology Studies	
A Single-Dose Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic (TK) Study of OTL-0038 with a 14-Day Recovery Period in Sprague Dawley Rats **((b) (4)963001)**	**Systemic exposure:** Systemic exposure increased as dosage increased from 3.1 to 46.3 mg/kg. The increase in systemic exposure, in terms of dose-normalized AUC_{last}, was greater than dose-proportional in males and females; From 30.8 to 46.3 mg/kg in females; exposure was nearly proportional. Based on AUC_{last}, exposure was similar between sexes at 30.8 and 46.3 mg/kg, but approximately 4-fold higher in females compared to males at 3.1 mg/kg. For half-life, systemic clearance, and apparent volume of distribution during the terminal phase, data were inadequate to determine trends regarding sex or dose level.
A Single-Dose Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic (TK) Study of OTL-0038 with a 14-Day Recovery Period in Sprague Dawley Dogs **((b) (4)963002)**	**Systemic exposure:** Systemic exposure to pafolacianine was not evident in most male and female dogs dosed at the 0.9 mg/kg low-dose level but was measurable in all animals dosed at the mid- (9.3 mg/kg) and high- (13.9 mg/kg) dose levels; at the 0.9 mg/kg low-dose level, plasma concentrations of pafolacianine were measurable in 2/6 males and 1/6 females only at 5 min post-dose. Exposure increased with increase in dose from 0.9 to 13.9 mg/kg. The increase in systemic exposure, in terms of mean dose-normalized AUC_{last}, was greater than dose-proportional in males and females. Systemic exposure to pafolacianine, in terms of AUC_{last}, was similar between sexes at all dosage levels evaluated. Half-life, systemic clearance, and apparent volume of distribution during the terminal phase, data were inadequate to determine trends regarding sex or dose level.
A 14-Day (Once Weekly) Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic Study of OTL-0038 in Sprague Dawley Rats **((b) (4)963018)**	**Plasma concentration**: Plasma concentrations of pafolacianine were measurable in all animals 5 min-24 hr. post-dose at all dose levels. Mean plasma concentrations decreased rapidly through 1 hr. post-dose and declined slowly from 1- 24 hr. **Systemic exposure**: Systemic exposure was dose-proportional from 3.1 to 46.3 mg/kg/dose in males and females. The increase in systemic exposure was similar between sexes. There was little to no accumulation after 3 repeated doses as the accumulation ratios ranged from 0.874-1.40. Systemic clearance (Cl) was low and the mean apparent volume of distribution at steady state (V_{ss}) was moderate following intravenous administration and was similar across doses and between sexes. The mean V_{ss} ranged from 0.773-1.70 L/kg resulting in a $t_{1/2}$ that ranged from approximately 6 -11 h. The NOAEL for pafolacianine under the conditions of this study was considered by the reviewer as 3.1 mg/kg/dose for males and females. Systemic exposure (C_0 and AUC_{last}) at the NOAEL was 240 µg/mL and 476 µg·hr/mL, respectively, for males and 210 µg/mL and 166 µg·hr/mL, respectively, for females.
A 14-Day (Once Weekly) Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic Study of OTL-0038 in Beagle Dogs **((b) (4)963019)**	**Plasma concentration:** Plasma concentrations of pafolacianine were measurable in all animals from 5 min-24 h post-dose at all dose levels on Days 0 and 13. Plasma concentrations decreased rapidly through 1 hr. post-dose and then more slowly from 1 hr. post-dose through 24 hr. on both evaluation days. **Systemic exposure:** Systemic exposure (by dose-normalized AUC_{last}) increased with increasing dose and was dose-

Type of Study	Major Findings
	proportional in males and females to a similar extent. Little to no accumulation was evident after weekly dosing as indicated by mean accumulation ratios that ranged from 0.952-1.19. **Mean systemic clearance: Cl** was low and the mean apparent volume of distribution at steady state (V_{ss}) was moderate following IV administration. Clearance and volume of distribution were similar across doses and between sexes. The mean Cl ranged from 1.59-2.39 mL/min/kg and the mean V_{ss} ranged from 1.47-2.42 L/kg. $T_{1/2}$ ranged from 13-18 hr.
TK Data From Reproductive Toxicology Studies	
An Intravenous Study of the Effects of OTL-0038 on Embryo-Fetal Development in Rats **((b) (4)963030)**	**Rat AUC:** Exposure increased with dose in a nearly dose-proportional manner in terms of C_0 and C_{max} and in a greater than dose-proportional manner in terms of AUC_{last}. Consequently, 100-fold increase in dose resulted in approximately 470- and 436-fold increases in AUC_{last} on GD 6 and GD 17, respectively. There was no notable accumulation of pafolacianine in plasma on GD 17 versus GD 6 (in terms of AUC_{last}) with accumulation ratio of 1.60, 1.04, and 1.47 at 0.015, 0.15, and 1.5 mg/kg/day, respectively.
An Intravenous Study of the Effects of OTL-0038 on Embryo-Fetal Development in Rabbits **(00963029)**	**Rabbit AUC:** Exposure, in terms of $AUC_{(0-t)}$ and C_{max}, increased with increasing dose in a greater than dose-proportional manner (12- to 17-fold increase across a 10-fold increase in dose) when comparing the 0.3 to 3 mg/kg/day dose levels on both evaluation days. Additionally, exposure increased in a dose-proportional manner across the 3-fold increase in dose from the mid to high (1 to 3 mg/kg/day) dose level.
TK Data From Carcinogenicity Studies	
No carcinogenicity study was conducted and none was needed.	

Abbreviations: ADME=absorption, distr bution, metabolism, excretion, AUC=area under the curve, C_{max}=maximum concentration, GD=gestation day, NOAEL=no-observed-adverse-effect level, PK=pharmacokinetics

5.5. Toxicology

5.5.1. General Toxicology

The rat and dog were selected as the rodent and nonrodent toxicology species, respectively, for the pafolacianine toxicology program. The clinically-relevant route of exposure (IV injection) was used in all in vivo toxicology studies, including single- and repeat-dose toxicology, genotoxicity studies, and embryo-fetal development studies. Single-dose intravenous (slow bolus) toxicity studies with 14-day recovery were conducted with pafolacianine formulated in phosphate-buffered saline (PBS [pH 7.4±0.1]) vehicle at up to 46.3 mg/kg in Sprague Dawley rats (Study #: (b) (4)963001) and at up to 13.9 mg/kg in Beagle dogs (Study #: (b) (4)963002). 14-day, once weekly repeat-dose (3 doses) intravenous (slow bolus) toxicity studies were conducted with pafolacianine formulated in PBS [pH 7.4±0.1]) vehicle at up to 46.3 mg/kg in Sprague Dawley rats and 13.9 mg/kg in Beagle dogs.

5.5.1.1. Single Dose Toxicity

Single Dose Toxicity Study in Rats

Study title/ number: A Single-Dose Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic (TK) Study of OTL-0038 with a 14-Day Recovery Period in Sprague Dawley Rats / (b) (4) 963001

- There were no mortalities and all rats survived to scheduled necropsies. Based on the findings, the no-observed-adverse-effect level (NOAEL) was 46.3 mg/kg, the highest dose level tested. Systemic exposure (C_0 and AUC_{last}) at the NOAEL was 174 µg/mL and 286 µg·hr/mL, respectively, for males and 231 µg/mL and 255 µg·hr/mL, respectively, for females.
- Test article-related clinical observations in males and females included a blue tail, dark green urogenital area, green extremities and green facial area, in the 30.8 mg/kg (mid-dose) and 46.3 mg/kg (high-dose) groups on study days 0-2. Test article-related clinical observation of blue urogenital area was noted in one 3.1 mg/kg group (low-dose) female.
- Neurobehavioral changes (slightly increased touch response and vocalization) occurred in males and females 5 min after administration at all dose levels of pafolacianine. Females were more sensitive than males.
- Green discoloration was observed in multiple tissues (injection site, skin, kidneys, and uteri) of main study animals (30.8 mg/kg and 46.3 mg/kg in both sexes) and in the kidney and skin in recovery animals (30.8 mg/kg and 46.3 mg/kg) at necropsy. Green discoloration was not detected in low dose (3.1 mg/kg) animals or in the vehicle control groups.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 6. Methods for Study No.: (b) (4) 963001

Methods	Details
Dose and frequency of dosing:	0 (vehicle), 3.1 (LD), 30.8 (MD), 46.3 (HD) mg/kg OTL-0038; single dose administration.
Route of administration:	Intravenous
Formulation/Vehicle:	Pafolacianine (Lot #13-038-41-15, % purity: 96.44% (at 270 nm) and 92.11% (at 774 nm (HPLC, achiral) was prepared in a phosphate-buffered saline (PBS), pH 7.4±0.1 / PBS
Species/Strain:	Sprague Dawley Crl:CD(SD) rats.
Number/Sex/Group:	10/sex/group
Age:	9-10 weeks of age at dosing
Satellite groups:	TK satellite, n=9/sex/group for pafolacianine, and n=3 for vehicle control.
Deviation from study protocol affecting interpretation of results:	None

Abbreviations: HD=high dose, HPLC=high-performance liquid chromatography, LD=low dose, MD=mid dose, TK=toxicokinetic

Table 7. Observations and Results: Changes From Control (Study No.: (b) (4) 963001)

Parameters	Major Findings
Mortality	There were no deaths. All animals survived to scheduled necropsies.
Clinical Signs	Test article-related blue tail, dark green urogenital area, green extremities, green facial area, and/or green material on the cage floor noted for males and females in the mid- and high- dose groups on days 0-2. One female in the low-dose group had a bluish discoloration of the urogenital area.
Body Weights	There were no treatment-related effects.
Ophthalmoscopy	No test article-related effects on ophthalmoscopic parameters.
Modified Irwin Assessment	Intravenous administration of 3.1, 30.8, or 46.3 mg/kg of OTL-0038 resulted in increases, albeit slight, in touch response in male and female rats. Increased vocalization were observed in females more frequently than in males especially at the mid to high doses. There were no concomitant changes in behavior. Effects did not appear dose related.
Hematology	No test article-related changes in hematology and coagulation parameters for males or females at any OTL-0038 dosage level at either the primary or recovery necropsies.
Clinical Chemistry	No treatment-related effects on serum chemistry.
Urinalysis	There was a higher urine pH noted in males and females in the MD and HD.
Gross Pathology	Test article-related gross findings included green discoloration in multiple organs for males and females in the MD and HD groups. These findings were more frequent at the primary compared to recovery necropsy. Discoloration was most frequently observed in the kidney and occurred in all males and females starting at the mid dose at primary and recovery sacrifices. At the primary sacrifice, green discoloration was also observed at the injection site in animals in both sexes at the mid and high doses. At recovery sacrifice, injection site discoloration was observed only in 1 MD male and 1 HD female. Other common sites for green discoloration were skin (primary and recovery sacrifices), urinary bladder (primary sacrifice) and all uteri in high dose group at primary sacrifice. No discoloration was observed in males or females administered the low dose at either primary or recovery sacrifice.
Organ Weights	No test article-related effect on organ weights.
Histopathology Adequate battery: Yes	Test article-related microscopic findings observed at primary sacrifice were limited to a slight increase in the severity of acute perivascular inflammation at the injection site of females across all dose levels and in a single male administered the MD in the recovery necropsy. No histopathology correlates were reported for tissue and organ discolorations.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose

Single Dose Toxicity Study in Dogs

Study title/ number: A Single-Dose Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic (TK) Study of OTL-0038 with a 14-Day Recovery Period in Beagle Dogs / (b) (4) 963002

- Notable test article-related clinical observations in males and females dogs dosed at the 9.3 and 13.9 mg/kg (high-dose) levels included urine containing green material and excessive salivation noted on study day (SD) 0. Pale body and extremities noted beginning on SD 6. In addition, pale gums were observed in two males and one female administered the mid dose (9.3 mg/kg). A higher mean urinary pH was observed in

females at the mid (9.3 mg/kg) and high (13.9 mg/kg) dose groups, and in males at the mid-dose level.

- Macroscopic findings at the primary necropsy were similar to those observed in rats and consisted of green discoloration in multiple organs including the kidneys at the mid and high dose groups; green discoloration of the urinary bladder in 1 male (HD; high dose) and 2 females (MD; mid dose) levels. Green discoloration was not observed in animals administered the low dose (LD; 0.9 mg/kg). There were no histologic correlates for the green discoloration in tissues.
- Histopathology revealed no remarkable test article-related microscopic findings at the primary and recovery sacrifices. However, there was a correlation between the red discolorations at the injection site with hemorrhage.
- Pafolacianine was well tolerated and resulted in a NOAEL of 13.9 mg/kg.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 8. Methods for Study No.: (b) (4) 963002

Methods	Details
Dose and frequency of dosing:	0 (vehicle), 0.9 (LD), 9.3 (MD), and 13.9 (HD) mg/kg OTL-0038; single-dose administration.
Route of administration:	Intravenous
Formulation/Vehicle:	Pafolacianine (Lot #13-038-41-15, % purity: 96.44% at 270 nm and 92.11% at 774 nm (HPLC, achiral) was prepared in a phosphate-buffered saline (PBS), pH 7.4±0.1 /PBS.
Species/Strain:	Dog/Beagle
Number/Sex/Group:	6/sex/group
Age:	7 months of age at receipt
Satellite groups:	None
Deviation from study protocol affecting interpretation of results	None

Abbreviations: HD=high dose, HPLC=high-performance liquid chromatography, LD=low dose, MD=mid dose

Table 9. Observations and Results: Changes From Control (Study No.: (b) (4) 963002)

Parameters	Major Findings
Mortality	No unscheduled deaths.
Clinical Signs	The urine in males and females administered the MD and HD (9.3 and 13.9 mg/kg), respectively, had a greenish discoloration. Excessive salivation was observed in MD and HD animals on SD 0, pale body extremities on days 6-9. Pale gums was observed in 2 males and 1 female administered the MD.
Body Weights	No test article-related effects.
Ophthalmoscopy	No test article-related effects on ophthalmoscopic parameters.
Hematology	No test article-related alterations in hematology and coagulation parameters.
Clinical Chemistry	No test article-related effects on serum chemistry at primary and recovery sacrifices.
Urinalysis	Higher mean urine pH values in the MD and HD group females and in the HD males at primary necropsy were considered test article related (change in urine pH in the females was statistically significant). At the recovery necropsy, only HD females had higher urine pH but the pH was not statistically significant when compared to vehicle control group.

Parameters	Major Findings
Gross Pathology	Green discoloration at the MD and HD in multiple organs including kidneys and urinary bladder. No green discoloration was observed in animals administered the LD. There were no histologic correlates for the green discoloration in tissues. There were no test article-related alterations in organ weights at either the primary or recovery necropsies.
Organ Weights	No test article-related effects on organ weight.
Histopathology Adequate battery: Yes	No test article-related microscopic findings.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose

5.5.1.2. Repeat-Dose Toxicity

Repeat-Dose Toxicology Study in Rats

Study title/ number: A 14-Day (Once Weekly) Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic Study of OTL-0038 in Sprague Dawley Rats / (b) (4) 963018

- There were no mortalities and all rats survived to scheduled necropsies. Based on the absence of toxicologically relevant findings, the no-observed-adverse-effect level (NOAEL) was set at 46.3 mg/kg, the highest dose level tested.
- Test article-related clinical observations in males and females included a blue tail, dark green urogenital area, green extremities and green facial area, in the 30.8 mg/kg (MD) and 46.3 mg/kg (HD) groups on study days 0-2. Test article-related clinical observation of blue urogenital area was noted in one 3.1 mg/kg group (low-dose) female.
- Neurobehavioral changes included a slightly increased touch response, vocalization in both males and females 5 min after i/v administration of the test article at all dose levels with females appearing more sensitive than males. Gross observations of green discoloration were observed in multiple tissues from the 30.8 mg/kg (MD and 46.3 mg/kg (HD) males and females at the primary necropsy. However, some green colored tissues (*i.e.*, kidney and skin) remained at the recovery necropsy in the 30.8 mg/kg (MD) and 46.3 mg/kg (HD) groups. Green discoloration was not detected in either the vehicle control or 3.1 mg/kg (LD) groups at either necropsy.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 10. Methods for Study No.: (b) (4) 963018

Methods	Details
Dose and frequency of dosing:	0 (vehicle), 3.1 (LD), 30.8 (MD), and 46.3 (HD) mg/kg of OTL-0038; once weekly for 14 days (3 doses). Frequency: Repeat-dose administration.
Route of administration:	Intravenous
Formulation/Vehicle:	The test article (pafolacianine; Lot #2289-2289-13-001H) was prepared in a phosphate-buffered saline (PBS; pH 7.4±0.1) vehicle.
Species/Strain:	Sprague Dawley Crl:CD(SD) rats.
Number/Sex/Group:	15/sex/group for vehicle control and HD, 10/sex/group for LD and MD.
Age:	38-days of age at receipt.
Satellite groups/ unique design:	TK satellite, n=9/sex for pafolacianine, and n=3/sex for vehicle control.
Deviation from study protocol affecting interpretation of results:	None.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose, TK=toxicokinetic

Table 11. Observations and Results: Changes From Control (Study No.: (b) (4) 963018)

Parameters	Major Findings
Mortality	No unscheduled deaths.
Clinical Signs	Dark green and/or blue urine or blue discolorations were noted in the 3.1, 30.8, and/or 46.3 mg/kg/dose group males and/or females throughout the dosing period. Discoloration was observed at physical examinations, on dosing days 0, 6 and 13 and/or non-dosing days; blue body and extremities were observed in the MD and HD group males and females. In the recovery period, blue tail and blue extremities were noted in the HD group males and females through SD 19 (i. e., 6 days after the last dose). Blue body was noted throughout the recovery period (SD 14-27) in the HD group males and females.
Body Weights	No treatment-related effects on body weight.
Ophthalmoscopy	No test article-related findings for ophthalmoscopic parameters.
Hematology	No test article-related changes in hematology and coagulation parameters.
Clinical Chemistry	No test article-related changes in serum chemistry parameters.
Urinalysis	Test article-related higher mean urine pH values were noted in the 30.8 mg/kg/dose group (mid-dose) and the 46.3 mg/kg/dose group (high-dose) males and females at the primary necropsy.
Gross Pathology	Test article-related gross findings consisted of blue and/or green discoloration in multiple organs including the kidney. This finding occurred at both primary and recovery sacrifices. At primary sacrifice, the discoloration was in all male and females at the mid (30.8 mg/kg/dose) and high (46.3 mg/kg/dose) doses. In the low dose group, 7/10 males and 1/10 females had discoloration.
Organ Weights	No treatment-related changes in organ weights.
Histopathology Adequate battery: Yes	Test article-related microscopic findings for males and females were present at primary necropsy and were limited to the cecum and injection sites. Submucosal edema and submucosal eosinophil and basophil infiltrates were present in the cecum at the primary necropsy in MD and HD group males. One high dose group female had infiltrates in the cecum. There were no histologic findings in the cecum at recovery sacrifice. No microscopic correlates were observed for the gross findings of discoloration.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose

Repeat-Dose Toxicology Study in Dogs

Study title/ number: A 14-Day (Once Weekly) Intravenous (Slow Bolus) Injection Toxicity and Toxicokinetic Study of OTL-0038 in Beagle Dogs / (b) (4) 963019

- Green urine was observed in males and females in all test article-treated groups. Green and blue gums and ear(s) were evident in males at the high dose. Green urine was also observed in animals administered the low dose (0.9 mg/kg/day).
- There was a test article-related increase in urinary pH as from the mid dose in the dosing period. At the Day 14 primary necropsy, test article-related green discoloration of multiple tissues were observed as from the mid dose level.
- Systemic exposure increased with increasing dose and was dose-proportional (by normalized AUC_{last}) and similar in both sexes. Little to no accumulation was evident after once per week dosing, as indicated by mean accumulation ratios that ranged from 0.952-1.19. The mean systemic clearance (Cl) was low and the mean apparent volume of distribution at steady state (Vss) was moderate following i/v administration, a finding that was similar across doses and sexes. $T_{1/2}$ was moderate and ranged from approx. 13 -18 h.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 12. Methods for Study No.: (b) (4) 963019

Methods	Details
Dose and frequency of dosing:	0 (vehicle control), 0.9 (LD), 9.3 (MD), and 13.9 (HD) mg/kg of pafolacianine; dosing once weekly for 14 days (3 doses).
Route of administration:	Intravenous
Formulation/Vehicle:	Pafolacianine (Lot #2289-2289-13-001H, % purity) in phosphate-buffered saline (PBS), pH 7.4±0.1 / PBS.
Species/Strain:	Dog / Beagle
Number/Sex/Group:	6/sex/group for vehicle control and HD, 4/sex/group for LD and MD.
Age:	6-7 months of age at study initiation.
Satellite groups/ unique design:	None.
Deviation from study protocol affecting interpretation of results:	None.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose

Table 13. Observations and Results: Changes From Control (Study No.: (b) (4) 963019)

Parameters	Major Findings
Mortality	No unscheduled deaths.
Clinical Signs	Tissue discoloration as previously reported in the single-dose toxicity studies in rats and dogs and in repeat-dose toxicity studies in rats was similarly observed in this study. Green urine was observed in treatment groups. Green and blue gums and ears were noted in both sexes at the MD and HD groups. Tissue discoloration persisted into the recovery period with observations of green and blue gums and ears and blue extremities and urogenital areas in males and females administered the HD. Green urine was also observed in animals administered the LD.
Body Weights	No test article--related effects on body weight.

Parameters	Major Findings
Ophthalmoscopy	No test article-related effects on ophthalmoscopic parameters.
ECG	No test article-related effects on ECG parameters on days 0 and 13.
Hematology	No test article-related alterations in hematology and coagulation parameters.
Clinical Chemistry	There was a dose-related occurrence of lipemic serum in males and females from the mid dose (9.3 mg/kg) level.
Urinalysis	Test article-related increase in urinary pH was observed in males and females as from the mid dose. The change did not persist into the recovery period.
Gross Pathology	Notable macroscopic findings included green discolorations of multiple organs in males and females at the MD and affected the bone, cecum, colon, duodenum, epididymides, esophagus, kidney, gingiva, ileum, injection site, jejunum, peripheral lymph nodes, sciatic nerve, prostate, skin, stomach, rectum, testes, tongue, and urinary bladder contents. Green discoloration of the kidney and urinary bladder was observed in males only at the LD. The discolorations persisted into the recovery period in the high dose group. The discoloration had no microscopic correlates.
Organ Weights	No test article-related changes in organ weights.
Histopathology Adequate battery: Yes	No test article-related microscopic findings.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose

General Toxicology; additional studies

Study title/ number: Single Dose Intravenous Administration Toxicity Study of OTL-0038 in Sprague-Dawley Rats with Near Infra-Red Light (NIR) Exposure / 20051173

- The aim of this study was to evaluate the response of internal organs to Near Infrared (NIR) exposure after a single IV administration of pafolacianine to Sprague -Dawley rats.
- Green discolored urine was observed in rats following dosing with either 30.8 (MD) or 40.3 mg/kg (HD) on the day of injection.
- Green and dark green kidneys were observed in male and female rats in the MD and HD groups. In the female rats, pale jejunum and/or duodenum were observed across all dose groups without a dose-response relationship

Conducting laboratory and location:

GLP compliance: Yes

Table 14. Methods for Study No.: 20051173

Methods	Details
Dose and frequency of dosing:	0 (vehicle control), 3.1 (LD), 30.8 (MD), and 46.3 (HD) mg/kg of pafolacianine; single dose administration.
Route of administration:	Intravenous (IV); slow bolus via lateral tail vein.
Formulation/Vehicle:	pafolacianine (Lot #13-038-41-15, % purity: 75.23%) in phosphate-buffered saline (PBS), pH 7.4±0.1 / PBS.
Species/Strain:	Rat / Crl:CD (Sprague-Dawley)
Number/Sex/Group:	5/sex/group
Age:	68 days of age at study initiation
Satellite groups/ unique design:	None
Deviation from study protocol affecting interpretation of results:	None

Abbreviations: HD=high dose, LD=low dose, MD=mid dose

Table 15. Observations and Results: Changes From Control (Study No.: 20051173)

Parameters	Major Findings
Mortality	One male rat administered the low dose (3.1 mg/kg) was euthanized due to an incision site that dehisced . All other rats survived to scheduled euthanasia.
Clinical Signs	Green discolored urine was observed in rats administered either MD or HD on the day of injection and the day after administration. Pale ears and hindlimbs/paws were observed on the day of surgery in the groups of female rats administered either MD or HD.
Body Weights	No test article-related effects on mean body weight or body weight change.
Hematology	No test article-related effects on mean hematology or coagulation parameters.
Clinical Pathology	There were statistically significant ($p > 0.05$) increases in serum bilirubin concentration in males and females administered MD or HD mg/kg pafolacianine/NIR when compared with the vehicle-treated/NIR group. Mean cholesterol increased significantly in MD females compared to vehicle control. Considering that there was no dose dependence in the liver, the result was determined as non-adverse. There was a significant reduction in mean creatinine concentration in females when compared to the vehicle/NIR group. However, given that there was no dose-dependence and a lack of microscopic findings in the kidney, the observation was considered non-adverse. Mean serum potassium concentration was significantly increased in HD (NIR treated) females compared with the vehicle control (NIR-treated). Due to the absence of effects on other electrolytes, this finding was not considered to represent an adverse effect. There was no evidence of phototoxicity as demonstrated by lack of adverse effects on clinical observations.
Urinalysis	Green colored urine was observed in animals administered test article/NIR exposure compared to vehicle/NIR-treated the group.
Gross Pathology	Green and dark green kidneys were observed in MD and HD males and females. Pale jejunum and/or duodenum were observed across all pafolacianine-treated females without a dose-response relationship, and there was a single observation of white mesentery in a single MD animal.
Organ Weights	No test article related effects on mean absolute or relative (to body weight) organ weights.
Histopathology Adequate battery: Yes	No test article-related histopathological findings. No noteworthy gross or histopathology findings were reported for the tissue and organ discolorations especially the kidneys.

Abbreviations: HD=high dose, LD=low dose, MD=mid dose, NIR=near infrared

5.5.1.3. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title/ number: Bacterial Reverse Mutation Assay / AD74ZM.502ICH. (b) (4)
Key Study Findings:

- The results of the bacterial mutagenicity assay indicated that under the conditions of the study, pafolacianine did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9.
- No positive increases in the mean number of revertants per OTL-0038-treated plate were observed with any of the tester strains in either the presence or absence of metabolic activation. Neither precipitate nor toxicity was observed. In conclusion, pafolacianine was negative (non-mutagenic) in the bacterial reverse mutation assay in both the presence and absence of metabolic activation.

GLP compliance: Yes

Test system: *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A in the presence and absence of Aroclor-induced rat liver.

Study is valid: Yes
In Vitro Assays in Mammalian Cell
Study title/ number: *In Vitro* Mammalian Cell Micronucleus Assay in Chinese Hamster Ovary (CHO) Cells / AD74ZM.368CRESTICH. (b) (4)

- Substantial cytotoxicity was not observed at any dose levels with any of the treatment conditions.
- The highest dose analyzed under each treatment condition was the highest dose tested in the micronucleus assay. This dose met limit as recommended by testing guidelines for this assay. Based on the findings, pafolacianine was negative for the induction of micronuclei in both non-nucleated and S9-activated test systems in the in vitro mammalian cell micronucleus assay in CHO cells.

GLP compliance: Yes

Test system: Chinese Hamster Ovary (CHO-K1) cells (repository number CCL 61) were obtained from (b) (4)

Study is valid: Yes

***In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)**

Study title/ number: *In vivo* Micronucleus Assay in Rats / AD74ZM.125M012ICH (b) (4)
Key Study Findings:

- Pafolacianine at doses up to and including 600 mg/kg did not show any genotoxic activity in this *in vivo* test for induction of chromosome damage.
- Based on the findings, pafolacianine was concluded to be negative in this assay.

GLP compliance: Yes

Test system: Sprague-Dawley (Hsd:SD) rats, 6-8 weeks of age at study initiation.

Study is valid: Yes

Other Genetic Toxicity Studies
None.

Conclusion based on Genotoxicity Studies:
No genotoxic hazards were identified when pafolacianine was evaluated in the standard International Conference on Harmonization (ICH)-compliant genotoxicity testing battery consisting of a bacterial reverse mutation assay, an *in vitro* micronucleus study conducted in Chinese Hamster Ovary (CHO) cells, and an *in vivo* rat bone marrow micronucleus study.

5.5.2. Carcinogenicity

Studies examining the carcinogenic potential of pafolacianine have not been conducted and are not needed.

5.5.3. Reproductive and Developmental Toxicology

Introduction:
Reproductive and Developmental Toxicology studies conducted with pafolacianine were limited to exploratory and definitive embryo-fetal toxicity studies in rats and rabbits. The Sponsor requested a waiver from conducting fertility and early embryonic development studies ((Segment I or FEED studies) and pre- and postnatal development studies (Segment III or PPND studies) based on the frequency of use, large safety margin based on the intended clinical dose, and absence of macroscopic or histopathologic findings in reproductive organs in single- and repeat-dose toxicity studies. A waiver from conducting FEED and PPND studies of pafolacianine was granted.

Embryo-fetal Development:
Brief Overview: By definition, in embryo-fetal development (EFD) studies, the test article was administered to animals from the day of implantation to the closure of the hard palate. The purpose of EFD studies is to provide information concerning the effects of prenatal exposure of the test article, pafolacianine on the pregnant animal and on the developing fetus.

In initial exploratory EFD studies conducted in rats and rabbits, excessive embryo-fetal toxicity was observed at all dose levels tested (> 15 mg/kg in both species) and a NOAEL with respect to the developing fetus could not be established. In an exploratory embryo-fetal development study conducted in rats with the unconjugated fluorescent dye component of pafolacianine (PPE-S0456), no evidence of embryo-fetal toxicity was demonstrated indicating that the embryo-fetal toxicity that occurred in both rats and rabbits at 15 mg/kg and higher was associated with the folate moiety component of pafolacianine. Consequently, a second set of dose range-finding studies was conducted in both species at lower dose levels prior to further evaluation in the definitive embryo-fetal development studies.

5.5.3.1. Dose Range-Finding Studies

Study title/ number: An Intravenous Dose Range-Finding Study of the Effects of OTL-0038 on Embryo/Fetal Development in Rats / (b) (4) 963028
Doses: 0 (vehicle); OTL-0038 (15, 50, 150, and 500 mg/kg/day.

Key findings:

- At 500 mg/kg/day, all females in the group were found dead or euthanized *in extremis* during gestation days 6-13, precluding evaluation of intrauterine parameters and fetal morphology at this dosage level. There was reduced food consumption and substantial loss of body weight (BW) and/or CNS-related clinical observations (clonic convulsions, cool body, and/or labored respiration) prior to death/euthanasia.
- 150 mg/kg/day: 1 female was euthanized *in extremis* on gestation day 8 following clonic convulsions. All females administered pafolacianine at 150 and 500 mg/kg/day had green body, excreta, urine, around nose and/or urogenital area from onset of administration to day of death/euthanasia.
- All animals administered the vehicle and pafolacianine at 15, 50 and 150 mg/kg/day survived but green body, urine, and/or feces generally throughout the treatment period.

Conclusion:
Based on these results, appropriate dosage levels for a definitive EFD toxicity study in rats could not be determined. Therefore, pafolacianine was evaluated at lower dose levels (0.015, 0.15, and 1.5 mg/kg/day) for a second dose range-finding (DRF) EFD study in timed pregnant Crl:CD(SD) rats.

Study title/ number: An Intravenous Dose Range-Finding Study of the Effects of OTL-0038 on Embryo/Fetal Development in Rats / (b) (4) 963033
Doses: 0 (vehicle), 0.015, 0.15, 1.5 mg/kg/day.

Key findings:

- All females in the vehicle control, 0.015, 0.15, and 1.5 mg/kg/day groups survived to scheduled necropsy on gestation day (GD) 20. Green tails were noted for all females

dosed at 1.5 mg/kg/day at the daily examinations and approx. 15 minutes following dosing through the day of necropsy.

- Mean maternal body weight, body weight gain, food consumption, net body weight, net body weight gain, and gravid uterine weight values in the 0.015, 0.15, and 1.5 mg/kg/day groups were similar to the respective control group values. Macroscopic findings in the 1.5 mg/kg/day group consisted of green discoloration of the kidneys and injection site; findings that were secondary to the green color of OTL-0038 and was not considered an adverse effect of pafolacianine.
- No other remarkable macroscopic observations were noted at dosage levels of 0.015, 0.15, and 1.5 mg/kg/day. Intrauterine growth and survival in the 0, 0.015, 0.15, and 1.5 mg/kg/day groups were comparable across all groups. No external developmental variations were noted for any fetuses in this study. Once-daily intravenous (slow bolus) administration of pafolacianine at dosage levels of 0.015, 0.15, and 1.5 mg/kg/day was well-tolerated with no evidence of maternal or developmental toxicity.

Conclusion:
Based on these findings and results of an initial DRF study conducted at higher OTL-0038 dose levels (15, 50, 150, and 500 mg/kg/day ([(b) (4)]963028), dosage levels of 0.015, 0.15, and 1.5 mg/kg/day were selected for a definitive EFD study of pafolacianine administered by intravenous injection to bred Crl:CD(SD) rats.

Study title/ number: An Intravenous Dose Range-Finding Study of the effects of PPE-S0456 on Embryo/Fetal Development in Rats / [(b) (4)]963034
Doses: 0 (vehicle), 15, 50, and 150 mg/kg/day.

Key findings:

- All animals survived to scheduled euthanasia on GD 20. Dose-dependent, increased incidences of green body, tail, urine, and/or feces were noted for all females in all test article-treated groups at the daily examinations and/or approximately 15 min following dosing. The test article (PPE-S0456; dye in pafolacianine) is a green compound, and the clinical observations were considered secondary to the color of the test article and not an adverse effect of PPE-S0456 administration.
- No other remarkable clinical observations were noted at 15, 50, and 150 mg/kg/day. Mean body weight, body weight gain, net body weight, net body weight gain, gravid uterine weight, and food consumption values at all dosage levels were similar to the respective control group values. Green discoloration of the amniotic sac, kidneys, injection site, and/or skin were noted for all animals in the 50 and 150 mg/kg/day groups and in a single 15 mg/kg/day group female.
- Intrauterine growth and survival were unaffected by maternal test article administration at all dosage levels. There were no fetal malformations or developmental variations observed at any dosage level.

Conclusion:
A once-daily intravenous (slow bolus) administration of PPE-S0456 at doses of 15, 50, and 150 mg/kg/day was not associated with maternal or embryo-fetal toxicity. In view that embryo-lethality was associated with pafolacianine administration to bred female rats under the same conditions and at the same dosage levels used in study (b) (4) 963028, it was concluded that the dye component of pafolacianine (PPE-S0456) was not responsible for the embryo-lethality noted in study (b) (4) 963028.

Study title/ number: An Intravenous (Bolus) Injection Study to Determine the Single-Dose Maximum Tolerated Dose of OTL-0038 in Non-pregnant New Zealand White Rabbits / (b) (4) 963026
Doses: 0 (vehicle), 25, 50, 75 and 150 mg/kg/day.

Key findings:

- All females survived to the scheduled euthanasia. All females in the 25, 50, 75, and 150 mg/kg groups had green or blue body, urine, and/or ears generally throughout the treatment period; these observations were considered secondary to the color of the test article and were not considered to represent adverse effects of pafolacianine.
- Food consumption, evaluated as g/animal/day and g/kg/day, at all dose levels was not affected by test article administration throughout the study. When the overall treatment period (study days 0-4) was evaluated, food consumption values for females in the 25, 50, 75, and 150 mg/kg groups (127 g/day to 194 g/day) were generally similar to pretest food consumption values (91 g/day to 244 g/day).
- Body weights, body weight gains, and food consumption were not affected by test article administration at all the doses explored.

Conclusions:
Under the conditions of this study, acute intravenous (slow bolus) administration of pafolacianine in nonpregnant New Zealand White rabbits was well-tolerated at dose levels of ≤150 mg/kg.

Study title/ number: An Intravenous (Bolus) Injection Dose Range-Finding Study of the Effects of OTL-0038 on Embryo/Fetal Development in Rabbits / (b) (4) 963027
Doses: 0 (vehicle), 15, 50, 150 mg/kg/day.

Key findings:

- One female (dosed at 50 mg/kg/day) and another (dosed at 150 mg/kg/day) aborted on GD 24 and GD 15, respectively. The female in the 150 mg/kg/day group lost body weight. A lower food consumption several days prior to abortion and 1 early resorption. The female in the 50 mg/kg/day group had a normal body weight gain and food consumption throughout a gestation period characterized by a late resorption and multiple external malformations similar to those observed in surviving litters.

- Green discoloration of the whole body was noted for both females at necropsy. All other females survived to scheduled necropsy. Green body was observed in females in 15, 50, and 150 mg/kg/day groups throughout the study. Necropsy findings of tissue discoloration were attributed to the test article color and were considered non-adverse.
- Decreased mean body weight and body weight gain noted for the 150 mg/kg/day group corresponded to lower mean food consumption values. Mean food consumption values for the 50 mg/kg/day group were similar to the concurrent control group values during the treatment period. Findings noted in all test article-treated groups consisted of open eyelid(s), ectrodactyly (split digits), adactyly (absence of digits), brachydactyly (disproportionately short digits), apodia (congenital absence of feet), absent claw, and cleft palate. Tarsal flexure was noted only in the 150 mg/kg/day group. Mandibular and maxillary micrognathia, hemimelia, mal-rotated paw, and syndactyly were noted only in the 15 mg/kg/day group.

Conclusion:
Marked embryo-fetal toxicity was observed at all pafolacianine doses evaluated. Embryofetal toxicity was manifested by increased post implantation loss, fewer viable fetuses at 50 and 150 mg/kg/day and lower mean fetal weights and increased external fetal malformations at all dosage levels. In addition, slight maternal toxicity, manifested by abortion, lower body weight gains, and/or food consumption, was noted at 50 and 150 mg/kg/day. Appropriate dosage levels for use in a definitive EFD study of pafolacianine administered by slow bolus intravenous injection in pregnant New Zealand White (NZW) rabbits could not be identified based on the findings in this study.

Study title/ number: An Intravenous (Bolus) Injection Dose Range-Finding Study of the Effects of OTL-0038 on Embryo-Fetal Development in Rabbits / (b) (4) 963036
Test animals: Time-mated NZW [Hra:(NZW)SPF rabbits.
Doses: 0 (vehicle), 0.3, 1 and 3 mg/kg.

Key findings:

- All rabbits in the vehicle control and the 0.3, 1, and 3 mg/kg/day test article groups survived to the scheduled necropsy on GD 29. Green ears were noted during the latter half of gestation in all females in the 3 mg/kg/day group at the daily examinations and/or approximately 15 min following dosing - an observation attributed to the color of the test article. It was not considered an adverse effect of pafolacianine.
- No other remarkable clinical observations were observed at any dosage level. Mean maternal body weight, body weight gain, net body weight, net body weight gain, gravid uterine weight, and mean food consumption values in the 0.3, 1, and 3 mg/kg/day groups were unaffected by test article administration.
- There were no remarkable macroscopic findings noted at the scheduled necropsy in the 0.3, 1, and 3 mg/kg/day groups. Intrauterine growth and survival in the 0.3, 1, and

3 mg/kg/day groups were unaffected by pafolacianine administration. There were no external malformations or developmental variations were noted at any dose level.

Conclusion:
Based on the lack of maternal and developmental toxicity noted at any pafolacianine dosage level evaluated, dosage levels of 1, 3, and 10 mg/kg/day were selected for a definitive EFD study of pafolacianine administered via slow bolus intravenous injection to time-mated New Zealand White rabbits.

5.5.3.2. Fertility and Early Embryonic Development

Studies on Fertility and Embryonic Development were not conducted and were not needed.

5.5.3.3. Embryo-Fetal Development

Definitive EFD studies were conducted in rats and rabbits to determine the potential of the test article (pafolacianine to induce developmental toxicity after maternal exposure during the critical period of organogenesis, to characterize maternal toxicity at the exposure levels tested, and to determine the NOAELs for maternal toxicity and developmental toxicity. In addition, a toxicokinetic assessment of OTL-0038 in plasma was performed. The NOAELs for maternal (F0) and developmental (F1) toxicity were each 1.5 mg/kg/day in rats and 3 mg/kg/day in rabbits, respectively. Steady-state maternal systemic exposure (AUC) to pafolacianine at the NOAEL was 9,630 ng·h/mL in rats and 34,800 ng·h/mL in rabbits.

Study title/ number: An Intravenous Study of the Effects of OTL-0038 on Embryo/Fetal Development in Rats / (b) (4) 963030

Key findings:

- All the female rats survived to the scheduled necropsy. A test article-related and non-adverse finding of green discoloration of the tails and urine was observed 15 min post-dose following dosing at the high dose of 1.5 mg/kg/day. This finding occurred sporadically over the treatment period (GD 6-17) and was not considered adverse. Greenish discoloration was also observed in the kidneys and observed in 24 of 25 rats administered the high dose. However, the discoloration was not considered treatment-related in view of the dark green fluorescent dye agent. There were no other test-article related clinical observations in the daily examinations or at 15 minutes following the end of the dosing.
- No article-related effects were reported on average maternal body weights, body weight gains, mean food consumption values, net body weights, net body weight gains, and gravid uterine weights in the 0.015, 0.15, and 1.5 mg/kg/day groups. No other test article-related findings were noted at the scheduled necropsy. Intrauterine growth and survival, and fetal morphology in all test article-treated groups were unaffected by maternal test article administration.

- Based on lack of adverse findings, 1.5 mg/kg/day was considered as the NOAEL for maternal toxicity and embryo/fetal development.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 16. Methods for Study No.: (b) (4) 963030

Methods	Details
Dose and frequency of dosing:	0 (vehicle control), 0.015 (LD), 0.15 (MD) and 1.5 (HD) mg/kg/day of OTL-0038.
Route of administration:	Intravenous injection by a slow bolus, over a 1-2 min period).
Formulation/Vehicle:	Pafolacianine (Batch #14-244-S3-B2-5.33C, purity #) prepared in phosphate-buffered saline (PBS), pH 7.4±0.1 / PBS.
Species/Strain:	Sprague Dawley Rat / Crl:CD(SD)
Number/Sex/Group:	25/mated females/group (1-4) dosed once daily from GDs 6-17.
Satellite groups:	TK satellite, n=3 or 9 mated females/group dosed once daily from GD 6-17.
Study design:	Main and TK groups: Test and control articles were administered as a single daily dose during the period of organogenesis (GDs 6-17). Laparohysterectomies were performed on GD 20 Main study group. TK animals were euthanized on GD 18.
Deviation from study protocol affecting interpretation of results:	None

Abbreviations: GD=gestation day, HD=high dose, LD=low dose, MD=mid dose, TK=toxicokinetic

Table 17. Observations and Results (Study No.: (b) (4) 963030)

Parameters	Major Findings
Mortality	No unscheduled deaths
Clinical Signs	There were no remarkable clinical signs in the gestation period (GD 6-17). Observed test article-related green tails and green urine noted at the HD following dose administration was not considered an adverse finding.
Body Weights	No adverse test article-related effects on mean maternal body weights, body weight gains, net body weights, gravid uterine weight gains at of the doses tested.
Necropsy findings Cesarean Section Data	No test article-related effects on mean maternal body weights, body weight gains and gravid uterine weights at any of the doses tested. At the scheduled necropsy on GD 20, test article-related green discoloration of the kidneys was noted for 24 of 25 females in the 1.5 mg/kg/day group. This finding was not considered adverse because the test article is a dark green fluorescent imaging agent (dye). No other test article-related findings were noted at the scheduled necropsy.
Necropsy findings Offspring	Intrauterine growth and survival, and fetal morphology in all test article-treated groups were unaffected by maternal test article administration.

Abbreviations: GD=gestation day, HD=high dose

Conclusions:

Test article-related but non-adverse clinical findings namely, green tails and green urine; and macroscopic findings at necropsy (green discoloration of the kidneys) were observed in the 1.5 mg/kg/day group and were attributed to the physical characteristics of the test article,

which is a dark green fluorescent imaging agent (dye). No adverse effects on survival, clinical conditions, body weight, food consumption, macroscopic findings, intrauterine growth and survival, and fetal morphology were noted for any test article-treated group. Based on the lack of adverse findings, the 1.5 mg/kg/day dose level was considered as the NOAEL for both maternal toxicity and embryo-fetal development. Systemic exposure (AUC_{last}) at the NOAEL on Gestation Day 17 was 9630 ng.h/mL.

Prenatal and Postnatal Development
Studies on Prenatal and Postnatal Development were not conducted.

5.5.4. Other Toxicology Studies

5.5.4.1. Local Tolerance Study

Study title/ Number: Acute Perivascular, Intramuscular and Subcutaneous Irritation Study of OTL-0038 in Rabbits / (b) (4) 963009

- No deaths or test article-related clinical signs were noted in this study conducted to determine the irritative potential of pafolacianine following a single perivascular, intramuscular and subcutaneous administrations to albino rabbits.
- Although a green discoloration was noted in the injection sites of all animals via perivascular route and a majority of the animals via subcutaneous route, there was no injection site irritation. In general, test article-related microscopic findings at 24 h post-injection included hyperkeratosis, squamous epithelial hyperplasia, subacute inflammation and necrosis. There was muscle degeneration and hemorrhage at intramuscular sites and subacute inflammation at subcutaneous sites.
- There were no test article-related microscopic findings at 96 h post-injection at any site (perivascular, intramuscular, or subcutaneous). At necropsy, test article-related green discoloration was noted macroscopically in the perivascular and subcutaneous injection sites at 24- and 96-h post-injection. The injection site reactions were not considered signs of irritation.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 18. Methods for Study No.: (b) (4) 963009

Methods	Details
Dose and frequency of dosing:	5 mg/mL of pafolacianine was administered as a single bolus injection (0.3 mL) via the perivascular space of the right ear marginal vein; single intramuscular injection (0.5 mL) at a site in the vastus lateralis muscle of the right leg; single subcutaneous injection (1.0 mL) at a site on right of the midline of the back. At all injection sites, vehicle in the corresponding volume was administered in the left ear, left leg and left of the midline of the back respectively for perivascular, intramuscular and subcutaneous injections, respectively.
Route of administration:	Perivascular, intramuscular, and subcutaneous injections.
Formulation/Vehicle:	pafolacianine was formulated in phosphate-buffered saline (PBS; pH 7.4±0.1)
Species/Strain:	Rabbit/ New Zealand White; Albino; Males
Number/Sex/Group:	3/sex/group
Age:	At least 12 weeks of age at study initiation
Satellite groups/ unique design:	None
Deviation from study protocol affecting interpretation of results:	None

Observations and Results: Changes From Control (Study No.: (b) (4) 963009

Summary of findings:
There were no test article-related clinical signs and all animals survived to scheduled necropsies. With the exception of a test article-related green discoloration at the perivascular injection site, there was no evidence of injection site irritation. Nonetheless, a slight erythema was observed perivascular sites in both control and treatment groups at the 1-, 24-, 48-, 72 or 96-hr post-dose observation periods. There were no remarkable test article-related changes on body weight. Organ weights were not evaluated. Test article-related microscopic findings were limited to the 24 h time point (Day 1 necropsy) and included hyperkeratosis, squamous epithelial hyperplasia, subacute inflammation and necrosis at the perivascular site, muscle degeneration and hemorrhage at the intramuscular site, and subacute inflammation at the subcutaneous site.

6. Clinical Pharmacology

6.1. Executive Summary

Pafolacianine is a fluorescent drug that targets the folate receptor (FR), which is overexpressed on ovarian cancer cells. Pafolacianine binds to FR-expressing cancer cells and is internalized via receptor mediated endocytosis. The proposed indication is for adult patients diagnosed with ovarian cancer as an adjunct for intraoperative identification of malignant lesions by fluorescence imaging. The recommended dose is 0.025 mg/kg to be administered (b) (4) before surgery.

The safety and efficacy of pafolacianine was evaluated in a randomized, multicenter trial (Study OTL-2019-OTL38-006; study 006 hereafter). The primary endpoint was the proportion of patients with at least one evaluable ovarian cancer lesion confirmed by central pathology that was detected with pafolacianine under fluorescent light but not under normal light or palpation and not otherwise identified for resection prior to surgery. The proportion of patients who met the primary endpoint was 29.6% (95%CI: 19.6%, 35.2%). The safety profile of pafolacianine was generally mild (Grade 1) with nausea, vomiting, and abdominal pain as the most frequent adverse reactions.

The proposed 0.025 mg/kg dose was determined based on one dose escalation study in healthy volunteers and one study in patients with ovarian cancer. Single doses ranging from 0.0125 mg/kg up to 0.2 mg/kg were investigated. Dose levels up to 0.05 mg/kg resulted in mild adverse reactions whereas dose levels of 0.1 and 0.2 mg/kg resulted in moderate adverse reactions. Additionally, higher doses were associated with lower tumor-to-background ratio. As such, the proposed 0.025 mg/kg achieved an acceptable balance of detection sensitivity and safety. The clinical pharmacology review focused on the adequacy of the proposed dosing and imaging window. Efficacy information (assay sensitivity and positive predictive value) obtained from study 006 could only support a 9-hour dosing window.

No dose modification is recommended based on intrinsic factors. Population pharmacokinetics (PK) analysis of data obtained across the development program did not identify a clinically meaningful change in exposure based on age, weight, mild to moderate renal or hepatic impairment, or disease state (healthy volunteers versus ovarian cancer patients). Additionally, the population PK analysis did not identify clinically meaningful changes in exposure based on sex or race; however, males represented only 5.6% (10/179), Blacks represented only 4.5% (8/179), and Asians represented only 3.9% (7/179) of subjects.

No dose modification is recommended based on drug-drug interactions. In vitro studies demonstrated that pafolacianine has low potential to inhibit CYP or UGT enzymes, or transporters. Additionally, the potential for enzyme induction is low because of the single-dose administration and the short half-life of pafolacianine. As a substrate, pafolacianine is not metabolized by CYP or UGT enzymes. Even though pafolacianine is a substate of OATP1B1, OATP1B3, and OAT1, the potential for interaction is likely insignificant given that it is a single-dose administration.

6.1.1. Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in NDA 214907. This NDA is approvable from a clinical pharmacology perspective. The key review highlights with specific recommendations are summarized below:

Table 19. Key Review Highlights

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	The evidence of effectiveness was obtained from a randomized trial in patients with ovarian cancer. The proportion of patients who had at least one lesion detected by pafolacianine under fluorescent light that was not detected under normal light or palpation was ~30%.
General dosing instructions	The recommended dosing is a single 0.025 mg/kg single dose to be administered within 9 hours prior to surgery.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose modification is recommended based on intrinsic or extrinsic factors.
Labeling	Labeling recommendations are generally acceptable, with changes to increase clarity.
Bridge between the to-be-marketed and clinical trial formulations	Not applicable.

6.1.2. Post-Marketing Requirements and Commitments

None.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Mechanism of action: Pafolacianine is a fluorescent marker that targets FR, which are overexpressed on ovarian cancer cells.

Distribution: The volume of distribution at the recommended 0.025 mg/kg dose is 17.1 L. The plasma protein binding of pafolacianine is 93.7%, with minimal partitioning into red blood cells.

Metabolism: Pafolacianine is not metabolized by cytochrome P450 enzymes. In a human ADME study, [^{14}C]-pafolacianine accounted for ~94% of the radioactivity in plasma.

Elimination: The elimination half-life of pafolacianine is 0.44 hours and mean plasma clearance is 28.6 L/hr. In the mass balance study, only 35% of the dose was recovered in urine (19.1%) and feces (15.8%) up to 30 days post administration due to cellular uptake.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The recommended dosage is 0.025 mg/kg administered over 60 minutes as an intravenous infusion to administered within 9 hours before surgery.

Therapeutic Individualization

No dose modifications are recommended based on intrinsic or extrinsic factors. Population PK analysis did not identify clinically meaningful differences in the pharmacokinetics of

pafolacianine based on age, sex, weight, mild to moderate renal impairment, mild to moderate hepatic impairment, or healthy subjects versus ovarian cancer patients.

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

Table 20. General Pharmacology and Pharmacokinetic Characteristics

Characteristic	Summary
Pharmacology	
Mechanism of action	Pafolacianine is a fluorescent drug that targets FR, which is overexpressed on ovarian cancer cells. Pafolacianine binds to FR-expressing cancer cells and is internalized via receptor mediated endocytosis. Pafolacianine absorbs light in the near-infrared region (NIR) with a range of 760 nm to 785 nm with absorption at 776 nm and emits fluorescence with a range of 790 nm to 815 nm with a peak emission of 796 nm.
Active moiety	Pafolacianine
QT prolongation	Based on nonclinical studies, there were no treatment-related qualitative or quantitative ECG-related changes.
General Information	
Molecular weight	1414.42 g/mol
Formulation development	The drug product is formulated as a single-use, sterile solution of 3.2 mg in 1.6 mL (2 mg/mL) solution of pafolacianine sodium.
Bioanalysis	Pafolacianine was measured using validated LC/MS/MS methods. A summary of the method validation report is included in Appendix 16.4.1.
Dose proportionality	The PK of pafolacianine exhibited more than dose-proportional increase in AUC and C_{max} after single doses ranging from 0.0125 mg/kg to 0.05 mg/kg. Mean C_{max} increased 7.3-fold and mean AUC increased 7.8-fold across the 4-fold increase in dose.
Accumulation	Pafolacianine is a single dose administration. Accumulation is not applicable.
Variability	Pharmacokinetic data obtained from the dose escalation study (b) (4) 1321A demonstrated that the variability in AUC was less than 20% when administered as a single dose.
Distribution	
Volume of distribution	The volume of distribution increased with dose, ranging from 15.9 to 38.8 L, and was notably larger than plasma volume. At the recommended dose of 0.025 mg/kg, the volume of distribution was 17.1 L.
Plasma protein binding	The plasma protein binding of pafolacianine is 93.7%.
Blood-to-plasma ratio	No notable partitioning into RBCs was observed. The partitioning ratio was 0.6.
Elimination	
Mean terminal half-life	The terminal half-life of pafolacianine was 0.44 hours.
Metabolism	
Primary metabolic pathways	At the recommended dose of 0.025 mg/kg, pafolacianine is primarily excreted as unchanged drug and appears to be marginally metabolized by O-dealkylation. CYP and UGT enzymes are not involved in its metabolism.
Transporter substrate	*In vitro,* pafolacianine is not a substrate of OATP1B1, OATP1B3, or OAT1.

Characteristic	Summary
DDI potential	Enzyme Inhibition In vitro, pafolacianine had low potential to inhibit CYP enzymes (CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5). Enzyme Induction Not applicable for a single-dose administration. Transporter Inhibition Pafolacianine also has low potential to inhibit P-gp, BCRP, OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1, MATE2-K, and BSEP *in vitro.*
Excretion	
Primary excretion pathway	In the mass balance study with a ^{14}C-pafolacianine, only 35% of the dose was recovered in urine (19.1%) and feces (15.8%). Therefore, both hepatic metabolism and renal excretion contribute to pafolacianine elimination.

Abbreviations: AUC=area under the curve, C_{max}=maximum concentration, DDI=drug-drug interaction, FR=folate receptor, LC-MS/MS= high-performance liquid chromatography with two mass spectrometry detectors, NIR=near infrared, PK=pharmacokinetics, RBC=red blood cells

6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

The clinical pharmacology program provides supportive evidence of effectiveness. The Applicant submitted results from several clinical trials to demonstrate the safety and efficacy of pafolacianine for the proposed indication and support dose selection (Table 21).

Table 21. A Summary Description of Clinical Studies Conducted to Support Dose Selection and Approval for the Proposed Indication

Trial	Description	Dosage	Main Objectives
(b) (4) 1321A (n=30)	Phase 1A: Placebo controlled study in healthy volunteers	0.025, 0.05, 0.1, 0.2 mg/kg	Assess safety, tolerability, and PK
(b) (4) 1321B (n=18)	Phase 1B: Patients with FR+ ovarian cancer	0.0125, 0.025, or 0.05 mg/kg	Assess safety, tolerability, and PK
OTL-2019-14COTL0038-001 (n=6)	Phase 1: Mass balance study in healthy volunteers	0.02 mg/kg	Assess mass balance, primary route and mechanism of elimination, plasma exposure, and metabolites
OTL-2014-OTL38-003 (n=48)	Phase 2: Single-dose, open label exploratory study in patients undergoing cytoreduction surgery	0.025 mg/kg	Assess safety and efficacy
OTL-2019-OTL38-006 (n=178)	Phase 3: Single-arm, single-dose, open-label in patients with ovarian cancer	0.025 mg/kg	Assess safety and efficacy

Abbreviations: FR=folate receptor, PK=pharmacokinetics

The safety and efficacy of pafolacianine was evaluated in a single-arm multicenter trial (Study 006). This study enrolled 178 patients with ovarian cancer scheduled to undergo surgical cytoreduction, debulking, or recurrent ovarian cancer surgery. A total of 134 patients received pafolacianine as well as normal light evaluation. The primary efficacy endpoint was the proportion of patients with at least one evaluable ovarian cancer lesion confirmed by central pathology detected with pafolacianine under fluorescent light but not under normal light or

palpation. The proportion of patients who met the primary endpoint was 0.269 (95% CI: 0.196, 0.352).

Dosing Window

In study 006, patients received pafolacianine up to 9 hours prior to the start of imaging. Based on a breakdown of efficacy by dosing window, it was noted that patients who received pafolacianine up between 6-9 hours before imaging did not achieve a response (Table 22).

Table 22. Proportion of Responders and Non-Responders Stratified by Time From End of Infusion to Start of Imaging

Infusion End Time to Imaging Start Times (Hrs.)	Proportion of Patients Meeting Primary Endpoint (%)		Total
	No	Yes	
min 0.38 to < 1 hr.	1	1	2
(Row %)	50	50	100.00
1 to 2 hrs.	20	15	35
(Row %)	57.14	42.86	100.00
> 2 to < 3 hrs.	20	4	24
(Row %)	83.33	16.67	100.00
3 to 6 hrs.	27	16	43
(Row %)	62.79	37.21	100.00
> 6 hrs. to max 8.95 hrs.	5	0	5
(Row %)	100.00	0	100.00
Total	73	36	109
	66.97	33.03	100.00

Source: Applicant's analysis submitted Feb 12, 2021 in response to information request. Data based on FAS from study 006.

To assess whether the lack of responses at later times (6-9 hours) was due to decreased sensitivity of the imaging modality, a breakdown of sensitivity – defined as true positive tumors detected out of the sum of all tumors (true positives and false negatives) – and positive predictive value – defined as the fraction of true positive tumors out of the sum of true positive and false positive tumors – by dosing window was analyzed.

In the 6-9 hours dosing window, the sensitivity and positive predictive value were similar to other dosing windows (Table 23), which indicates that the imaging modality is capable of detecting tumors within this dosing window.

Table 23. Sensitivity and Positive Predictive Value Stratified by Dosing Window

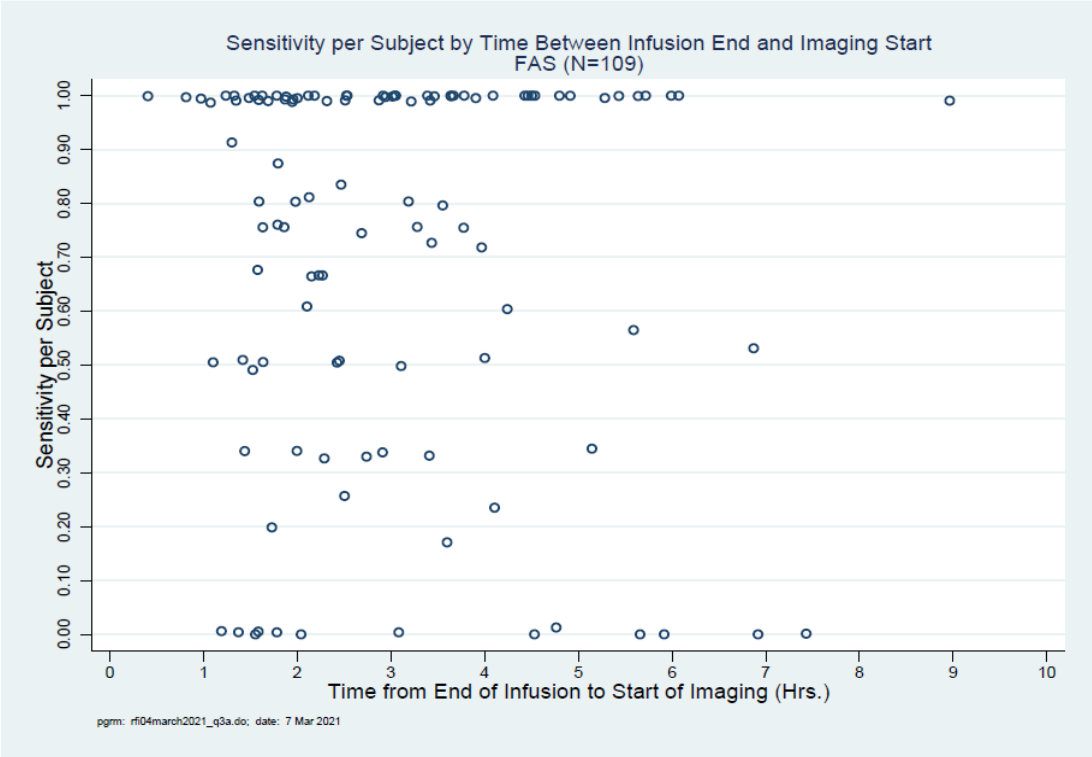
Dosing Windows Hrs.	Lesion Count Over All FAS Subjects				Positive Predictive Value
	TP Lesions	FN Lesions	FP Lesions	Sensitivity	
< 1	13	0	0	1.00	1.00
1 to 2	155	46	79	0.77	0.66
> 2 to < 3	66	31	63	0.68	0.51
3 to 6	146	46	72	0.76	0.67
> 6 to 9	15	13	7	0.54	0.68
Total	**395**	**136**	**221**	**0.74**	**0.64**

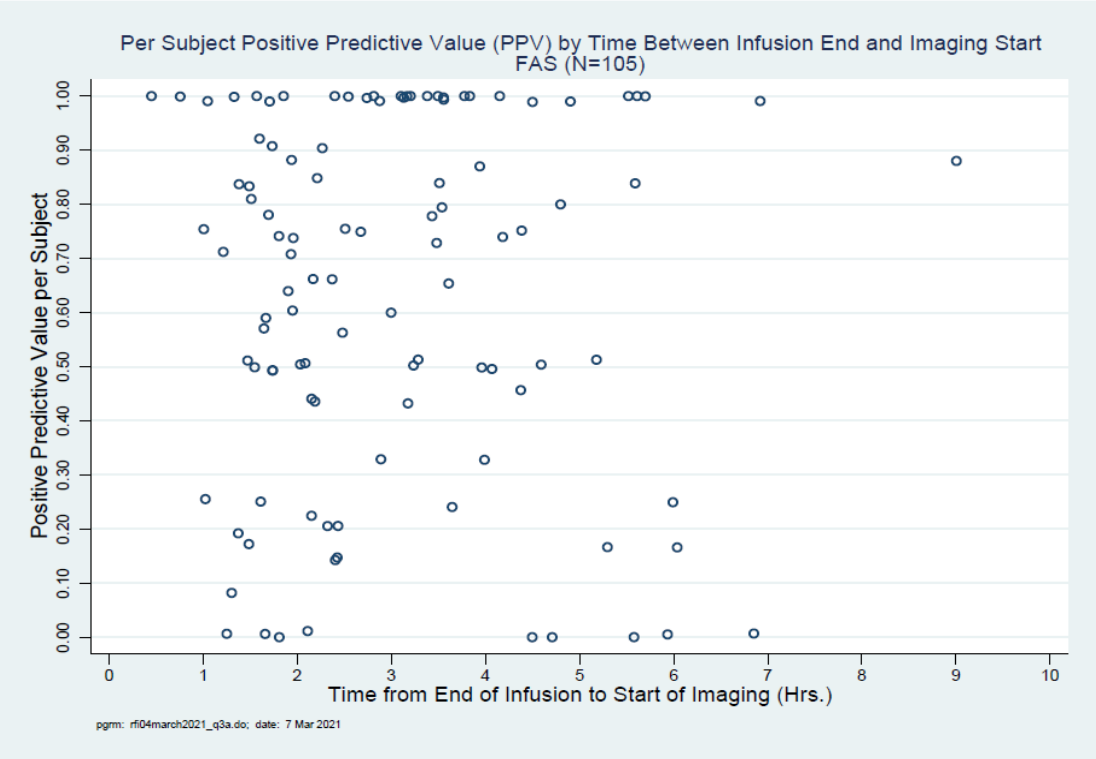
Sensitivity and Positive Predictive Value is calculated across all lesions irrespective of individual subjects.
Sensitivity = TP / (TP+FN)
Positive Predictive Value = TP / (TP+FP)
pgrm: rfi04march2021_q3b.do

Source: Applicant's analysis submitted on March 11, 2021 in response to information request. Data based on FAS from Study 006.
Abbreviations: FAS=full analysis set, FN=false negative, FP=false positive, TP=true positive

The lower sensitivity (0.54) and positive predictive value (0.68) in 6-9 hour window compared to earlier times could be attributed to the lower number of patients in this window (Figure 3). Of note, only 5 patients with a total of 28 lesions were captured in this window. As such, data obtained from study 006 supports the administration of pafolacianine up to 9 hours before the start of surgery.

Figure 3. Sensitivity (Top Panel) and Positive Predictive Value (Bottom Panel) Per Subject by Time Between the End of Infusion and Start of Imaging





Source: Applicant's analysis submitted on March 11, 2021 in response to information request. Data based on FAS from study 006.
Abbreviations: FAS=full analysis set

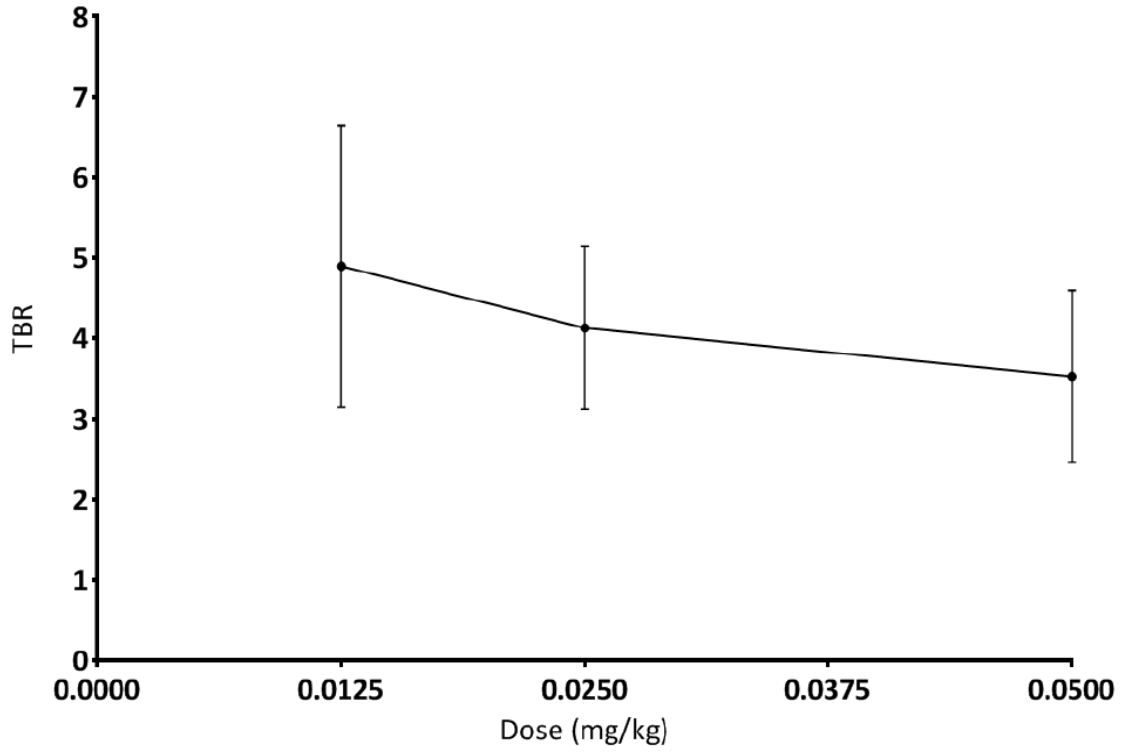
Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

The proposed dosing regimen is appropriate for the general patient population for which the indication is being sought.

Dose selection was supported by data obtained from study (b) (4) 1321A and study (b) (4) 1321B. Study (b) (4) 1321A was a first-in-human study in healthy volunteers. The dose levels that were assessed were 0.025 mg/kg (n=6), 0.05 mg/kg (n=5), 0.1 mg/kg (n=5), and 0.2 mg/kg (n=9). The most frequent adverse reactions were nausea, pruritus, abdominal pain, and somnolence. Moderate (Grade 2) adverse reactions were observed only in the 0.1 and 0.2 mg/kg cohorts; subjects in the lower dose cohorts experience only mild (Grade 1) adverse reaction.

Based on results from study (b) (4) 1321A, the lower dose levels were investigated in patients with ovarian cancer with FR+ lesions in study (b) (4) 1321B. The dose levels studied were 0.0125 mg/kg (n=12), 0.025 mg/kg (n=3), and 0.05 mg/kg (n=3). Similar to the safety profile in healthy volunteers, adverse reactions were generally mild (Grade 1) at the 0.025 mg/kg only 1 incidence of nausea was observed. The tumor-to-background ratio decreased with increasing doses, likely due to higher background at the higher doses (Figure 4).

Figure 4. Tumor-to-Background Ratio as Function of Dose in Patients With FR+ Ovarian Cancer



Source: Study (b) (4) 1321B CSR.
Abbreviations: TBR=tumor to background ratio, FR=folate receptor

Given the favorable safety profile of the 0.025 mg/kg dose and the fact that the tumor-to-background ratio was not substantially different compared to the lower dose, the 0.025 mg/kg was selected for evaluation in the phase 3 trial .

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

An alternative dosing regimen or management strategy is not required for subpopulations based on intrinsic factors. Population PK assessed the effect of age, weight, liver function, renal function (creatinine clearance; ClCr), race, sex, and disease state. None of evaluated intrinsic factors affected the PK of pafolacianine.

The analysis dataset included patients with mild (n=59) to moderate (n=13) renal impairment but not patients with severe or end-stage renal disease. Also, the dataset included patients with mild (n=12) to moderate (n=3) hepatic impairment with no information regarding severe hepatic impairment. Given that pafolacianine is a single-dose administration and that there is no trend of change in exposure with renal or hepatic function, additional characterization of PK in patients with severe organ impairment is not be required.

Patients with higher body weight had slightly higher incidence of adverse reactions such as mild (Grade 1) to moderate (Grade 2) nausea and vomiting; however, the plasma exposure in patients with high body weight is similar to that in patients with lower body weight. The increased incidence of adverse reactions cannot be explained based on plasma exposure; therefore, dose modifications based on body weight are not necessary.

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

There are no clinically relevant drug-drug interactions. In vitro studies show that pafolacianine is not metabolized by CYP or UGT enzymes. Additionally, pafolacianine did not inhibit CYP enzymes or UGT1A1 in vitro, and pafolacianine is administered as a single dose with a very short half-life so the potential for enzyme induction is very low.

Pafolacianine did not inhibit OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE-2K, P-gp, or BCRP. Even though pafolacianine is a substrate of OATP1B1, OATP1B3, and OAT1 in vitro, the in vivo relevance of a potential interaction is low given that pafolacianine is administered as a single dose.

Pafolacianine binds to the folate receptor, and folate, folic acid, or folate-containing supplements may compete with pafolacianine for folate receptors and reduce its binding, leading to reduced sensitivity of detection. To mitigate the risk of this interaction, the recommendation to avoid folate, folic acid, or folate containing supplements 48 hours prior to

pafolacianine administration has been included in the United States Prescribing Information (USPI).

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

To support this NDA 214907, the Applicant submitted the following:

- Report from a Phase 3 clinical study 006 in patients with ovarian cancer.
- Report from a Phase 2 clinical study 003 in patients with ovarian cancer
- Additional safety data from a Phase 2 clinical study 005 in patients with lung cancer.

The efficacy evaluation for use of Cytalux was based on the results of Study 006, the Phase 3, single dose, open label study conducted across multiple centers. A dose of 0.025 mg/kg of Cytalux was administered intravenously (IV) from 1 to 9 hours prior to the start of surgery. A modified randomization scheme was used with both the investigators and the study staff blinded until after the completion of standard of care (SOC) surgery. Pathologists from central pathology location were also blinded to the outcome of surgery.

Table 24. Details of Phase 3 Study 006

Study Design	Study Start, Enrollment Status, Date Total Enrollment/ Enrollment Goal	Primary Efficacy Endpoint	Dose Route Regimen	Diagnosis/ Inclusion Criteria	Number Enrolled/ Dosed/ Completed	Number by Study Analysis Population	Gender M/F Median Age (Range)
Phase 3, single-arm, single dose, open-label, multicenter investigational sites (10 sites in the US and 1 site in the Netherlands)	Study start: 5/16/2017 Study completed: 4/16/2020 Total enrollment: 178 (signed consent) Enrollment goal: ~170 patients to achieve a FAS of ≥100	Proportion of patients with ≥1 evaluable FR+ ovarian cancer lesion confirmed by central pathology detected using pafolacianine sodium plus NIR fluorescent light but not under normal light or palpation	Pafolacianine sodium 0.025 mg/kg 60-minute IV infusion, to be completed at least 1 hour prior to initiation of fluorescence imaging Single dose	FR+ ovarian cancer Female patients with primary diagnosis, or high clinical suspicion, of primary epithelial ovarian cancer, planned for primary surgical cytoreduction, interval debulking, or have recurrent ovarian cancer surgery, and scheduled to undergo laparotomy for the debulking surgery, scheduled to undergo laparoscopy and laparotomy for the debulking surgery if cancer detected on laparoscopy	Enrolled: 178 Dosed: 150 Completed: 134/150 (89.3%)	FAS 109/150 (72.7%) PP 106/150 (70.7%) SAS 150	F 62y (29, 89)

Source: Applicant submission 2.7.3 Summary of Clinical Efficacy, Table 3, Page 22
Evaluable lesions were defined as lesions that did not appear on an organ or tissue that was intended for removal regardless of the absence or presence of tumor based on the Pre-Fluorescence Surgical Plan.
Abbreviations: F=female, FAS=full analysis set, FR=folate receptor, mg/kg=milligrams per kilogram, NIR=near-infrared, PP=per protocol,, SAS=safety analysis set, y=year

Table 25. Details of Phase 2 Study 003

Study Design	Study Start Enrollment Status, Date Total Enrollment/ Enrollment Goal	Primary Efficacy Endpoint	Dose Route Regimen	Diagnosis / Inclusion Criteria	Number Enrolled/ Dosed/ Completed	Number by Study Analysis Population	Gender M/F Median Age (Range)
Phase 2, single dose, open-label multicenter (4 US sites)	Study start: 12/15/2014 Study completed: 11/16/2015 Total enrollment: 48 Enrollment goal: up to 45 patients with FRα+ ovarian cancer in order to obtain data from 40	Sensitivity and PPV of pafolacianine sodium for the detection of FRα+ ovarian cancer lesions	Pafolacianine sodium 0.025 mg/kg IV infusion, over 60 minutes Single dose	Primary diagnosis, or high clinical suspicion, of epithelial primary ovarian cancer. Female adult patients planned for primary debulking or interval debulking surgery, and: scheduled to undergo laparotomy for the debulking surgery or scheduled to undergo laparoscopy and then undergo laparotomy for the debulking surgery, if cancer was detected.	Enrolled: 48 Dosed: 44/48 (91.7%) Completed: 43/48 (89.6%)	mITT 29/48 (60.4%) Safety: 44/48 (91.7%)	F 64y (37, 82)

Source: Applicant submission 2.7.3 Summary of Clinical Efficacy, Table 3, Page 22;Evaluable lesions were defined as lesions that did not appear on an organ or tissue that was intended for removal regardless of the absence or presence of tumor based on the Pre-Fluorescence Surgical Plan.
Abbreviations: F=female, FR=folate receptor, mg/kg=milligrams per kilogram, mITT=modified intent to treat (population), , PPV=positive predictive value; y=year

Table 26. Listing of Clinical Trials Relevant to This NDA

Trial Identity	Trial Design	Study Endpoints	Treatment/ Follow Up	No. of Subjects Enrolled	Study Population	No. of Centers and Countries
Study to Support Safety						
OTL-005 NCT 02872701	Phase 2, multicenter, single dose, open label	Safety	Single dose OTL38 (0.025 mg/kg)	110	Patients with lung cancer	5 US sites
Other Studies Pertinent to the Review of Efficacy or Safety (e.g., Clinical Pharmacological Studies)						
OTL-2019-14COTL-001	Phase 1, ADME open label	Assess mass balance, primary route and mechanism of excretion, metabolites	[^{14}C]-tyrosine-OTL-0038 (105 µCi, ~0.02 mg/kg	6	Healthy volunteers	The Netherlands C. Voors-Pette MD
(b) (4) 1321-A	Phase 1, double blind, single ascending dose, randomized, placebo controlled	Assess safety, tolerability, efficacy and PK	Single ascending dose of OTL38 (0.025, 0.05, 0.1 and 0.2 g/kg); and placebo IV	30	Healthy volunteers	Single site, The Netherlands J. Burggraaf MD
(b) (4) 1321-B	Single ascending dose, open label, exploratory study.	Assess safety, tolerability, and PK	Single ascending dose of OTL38 (0.0125, 0.025 and 0.05 mg/kg); Added dose of 0.0125 mg/kg IV	18	Patients with ovarian cancer	Single site, The Netherlands J. Burggraaf MD

Source: Adapted from Applicant submitted Summary of Clinical Studies 2.7.4 of NDA Page 28 and Table 5.
Abbreviations:ADME=administration,distr bution,metabolism,excretion, FR+=folate receptor positive; kg=kilogram, mg=milligram; NCT=national clinical trial, NDA=new drug application, PK=pharmacokinetics

7.2. Review Strategy

Primary evidence of effectiveness and safety for the use of Cytalux as an adjunct during debulking surgery in patients with ovarian cancer was provided from the Phase 3 multi-center trial Study 006 with supporting data from the Phase 2 ovarian cancer trial Study 003. Additional safety data were provided from a Phase 2 lung cancer trial Study 005. The two ovarian cancer trials were conducted in similar populations i.e. patients with ovarian cancer undergoing primary or interval debulking surgery.

Assessment of Efficacy consisted of:

- Review of phase 3 clinical Study 006 (**OTL-2016-OTL006) – confirmatory trial**
- Review of phase 2 clinical Study 003 (**OTL-2014-OTL003) – supporting data**
- Included data verification, evaluation of the trial design, primary and secondary endpoints and patient populations..

Assessment of Safety:

- Safety results from phase 3 clinical Study 006 and phase 2 clinical studies - Study 003 and Study 005.

Generally, all the results of these trials reported by the Applicant and summarized in Section 8 of the review were reviewed by the clinical and statistical team and additional analysis was performed by the statistical team using the data submitted by the Applicant. In addition to the results of the primary, secondary and exploratory end points, the review of the application included consultations with Oncology - regarding the current status of ovarian cancer management including the role of Folate Receptor assay status (Oncology Consultation Report: 4839234 July 10, 2021); CDRH – on the suitability and role of NIR imaging devices as well as training for the imaging devices and the regulatory status of the IHC assay method for Folate Receptor expression that was used for the trials (CDRH Consultation Reports: ICCR00084169 and ICC2100438 2021); Division of Pediatrics and Maternal Health (DPMH) – regarding the safety concerns of pafolacianine to the fetus (b) (4) Additionally, the Office of Scientific Investigations (OSI) conducted inspections regarding matters pertaining to the conduct of relevant studies. Table 27 below provides a comparison of the basic characteristics of the two ovarian cancer studies.

Table 27. Narrative Descriptions of Clinical Efficacy Studies 006 and 003

Description	OTL-2016-OTL006	OTL-2014-OTL003
Phase	3	2
Start date	3/14/2018	12/15/2014
End date	4/16/2020	11/16/2015
Type of study	Open label, single-arm, multicenter, single dose, pivotal	Open label, single-arm, multicenter
FRα	Positive	Positive
OTL38 dose	0.025 mg/kg	0.025 mg/kg
Imaging device	[redacted] (b) (4)	
Primary endpoint	Patients with at least 1 FR+ lesion	Sensitivity, PPV
Secondary endpoint	FPRp	-
Exploratory	Inoperability rate	-
Demographic baseline characteristics	Age=60.8 Enrolled=178 Not completed=28 Pafolacianine=150 NL only=6 Median BMI=28.05 kg/m^2 Min, max=18.8, 50.7 Not randomized=10 White=84.7% Reproductive potential=15.3%	Age=63.8 Enrolled=48 Not completed=5 Pafolacianine=44 Median BMI=25.3 kg/m^2 Min, max=19.1, 32 White=79.5% Reproductive potential=4.5%
Exposed to FL imaging	1-41 min	2-32 min
Infusion interrupted	25 patients (18.7%)	7 (15.9%)
Death	2 patients, not drug related	No deaths

Source: Applicant supplied 2.7.3.2.1 Summary of Clinical Efficacy
Abbreviations: BMI=body mass index, FL=fluorescent light, FR=folate receptor, FPRp=false positive rates at the patient level, kg=kilogram, mg=milligram, NL=normal light, PPV=positive predictive value

8. Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study OTL38-2016-006

A Phase 3, Randomized Single Dose, Open Label Study to Investigate the Safety and Efficacy of OTL38 Injection for Intraoperative Imaging of Folate Receptor Positive Ovarian Cancer

Trial Design

OTL-006 was a phase 3, multicenter, prospective, single-arm, open-label study of single dose of Cytalux for the intraoperative visualization of folate receptor positive epithelial ovarian cancer as an adjunct during debulking surgery. This was the confirmatory study used for this NDA. The trial was designed to establish the utility of Cytalux with NIR fluorescence imaging in identifying additional ovarian cancer lesions compared to evaluation under normal light and

palpation by the surgeon. In order to minimize bias, all patients identified to proceed to the study were exposed to pafolacianine but were randomized to surgery with NIR light or normal light only.

The trial was performed at 13 US sites and 1 site in the Netherlands and the Central Pathology lab was located at (b) (4) The Sponsor contracted with (b) (4) as the Contract Research Organization (CRO) for the study.

The diagnostic criteria that were used to enroll patients generally reflect the incidence and natural history of ovarian cancer and are applicable to the target US population.

Key inclusion/Exclusion criteria: Apart from the standard IC/EC the following can be considered as important criteria that are applicable to the specific patient population directed for the adjunct use of Cytalux.

- The inclusion criteria comprised patients with a diagnosis or a high clinical suspicion of primary ovarian cancer of epithelial type or with recurrent ovarian cancer and planned debulking surgery.
- FR negative ovarian cancer was an exclusion criteria
- Study 006 was conducted with two NIR devices. (b) (4) - (FDA reference 510K (b) (4) and 2. (b) (4) (FDA reference (b) (4) has been cleared by FDA's Center for Devices and Radiation Health (CDRH) for use with Cytalux.

Intravenous administration of Cytalux at a dose of 0.025 mg/kg body weight was established on the basis of Study CHCR1321A, because that dose provided optimal uptake in ovarian cancer lesions with overall acceptable adverse safety. In this NDA, the Applicant has proposed to (b) (4) However, data from Study 006 showed that administration of Cytalux achieved effective visualization when administered between 1 to 9 hours prior to surgery as supported by the tumor to background ratio (TBR) measurements at multiple time points. There was a decrease in TBR and image contrast beyond 9 hours.

Treatment compliance and the overall conduct of the Study were monitored by the CRO (b) (4). Study 006 started on May 14, 2018, was completed on April 16, 2020, and enrolled adult patients with known or suspected of ovarian cancer. Patients had primary debulking surgery or interval debulking surgery (with neoadjuvant chemotherapy) along with total abdominal hysterectomy and bilateral salpingo-oophorectomy based on the clinical status.

Each patient received a single infusion of Cytalux (0.025 mg/kg) over 60 minutes about 1 to 9 hours prior to intraoperative imaging. When Cytalux is injected, it binds to the FRs and absorbs light at maximum wavelengths of (b) (4) 776 nm and emits light at wavelengths in the NIR spectrum with maximum emission (b) (4) 796 nm. The following two near infrared (NIR) imaging

devices were used as part of Study 006 - (b) (4)® and (b) (4) (b) (4) Inclusion criterion was that patients have a primary diagnosis, or a high clinical suspicion, of ovarian cancer (of epithelial type) and were to undergo surgery or laparoscopy followed by laparotomy for the debulking surgery if cancer was detected during laparoscopy. The NIR imaging devices (b) (4) and (b) (4) were used in the study and are currently being reviewed for 510 (k) clearance. (b) (4) has been cleared by the FDA for NIR fluorescent imaging with Cytalux . The Applicant states that these two devices have the critical operational features expected in a fluorescent camera system i.e. stable light source capable of providing consistent and uniform illumination of the surgical field, appropriate excitation and emission barriers in the light path for Cytalux fluorescence, image collection and recording software and hardware in a system that does not impede the flow of surgery or compromise the sterility of the surgical field. (FDA reference numbers - 510K (b) (4) and 510K (b) (4), respectively) See Table 28 below.

Table 28. Study Investigational Devices (Study 006)

Investigational Devices	
Name Description Specifications	(b) (4)
Name Description Specifications	

Source: Table 2 in Study 006 CSR supplied by Applicant
Abbreviations: FDA=food and drug administration, NIR=near infrared, nm=nano-meter

In Studies 006 and 003 (described below), the patient population used for analysis of efficacy was the modified intent-to-treat (mITT) population or full analysis set (FAS). In Study 006, the FAS was also referred to as the mITT analysis set. A per protocol analysis set (PPAS) was also defined for Study 006. The primary end point in Study 006 was the proportion of patients in whom additional lesions were identified using Cytalux injection with fluorescence imaging and subsequently conformed by pathology/IHC analysis as FR+ ovarian cancer compared to normal light and/or palpation to identify such lesions. The estimated sample size in Study 006 was 100 patients in the FAS to achieve 80% power on the primary efficacy endpoint of a threshold of 10%; 12 sites were selected to enroll the 100 FAS patients.

Randomization:
Conventional randomization was not employed because of inability to use a placebo during surgery for comparison. A modified randomization scheme was used to maintain the integrity of the trial. In Study 006, a small cohort of subjects receiving Cytalux injection were randomized to undergo lesion assessment under normal light only with standard of care surgery without the use of fluorescent imaging, to guard against possible “under-calling” of lesions during normal

light assessment and palpation. The allocation ratio of randomization to fluorescent imaging versus normal light only was 20:1, under agreement between the Applicant and the FDA during the Special Protocol Assessment (SPA) request from the Sponsor and agreement dated June 2, 2017 (FDA Reference ID 410619). A subsequent modification dated January 22, 2019 included the use of another imaging system, recalculation of sample size, and an increase in the number of study sites to include non-US sites (FDA Reference ID 4635243). Of note, investigators and their staff, and patients were blinded to the randomized allocation ratio per protocol.. All participating patients received Cytalux. However, 5% of patients receiving Cytalux were randomly assigned to undergo lesion assessment under normal light only and did not undergo fluorescent imaging, thus were not included in the FAS.

Folate Receptor Status and Histopathology:

The FR positivity was always assessed in conjunction with histopathology confirmation of ovarian cancer in establishing the standard of truth for the efficacy endpoints.

Study Endpoints

Primary Efficacy Endpoint:
The primary efficacy endpoint was the proportion of patients with at least one FR+ ovarian cancer lesion confirmed by central pathology (Standard of Truth) that was detected using Cytalux plus NIR fluorescent light but not under normal light or palpation. Evaluable lesions were lesions that would not otherwise be removed regardless of the absence or presence of tumor.

Secondary Efficacy Endpoints:
False Positive Rate at the patient level (FPRp) - the proportion of FR+ ovarian cancer patients in whom all lesions, whether or not they had been intended for removal according to the Pre-Fluorescence Surgical Plan, detected by NIR fluorescent light only were histologically negative.

Sensitivity or True Positive Rate (TPR) for Cytalux plus NIR fluorescent light - the proportion of fluorescent-light-positive lesions that were histologically confirmed to be FR+ and ovarian cancer by central pathology relative to the total number of lesions confirmed to be FR+ and ovarian cancer by central pathology whether or not they had been intended for removal according to the Pre-Fluorescence Surgical Plan.

FPR for Cytalux plus NIR fluorescent light: The FPR was calculated as the Positive Predictive Value (PPV) and was defined as the proportion of fluorescent light positive lesions removed that are histologically confirmed to be non-cancerous, or if cancerous, not FR+ and ovarian cancer, by central pathology relative to the total number of lesions removed with fluorescent light imaging whether or not they had been intended for removal according to the Pre-Fluorescence Surgical Plan.

Exploratory Endpoints:

- Sensitivity, false positive rate, and inoperability rate (all at the lesion level) excluding lymph nodes.
- Positive predictive value, true positive (TP)/TP+ false positive (FP), for OTL38 whether or not they had been intended for removal according to the Pre-Fluorescence Surgical Plan.
- Proportion of patients who have at least one FR+ ovarian cancer lesion confirmed by central pathology whether or not lesions would not otherwise be removed.
- Proportion of patients who have at least one FR+ cancer lesion (ovarian or other cell type) confirmed by central pathology that was detected with OTL38 and NIR fluorescent light and not detected under normal light that would not otherwise be removed.
- Proportion of patients who have at least one cytologically abnormal FR+ lesion that would not otherwise be removed.
- Number, per patient, of additional NIR fluorescent light positive lesions that are confirmed to be ovarian cancer and FR+ by central pathology that were not detected under normal light that would not otherwise be removed.
- Number, per patient, of additional fluorescent light positive lesions that would not otherwise be removed that are confirmed to be ovarian cancer and FR+ by central pathology that were not detected under normal light, for the subset of patients with at least one additional such lesion detected by fluorescent light only.
- Number, per patient, of additional fluorescent light positive lesions that would not otherwise be removed that are confirmed to be cancer (ovarian or other cell type) by central pathology and were not detected under normal light.
- Number, per patient, of additional fluorescent light positive lesions that would not otherwise be removed that are confirmed to be cancer (ovarian or other cell type) by central pathology and were not detected under normal light for the subset of patients with at least one additional such lesion detected.
- Sensitivity, FPR and the PPV whether or not lesions had been intended for removal according to the Pre-Fluorescence Surgical Plan
- Cancer (ovarian or other cell type) that are FR+
- Cytologically abnormal lesions that are FR+
- Proportion of patients in whom the Pre-Fluorescence Surgical Plan was changed based on fluorescent imaging both prior to initiation of the surgical procedure and upon reimaging of the surgical field after surgical procedure prior to surgical closure.
- CA-125 levels baseline (before surgery) and at 6 months post-surgery and the Integrated Summary of Efficacy, for data source.

Statistical Analysis Plan

The following analysis populations were used in the efficacy analyses:

Full Analysis Set:
Patients exposed to OTL38 and who were randomly assigned to undergo NIR fluorescent imaging (OTL38+imaging) and who:

- Were evaluated under both normal light and NIR fluorescent light imaging
- Had central pathology and histology confirmation for at least one FR+ ovarian cancer lesion detected under normal light or NIR fluorescent light imaging

For the evaluation of the primary efficacy endpoint, only the evaluable lesions contributed by FAS patients were considered. The FAS was also referred to as the modified Intent-to-Treat analysis set.

Per Protocol Analysis Set (PPAS):
All patients who met the FAS criteria in addition to the following criteria:

- Met all inclusion criteria and none of the exclusion criteria and had no major protocol deviations
- Received at least half of the OTL38 dose and Initiated NIR fluorescent imaging no earlier than 1 hour after completing their OTL38 dose

The primary endpoints were analyzed using the patients from the intersection of the Full Data Analysis Set. The primary analysis of the primary efficacy endpoint was a one-sample test for a proportion via an exact binomial test conducted at the two-tailed alpha level of 0.05. The test compared the observed proportion against a threshold of 0.10. The efficacy of Cytalux in combination with NIR fluorescent imaging was confirmed if the null hypothesis was rejected and the observed proportion was greater than 0.10. The exact (Clopper-Pearson) two-sided 95% CI was also calculated. This analysis was conducted using the evaluable lesions contributed by patients in the FAS.

Sensitivity analyses for the primary efficacy endpoint were conducted separately for the PPAS. Subgroup analysis was also performed for exploratory purpose, using the FAS, for descriptive summary statistics for the primary efficacy endpoint. For each subgroup, the point estimate and the exact 95% CI for the primary efficacy endpoint was to be provided.

The sample size of 100 evaluable patients was chosen to achieve an approximate power of 80% to reject the null hypothesis in favor of the alternative assuming the true proportion was 0.20 (20%) or more. Rejection of the null hypothesis in favor of the alternative hypothesis provided confirmatory evidence of the efficacy of OTL38 in combination with NIR fluorescent light to detect additional FR+ ovarian cancer lesions over and beyond that detected by normal light in greater than 0.10 (10%) of patients. The sample size estimation was calculated assuming the analysis was conducted via a one-sample test for a proportion via an exact binomial test with a two-tailed alpha level of 0.05.

The secondary objectives of the study were to estimate the proportion of FR+ ovarian cancer patients in whom all lesions, without regard to evaluable lesion status, detected by NIR fluorescent light only were histologically negative (the FPRp), and to estimate the sensitivity and FPR for OTL38 in combination with fluorescent light with respect to the detection of FR+ ovarian cancer lesions confirmed by central pathology.

Analyses of secondary efficacy endpoints were based on the FAS. The FPRp was analyzed descriptively, providing the point estimate and the two-sided 95% Wilson (score) CI. The analysis was conducted using the FAS. The a priori Not-to-Exceed Threshold was 17% for the FPR at the patient level. The analysis was to be descriptive. The sensitivity endpoint was analyzed using a generalized linear mixed model (GLMM) for a binomial outcome with a logit link function. The model contained a random effect for patient to allow for intra-cluster dependence among lesions within the same patient and a fixed effect for the constant. Sensitivity was estimated from the fixed effect portion of the model. A similar GLMM was conducted for the FPR.

The analyses for the sensitivity and FPR endpoints were conducted using the FAS without regard to the evaluable status of the lesions; however, patients with no NIR fluorescent light lesions evaluated by central pathology (TP + FP=0) were excluded from the FPR analysis. Similarly, patients with no FR+ and ovarian cancer lesions evaluated by central pathology (TP)+ false negative (FN)=0) were excluded from the sensitivity endpoint analysis. Point estimates and 95% CIs are provided for both endpoints.

Protocol Amendments

The original Study protocol was dated May 16, 2017 and included clinical study protocol version1, and statistical analysis plan version 1. This formed the basis for the Special protocol assessment agreement dated June 2, 2017 (FDA Ref ID 4106198). The subsequent Protocol Amendment Version 2, dated November 27, 2018 focused primarily on the addition of (b) (4) (b) (4) increasing study duration/patient accrual, making the language consistent for reporting AEs, adjusting the exclusion criteria related to miliary disease, increasing the percentage of patients not expected to meet FAS criteria, increasing study sites to a total of 16, and clarifying the pre-fluorescence surgical plan details. This modification was agreed on January 22, 2019 (FDA reference ID4378474). On February 28, 2020 a revised case report form (CRF) Version 5, was submitted. See Table 29 below.

Table 29. Protocol Amendments – Study 006

Protocol/Amendments	Details
Protocol	
OTL-2016-OTL38-006 clinical study protocol Version 1, dated 5/16/2017	Appendix 16.1.1; Module 5.3.5.1; OTL-2016-OTL38-006 CSR
Protocol amendments	
OTL-2016-OTL38-006 clinical study protocol Version 2, dated 11/27/2018	Appendix 16.1.1; Module 5.3.5.1; OTL-2016-OTL38-006 CSR
Annotated case report form	
OTL-2016-OTL38-006 case report form Version 5, dated 2/28/2020	Appendix 16.1.2; Module 5.3.5.1; OTL-2016-OTL38-006 CSR

Source: Adapted from Sponsor supplied Table 3, BIMO Reviewers Guide with NDA.
Abbreviation: CSR=clinical study report, OTL=On Target Lab Inc

Study OTL38-2014-003

A Phase 2, Single Dose, Open-Label Study to Investigate the Safety and Efficacy of OTL38 Injection (OTL38) for Intra-operative Imaging of Folate Receptor alpha Positive Ovarian Cancer

The results from this study, conducted between December 15, 2014 and November 14, 2015 were provided as supporting data in this submission. Inclusion and exclusion criteria, imaging with NL and F, pathology assessments and conduct of the study were identical to Study 006. See Table 27. No blinding procedures were used for Study 003.

Trial Design:
Study 003 is a phase 2, single dose, open label study to investigate the safety and efficacy of 0.025 – 0.050 mg/kg of pafolacianine injection 2 to 3 hours prior to cytoreduction surgery for intraoperative imaging of ovarian cancer. The study was designed to evaluate the sensitivity and specificity (PPV) of OTL38 to detect FRα+ ovarian cancer lesions during cytoreductive surgery, when visualized by an intraoperative fluorescence imaging system.

Sensitivity for the detection of FRα+ ovarian cancer lesions was defined as the ratio (multiplied by 100) of the number of FRα+ ovarian cancer lesions confirmed by both fluorescent light and by pathology and/or immunohistochemistry (True Positive=TP) over the number of FRα+ ovarian cancer lesions confirmed by the pathology and/or immunohistochemistry (TP+FN, where FN=False Negative).

Since only lesions testing positive as visualized by normal light or OTL38 were removed, no test negative lesions were removed. As such PPV was calculated in lieu of specificity. PPV for the detection of FRα+ ovarian cancer lesions was defined as the ratio (multiplied by 100) of the number of FRα+ ovarian cancer lesions confirmed by both fluorescent light and the pathology and/or immunohistochemistry (TP) over the number of FRα+ ovarian cancer lesions confirmed by fluorescent light (TP + FP, where FP=False Positive).In addition, the safety and tolerability of OTL38 administered as a single IV dose, was also evaluated.

The use of OTL38 was investigated in the FRα+ ovarian cancer during primary surgical cytoreduction surgery. Thus, female patients (>18 years of age) with a primary diagnosis or at high clinical suspicion of primary ovarian cancer (of epithelial type), who were candidates for primary debulking or interval debulking surgery were chosen as candidates for this study. Patients were considered primarily eligible (a) if they were scheduled to undergo laparotomy for the debulking surgery or, (b) if they were scheduled to undergo laparoscopy and had preauthorized to undergo laparotomy for the debulking surgery, if cancer was detected on the laparoscopy.

Primary Objectives

- To assess the safety and tolerability of single IV doses of OTL38
- To assess the sensitivity and Positive Predictive Value (PPV) of OTL38 in detecting Folate Receptor-alpha positive (FRα+) ovarian cancer during surgery

Secondary Objective

- To assess the safety of using the (b) (4) the (b) (4), and the (b) (4) for intraoperative imaging with OTL38

Exploratory Objective

- To assess the number of FRα+ ovarian cancer lesions detected with fluorescent light compared to usual visual/tactile conditions and/or palpation

Study Endpoints

Primary Efficacy Endpoint

- Sensitivity and PPV of OTL38 for the detection of FRα+ovarian cancer lesions

Exploratory Endpoints

- Sensitivity and PPV of OTL38 for the detection of FRα+ ovarian cancer lesions, including all other lesions
- Sensitivity and PPV of OTL38 for the detection of ovarian cancer lesions.
- Sensitivity and PPV of OTL38 for the detection of o FR+ or FRβ+ in ovarian cancer lesions.
- Sensitivity and PPV of OTL38 for the detection of FR+ or FRβ+ in cytologically abnormal (including ovarian cancer) lesions.
- Sensitivity and PPV of OTL38 for the detection of FR+ ovarian cancer lesions including untested lesions.
- Number of FR+ovarian cancer lesions per patient identified under usual visual/tactile conditions compared with such lesions identified using OTL38 and fluorescence imaging.
- Proportion of patients in whom additional lesions were identified using OTL38 with fluorescence imaging and subsequently confirmed by pathology/

immunohistochemistry analysis as FRα+ ovarian cancer compared with using normal light and/or palpation to identify such lesions.
- Miliary Disease: Proportion of FRα+ ovarian cancer lesions identified by Fluorescence only compared with using visual/tactile conditions to identify such lesions were

Tumor lesion detection was performed using the above indicated NIR imaging devices at pre- and post-cytoreduction surgery. Investigators used normal and fluorescence imaging to detect lesions. In addition pathology and immunohistochemistry for folate receptors were performed for all samples. Adverse events were recorded for all enrolled patients from the time of start of study drug administration through visit 4 (28± 4 days) and were classified as mild, moderate and severe grades as observed and whether they were related or unrelated to the study drug.

Statistical Analysis Plan for Study 003:

The following analysis populations were used in the efficacy analyses for Study 003:

1. Safety Population: Safety population consisted of all patients who received OTL38
2. Intent-To-Treat (ITT) Population: The ITT population consisted of all patients who signed the Informed Consent Form at Visit 1.
3. Modified Intent-To-Treat (mITT) Population: The 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 FRα+ target ovarian cancer lesion. Patients enrolled to the study under protocol amendment 4 were not included in the mITT population.

Efficacy and exploratory analyses were conducted using the mITT population. Sensitivity for the detection of FRα+ ovarian cancer lesions was defined as the ratio (multiplied by 100) of the number of FRα+ ovarian cancer lesions confirmed by both fluorescent light and by pathology and or immunohistochemistry(IHC) (True positive=TP) over the number of FRα+ ovarian cancer lesions confirmed by pathology and or IHC (TP+FN). FN = false negative.

- Sensitivity = TP/ (TP+FN) x 100%;
- PPV= TP/(TP+FP) x 100%

Healthy normal tissue was not removed as part of this study, hence a true negative sample was not available. For sensitivity, TP was assumed to follow a binomial distribution B (P, N) with P=true sensitivity and N=TP+FN. For PPV, TP was assumed to follow a binomial distribution B (P, N) with P=true PPV and N=TP+FP. Sensitivity and PPV were estimated using the Glimmix procedure for a binomial distribution in SAS.

Sensitivity and PPV of OTL38 to detect other types of lesions were determined using methods similar to those used for the primary efficacy analysis. The number of lesions per patient

detected by usual visual/tactile methods vs. fluorescent imaging was analyzed using a paired t-test. The p-value was calculated and the mean difference in number of lesion and its 95% CI was provided.

The proportion of patients in whom more lesions were identified using OTL38 with fluorescence imaging and subsequently confirmed by pathology/immunohistochemistry analysis as FRα+, ovarian cancer compared with using usual visual/tactile conditions to identify such lesions was estimated using Proc Freq in SAS, and its 95% CI was provided.

For miliary disease, the proportion of FRα+ ovarian cancer lesions identified by fluorescence only compared to the lesions observed using visual/tactile methods was provided.

8.1.2. Study Results (Study 006):

Compliance with Good Clinical Practices

The Applicant indicated that the study was performed in compliance with good clinical practice (GCP) and with oversight from the local institutional review board (IRB), guided by ethical considerations and provisions of Title 21 CFR Part 56 and 21 CFR Part 50. In this regard, please refer to Section 4.1 and Table 3 or discussion on GCP related to central pathology reads.

Financial Disclosure

There was no disclosable information from all study principal investigators and sub-investigators.

Patient Disposition:

A total of 178 subjects were enrolled after being assessed for eligibility; 28 subjects were excluded for a variety of reasons – screen failure 9; subject withdrawal 8; cancelled surgery 5; non-compliance 2; missed appointment 2; investigator decision to perform laparoscopy 1; and Sponsor decision/no camera training 1. Of the remaining 150 patients who received pafolacianine, 134 were randomized to fluorescent and normal light ; 6 were randomized to normal light (NL) only; and not-randomized were 10 (disease too extensive 7; adverse event (AE) 2; benign biopsy 1). This resulted in the Full Analysis Set (FAS) of 109 patients after excluding 25 subjects who did not have central pathology and histological confirmation. The Per Protocol Analysis Set (PPAS) was 106 and excluded 3 patients because the fluorescent light (FL) imaging was initiated earlier than one hour after completing the pafolacianine dose= 2; and did not receive at least half the pafolacianine dose =1. Table 30 summarizes disposition for all enrolled subjects.

Table 30. Study 006 Subject Disposition for All Enrolled Subjects

Definition	FL & NL n (%)	NL Only n (%)	Not Randomized n (%)	Overall n (%)
Signed informed consent				178
Screen failure				28
Safety analysis set	134	6	10	150
Full analysis set	109	-	-	109
1 evaluable lesion	57 (52.3)	-	-	57 (52.3)
No. of evaluable lesions	149	-	-	149
PP analysis set	106 (79.1)	-	-	106 (79.1)
PK analysis set	122 (91.0%)	6 (100)	8 (80.0)	136 (90.7)
Study completion status				
Completed	128 (95.5%)	6 (100)	0	134 (89.3)
Did not complete	6 (4.5%)	6 (100)	10 (100)	16 (10.7)
Reasons early withdrawal				
Withdrawal by subject	0	0	0	0
Non-compliance	0	0	0	0
Lost to follow up	2 (1.5)	0	0	2 (1.3)
Investigator decision	0	0	0	0
AE	0	0	2 (20)	2 (1.3)
Stopped by Sponsor	0	0	0	0
Death	1 (0.7)	0	0	1 (0.7)
Other	3 (2.2)	0	8 (80)	11 (7.3)

Source: Adapted from the Applicant supplied Table 7; 2.7.3 Summary of Clinical Efficacy, Page 33.
Abbreviations: AE=adverse event, FL=fluorescent light, NL=normal light, PK=pharmacokinetics, PP=per protocol

A total of 178 patients were enrolled for participation in this study. Of these, 28 patients did not receive pafolacianine sodium resulting in a total of 150 receiving drug and were included in the SAS population. From the 150 patients receiving pafolacianine sodium, 134 patients were randomly assigned to the fluorescent and normal light group, 6 patients to normal light only, and 10 patients were not randomly assigned to an imaging group. Thus there were 109 patients in the FAS group. Twenty five patients were excluded from the analysis; See Table 31 below for the summary details on these 25 patients.

Table 31. Twenty Five Patients Receiving Study Drug Excluded From FAS Study 006

Sub. ID	Safety	FAS	Completed	Planned Treatment for Period 01	Reason for Discontinued From Study	Reason for Exclusion
(b) (6)	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	N	OTL38 with FL & NL	Device failure-patient not imaged.	Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL

Sub. ID	Safety	FAS	Completed	Planned Treatment for Period 01	Reason for Discontinued From Study	Reason for Exclusion
(b) (6) [redacted]	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	N	OTL38 with FL & NL	Dis. too extensive, camera not used	Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL
	Y	N	Y	OTL38 with FL & NL		Negative for at least 1 CP and HP confirmation of FR+ ovarian cancer under NL & FL

Source: Applicant supplied Listing of Intent-to-image Set, in the attachment in response to IR dated June 1, 2021.
Abbreviations: CP=central pathology, dis.=discontinued; FAS=full analysis set, FL=fluorescent light, FR=folate receptor, HP=histopathology, NL=normal light; Dis= distance

Protocol Violations/Deviations

Among the protocol deviations reported by the Applicant, the majority were minor in nature. There were 3 major deviations in the Safety Analysis Set (SAS) (2%); consisting of Assessment at incorrect time point 2 (1.3%) and Missed assessment 1 (0.7%) that were reported for site 2. The three patients were in the NIR fluorescent and normal light group (Patient [redacted] (b) (6) [redacted]). See Table 32 below.

Table 32. Study 006 Major Protocol Deviations

Subject No.	Date of Deviation	Deviation Description	Action Taken	Category	Major/ Minor
(b) (6)		Full hour not passed prior to camera turned on	PI and staff worked to prevent recurrence of the incident	Assessment at incorrect time point	Major
		Full hour not passed prior to camera turned on	Deviation noted by (b) (4) aware of deviation, sub-investigator. Surgeons re-trained, (b) (4) training completed.	Assessment at incorrect time point	Major
		Technical issue with camera	Subject not evaluable. New equipment provided	Missed assessment	Major

Source: Applicant supplied Listing 16.2.2.1 Protocol deviations in BIMO Appendix-1.
Abbreviations: CRO=contract research organization, PI=principal investigator,

In addition, the following observations triggered a consultation to the Office of Scientific Investigation (OSI).

a. Site #2 showed an overall lower number of evaluable lesions despite having high-volume surgeons and an onsite pathology laboratory accessible.

b. Site # 5 had a high number of protocol violations (*albeit* minor) and it is unclear if training, or lack thereof, contributed to these events.

c. Administered dose: Intended versus final: The study drug pafolacianine was administered based on total body weight (mg/kg) of individual patients. For the combined ovarian cancer population (N=194), the mean planned dose was 1.8 mg (SD 0.384) and min & max (1.0, 3.3) mg. The total administered dose was a mean of 1.79 mg (SD 0.495) and min & max of (0.1, 3.8) mg. The total infusion duration was a mean of 61.32 min (SD 12.95) min & max (4.0, 137) min. A total of 35 patients (18%) had their infusions interrupted. For this population (N=294), 185 patients received a complete dose. The data were further analyzed on the basis of subgroups of age, BMI and other factors.

Final clinical Inspection Summary from the Office of Scientific Inspections was received on July 14, 2021. The report satisfactorily addressed the issues raised. See the summary and Table 3 in Section 4.1.

Demographics and Other Baseline Characteristics

Table 33 below summarizes the demographic and other baseline characteristics for the primary efficacy analysis.

Table 33. Demographic and other Baseline Characteristics of the Primary Efficacy Analysis

	Not Randomized N=10 n (%)	Treatment Group N=140 FL & NL N=134 n (%)	Treatment Group N=140 NL Only N=6 n (%)	Total N=150 n (%)
Sex				
Male				
Female	10	134	6	150 (100)
Age (years)				
Mean (SD)	65.2 (9.98)	60.4 (10.91)	62.8 (21.77)	60.8(11.39)
Median	66.0	61.0	66.5	62.0
Min, max	43, 78	33, 81	29, 89	29. 89
Age group				
<70 years	6 (60)	101 (75.4)	4 (66.7)	111 (74)
≥70 years	4 (40)	33 (24.6)	2 (33.3)	39 (26)
Race				
White	8 (80)	114 (85.1)	5 (83.3)	127 (84.7)
Black or African American	0	7 (5.2)	0	7 (4.7)
Asian	1(10)	5 (3.7)	1 (16.7)	7 (4.7)
American Indian or Alaska Native		4 (3.0)	0	4 (2.7)
Native Hawaiian or other Pacific Islander		0	0	0
Other[1]	1(10)	4 (3.0)	0	4 (2.7)
Ethnicity				
Hispanic or Latino	1 (10)	15 (11.2)	2 (33.3)	18 (12)
Not Hispanic or Latino	9 (90)	116 (86.6)	4 (66.7)	129 (86)
Weight (kg)				
n	10	134	6	150
Mean	71.65 (14.55)	74.17 (16.23)	75.35 (16.00)	74.06 (16.03)
Median	68.15	73.7	73.30	73.35
Min, max	52.8, 94.8	41.6, 133.1	60.5, 99.7	41.6, 133.1
BMI (kg/m^2)				
n	7	122	3	132
Mean	30.51 (5.35)	27.93 (5.831)	26.5 (3.64)	28.03 (5.8)
Median	29.50	28.05	24.40	28.05
Min, max	24.8, 38.9	18.8, 50.7	24.4, 30.7	18.8, 50.7

Source: Adapted from Sponsor supplied Table 7, Clinical Study Report OTL-006, Page 51.
[1] Definition of Other = Race non-specified or declined to answer or not listed.
Abbreviations: FL=fluorescent light, NL=normal light, SD=standard deviation

A total of 194 patients with known or high suspicion for ovarian cancer who underwent surgical debulking were studied. All patients were included in the Safety Population, the ITD Efficacy Population and the Per Protocol Population.

Age Distribution:
Overall, patient's age ranged from 29 to 89 years with a mean of 60.8 years of age; 111 patients (74%) were younger than 70 years while 39 patients (26%) were 70 years of age or older. Of the 134 patients in the NIR fluorescent light imaging group, the minimum and maximum ages were 33 and 81 years, respectively, and 101 patients (75.4%) were younger than 70 years of age. For the 6 patients randomized to normal light only, the minimum and maximum ages were 29 and 89 years, respectively, and 4 patients (66.7%) were younger than 70 years of age.

Ethnicity Distribution:
The majority (129 patients [86%]) were not Hispanic or Latino, while 18 patients (12%) were Hispanic or Latino, and 3 patients (2%) did not report their ethnicity. In the NIR fluorescent light imaging group, 116 patients (86.6%) were not Hispanic or Latino, and, in the normal light group, 4 patients (66.7%) were not Hispanic or Latino.

Patients were White (127 [84.7%]), Black or African American (7 [4.7%]), Asian (7 [4.7%]), American Indian or Alaska Native (4 [2.7%]), and other (4 [2.7%]), and race was not reported for 1 patient (0.7%). In the NIR fluorescent light imaging group, 114 patients (85.1%) were White, 7 patients (5.2%) were Black or African American, 5 patients (3.7%) were Asian, 4 patients (3.0%) were American Indian or Alaska Native, and 4 patients (3.0%) were other. In the normal light group, 5 patients (83.3%) were White, and 1 patient (16.7%) was Asian.

Reproductive Potential:
The majority of patients (102 [68.0%]) were considered post-menopausal, with 23 patients (15.3%) considered to be of childbearing potential, and 25 patients (16.7%) were considered sterile.

Safety Analysis Set:

The Safety Analysis Set consisted of 150 patients, FL and NL 134 patients; NL only 6; Not Randomized 10 patients. All patients were diagnosed with advanced ovarian cancer, and were imaged during the debulking surgery. The majority of patients were White (n= 108; 85%). Distribution of patients as per clinical stage was Stage I=4.4%; Stage II=2.2%; Stage III= 25.7%; Stage IV=22.5% and Stage Unknown= 44.9%.

Table 34. Cancer Type at Study Entry:

	Study 006 Safety N=150	Study 006 FAS N=109
	Initial Diagnosis	Post-Surgery
Statistics	**n (%)**	**n (%)**
Ovarian cancer	77 (51.3)	92 (84.4)
Primary peritoneal cancer	5 (3.3)	7 (6.4)
Fallopian tube cancer	0	3 (2.8)
High clinical suspicion	60 (40)	N/A

Source: Adapted from Sponsor Table 4; 2.7.3 Summary of Clinical Efficacy Page 23
Abbreviations: FAS=full analysis set

Surgery Types:
The 109 FAS patients underwent the following types of surgeries: Primary cytoreduction surgery 39 (35.8%; Interval debulking surgery 58 (53.2%); recurrent ovarian cancer surgery 2 (1.8% and other 10 (9.2%). Surgery was stopped for 5 patients (4.6%). The distribution reflects the current trends in the management of ovarian cancer.

Stage at Presentation:
Proportion of patients with at least one confirmed FR+ ovarian cancer evaluable lesion by staging were Stage I (20%); Stage II (33.3%); Stage III (34.3%); Stage IV (31.3%); and Stage Unknown 33.3%).

Cancer Types:
For the Full Analysis Set (FAS) of 109 patients, 59 (54.1%) were ovarian cancer; 4 (3.7%) primary peritoneal cancer; 41 (37.6%) high clinical suspicion for ovarian cancer; other 5 (4.6%) (1 Mullerian tumor; 1 carcinosarcoma; 1 endometroid; 2 tubo-ovarian cancer).

Patients with Reproductive Potential:
For the combined ovarian cancer patient population in Studies 003 and 006 (N=194), there were 25 subjects (12.9%) that were potentially able to bear children; 102 (52.6%) were post-menopausal; 25 (12.9%) were sterile and for 42 subjects (21.6%) data were not collected/applicable/reported.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The Applicant did not conduct any clinical drug-drug interaction studies with pafolacianine injection. During all clinical studies, the clinical protocols required the study drug not to be infused concomitantly in the same intravenous line with any other agent. Because of folic acid binds competitively binding to folate receptors, all folate, folic acid or folate containing supplements were withheld for 48 hours prior to the administration of pafolacianine sodium. Treatment compliance and concomitant medications use were not issues, as the single use drug administration was done in a controlled environment prior to surgery. Likewise, any use of rescue medication such as anti-emetics, anti-nausea drugs and or interruption of infusion were carried out under the supervision of the study investigators and as deemed clinically necessary at the time of study drug infusion.

Efficacy Results – Primary Endpoint

The Applicant defined the primary efficacy endpoint as the proportion of patients with at least one evaluable FR+ ovarian cancer lesion confirmed by central pathology that was detected with Cytalux under NIR fluorescent light but not under normal light or palpation and not otherwise identified for resection prior to surgery. The Sponsor's analysis of the primary efficacy endpoint resulted in 36 of 109 women (33.0%) (95% CI [24.3, 42.7]) with at least one additional evaluable

FR+ ovarian cancer lesion confirmed by central pathology that was detected with the OTL38 injection and NIR fluorescent light but not under normal light or palpation.

See Section 8.3 on Statistical Issues for the additional analysis conducted and for the appropriate reporting of the primary efficacy endpoint.

Data Quality and Integrity

The data sets provided by the Applicant were reviewed by the review team and where appropriate, requests for additional information or clarification were sent to the Applicant as indicated. Refer also to the final Clinical Summary Report from the OSI provided in Section 4.1.

Efficacy Results – Secondary and Other Relevant Endpoints

All analyses in this section for secondary and other endpoints were also performed on the FAS. The Applicant reported lesion level sensitivity (true positive rate), defined as detecting ovarian cancer and FR+ lesions by NIR fluorescent light. The estimated lesion-level sensitivity reported by the Applicant was 83.0%, with a 95% CI [73.9, 89.4]). The Applicant also reported lesion level FPR (defined as one minus the PPV) with respect to the detection of ovarian cancer and FR+ lesions by NIR fluorescent light. The estimated lesion level FPR reported by the Sponsor was 32.7% with a 95% CI [25.6, 40.7].

Dose/Dose Response

A single dose of 0.025 mg/kg of Cytalux was injected intravenously for intraoperative guidance 1 to 9 hours prior to surgery.

Efficacy Results – Secondary or Exploratory endpoints

Key Secondary Endpoints:

False Positive Rate at the Patient Level

The FPRp was a secondary efficacy endpoint and was defined as the proportion of folate positive ovarian cancer patients in whom all lesions, without regard to evaluable lesion status, detected by NIR fluorescent light only (and not detected by normal light or palpation) were histologically negative. There were 27 patients meeting the criteria, resulting in a FPRp of 24.8% (95% CI [17.6, 33.6]). The pre-defined Not-to-Exceed Threshold was 17%.

Sensitivity (True Positive Rate)

Sensitivity (TPR) was defined as the proportion of NIR fluorescent light positive lesions that were histologically confirmed to be FR+ and ovarian cancer by central pathology relative to the total number of lesions confirmed to be FR+ and ovarian cancer by central pathology without

regard to evaluable lesion status. The GLMM analysis for the 109 FAS patients contributing to this analysis yielded an estimated sensitivity (TPR) of 83%.

Key Exploratory end points from Study 006

For Study 006, 26.9% of patients (95% CI [24.3,42.7]); *P* < 0.001) met the primary efficacy criteria (i.e. the proportion of patients with at least 1 FR+ ovarian cancer lesion confirmed by central pathology that was detected using pafolacianine sodium plus NIR fluorescent light but not under normal light and/or palpation). Primary endpoint analysis based on stage of cancer at surgery showed average of about 30% for stages II-IV and 45.5% for those with miliary disease and 31.6% for non-miliary disease.

-For the exploratory endpoint of inoperability rate at the lesion level, 16 lesions contributed by 109 patients in the FAS were identified by NIR only and could not be removed because of lesion location or other surgical factors, the lesion inoperability rate was 0.1%.

- At the lesion level, for pafolacianine sodium combined with NIR for the detection of lesions that were determined to be both FR+ and ovarian cancer, excluding lymph nodes, sensitivity was 83.2%.

-At the lesion level, for pafolacianine sodium combined with NIR, excluding lymph nodes, False Positive Rate (FPR) was 29.1% (95% CI [22.0,37.4]).

- Positive Predictive Value (PPV) for pafolacianine sodium combined with NIR was 67.3% (95% CI [59.3,74.4]).

-Number and rate per patient of additional evaluable confirmed FR+ ovarian cancer lesions detected by the combination of pafolacianine sodium and NIR but not under normal light and/or palpation were 1.7 (95% CI[1.3,2.2]) for the mean count per patient and 0.2 for additional lesion rate per patient.

-At the lesion level, True Positive Rate (TPR) for pafolacianine sodium combined with NIR (all FR+ cancer lesions or FR+ cytologically abnormal lesions) was 83.0% (95% CI [73.9,89.4]) for FR+ cancer lesions (ovarian or other cell type) and 81.3% (95% CI- [72.4,-87.8) for FR+ cytologically abnormal lesions.

-Proportion of patients for whom the pre-fluorescence surgical plan was changed based on fluorescent imaging was 24.8% for before initiation of surgical procedure, and 42.2% upon reimaging of the surgical field after the surgical procedure immediately before surgical closure and 54.1% overall.

-Distribution of patients in trial 006 under surgery type – Primary debulking=36%, Interval debulking= 53%, other=11% indicates the existing trends in managing ovarian cancer.

In an analysis of a subset of patients with all evaluable lesions (XN and P), excluding non-evaluable (XS) lesions (i.e. lesions that were targeted for removal regardless of fluorescence status), the FPRp was 19.3% (Table 35). Additional post hoc analysis of FPRp in patients in whom all lesions i.e. all lesions detected by NIR fluorescent light and normal light/palpation (⊗, XN, XS,P – see Table 37) were histologically negative the FPRp was 8.3%, as indicated in Table 36. At the lesion level, sensitivity (without regard to evaluable lesion status) was estimated as 83%.

Table 35. Study 006 Post Hoc Analysis - Patient-Level FPR for OTL38 and FL Evaluable Lesions (FAS)

	Assessment Statistic FAS N=109
Patients with all evaluable lesions detected by OTL38 & FL (XN, P) being histologically negative.	
n	21
Proportion	0.193 (19.3%)
95% CI (%)	[13.0, 27.7]

Source: Applicant supplied Table 6, ISE Module 5.3.5.3
Abbreviations: CI=confidence interval, FAS=full analysis set, FL=fluorescent light, FPR=false positive rate, P,XN=refer to Table 37

Table 36. Study 006 Post Hoc Analysis: Secondary Efficacy Endpoint. Patient-Level FPR for OTL38 and FL - all lesions detected by FL in FAS

	Assessment Statistic FAS N=109
All lesions detected by OTL38 & FL (OX, XN, XS, P) being histologically negative.	
n	9
Proportion	0.083 (8.3%)
95% CI (%)	[4.4, 15.0]

Source: Applicant supplied Table 7 ISE Module 5.3.5.3
Abbreviations: CI=confidence interval, FAS=full analysis set, FL=fluorescent light, FPR=false positive rate, OX,P,XN,XS=refer to Table 37.

Table 37. Key to Lesion Identifiers

Identifier	Condition Under Which Lesion Detected; Action Taken
O	Normal light only
⊗	Both normal light and fluorescence imaging
P	Post-resection lesion detection under fluorescence imaging only
X	Fluorescence imaging only (pre-resection)
XN	Fluorescence imaging only; lesion removed, not part of the surgical plan
XS	Fluorescence imaging only; lesion removed as part of the surgical plan

Source: Applicant supplied Study 006 CSR Module 5.3.5.1.
These abbreviations and codes form the basis for the several tables used in this Unireview document.

Study Results (Study 003):

The Applicant is using Study 003 (N = 44) to support the efficacy results from study 006. Table 38 below provides the summary of the patient disposition for Study 003.

Compliance with Good Clinical Practices:
Applicant assures that the study was conducted in compliance with International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines, the IHC6 Good Clinical Practices (GCP) guideline.

Financial Disclosure
There was no disclosable information from all study principal investigators and sub-investigators.

Patient Disposition:
There were 48 patients who provided informed consent and were enrolled in the study. A total of 44 patients (44/48; 91.7%) received OTL38 and were included in the Safety population. The 4 patients who did not receive OTL38 included 2 patients who voluntarily withdrew from the study, 1 patient was withdrawn based on Investigator decision and 1 patient due to reason listed as "Other: drug not useable". All but 1 patient in the Safety population completed the study per protocol. The sponsor indicated that patient who did not complete the study withdrew early from the study (4 days after receiving OTL38) and did not attend any follow-up visits. No patients died during the study, and there were no AEs related to drug or device that led to discontinuation of any patient from the study. Table 38 below provides these details.

Table 38. Patient Disposition – Study 003

Disposition	Number of Patients (%)
Patients Enrolled	48
Safety Population [1]	44 (91.7%)
mITT Population [2]	29 (60.4%)
Completed Study	
Yes	43 (89.6%)
No	5 (10.4%)
Primary Reason for Discontinuation	
Non-compliance	0 (0%)
Lost to Follow-up	0 (0%)
Adverse Event/Adverse Device Effect	0 (0%)
Study Terminated by Sponsor	0 (0%)
Death	0 (0%)
Withdrawal by Subject	3 (6.3%)
Investigator Decision	1 (2.1%)
Other	1 (2.1%)

Source: Adapted from Listing 16.2.1.1. Section 14.1 – Table 14.1.1
[1] All patients who received pafolacianine

Protocol Violations/Deviations:

There were 16 (16/44; 36.4%) patients with major protocol deviations in the Safety population. The summary below explains the major protocol deviations:

- The most common major deviation in patients was failure to promptly report an SAE or an unexpected AE (13/44 patients; 29.5%). These included SAEs of hematoma, infections, urinary tract infection (UTI), sepsis, surgical wound infection, hypotension, constipation, hemorrhage, pleural effusion, pneumonia, deep vein thrombosis (DVT), septic shock, anemia and small bowel obstruction.
- Surgery was performed outside of the protocol-defined window (2 to 3 hours) after study drug administration for 7/44 (15.9%) patients. There was no subject excluded from mITT due to surgery being performed out of the protocol-defined window.
- Two patients (b) (6) had issues associated with the informed consent process (a certified translator was not used for these subjects with native languages other than English).

Demographics and other Baseline Characteristics:

Table 39 below provides a summary of the demographics and baseline characteristics for the safety population in Study 003.

Table 39. Demographics and Other Baseline Characteristics

Characteristic	Number of Patients N=44 n (%)
Age (years)	
n	44
Mean (SD)	63.8 (10.19)
Median	64
Min, max	37, 82
Sex	
Female	44 (100)
Childbearing potential	
Yes	2 (4.5)
No	42 (95.5)
Post menopause	31 (70.5)
Sterile	11 (25.0)
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)
Height (cm)	
n	36
Mean (SD)	16.03 (6.47)
Median	160
Min, max	140.4, 170.0
Weight (kg)	
n	44
Mean	64.68 (10.09)
Median	63.3
Min, max	46.6, 85.4
BMI (kg/m^2)	
n	36
Mean	24.94 (3.69)
Median	25.3
Min, max	19.1, 32.0

Source: Sponsor submitted Study 003, CSR Table 14.1.3
All patients who received pafolacianine, underwent cytoreductive surgery, were exposed to fluorescent light and had at least one FRα+FRa+ target lesion.
Abbreviations: BMI=body mass index, mITT=modified intent to treat population

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The Applicant did not conduct any clinical drug-drug interaction studies with pafolacianine injection. During all clinical studies, the clinical protocols required the study drug not to be infused concomitantly in the same intravenous line with any other agent. Because folic acid competitively binds to folate receptors, all folate, folic acid or folate containing supplements were withheld for 48 hours prior to the administration of pafolacianine sodium. Treatment compliance and concomitant medications use were not issues, as the single use drug administration was done in a controlled environment prior to surgery. Likewise, any use of rescue medication such as anti-emetics, anti-nausea drugs and or interruption of infusion were carried out under the supervision of the study investigators and as deemed clinically necessary at the time of study drug infusion.

Efficacy Results:
The efficacy data (sensitivity and positive predictive value) from study 003 are listed in Table 40, Table 41 and Table 42 below. Of note, Study 003 used the modified intent to treat population (mITT) and the efficacy was based on the lesion level analysis. The sensitivity of pafolacianine in detecting FRα+ ovarian cancer, with patient as random effect was 97.97% with a lower one-sided 95% CI = 87.75. The estimate for PPV was 94.93%. Similarly, the sensitivity of pafolacianine in detecting ovarian cancer lesions was 96.82% and the PPV was 92.62% and the sensitivity in detecting FR+ (both α and β) was 97.2% with a PPV of 95.02%.

Table 40. Sensitivity and PPV of Pafolacianine in Detecting FRa+ Ovarian Cancer Lesions Study 003

	OTL38 mITT Population (N=29)		
	Number of Lesions	Estimate (lower one-sided 95% CI) [1] (with patient as random effect)	Estimate (lower one-sided 95% CI) [2] (without patient as a random effect)
TP	171		
FP	23		
FN	28		
TN	3		
Sensitivity[3]		97.97 (87.75)	85.93 (81.19)
PPV[4]		94.93 (86.13)	88.14 (83.59)

Source: From Listing 16.2.1.1, Listing 16 Section 14.1 – Table 14.2.1.1 & Table 14.2.1.2
[1] Estimated using Proc Glimmix in SAS for binomial distribution with patient as a random effect
[2] Estimated using Proc Glimmix in SAS for binomial distribution
[3] Sensitivity of OTL38 for the detection of FRα+ ovarian cancer lesions
[4] Positive predictive value of OTL38 for the detection of FRα+ovarian cancer lesions.
Abbreviations: CI=confidence interval, FN=false negative, FP=false positive, mITT=modified intent to treat population, PPV=positive predictive value, TN=true negative, TP=true positive

Table 41. Sensitivity and PPV of Pafolacianine in Detecting Ovarian Cancer Lesions

	OTL38 mITT Population (N=29)		
	Number of Lesions	Estimate (lower one-sided 95% CI) [1] (with patient as random effect)	Estimate (lower one-sided 95% CI) [2] (without patient as a random effect)
TP	198		
FP	34		
FN	38		
TN	7		
Sensitivity[3]		96.82 (86.09)	83.90 (79.40)
PPV[4]		92.62 (83.35)	85.34 (80.93)

Source: Adapted from Applicant submission 003 Table 6 of CSR, 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.
[1] Estimated using Proc Glimmix in SAS for binomial distr bution with patient as a random effect
[2] Estimated using Proc Glimmix in SAS for binomial distr bution
[3] Sensitivity of OTL38 for the detection of ovarian cancer lesions
[4] Positive Predictive Value (PPV) of OTL38 for the detection of ovarian cancer lesions
Abbreviations: TP = true positive; FP =false positive; FN =false negative; TN =true negative

Table 42. Sensitivity and PPV of Pafolacianine in Detecting FRα or FRβ in Lesions With Cytologic Abnormalities (Including Ovarian Cancer)

	OTL38 mITT Population (N=29)		
	Number of Lesions	Estimate (lower one-sided 95% CI) [1] (with patient as random effect)	Estimate (lower one-sided 95% CI) [2] (without patient as a random effect)
TP	191		
FP	10		
FN	29		
TN	4		
Sensitivity[3]		97.20 (88.62)	86.82 (82.43)
PPV[4]		95.02 (91.66)	95.02 (91.66)

Source: Applicant submission Study 003 CSR. Note: Includes all lesions with pathology and immunohistochemistry results.
TP: Lesions that fluoresced and tested positive for either FRα or FRβ and tested positive for cytologic abnormality.
FP: Lesions that fluoresced but tested negative for both FRα or FRβ and test positive for cytologic abnormality; or lesions that fluoresced and test positive for either FRα or FRβ but test negative for cytologic abnormality.
FN: Lesions that did not fluoresce but tested positive for either FRα or FRβ and tested positive for cytologic abnormality.
TN: Lesions that did not fluoresce and tested negative for both FRα or FRβ but tested positive for cytologic abnormality; or lesions that did not fluoresce but tested positive for either FRα or FRβ and tested negative for cytologic abnormality.
[1] Estimated using Proc Glimmix in SAS for binomial distr bution with patient as a random effect
[2] Estimated using Proc Glimmix in SAS for binomial distr bution
[3] Sensitivity of OTL38 for the detection of FRα or FRβ in cytologically abnormal lesions (including ovarian cancer)
[4] Positive Predictive Value (PPV) of OTL38 for the detection of FRα or FRβ in cytologically abnormal lesions (including ovarian cancer)
Abbreviations: FN=false negative, FP=false positive, TN=true negative, TP=true positive

Data Quality and Integrity:

The data sets provided by the Applicant were reviewed by the review team and where appropriate, requests for additional information or clarification were sent to the Applicant as indicated. Refer to the final Clinical Summary Report from the OSI provided in Section 4.1.

Additional Analyses Conducted on the Individual Trial

Please refer to Section 8.3 Statistical Issues.

Integrated Review of Effectiveness

8.1.3. Assessment of Efficacy Across Trials

Not applicable.

Additional Efficacy Considerations

Overall Assessment of Test Performance:

The pafolacianine phase 3 study met its primary endpoint goal for the lower bound of the 95% CI of sensitivity to be greater than 10%, with an observed point estimate of 26.9 % and a 95% CI of [19.6, 35.2] The data presented are to be viewed in the context of the need for the adjunctive role of fluorescence guided surgery (FGS) in debulking surgery for ovarian cancer that is a serious disease with largely unmet needs for effective management approaches. The False Positive Rate of 32.7% . indicates that nearly one third of the time, the lesion fluoresces without being FR+. This indicates the need for surgeons to use caution in going after fluorescent positive lesions in situations where deviation from the pre-surgical planning is contemplated that would result in the removal of additional tissue that might jeopardize patient safety. However, in both situations the chances for compromised patient safety are mitigated as surgeons are governed by pre-surgical plan and standard of care surgical practice. Along these lines, the Applicant is being advised to insert a statement in Section 5: Warnings and Precautions of the proposed label for Cytalux to indicate the risk of FP (false positive) and FN (false negative) situations as a general guide to the treating surgeon- **"**Errors may occur with the use of Cytalux during intraoperative fluorescence imaging to detect ovarian cancer, including false negatives and false positives. Non-fluorescing tissue in the surgical field does not rule out the presence of ovarian cancer [see Clinical Studies (14)]. Fluorescence may be seen in non-cancerous tissue including areas of the bowel, lymph nodes and inflamed tissue."

The Applicant's request for Orphan Drug Designation of OTL38 to "*detect alpha receptor positive lesions in ovarian cancer patients*" was approved by the Office of Orphan Product Development on December 23, 2014 . (b) (4) To address this, additional analysis of

study 006 data were conducted by the FDA statistical team to calculate the primary efficacy endpoint as the proportion of patients with at least one evaluable ovarian cancer lesion confirmed by central pathology that was detected with Cytalux under fluorescent light but not under normal light or palpation and not otherwise identified for resection prior to surgery. The detection proportion was estimated in women who underwent both normal light and fluorescent light (Intent-to-Image Set). The detection performance met the pre-specified success threshold of 10%. Based on these results, it was decided against the need for evaluating the FR+ status prior to Cytalux administration. (b) (4)

8.1.4. Assessment of Effectiveness

The goal of ovarian cancer debulking surgery is to achieve as close to R0 [no visible disease] as possible. Overall, the results from Phase 3 study (OTL-006) with support from results of the Phase 2 study (OTL-003) demonstrate that 0.025 mg/ kg dose of Cytalux injection and NIR fluorescent light used as an adjunct during ovarian cancer surgery can enhance the identification of malignant lesions..

The surgeon investigators participating in Study 006 were asked to answer two questions in the post-surgery questionnaire pertaining to completeness of the surgical resection achieved. that aspect. Overall, the responses were favorable. See Table 43 for additional details.

Table 43. Summary of Achieving R0 From Post-Fluorescence Surgical Reports

Characteristic Category/Statistics	Full Analysis Set N=109 n (%)
Did the use of the Fluorescent Camera System achieve more complete debulking (closer to R0)?	
Yes	55 (50.5)
No	44 (40.4)
Not applicable	10 (9.2)
Achieved R0 (no visible disease, regardless of disease) after resection?	
Yes	68 (62.4)
No	23 (21.1)
Not applicable	18 (16.5)

Source: Adapted from Applicant supplied Table 23, 14.2.5
Abbreviations: R0=zero residual disease

8.2. Review of Safety

8.2.1. Safety Review Approach

Safety review evaluation for this NDA included:

A review of the following information submitted by the Applicant -

- Safety data from studies in the OTL Integrated Clinical Study Safety Database. Safety data were collected in the Pivotal Study 006 and supporting Study 003 (in patients with

ovarian cancer) and Study 005 (in patients with Lung cancer) for a combined total of 294 subjects.

8.2.2. Review of the Safety Database

Overall Exposure

Safety findings included data primarily from Study 006 with supporting data from Study 003 in patients with ovarian cancer and Study 005 in patients with lung cancer that all involved a single dose administration of the study drug pafolacianine at 0.025 mg/ kg. These include a total of 194 adult patients with ovarian cancer. The details of these studies are provided in Table 24, Table 25, and Table 26.

Adequacy of the safety database
Collectively, the safety data submitted by the Applicant are adequate to evaluate the safety of the use of pafolacianine as an adjunct optical imaging agent during debulking surgery in patients with ovarian cancer.

8.2.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

The Applicant presents safety data for adult patients with ovarian cancer and lung cancer who participated in studies 006, 003, and 005. Collectively these data provide an overview of adverse events for pafolacianine and allow exploration of patients with ovarian cancer based on age groups, race, ethnicity, BMI, disease stage, and tumor types . Descriptive statistics are used to describe demographic and baseline characteristics.. The Applicant provided case report forms (CRF) and narratives for the deaths in the pivotal trial.

In Study 006, in the 150 patients included in the safety analysis set, 134 were randomized to NIR fluorescent and normal light for analysis. Overall, 132 of these patients (98.5%) received 50% to 100% of the planned dose. Infusions were interrupted in 25 patients (18.7%) and restarted in 21 patients (84.0%). Length of time of exposure to fluorescence imaging ranged from 1 to 41 minutes.

Categorization of Adverse Events

AEs were classified using standard terminology from the verbatim description according to Medical Dictionary for Regulatory Activities (MedDRA) Coding Dictionary (Version 20.1. TEAEs were reported as mild, moderate, severe, life-threatening, or fatal and classified as short-term (within 1 week of surgery), mid-term (within the first 6 weeks) and long-term.

The adverse events could be divided into drug-related events and procedure-related events and were reported at the patient level. The adverse events were tabulated by MedDRA system

organ class and preferred term, by relationship to investigational product and by intensity. Subgroup analyses of adverse events were performed by gender, age group, race, ethnicity, BMI, study sites as specified in Table 44 below.

Table 44. Subgroup Analyses of AEs by Study Population in 006 Through April 16, 2020

Parameter	Details
Gender	Male, female
Age group	Adult patients
	< 65 years
	>65 years
Race	Caucasian, Black, Hispanic, Asian, Other, Unknown, Missing
Study sites	Sites 1 through 14
Weight	Underweight, normal weight, overweight, obese
BMI	UW: <18.5; Nl: 18.5-25; OW: 25.0-30.0; Obese: ≥30.0
Disease stage	Stage I, II, III, IV
Surgery type	Primary debulk., interval debulking, recurrent ovarian cancer, other.
Disease type	Miliary vs non-miliary

Source: Applicant supplied tables ISS 14.1.2 Demographic and Baseline Characteristics.
Abbreviations: AE=adverse event, BMI=body mass index, Nl=normal weight, OW=overweight, UW=underweight

Routine Clinical Tests

All patients underwent general anesthesia for the debulking surgery with routine patient monitoring. Information on vital sign changes and lab results are presented from Study 006 supported by 003 and 005. In addition, to assessing the safety, the following tests were performed at the times specified in the tabular study schedule shown for Study 006 in Table 45 below. The investigators recorded vital signs (heart rate, temperature, blood pressure (BP), respiration rate) immediately prior to and following pafolacianine injection at 15 min, 30 min, 45 min, 1 hr. and 2 hr. Laboratory evaluation included complete blood count and, clinical chemistry panel, pregnancy test, along with PK sampling, CA-125 assay, as applicable. See Table 45 below.

Table 45. Tabular Schedule for Study 006

Study Procedure	Visit 1 Screening (Up to Day -28)	Visit 2 Day of Admission (Day 0) or Day of Surgery (Day 1)	Visit 3 Safety (Day 7 ± 4 days)	Visit 4 [a] Safety (Day 28 ± 4 days) [b]	Follow-up (Month 6)
Informed Consent	x				
Inclusion/Exclusion Criteria Met	x				
Established Diagnosis or High Clinical Suspicion of Primary Ovarian Cancer	x				
Clinical Chemistry [c]	x	x	x		
Urinalysis	x				
CBC with Differential	x	x	x		
Weight	x				
Pregnancy Test [d]	x	x			
Medical History	x	x			
Vital Signs [e]	x	x [f]	x		
Physical Examination [g]	x	x	x		
12-lead ECG	x		x		
Study Drug Administration [h]		x			
PK Sampling [i]		x			
Randomization (Sealed Envelope Opening by the Investigator)		x [j]			
Pre-Fluorescence Surgical Plan based on Normal White Light and Palpation		x			
Surgical Plan based on Near-infrared Fluorescent Light Imaging		x			
Surgery with Associated Procedures Including Intra-Operative Imaging. [k]		x			
Tissue Sample Collection		x			
Investigator Questionnaires		x			
Adverse Event Assessments		x	x	x	
ADE Assessments		x	x	x	
Concomitant Medications	x	x	x	x	
Disease State (CA-125)		x			x [l]

Source: Table 3, in the Applicant supplied CSR OTL-006.
Abbreviations: ADE=adverse device reaction, CA-125=cancer antigen 125, CBC=complete blood count, ECG=electrocardiogram, PK=pharmacokinetics

8.2.4. Safety Results

Table 46. Overall Summary of Treatment-Emergent Adverse Events (Safety Population) Study 003

	n (%) Patients (N=44)
Patients Reporting at Least One TEAE	44 (100.0%)
Patients Reporting at Least One SAE	13 (29.5%)
Patients Reporting at Least One TEAE Leading to Death	0
Maximum Severity [1]	
Mild	6 (13.6%)
Moderate	26 (59.1%)
Severe	12 (27.3%)
Closest Relationship to Study Drug [2]	
Related [3]	8 (18.2%)
Not Related [4]	36 (81.8%)
Closest Relationship to Imaging System [5]	
Related [6]	0
Not Related [7]	44 (100.0%)

Source: Adapted from Table 10; Section 14.3.1 – Table 14.3.1.1, Listing 16.2.7.1
[1] Patients reporting more than one adverse event are counted only once using the highest severity; who received pafolacianine
[2] and [5] Patients reporting more than one adverse event are counted only once using the closest relationship.
[3] and [6] Includes all events reported as "Possible", "Probable", or missing relationship.
[4] and [7] Includes all events reported as "Unlikely", or "Not Related" relationship.
Abbreviations: mITT=modified intent to treat population, SAE=serious adverse event, TEAE=treatment emergent adverse events

Deaths

There were two deaths (Patient numbers: (b) (6)) in the Phase 3 Study 006. Both were reported to be not attributable to the study drug but related to other factors. One patient who was randomized to the fluorescent and normal light imaging group died due to septic shock. The second patient who received the study drug but was not randomly assigned to any imaging group died due to worsening renal dysfunction. Neither death was reported by the investigator to be related to the administration of pafolacianine or to imaging system. See Table 47 for the summary.

Table 47. Details of Two Deaths Encountered in Study 006

Patient Details	Date	Recorded Events
Patient 1, (b) (6)	(b) (6)	65 year old, ovarian cancer, Stage IIIC, comorbidities, concurrent meds
		Pafolacianine administered, primary debulking extensive disease, no surgery, camera not used
		per oral day 17, readmitted with AKI, and associated metabolic acidosis
		Patient stabilized, discharged
		Hospice care
		Patient expired
Patient 2, (b) (6)		60 year old, ovarian cancer, Stage IIIC, primary debulking surgery, pafolacianine administered, (b) (4) camera used
		Readmitted with hypoalbuminemia; (b) (6) abdominal pain
		Admitted to ICU with hypotension
		Transferred to hospice care
		Patient expired

Source: Applicant supplied information ISS 14.3.3.1 Narratives of Deaths submitted in the NDA package.
Abbreviations: AKI=acute kidney injury, ICU=intensive care unit

Serious Adverse Events

No drug related serious AE was reported for the safety population. See Table 48 below.
Significant Adverse Events: None reported

Treatment Emergent Adverse Events and Adverse Reactions

Table 48 below summarizes the TEAEs for the entire Safety Population of patients with ovarian and Lung cancer (N=294) and the results show consistency between the two tumor types.

Table 48. Overall Summary of Treatment-Emergent Adverse Events - Safety Population

Subjects With at Least One TEAE in Category	Combined Ovarian Cancer N=194 n (%)	Combined Ovarian and Lung Cancer N=294 n (%)
Any TEAE	176 (90.7)	270 (91.8)
Any drug related TEAE	53 (27.3)	73 (24.8)
Any severe TEAE	41 (21.1)	70 (23.8)
Any TEAE leading to drug withdrawal	5 (2.6)	11 (3.7)
Any TEAE leading to death	2 (1.0)	2 (0.7)
Any serious TEAE	35 (18.0)	49 (16.7)
Any drug related serious TEAE	0	0
Any adverse device effect	0	0

Source: Applicant supplied ISS- Appendix-1 Table 14.3.1.1.1
The safety analysis set includes all subjects who received any amount of study drug.
A drug related TEAE is a TEAE with a relationship to study drug of possibly, probably, or definitely related as determined by the principal investigator.
The denominator for percentages is the number of subjects in the safety analysis set for each subgroup.
Abbreviations: TEAE=treatment-emergent adverse event

Table 49 below summarizes the treatment-emergent adverse events (TEAEs) for the entire Safety Population of patients with ovarian and lung cancer (N = 294) and the patients with

ovarian cancer only. The results show consistency between the two tumor types and no significant differences between the two broader age groups of <65 and ≥65 years.

Table 49. Overall Summary of Treatment-Emergent Adverse Events for Subgroup Age - Safety Population

	Combined Ovarian Cancer N=194 n (%)		Ovarian and Lung Cancer N=294 n (%)	
Subjects With at Least One TEAE in Category	**<65 N=116**	**≥65 N=78**	**<65 N=144**	**≥65 N=150**
Any TEAE	107 (92.2)	69 (88.5)	135 (93.8)	135 (90.0)
Any drug related TEAE	38 (32.8)	15 (19.2)	40 (30.6)	29 (19.3)
Any severe TEAE	19 (16.4)	22 (28.2)	26 (18.1)	44 (29.3)
Any TEAE leading to drug withdrawal	4 (3.4)	1 (1.3)	7 (4.9)	4 (2.7)
Any TEAE leading to death	1 (0.9)	1 (1.3)	1 (0.7)	1 (0.7)
Any serious TEAE	18 (15.5)	17 (21.8)	20 (13.9)	29 (19.3)
Any drug related serious TEAE	13 (11.2)	13 (16.7)	22 (15.3)	28 (18.7)
Any adverse device effect	0	0	0	0

Source: Applicant supplied ISS- Appendix-1 Table 14.3.1.1.2
The safety analysis set includes all subjects who received any amount of study drug.
A drug related TEAE is a TEAE with a relationship to study drug of possibly, probably, or definitely related as determined by the Principal Investigator.
The denominator for percentages is the number of subjects in the Safety Analysis Set for each subgroup
Abbreviations: TEAE=treatment-emergent adverse event

Table 50 below summarizes the TEAEs for the entire Safety Population of patients with ovarian and lung cancer (N=294) and patients with ovarian cancer only. The results show consistency between the two groups. If the patients are classified into two groups, 'normal' and 'higher' BMI groups, there appears to be a trend for numerically higher incidence of drug-related adverse events in the higher BMI groups.

Table 50. Overall Summary of Treatment-Emergent Adverse Events for Subgroup BMI Safety Analysis Set

	Combined Ovarian Cancer N=194				Combined Ovarian and Lung Cancer N=294			
Subjects With at Least One TEAE in Category	**UW**	**Normal N=66 n (%)**	**OW N=48 n (%)**	**Obese N=54 n (%)**	**UW N=2 n (%)**	**Normal N=101 n (%)**	**OW N=84 n (%)**	**Obese N=75 n (%)**
Any TEAE	0	60 (90.9)	43 (89.6)	49 (90.7)	1 (50.0)	94 (93.1)	76 (90.5)	49 (92.6)
Any drug related TEAE	0	12 (18.2)	11 (22.9)	21 (38.9)	0	16 (15.8)	18 (21.4)	30 (37.0)
Any severe TEAE	0	13 (19.7)	12 (25.0)	14 (25.9)	1 (50.0)	22 (21.8)	22 (26.2)	23 (28.4)
Any TEAE leading to drug withdrawal	0	0	1 (2.1)	3 (5.6)	0	0	3 (3.6)	7 (8.6)
Any TEAE leading to death	0	2 (3.0)	0	0	0	2 (2.0)	0	0
Any serious TEAE	0	11 (16.7)	10 (20.8)	11 (20.4)	15 (14.9)	16 (19.0)	14 (17.3)	3 (11.5)
Any drug related serious TEAE	0	0	0	0	0	0	0	0
Any adverse device effect	0	0	0	0	0	0	0	0

Source: Adapted from Applicant supplied ISS- Appendix-1 Tables 14.3.1.1.5 and 14.3.6.2.
The safety analysis set includes all subjects who received any amount (range 0.1 – 3.8 mg) of pafolacianine.
A drug related TEAE is a TEAE with a relationship to study drug of possibly, probably, or definitely related as determined by the Principal Investigator.
The denominator for percentages is the number of subjects in the safety analysis set for each subgroup.
Abbreviations: BMI=body mass index, mg=milligram, OW=overweight, TEAE=treatment-emergent adverse event, UW=underweight

Table 51 below summarizes the incidence of drug related adverse reactions with an incidence of over 1% in the safety analysis set.

Table 51. Treatment Emergent Adverse Events – Incidence >1%, Safety Analysis Set

Drug Related Adverse Reaction	N=294 n (%)
Nausea	44 (15.0)
Vomiting	17 (5.8)
Abdominal pain	8 (2.7)
Infusion related reactions (includes injection site)	7 (2.4)

Source: Adapted from Applicant supplied ISS- Appendix-1 Multiple Tables 14.3.1.1.1 and 14.3.6.2
The Safety Analysis Set includes all subjects who received any amount (range=0.1 to 3.8 mg) of pafolacianine.
A drug related TEAE is a TEAE with a relationship to study drug of possibly, probably, or definitely related as determined by the Principal Investigator.

Dropouts and/or Discontinuations Due to Adverse Effects

There were a total of 6 instances of TEAEs in 4 patients who discontinued or dropped out due to adverse effects. See Table 52 below.

Table 52. Listing of Adverse Events That Lead to Study Drug Withdrawal - Study 006

Subject ID	MedDRA Organ Class Preferred Term Verbatim Term	Start Date/ End Date	Study Day/ Start End	TEAE/ SAE
(b) (6)	GI disorders Vomiting	(b) (6)	1/1	Y/N
	GI disorders Nausea		1/3	Y/N
	Immune system disorder Hypersensitivity		1/2	Y/N
	GI disorders Nausea		1/1	Y/N
	GI disorders Vomiting		1/1	Y/N
	Skin & subcutaneous tissue disorders Rash		1/1	Y/N

Source: Adapted from Applicant supplied ISS- Appendix-1 Tables 14.3.6.2 and 16.2.7.3.
The safety analysis set includes all subjects who received any amount range (0.1-3.8 mg) of pafolacianine.
A drug related TEAE is a TEAE with a relationship to study drug of possibly, probably, or definitely related as determined by the Principal Investigator.
Abbreviations: GI=gastrointestinal, MedDRA=Medical Dictionary for Regulatory Activities, mg=milligram, SAE=serious adverse event, TEAE=treatment-emergent adverse event

Laboratory Findings

The following laboratory tests were performed at the local labs for each participating subject at baseline and on Day 7- Complete Blood Count (CBC), Hematocrit (Hct), Basic Metabolic Panel

(BMP), Liver Function Tests (LFTs) for the exposed population. Safety analysis set (N=294) included combined patients with ovarian cancer from two studies (Study 006 and Study 003)-N=194; and patients with lung cancer from Study 005 – (N=100). A review of Tables 14.3.4.1 – 4 in the Integrated Summary of Safety submitted by the Applicant as part of the NDA, did not show any significant changes from the baseline values of the above indicated laboratory tests.

Vital Signs

Vital signs were monitored during the pafolacianine infusion in all three studies of the safety analysis. Vital signs were measured at Visit 1, Visit 2, and Visit 3 in each study. On the day of study drug infusion, vital signs were measured at baseline and at 15, 30, 45, 60 minutes after the start of the infusion followed by measurements at 1hour and 2 hours at the end of the infusion, and on Day 7.

Table 53 and Table 54 below summarize the pulse rate and systolic blood pressure at multiple time points beginning at baseline and during and after the infusion of pafolacianine. There were no significant shifts from the baseline values.

Table 53. Vital Signs Summary Statistic and Change From Baseline – Pulse Rate

Parameter	Visit	Assessment Time	Statistic	Combined Ovarian Cancer N=194	Combined Ovarian and Lung Cancer N=294
Pulse rate (beats/min)	Baseline	Baseline	n	194	294
			Mean (SD)	78.27 (12.834)	76.14 (13.096)
			Median	77.50	75.00
			Min, max	52.00, 111.00	43, 121.00
Pulse rate (beats/min)	15 minute post infusion start	Visit	n	184	268
			Mean (SD)	77.74 (13.180)	74.68 (13.343)
			Median	76.00	72.00
			Min, max	51, 125.00	41.00, 125.00
		Visit change from BL	n	184	268
			Mean (SD)	-0.67 (8.829)	-1.29 (8.843)
			Median	-1.00	-1.00
			Min, max	-33.00, 37.00	-64.00, 37.00
Pulse rate (beats/min)	30 minute post infusion start	Visit	n	182	260
			Mean (SD)	77.03 (12.97)	74.25 (12.88)
			Median	77.00	73.00
			Min, max	50.00, 121.00	47.00, 121.00
		Visit change from BL	n	182	260
			Mean (SD)	-1.36 (8.485)	-1.70 (8.652)
			Median	-1.00	-1.50
			Min, max	-34.00, 30.00	-65.00, 30.00
Pulse rate (beats/min)	1 hour post infusion end	Visit	n	169	242
			Mean (SD)	76.51 (12.760)	73.70 (12.897)
			Median	76.00	72.00
			Min, max	42.00, 112.00	42.00, 120.00
		Visit change from BL	n	169	242
			Mean (SD)	-1.59 (8.484)	-1.89 (8.834)
			Median	-2.00	-2.00
			Min, max	-34.00, 42.00	-65.00, 42.00
Pulse rate (beats/min)	2 hour post infusion end	Visit	n	57	74
			Mean (SD)	80.95 (12.597)	77.61 (13.163)
			Median	81.00	76.5
			Min, max	58.00, 111.00	52.00, 111.00
		Visit change from BL	n	57	74
			Mean (SD)	1.12 (10.471)	0.49 (10.073)
			Median	1.00	0.00
			Min, max	-24.00, 29.00	-24.00, 29.00

Source: Applicant supplied Table 14.3.5.3 of the Integrated Summary of Safety (ISS) submitted with NDA 214907.
Abbreviations: BL=baseline, SD=standard deviation

Table 54. Vital Signs Summary Statistics and Change From Baseline – Systolic BP

Parameter	Visit	Assessment Time	Statistic	Combined Ovarian Cancer N=194	Combined Ovarian and Lung Cancer N=294
Syst. BP (mm Hg)	Baseline	Baseline	n	194	294
			Mean (SD)	126.99 (17.241)	131.35 (20.889)
			Median	126.00	130.00
			Min, max	86.00, 174.00	10.00, 192.00
Syst. BP (mm Hg)	15 minute post infusion start	Visit	n	184	272
			Mean (SD)	125.99 (16.829)	129.34 (17.363)
			Median	124.00	129.50
			Min, max	85.00, 179.00	85.00, 179.00
		Visit change from BL	n	188	272
			Mean (SD)	-1.14 (12.107)	-2.06 (13.082)
			Median	-0.50	-1.00
			Min, max	-39.00, 40.00	-48.00, 40.00
Syst. BP (mm Hg)	30 minute post infusion start	Visit	n	185	263
			Mean (SD)	123.32 (17.085)	126.74 (18.098)
			Median	122.00	126.00
			Min, max	90.00, 174.00	90.00, 190.00
		Visit change from BL	n	185	263
			Mean (SD)	-3.99 (14.193)	-4.76 (15.485)
			Median	-4.00	-4.00
			Min, max	-49.00, 44.00	-69.00, 44.00
Syst. BP (mm Hg)	1 hour post infusion end	Visit	n	114	145
			Mean (SD)	121.48 (18.499)	125.39 (19.963)
			Median	119.00	124.00
			Min, max	75.00, 164.00	75.00, 166.00
		Visit change from BL	n	114	145
			Mean (SD)	-5.40 (17.577)	-4.82 (16.880)
			Median	-4.00	-4.00
			Min, max	55.00, 45.00	55.00, 45.00
Syst. BP (mm Hg)	2 hour post infusion end	Visit	n	74	92
			Mean (SD)	118.01 (16.839)	122.27 (19.288)
			Median	118.50	121.00
			Min, max	87.00, 176.00	87.00, 176.00
		Visit change from BL	n	74	92
			Mean (SD)	-8.43 (20.338)	-5.83 (22.689)
			Median	-6.00	-5.00
			Min, max	-60.00, 38.00	-60.00, 99.00

Source: Applicant supplied Table 14.3.5.3 of the Integrated Summary of Safety (ISS) submitted with NDA 214907.
Abbreviations: BL=baseline, BP=blood pressure, Hg=mercury, SD=standard deviation

Overall, while TEAEs related to vital signs were reported, according to the Applicant, none of them were deemed to be related to pafolacianine administration.

Electrocardiograms (ECGs)

Electrocardiograms were performed at Visit 1 and Visit 3 in Study 006. Of the 111 patients for whom data were available, 71/111 (64%) had normal ECGs at baseline; 40/11 (36%) had

abnormal ECG that were considered clinically non-significant at baseline. No patient had an abnormal ECG that was clinically significant. At Day 7, five patients (4.5%) had an abnormal ECG that were considered clinically significant; 3 of them (2.7%) had a normal ECG at baseline and 2 (1.8%) had abnormal ECG that was considered not clinically significant at baseline. Eighteen patients (16.2%) with a normal ECG at baseline had a Day 7 ECG, that was abnormal but not clinically significant, while 16 patients (14.4%) who had an abnormal but not clinically significant ECG at baseline had a normal ECG on the Day 7 visit. Overall, 72 patients (64.8%) had no ECG change from baseline on the Day 7 visit. See Table 55 below for detailed summary of ECG analysis.

Table 55. ECG Parameters - Changes From Baseline

Parameter	Visit	Assessment Time	Statistic	Combined Ovarian Cancer N=194
PR interval	Baseline	Baseline	n	188
			Mean (SD)	154.41 (28.350)
			Median	152.00
			Min, max	99.00, 352.00
	Day 7	Visit	n	112
			Mean (SD)	148.13 (19.175)
			Median	148.00
			Min, max	108.00, 204.00
		Visit change from BL	n	109
			Mean (SD)	-7.97 (17.169)
			Median	-6.00
			Min, max	-75.00, 24.00
QT interval (msec)	Baseline	Visit	n	189
			Mean (SD)	390.95 (34.414)
			Median	390.00
			Min, max	272.00, 488.00
	Day 7	Visit change from BL	n	114
			Mean (SD)	374.34 (38.019)
			Median	382.00
			Min, max	256.00, 450.00
QTc interval (msec)	Baseline	Visit	n	147
			Mean (SD)	432.12 (25.761)
			Median	432.00
			Min, max	368.00, 503.00
		Visit change from BL	n	109
			Mean (SD)	0.16 (26.393)
			Median	5.0
			Min, max	-77.00, 93.00
QRS duration (msec)	Baseline	Baseline	n	189
			Mean (SD)	84.52 (14.896)
			Median	84
			Min, max	0.00, 160.00
	Day 7	Visit	n	114
			Mean (SD)	86.27 (13.122)
			Median	84.50
			Min, max	64.00, 160.00
		Visit change from BL	n	111
			Mean (SD)	2.04 (7.636)
			Median	0.00
			Min, max	-12.00, 38.00

Source: Applicant supplied Table 14.3.5.3 of the Integrated Summary of Safety (ISS) submitted with NDA 214907.
Abbreviations: BL=baseline, ECG=electrocardiogram, PR=P-R waves in ECG, QRS=QRS wave in ECG, QT=Q -T waves, QTc=corrected QT interval, SD=standard deviation

QT

No patient had a QTc > 500 msec. One subject (b) (6) in Study 006 had a QTc of 500 msec on the Day 7 visit that was different from the baseline QTc of 420 msec – this was considered abnormal but not clinically significant.

Immunogenicity

Not applicable to pafolacianine.

8.2.5. Analysis of Submission-Specific Safety Issues

Gastrointestinal (GI) Reactions

GI reactions such as nausea and vomiting were the most common AEs observed in patients who were exposed to pafolacianine. Patients with higher BMI had a numerically higher incidence of these AEs resulting in the interruption of study drug infusion. A majority of patients in this group received pre-medication with anti-emetics or anti- histamines. These observations prompted the recommendation in Section 2 (Dosage and Administration) and Section 5 (Warnings and Precautions) of the PI (prescribing information) for pretreatment of patients with antihistamines and/or anti-nausea medications prevent/mitigate the occurrence of GI related AEs.

8.2.6. Safety Analyses by Demographic and Other Baseline Characteristics

Table 56 and Table 57 summarize the prevalence of TEAEs by sub-groups (age, BMI) in the safety population (N=294). No differences by age are noted. Numerically higher treatment-related adverse events were noted in patients with higher BMIs.

Table 56. Any TEAE, Serious TEAE, Related TEAE and AESI, by Subgroup – Age

Characteristic Category/Statistic	Combined Ovarian Cancer N=194 n (%)	Combined Ovarian and Lung Cancer N=294 n (%)
Age group		
<65 years	116 (59.8)	144 (49.0)
≥65 years	78 (40.2)	150 (51.0)
Subjects with at least one TEAE		
<65	107 (92.2)	135 (93.8)
≥65	69 (88.5)	135 (90.0)
Total number of TEAE		
<65	551	679
≥65	423	887
Subject with at least one serious TEAE		
<65	18 (15.5)	20 (13.9)
≥65	17 (21.8)	29 (19.3)
Subjects with at least one AESI		
<65	22 (19.0)	28 (19.4)
≥65	11 (14.1)	23 (15.3)
Subjects with at least one related TEAE[1]		
<65	38 (32.8)	44 (30.6)
≥65	15 (19.2)	29 (19.3)

Source: Adapted from Applicant submitted Integrated Summary of Safety, Tables- 14.3.2.1.3, 14.3.2.2.1, 14.2.3.2.3.1, and 14.3.2.5.1.
[1] Related to pafolacianine.
Abbreviations: AESI=adverse effects of special interest, TEAE=treatment-emergent adverse effect

Table 57. Any TEAE, Serious TEAE, Related TEAE and AESI, by Subgroup – BMI

Characteristic Category/Statistic	N	Combined Ovarian Cancer N=194 n(%)	N	Combined Ovarian and Lung Cancer N=294 n(%)
BMI (kg/m^2)				
n		168		268
Mean (SD)		27.37 (5.532)		27.37 (5.46)
Median		26.8		26.6
Min. max		18.8, 50.7		16.8, 50.7
Subjects with at least one TEAE				
UW	0	0 (0.0)	2	1 (50.0)
Normal	66	60 (90.9)	101	94 (93.1)
OW	48	43 (89.6)	84	76 (90.5)
Obese	54	49 (90.7)	81	75 (92.6)
Missing	26	24 (92.3)	26	24 (92.3)
Subjects with at least one serious TEAE				
UW	0	0 (0.0)	2	1 (50.0)
Normal	66	11 (16.7)	101	15 (14.9)
OW	48	10 (20.8)	84	16 (19.0)
Obese	54	11 (20.4)	81	14 (17.3)
Missing	26	3 (11.5)	26	3 (11.5)

Characteristic Category/Statistic	N	Combined Ovarian Cancer N=194 n(%)	N	Combined Ovarian and Lung Cancer N=294 n(%)
Subjects with at least one related TEAE[1]				
UW		0		2
Normal	66	12 (18.2)	101	16 (15.8)
OW	48	11 (22.9)	84	18 (21.4)
Obese	54	21 (38.9)	81	30 (37.0)
Missing	26	9 (34.6)	26	9 (34.6)
Subjects with at least one AESI				
UW	0	0	2	0
Normal	66	7 (10.6)	101	10 (9.9)
OW	48	5 (10.4)	84	12 (14.3)
Obese	54	15 (27.8)	81	23 (28.4)
Missing	26	6 (23.1)	26	6 (23.1)

Source: Adapted from Applicant submitted Integrated Summary of Safety, Tables- 14.3.2.1.3, 14.3.2.2.1, 14.2.3.2.3.1 and 14.3.2.5.1.
[1] Related to pafolacianine
Abbreviations: AESI=adverse effects of special interest, BMI=body mass index, OW=overweight, TEAE=treatment emergent adverse effect, UW=underweight

8.2.7. Specific Safety Studies/Clinical Trials

The Applicant provided report of the safety from Study 006 with supporting data from Study 003 and Study 005. No serious drug-related adverse reactions were reported during the course of these studies. Most of the reported AEs were of mild to moderate severity.

8.2.8. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

There are no human data available to evaluate for risk for drug-associated carcinogenicity or tumor development and none are needed.

Human Reproduction and Pregnancy

There are no human data available to evaluate for a drug-associated risk of major birth defects, miscarriage, or other adverse maternal or fetal outcomes.

Pediatrics and Assessment of Effects on Growth

These data are not applicable. Following a request from the Applicant for IND118215, the FDA granted a waiver for pediatric studies on January 31, 2020. See discussion under Section 10 Pediatrics.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Pafolacianine is a single use drug given prior to surgery in patients with ovarian cancer. The risks for drug abuse potential and withdrawal do not apply.

8.2.9. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

Cytalux is currently not marketed.

Expectations on Safety in the Postmarket Setting

No specific safety signals are noted in the review of the clinical studies that warrant further investigation.

8.2.10. Integrated Assessment of Safety

Review of the safety data submitted by the Applicant indicates pafolacianine has an acceptable safety profile when used in the prescribed manner in adult patients with ovarian cancer. The integrated analyses of safety data were conducted using the integrated Safety Analysis Set (SAS), defined as all patients exposed to pafolacianine sodium injection in the following pooled datasets-

- Ovarian Combined 003 and 006)
- Ovarian and Lung Combined (003, 006, and 005).

Aspects of the safety of pafolacianine sodium injection in the pooled datasets were assessed by extent of exposure, the occurrence of TEAEs, serious TEAEs (including death), clinical laboratory results, vital sign measurements, ECG results, and adverse device effects (ADE).

The following observations were made on the safety of pafolacianine injection in these studies:

- There were 13 patients (4.4%) who received less than 85% of the planned dose. Overall, a total of 41 patients (13.9%) had an infusion interruption. The infusion interruptions were due to AEs in 32 of the 41 patients (78%).
- Overall, a total of 1566 TEAEs were reported in 270 patients (91.8%). Of these, 73 patients (24.8%%) reported 136 TEAEs considered related to the administration of the pafolacianine sodium Injection; 76 SAEs were reported in 49 patients (16.7%) and none were considered related to the administration of the pafolacianine sodium injection.
- Two deaths occurred in the clinical program (1 due to septic shock and 1 due to worsening of renal impairment). Neither of these deaths or SAEs leading up to the death were considered related to the administration of the pafolacianine sodium or the use of the (b) (4) in the opinion of the Investigator.
- Two of the 294 patients exposed to study drug were withdrawn from the study due to TEAEs of vomiting (1 mild event and 1 moderate intensity event). Patient (b) (6) experienced vomiting of mild intensity after receiving 0.19 mg of OTL38 5 minutes into the IV infusion and Patient (b) (6) experienced vomiting of moderate intensity after receiving 0.117 mg of OTL38 4 minutes into the IV infusion. Neither of the patients'

infusions were restarted and both events were considered resolved on the day of their withdrawal. These events were not considered related to the use of the [redacted] (b) (4)

- Nausea (31 patients [10.5%]), vomiting (16 patients [5.4%]) were the most frequently reported Adverse Events of Special Interest (AESIs) and were similar to the most frequently reported overall TEAEs.

Adverse Events of Special Interest (AESI):

Overall, 35 AESIs were reported by 24 patients (16%). AESIs are infusion reactions that met one of following 4 criteria: (1) AEs that resulted in interruption of study drug infusion in the study drug administration data; (2) AEs that resulted in study drug interruption or study drug withdrawal in the AE data; (3) AEs that started during the study drug infusion as per the recoded start time (AEs that had the same start time as the study drug infusion were not counted); (4) AEs without a recorded start time that had both a start date and stop date of Study Day 1 and were indicated as drug related. The occurrences of these AESIs were in general similar to the most frequently reported TEAEs. See Table 58 below.

Table 58. Adverse Events of Special Interest (AESI)

Patients with at least One TEAE in Category Preferred Term	Fluorescent and Normal Light Normal Light (N=134)	Normal Light Only (N=6)	Not Randomized (N=10)	Overall (N=150)
Patients with ≥ 1 AESI	21 (15.7%)	0	3 (30.0%)	24 (16.0%)
Nausea	12 (9.0%)	0	2 (20.0%)	14 (9.3%)
Vomiting	4 (3.0%)	0	3 (30.0%)	7 (4.7%)
Abdominal pain	2 (1.5%)	0	0	2 (1.3%)
Pruritus	1 (0.7%)	0	0	1 (0.7%)
Flushing	1 (0.7%)	0	0	1 (0.7%)
Oral dysaesthesia	1 (0.7%)	0	0	1 (0.7%)
Throat irritation	1 (0.7%)	0	0	1 (0.7%)
Upper airway cough syndrome	1 (0.7%)	0	0	1 (0.7%)
Feeling hot	1 (0.7%)	0	0	1 (0.7%)
Anxiety	1 (0.7%)	0	0	1 (0.7%)
Hypersensitivity	1 (0.7%)	0	0	1 (0.7%)
Dyspepsia	1 (0.7%)	0	0	1 (0.7%)
Paraesthesia oral	1 (0.7%)	0	0	1 (0.7%)
Hot flush	1 (0.7%)	0	0	1 (0.7%)
Rash	0	0	1 (10.0%)	1 (0.7%)

Source: Sponsor supplied Table 32, OTL-006 CSR in the NDA.
Abbreviations: AESI=adverse event of special interest, TEAE=treatment emergent adverse event

In general, no trend was established for the observation of abnormal findings in the safety laboratory assessments (hematology and clinical chemistry), ECGs, or physical examinations. Similarly, no AEs were attributed to the use of NIR imaging systems- [redacted] (b) (4) [redacted] (b) (4) ®. Overall, 37 SAEs were reported for 22 patients (14.7%) Table 59; however, in the opinion of the Investigator, none were considered related to the administration of the pafolacianine injection or the use of either the [redacted] (b) (4) [redacted] (b) (4)

Table 59. Serious Treatment-Emergent Adverse Events (Safety Analysis Set)

MedDRA SOC Preferred Term	FL and NL N=134 n(%)	NL Only N=6 n(%)	Not Randomized N=10 n(%)	Overall N=150 n (%)
Patients with at least one serious TEAE	18 (13.4)	1 (16.7)	3 (30.0)	22 (14.7)
Total number of serious TEAEs	31	1	5	37

Source: Adapted from Table 31 of Study 006 CSR in the submitted NDA Page 89/528.
Abbreviations: FL=fluorescent light, MedDRA=Medical Dictionary for Regulatory Activities, NL=normal light, SOC=system organ class, TEAE=treatment emergent adverse event

Overall, the safety data show that a single dose of 0.025 mg/ kg pafolacianine sodium injection has an acceptable safety profile when used as an adjunct during debulking surgery in adult patients with ovarian cancer.

8.3. Statistical Issues

A total of 134 women were evaluated under both normal light and NIR fluorescent light imaging. Twenty five women did not have central pathology confirmation for at least one ovarian cancer lesion detected under normal light or NIR fluorescent light imaging. (b) (4)

In the Applicant's original efficacy analysis, these 25 women were excluded from the FAS dataset, see Section 8.1. Given that these 25 women could not be confirmed to have ovarian cancer, they were considered 'not detected' for the purpose or primary efficacy endpoint analysis. Thus, the detection proportion should be estimated in women who underwent both normal light and NIR fluorescent light visualization (Intent-to-Image Set), i.e., n=134, see Table 60.

Table 60. Detection Proportion With Cytalux Under Fluorescent Light but Not Under Normal Light or Palpation

	N=134
Women with at least one confirmed ovarian cancer lesion	
Number (n)	36
Proportion (%)	0.269 (26.9%)
95% CI (proportion)[1]	[0.196, 0.352][2]

Source: FDA Statistical Reviewer's Analysis Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
[2] The lower bound of the 95% confidence interval based on exact binomial exceeds the prespecified proportion of 0.10.
Abbreviations: CI=confidence interval

The primary efficacy endpoint met its pre-specified threshold of 0.1. That is, the lower bound of the 95% CI for detection rate is above 0.1.

FDA statistical reviewer performed an exploratory analysis of the patient-level false positive rate (FPRp), i.e., percent of women with all lesions detected by OTL38 and NIR fluorescent light only are histologically negative based on all 134 imaged women. The FPRp was 20.2% (95% CI,

[13.7%, 28.0%]). The upper bound of FPRp was greater than the pre-defined Not-to-Exceed Threshold of 17%. Therefore, the FPR at patient level failed to meet the pre-specified criterion.

Table 61. Patient-Level False Positive Rate With Cytalux Under Fluorescent Light but Not Under Normal Light or Palpation

	N=134
Women with all her lesions detected by OTL38 and NIR fluorescent light only are histologically negative	
Number (n)	27
Proportion (%)	0.202 (20.2%)
95% CI (proportion)[1, 2]	[0.137, 0.280]

Source: FDA Statistical Reviewer's Analysis Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
[2] The upper bound of the 95% confidence interval based on exact binomial exceeds the prespecified proportion of 0.17.
Abbreviations: CI=confidence interval, NIR=near infrared

Table 62.Lesion-Level True Positive Rate and Lesion-Level False Positive Rate for OTL38 Combined With Fluorescent Light[1]

	Number of Lesions
Number of lesions identified as fluorescent light positive, but negative for ovarian cancer by central pathology (FP)	219
Number of lesions identified as fluorescent light positive and confirmed positive for ovarian cancer by central pathology (TP)	397
Number of lesions identified that were fluorescent light negative, but confirmed to be ovarian cancer by central pathology (FN)	136
Lesion level true positive rate (95% CI)[2]	74.5% [70.6%, 78.1%]
Lesion level false positive rate (95% CI)[3]	35.6% [31.8%, 39.5%]

Source: FDA Statistical Reviewer's Analysis Results.
Notes: Lesion Level True Positive Rate was the ratio TP/(TP+FN). Lesion Level False Positive Rate was the ratio FP/(TP+FP).
[1] The Sponsor reported lesion level data based on 109 women.
[2] 95% CIs are calculated using the exact Clopper-Pearson method.
[3] 95% CIs are calculated using the exact Clopper-Pearson method.
Abbreviations: CI=confidence interval, FN=false negative, FP=false positive, TP=true positive

From the lesion level analysis, it appeared that the lower bound of the 95% confidence interval for lesion-level true positive rate was 70.6%. The upper bound of the 95% confidence interval for lesion-level false positive rate was 39.5%

Subgroup analysis

FDA statistical reviewer performed subgroup analyses of the detection rates with subgroups derived using all imaged women. The results of these subgroup analyses are presented in Table 63 to Table 67. The subgroup analysis results appear to be consistent with that reported for the primary efficacy analysis in Table 60 (Section 8.3).

Table 63. Primary Endpoint by Subgroup Age

Assessment Statistic	<65 years N=87	≥65 years N=47
Patients with at least 1 confirmed ovarian cancer evaluable lesion		
Number (n)	24	12
Proportion (%)	0.276 (27.6)	0.255 (25.5)
95% CI (proportion)[1]	[0.185, 0.382]	[0.140, 0.404]

Source: FDA Statistical Reviewer's Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
Abbreviations: CI=confidence interval

Table 64. Primary Endpoint By Subgroup Race

Assessment Statistic	American Indian/ Alaska Native N=4	Asian N=5	Black or African American N=7	White N=114	Other N=4
Patients with at least 1 confirmed ovarian cancer evaluable lesion					
Number (n)	1	1	1	32	1
Proportion (%)	0.25 (25)	0.2 (20)	0.143 (14.3)	0.281 (28.1)	0.25 (25)
95% CI (Proportion)[1]	[0.006, 0.806]	[0.005, 0.716]	[0.004, 0.579]	[0.201, 0.373]	[0.006, 0.806]

Source: FDA Statistical Reviewer Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
Abbreviations: CI=confidence interval

Table 65. Primary Endpoint By Subgroup Surgery Type

Assessment Statistic	Primary Cytoreduction Surgery N=46	Interval Debulking Surgery N=69	Recurrent Ovarian Cancer Surgery N=2	Other N=17
Patients with at least 1 confirmed ovarian cancer evaluable lesion				
Number (n)	7	23	1	5
Proportion (%)	0.152 (15.2)	0.333 (33.3)	0.5 (50)	0.294(29.4)
95% CI (proportion)[1]	[0.063, 0.289]	[0.224, 0.457]	[0.013, 0.987]	[0.103, 0.560]

Source: FDA Statistical Reviewer Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
Abbreviations: CI=confidence interval

Table 66. Primary Endpoint By Subgroup Stage of Cancer

Assessment Statistic	Stage I N=7	Stage II N=6	Stage III N=70	Stage IV N=20	Unknown N=31
Patients with at least 1 confirmed ovarian cancer evaluable lesion					
Number (n)	1	2	23	5	5
Proportion (%)	0.143 (14.3)	0.333 (33.3)	0.329 (32.9)	0.25 (25.0)	0.161 (16.1)
95% CI (proportion)[1]	[0.004, 0.579]	[0.043, 0.777]	[0.221, 0.451]	[0.087, 0.491]	[0.055, 0.337]

Source: FDA Statistical Reviewer Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
Abbreviations: CI=confidence interval

Table 67. Primary Endpoint by the Presence of Miliary vs. Non-Miliary Disease

Assessment Statistic	Miliary Disease N=13	Non-miliary Disease N=121
Patients with at least 1 confirmed ovarian cancer evaluable lesion		
Number (n)	5	31
Proportion (%)	0.385 (38.5%)	0.256 (25.6%)
95% CI (proportion)[1]	[0.139, 0.684]	[0.181, 0.344]

Source: FDA Statistical Reviewer Results
[1] 95% CIs are calculated using the exact Clopper-Pearson method
Abbreviations: CI=confidence interval

8.4. Conclusions and Recommendations

We recommend approval of this New Drug Application (NDA 214907) for the use of Cytalux as an adjunct for intraoperative identification of malignant lesion in adult patients with ovarian cancer. Our recommendation is based on the review of the Applicant's pharmacokinetic, clinical safety and efficacy data on the usage of pafolacianine in the referenced clinical trials - Phase 3 trial (Study 006) with supporting evidence from a Phase 2 trial (Study 003). The data presented were reviewed in the context of the need for the adjunct role of FGS in debulking surgery for ovarian cancer – a serious disease with no effective early detection methods and the largely unmet medical need for more effective management approaches. Study 006 assessed the proportion of patients with at least one evaluable ovarian cancer lesion confirmed by central pathology that was detected with Cytalux under fluorescent light but not under normal light or palpation and not otherwise identified for resection prior to surgery. The results show that the lesion detection performance met the pre-specified success threshold of 0.10 in the whole patient group as well as in all the sub-groups of the study population of Study 006.

Due to predominantly late presentations of ovarian cancer, the primary approach in its management remains cytoreduction with debulking surgery which aims to achieve better survival. Intraoperative fluorescent image guidance while improving the efficacy of SOC debulking surgery may also reduce the risk associated with the potential removal of normal vital tissue.

The selection of a 0.025 mg/kg dose for Cytalux was based on the Applicant's early phase and PK studies. The absence of severe treatment-emergent adverse event (TEAE) in the safety population provides overall confidence for the safe use of Cytalux at this dose level.

In Study 006, the Applicant used two imaging devices which excite at 760 nm to 785 nm and detect emission at 794 nm to 796 nm – 1) (b) (4) (FDA reference 510K (b) (4) and 2) (b) (4) (FDA reference 510K (b) (4) Both these devices are currently under review for 510 (k) clearance and it is expected that (b) (4) (b) (4) will obtain clearance from the FDA for use with Cytalux. The following text was included in Section 2 (Dosage and Administration) of the PI to inform the users that Cytalux can be used only with FDA cleared imaging devices –

- Cytalux is to be used with an imaging system cleared by the FDA for specific use with Cytalux
- Cytalux should only be used by surgeons who have completed a training program on use of imaging systems for fluorescence imaging during surgery. Training is provided by the device manufacturer.

(b) (4)

(b) (4) Based on this analysis, it was deemed that the PI should indicate the use Cytalux in *"adult patients with ovarian cancer*" without the prerequisite for knowing the FR status. The use of Cytalux infusion could lead to the removal of tissue that fluoresces under NIR imaging but is non-cancerous on histopathological examination (False Negative lesions). Therefore, to address the potential for False Negatives as well as the reported false positive rate in lesion identification with Cytalux, the following cautionary text regarding the risk of misinterpretation was added in Section 5 (Warnings and Precautions) of the PI -

- Errors may occur with the use of Cytalux during intraoperative fluorescence imaging to detect ovarian cancer, including false negatives and false positives. Non-fluorescing tissue in the surgical field does not rule out the presence of ovarian cancer. Fluorescence may be seen in non-cancerous tissue including areas of the bowel, kidneys, lymph nodes and inflamed tissue.

To address potential adverse reactions such as nausea, vomiting, abdominal pain, flushing, dyspepsia, chest discomfort, pruritus and hypersensitivity that were reported during Cytalux infusion, the following recommendations were included in Section 2 (Dosage and Administration) and Section 5 (Warnings and Precautions) of the PI-

- Treatment with antihistamines and/or anti-nausea medications
- Interruption of the infusion if an adverse reaction occurs during infusion, treatment as necessary, and completion of the infusion within 3 hours of the start of the administration

To inform the users that folic acid may block access of pafolacianine to folate receptors, cautionary text was added in the PI in Section 2 (Dosage and Administration), and Section 7 (Drug Interactions) to indicate that folate, folic acid, or folate containing supplements should not be taken by patients within 48 hours before administration of Cytalux.

9. Advisory Committee Meeting and Other External Consultations

No advisory committee meeting or other external consultation were deemed necessary for this NDA.

10. Pediatrics

Ovarian cancer incidence is essentially zero in the pediatric population. Thus, it is not possible to conduct studies for the proposed indication in the pediatric population and as such the inclusion criteria for the Applicant conducted clinical trials in ovarian cancer specifically were limited to patients above 18 years of age. In addition, Cytalux was granted Orphan Drug Designation (ODD#14-4587). The Sponsor, On Target Laboratories LLC, submitted an initial Pediatric Study Plan and a request for full waiver for OTL38 and Division of Imaging and Radiation Medicine (DIRM) consulted with the Division of Pediatric and Maternal Health (DPMH) on September 25, 2019. Written response (December 11, 2019) and initial agreement for a PSP (January 31, 2020) were completed. It was determined that pediatric studies are not required for Cytalux for intraoperative imaging in patients with ovarian cancer because of its Orphan Drug Designation status.

11. Labeling Recommendations

11.1. Prescription Drug Labeling

<u>Prescribing information (PI):</u>
The following points/changes have been addressed in the prescribing information.

- Dosage and Administration
  - Dose to be administered within 1 to 9 hours
  - Infusion-related reactions and their management
  - User Training
  - False positive and false negative assessments.

- Warnings and Precautions
  - Potential embryo-fetal toxicity
  - (b) (4)
  - Risk of misinterpretation
  - Infusion-related reactions
- Pregnancy and Lactation
  - Limitations of available evidence

Statements related to pertinent points in the PI are provided below:

- Indications and Usage:
  - "Cytalux is an optical imaging agent indicated in adult patients with ovarian cancer as an adjunct for intraoperative identification of malignant lesions ."
- Dosage and Administration:
  - A statement to minimize risk of aggregation of pafolacianine: "Only use of 5% Dextrose Injection, USP for dilution and do not to use other diluents due to incompatibility".
  - A statement to minimize the risk of folate supplements interfering with Cytalux: "Discontinue folate, folic acid, or folate containing supplements 48 hours before administration of Cytalux".
- Warnings and Precautions:
  - Warning about infusion reactions. Treatment with antihistamines and/or anti-nausea medication are to be considered for infusion-related reactions; the infusion may also be interrupted.
  - Warning about the risk of misinterpretation and the potential for errors that may occur , including false negatives and false positives.
  - Warning about the embryo-fetal toxicity- The need to verify pregnancy status of females of reproductive potential prior to initiating Cytalux treatment is emphasized.
  - Warning about the use of incorrect diluent to prepare the Cytalux infusion and the potential for causing aggregation of pafolacianine that may induce infusion reactions. Emphasis on the use of 5% Dextrose Injection, USP to prepare the Cytalux infusion solution.
- Adverse Reactions:
  - Adverse reactions that occurred in $\geq$ 1 % of patients are reported Statement included that some of these adverse reactions occurred during the intravenous administration of Cytalux.

12. Risk Evaluation and Mitigation Strategies (REMS)

Given the generally favorable safety profile of pafolacianine in the intended patient population, there are no additional risk evaluation and management strategies required beyond those recommended in the labeling.

13. Postmarketing Requirements and Commitments

None are needed.

14. Division Director (Clinical) Comments

I concur with the assessment that the benefit vs. risk of Cytalux is favorable for adjunctive use in adult patients with ovarian cancer for the intraoperative identification of malignant lesions. I concur with the unanimous recommendation by the NDA reviewers that this application be approved.

15. Deputy Director (OSM) Comments

The review team recommends approval of Cytalux for intraoperative use in patients with ovarian cancer as an adjunct in identifying malignant lesions. In the setting of a life-threatening illness and an unmet medical need, for establishing substantial evidence of effectiveness, the team is relying on data from an adequate and well-controlled clinical trial demonstrating the added value of using this drug for identifying lesions not seen without the drug in at least approximately 20% (as measured by the lower bound of 95% Confidence Interval) of ovarian cancer patients undergoing surgery. This is a clinically meaningful finding. The finding is further supported by data from a smaller efficacy study demonstrating adequate intraoperative imaging performance. The risk of false positive detections is being mitigated by labeling. The safety profile of Cytalux appears acceptable. I therefore support the team's overall assessment that the drug's benefit outweighs its risk and agree with the approval recommendation.

16. Appendices

16.1. References

874961, Rid, October 19, 2021, Office of Pharmaceutical Quality Integrated Quality Review, DARRTS.

4825762, Rid, July 14, 2021, FDA Consult, Office of Scientific Investigations, DARRTS.

4839234, Rid, July 10, 2021, FDA Consult, Division of Oncology 1 (DO1)/Office of Oncologic Diseases (OOD).

4868034, Rid, October 5, 2021, CDRH Summary, Folate Receptor (FR, alpha and beta isoforms), DARRTS.

(b) (4)

Al Rawahi, T, AD Lopes, RE Bristow, A Bryant, A Elattar, S Chattopadhyay, and K Galaal, 2013, Surgical cytoreduction for recurrent epithelial ovarian cancer, Cochrane Database Syst Rev, (2):CD008765.

Boogerd, LSF, CES Hoogstins, KN Gaarenstroom, CD de Kroon, JJ Beltman, T Bosse, E Stelloo, J Vuyk, PS Low, J Burggraaf, and AL Vahrmeijer, 2018, Folate receptor-alpha targeted near-infrared fluorescence imaging in high-risk endometrial cancer patients: a tissue microarray and clinical feasibility study, Oncotarget, 9(1):791-801.

Bottoni, P and R Scatena, 2015, The Role of CA 125 as Tumor Marker: Biochemical and Clinical Aspects, Adv Exp Med Biol, 867:229-244.

Brand, AH, PA DiSilvestro, J Sehouli, and JS Berek, 2017, Cytoreductive surgery for ovarian cancer: quality assessment, Ann Oncol, 28(suppl_8):viii25-viii29.

Bristow, RE, RS Tomacruz, DK Armstrong, EL Trimble, and FJ Montz, 2002, Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis, J Clin Oncol, 20(5):1248-1259.

Charlotte E. S. Hoogstins M.D., Henricus J. M. Handgraaf M.D., Leonora S. F. Boogerd M.D., Jacobus Burggraaf M.D., and ALV M.D., 2017, Image guided surgery using near-infrared

fluorescence: road to clinical translation of novel probes for real time tumor visualization, Proc. SPIE 10049, Molecular-Guided Surgery: Molecules, Devices, and Applications III, 100490X.

Chiofalo, B, S Bruni, C Certelli, I Sperduti, E Baiocco, and E Vizza, 2019, Primary debulking surgery vs. interval debulking surgery for advanced ovarian cancer: review of the literature and meta-analysis, Minerva Med, 110(4):330-340.

Crane, LM, M van Oosten, RG Pleijhuis, A Motekallemi, SC Dowdy, WA Cliby, AG van der Zee, and GM van Dam, 2011, Intraoperative imaging in ovarian cancer: fact or fiction?, Mol Imaging, 10(4):248-257.

De Jesus, E, JJ Keating, SA Kularatne, J Jiang, R Judy, J Predina, S Nie, P Low, and S Singhal, 2015, Comparison of Folate Receptor Targeted Optical Contrast Agents for Intraoperative Molecular Imaging, Int J Mol Imaging, 2015:469047.

Gadducci, A, V Guarneri, FA Peccatori, G Ronzino, G Scandurra, C Zamagni, P Zola, and V Salutari, 2019, Current strategies for the targeted treatment of high-grade serous epithelial ovarian cancer and relevance of BRCA mutational status, J Ovarian Res, 12(1):9.

Gourley, C and MA Bookman, 2019, Evolving Concepts in the Management of Newly Diagnosed Epithelial Ovarian Cancer, J Clin Oncol, 37(27):2386-2397.

Gunakan, E, YA Tohma, M Tunc, H Akilli, H Sahin, and A Ayhan, 2020, Factors associated with surgical morbidity of primary debulking in epithelial ovarian cancer, Obstet Gynecol Sci, 63(1):64-71.

Ibeanu, OA and RE Bristow, 2010, Predicting the outcome of cytoreductive surgery for advanced ovarian cancer: a review, Int J Gynecol Cancer, 20 Suppl 1:S1-11.

ICCR00084169 and ICC2100438, 2021, ICCR Folate Receptor DARRTS.

Jelovac, D and DK Armstrong, 2011, Recent progress in the diagnosis and treatment of ovarian cancer, CA Cancer J Clin, 61(3):183-203.

Jochum, F, M Vermel, E Faller, T Boisrame, L Lecointre, and C Akladios, 2020, Three and Five-Year Mortality in Ovarian Cancer after Minimally Invasive Compared to Open Surgery: A Systematic Review and Meta-Analysis, J Clin Med, 9(8).

Kalli, KR, AL Oberg, GL Keeney, TJ Christianson, PS Low, KL Knutson, and LC Hartmann, 2008, Folate receptor alpha as a tumor target in epithelial ovarian cancer, Gynecol Oncol, 108(3):619-626.

Khiewvan, B, DA Torigian, S Emamzadehfard, K Paydary, A Salavati, S Houshmand, TJ Werner, and A Alavi, 2017, An update on the role of PET/CT and PET/MRI in ovarian cancer, Eur J Nucl Med Mol Imaging, 44(6):1079-1091.

Kim, HS, JY Kim, YJ Lee, SH Kim, JY Lee, EJ Nam, S Kim, SW Kim, and YT Kim, 2018, Expression of programmed cell death ligand 1 and immune checkpoint markers in residual tumors after neoadjuvant chemotherapy for advanced high-grade serous ovarian cancer, Gynecol Oncol, 151(3):414-421.

Kobal, B, M Noventa, B Cvjeticanin, M Barbic, L Meglic, M Herzog, G Bordi, A Vitagliano, C Saccardi, and E Skof, 2018, Primary debulking surgery versus primary neoadjuvant chemotherapy for high grade advanced stage ovarian cancer: comparison of survivals, Radiol Oncol, 52(3):307-319.

Kumar, A, JM Janco, A Mariani, JN Bakkum-Gamez, CL Langstraat, AL Weaver, ME McGree, and WA Cliby, 2016, Risk-prediction model of severe postoperative complications after primary debulking surgery for advanced ovarian cancer, Gynecol Oncol, 140(1):15-21.

Kurman, RJ and M Shih Ie, 2016, The Dualistic Model of Ovarian Carcinogenesis: Revisited, Revised, and Expanded, Am J Pathol, 186(4):733-747.

Lee, JYK, SS Cho, W Stummer, JL Tanyi, AL Vahrmeijer, E Rosenthal, B Smith, E Henderson, DW Roberts, A Lee, CG Hadjipanayis, JN Bruce, JG Newman, and S Singhal, 2019, Review of clinical trials in intraoperative molecular imaging during cancer surgery, J Biomed Opt, 24(12):1-8.

Liang, MI, EN Prendergast, JN Staples, CH Holschneider, JG Cohen, and I Cass, 2019, Prognostic role of pathologic response and cytoreductive status at interval debulking surgery after neoadjuvant chemotherapy for advanced epithelial ovarian cancer, J Surg Oncol, 120(4):779-785.

Mahalingam, SM, SA Kularatne, CH Myers, P Gagare, M Norshi, X Liu, S Singhal, and PS Low, 2018, Evaluation of Novel Tumor-Targeted Near-Infrared Probe for Fluorescence-Guided Surgery of Cancer, J Med Chem, 61(21):9637-9646.

Markert, S, S Lassmann, B Gabriel, M Klar, M Werner, G Gitsch, F Kratz, and A Hasenburg, 2008, Alpha-folate receptor expression in epithelial ovarian carcinoma and non-neoplastic ovarian tissue, Anticancer Res, 28(6A):3567-3572.

Nishio, S and K Ushijima, 2020, Clinical significance of primary debulking surgery and neoadjuvant chemotherapy-interval debulking surgery in advanced ovarian cancer, Jpn J Clin Oncol, 50(4):379-386.

Parker, N, MJ Turk, E Westrick, JD Lewis, PS Low, and CP Leamon, 2005, Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay, Anal Biochem, 338(2):284-293.

Rizzo, S, M Del Grande, L Manganaro, A Papadia, and F Del Grande, 2020, Imaging before cytoreductive surgery in advanced ovarian cancer patients, Int J Gynecol Cancer, 30(1):133-138.

Scaranti, M, E Cojocaru, S Banerjee, and U Banerji, 2020, Exploiting the folate receptor alpha in oncology, Nat Rev Clin Oncol, 17(6):349-359.

Sharma, SK, B Nemieboka, E Sala, JS Lewis, and BM Zeglis, 2016, Molecular Imaging of Ovarian Cancer, J Nucl Med, 57(6):827-833.

Shetty, M, 2019, Imaging and Differential Diagnosis of Ovarian Cancer, Semin Ultrasound CT MR, 40(4):302-318.

Shih, KK and DS Chi, 2010, Maximal cytoreductive effort in epithelial ovarian cancer surgery, J Gynecol Oncol, 21(2):75-80.

Sioulas, VD, MB Schiavone, D Kadouri, O Zivanovic, KL Roche, R O'Cearbhaill, NR Abu-Rustum, DA Levine, Y Sonoda, GJ Gardner, MM Leitao, Jr., and DS Chi, 2017, Optimal primary management of bulky stage IIIC ovarian, fallopian tube and peritoneal carcinoma: Are the only options complete gross resection at primary debulking surgery or neoadjuvant chemotherapy?, Gynecol Oncol, 145(1):15-20.

Summary, CI, 2021, Clinical Inspection Summary

Torre, LA, B Trabert, CE DeSantis, KD Miller, G Samimi, CD Runowicz, MM Gaudet, A Jemal, and RL Siegel, 2018, Ovarian cancer statistics, CA Cancer J Clin, 68(4):284-296.

van Dam, GM, G Themelis, LM Crane, NJ Harlaar, RG Pleijhuis, W Kelder, A Sarantopoulos, JS de Jong, HJ Arts, AG van der Zee, J Bart, PS Low, and V Ntziachristos, 2011, Intraoperative tumor-

specific fluorescence imaging in ovarian cancer by folate receptor-alpha targeting: first in-human results, Nat Med, 17(10):1315-1319.

Vergote, I, CG Trope, F Amant, GB Kristensen, T Ehlen, N Johnson, RH Verheijen, ME van der Burg, AJ Lacave, PB Panici, GG Kenter, A Casado, C Mendiola, C Coens, L Verleye, GC Stuart, S Pecorelli, NS Reed, R European Organization for, G Treatment of Cancer-Gynaecological Cancer, and NCT Group, 2010, Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer, N Engl J Med, 363(10):943-953.

Wakabayashi, MT, PS Lin, and AA Hakim, 2008, The role of cytoreductive/debulking surgery in ovarian cancer, J Natl Compr Canc Netw, 6(8):803-810; quiz 811.

16.2. Financial Disclosure

Table 68. Covered Clinical Study (Name and/or Number): OTL-2016-OTL38-006

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 14		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 0 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in S Sponsor of covered study: On Target Laboratories Inc		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

16.3. Nonclinical Pharmacology/Toxicology

Not applicable.

16.4. OCP Appendices (Technical Documents Supporting OCP Recommendations)

16.4.1. Bioanalytical Methods

The plasma concentrations of pafolacianine were determined using liquid chromatography tandem mass spectrometry. Human K_2EDTA plasma (0.2 mL) containing OTL-0038 and the internal standard, OTL-0038-$^{13}C_9$, was extracted using off-line solid-phase extraction and

analyzed by LC-MS-MS. The peak area of the m/z 441.0 → 395.7 OTL-0038 (triply charged) MRM transition was measured against the peak area of the m/z 444.0 → 398.7 OTL0038-$^{13}C_9$ internal standard (triply charged) MRM transition. Quantitation was performed using a weighted $1/x^2$ linear least squares regression analysis generated from calibration standards prepared on the day of extraction. Negative electrospray ionization was used.

OTL-0038 was also quantitated by a human urine assay analogous to the validated plasma assay. Extractions from buffered urine utilized weak anion exchange cartridges. LCMS/ MS (using the same triply charged MRM transitions) was used for the quantification of OTL-0038 concentrations. Negative electrospray ionization was used. A summary of the methods is summarized in Table 69.

Table 69. Summary of Bioanalytical Methods Used to Quantify Pafolacianine (OTL-0038)

Analyte / Matrix (Report No.)	LLOQ (ng/mL)	ULOQ (ng/mL)	Precision	Accuracy	Stability
OTL-0038 / Human Plasma (R13355)	2	500	Passed	Passed	Freeze/Thaw, 3 cycles (-18°C / room temp) Bench Top, 26 hours (room temp) Long Term, 276 days (-18°C)
OTL-0038 / Human Plasma ((b) (4) 963015)	2	500	Passed	Passed	Freeze/Thaw cycle, 5 cycles (-70°C / wet ice) Bench Top, 19 hours (wet ice) Long Term, at least 22 days (-70°C)
OTL-0038 / Human Urine ((b) (4) 963017)	5	1000	Passed	Passed	Freeze/Thaw cycle, 5 cycles (-70°C / wet ice) Bench Top, 20.5 hours (wet ice) Long Term, at least 30 days (-70°C, -20°C)
OTL-0038 / Human Plasma ((b) (4) 4006009)	1	500	Passed	Passed	Freeze/Thaw cycle, 5 cycles (-70°C / wet ice) Bench Top, 24 hours (wet ice) Long Term, 547 days (-70°C)
M5 / Human Plasma ((b) (4) 4007084)	0.2	20	Passed	Passed	Freeze/Thaw cycle, 4 cycles (-70°C / wet ice) Bench Top, 24 hours (wet ice) Long Term, 177 days (-70°C, -20°C)

Source: Summary of Biopharmaceutics and Associated Bioanalytical Methods (Module 2.7.1)
Abbreviations: LLOQ=lower limit of quantification, ULOQ=upper limit of quantification

16.4.2. Pharmacometrics Review

16.4.2.1. Population PK analysis

16.4.2.1.1. Review Summary

In general, the Applicant's population pharmacokinetics (PK) analysis is considered acceptable for the purpose of identifying intrinsic and extrinsic factors as potential covariates affecting the PK of pafolacianine sodium injection in patients and healthy volunteers. The Applicant's analyses were verified by the reviewer, with no significant discordance identified. More specifically, the developed model was used to support the current submission as outlined in Table 70.

Table 70. Specific Comments on Applicant's Final Population PK Model

Utility of the Final Model			Reviewer's Comments
Support Applicant's proposed labeling statements about intrinsic and extrinsic factors	Intrinsic factor	No clinically significant differences in pharmacokinetics of pafolacianine were identified based on age (18-89 years), weight (41.6-134 kg), mild to moderate renal impairment (ClCr 30 to 89 mL/min), mild to moderate hepatic impairment (total bilirubin <3 ULN and AST>ULN).	The statement is acceptable. Covariate analysis using the Sponsor's basic model demonstrates that no evident difference (greater than 80-125%) exists based on age, body weight, gender, and race (Table 72).

Abbreviations: AST=aspartate aminotransferase, PK=pharmacokinetic, ULN=upper limit of normal

16.4.2.1.2. Introduction

The primary objectives of Applicant's analysis were to characterize the PK and potential effect of various intrinsic and extrinsic factors as potential covariates affecting the PK of pafolacianine sodium injection in patients and healthy volunteers.

16.4.2.1.3. Model development

Data

The analysis was based on PK data from 3 studies. The study design, study population, and timing of blood samples varied among the 3 clinical studies. Brief descriptions of the studies included are presented in Table 71. The final NONMEM data file for analysis contained 784 PK observations from 179 subjects. Table 72 provides summary statistics of the baseline demographic covariates in the analysis dataset.

Table 71. Summary of Studies With PK Sampling Included in Population PK Analysis

Protocol # & Study Design	Dosage Regimen & Study Description	Number of Subjects in Population PK Analysis, Subject Type and Food Status	Dose (mg/kg)
(b) (4) 1321A: Phase 1A, double-blind, single dose, randomized, placebo-controlled study in healthy subjects	A single dose of 0.025, 0.05, 0.1 or 0.2 mg/kg by IV infusion	23 healthy subjects	0.025 (n=8) 0.05 (n=4) 0.1 (n=4) 0.2 (n=7)
(b) (4) 1321B: Phase 1, single dose, open-label exploratory study in patients	A single dose of 0.0125, 0.025, or 0.05 mg/kg by IV infusion	16 patients with folate receptor alpha positive ovarian cancer	0.0125 (n=10) 0.025 (n=3) 0.05 (n=3)
OTL-2016-OTL38-006: Phase 3, randomized, single dose, open-label study to investigate the safety and efficacy for intraoperative imaging of folate receptor positive ovarian cancer	A single IV infusion of 0.025 mg/kg OTL38 IV at least 1 hour prior to intraoperative imaging	140 patients with folate receptor positive ovarian cancer	0.025

Source: Table 1 of Applicant's Population PK report and CSRs of the 3 protocols, and FDA reviewer's analysis based on population PK data dosing.xpt and pk.xpt
Abbreviations: IV=intravenous, PK=pharmacokinetics

Table 72. Summary of Baseline Demographic Covariates for Analysis

Covariate	Statistic
Baseline body weight (kg)	
N	179
Mean (SD)	73.6 (15.6)
Median (min, max)	73.1 (41.6,134)
Age (year)	
N	179
Mean (SD)	57.0 (15.4)
Median (min, max)	60.0 (18.0,89.0)
Baseline CRCL (mL/min)	
N	179
Mean (SD)	102.5 (34.8)
Median (min, max)	98.3 (37.2,234.1)
Baseline ALT (U/L)	
N	179
Mean (SD)	21.4 (22.2)
Median (min, max)	17 (4,278)
Baseline Bilirubin (mg/dL)	
N	179
Mean (SD)	0.492 (0.306)
Median (min, max)	0.400 (0.117,2.29)
Sex n (%)	
Male	10 (5.6)
Female	169 (94.4)
Race n (%)	
White	151 (84.4)
Black	8 (4.5)
American Indian or Alaska Native	4 (2.2)
Asian	7 (3.9)
Mixed	1 (0.6)
Other	7 (3.9)
Not Known	1 (0.6)
Patient status n (%)	
Healthy	23 (12.8)
Cancer	156 (87.2)
Renal impairment n (%)	
Normal	107 (59.8)
Mild	59 (33.0)
Moderate	13 (7.2)
Hepatic impairment n (%)	
Normal	164 (91.6)
Mild	12 (6.7)
Moderate	3 (1.7)

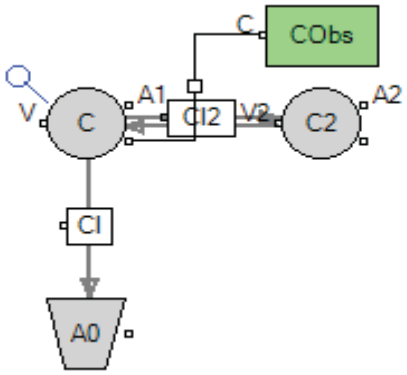
Source: FDA reviewer's analysis based on population PK dataset dosing.xpt and pk.xpt
Abbreviations: ALT=alanine aminotransferase, CRCL=creatinine clearance, SD=standard deviation

Base Model

After an overall evaluation of different structural models which included 1-, 2-, and 3-compartment models, the selected base model was a two-compartment PK model with first-order elimination from the central compartment (Figure 5). Inter-individual variability (IIV) was

modelled for on CL (clearance from central compartment), V (volume of central compartment) and V2 (volume of peripheral compartment), assuming a log-normal distribution for patient level random effects. The IIV on CL2 (inter-compartment clearance) was not included due to high overall ETA shrinkages. A proportional error model was selected to account for the residual variability. Additive and mixed (additive and proportional) error models were also applied but resulted in higher OFVs.

Figure 5. Scheme of Model Structure



Source: Figure 1 of Applicant's PPK report

Covariate analysis

Age, ALT, bilirubin, weight, CLCR, race, classifications of renal impairment, classification of hepatic impairment, sex and disease state were added as potential covariates for CL, V and V2 model parameters in SCM analysis. No covariates were identified to have any significant effect on PK of pafolacianine sodium.

Final Model

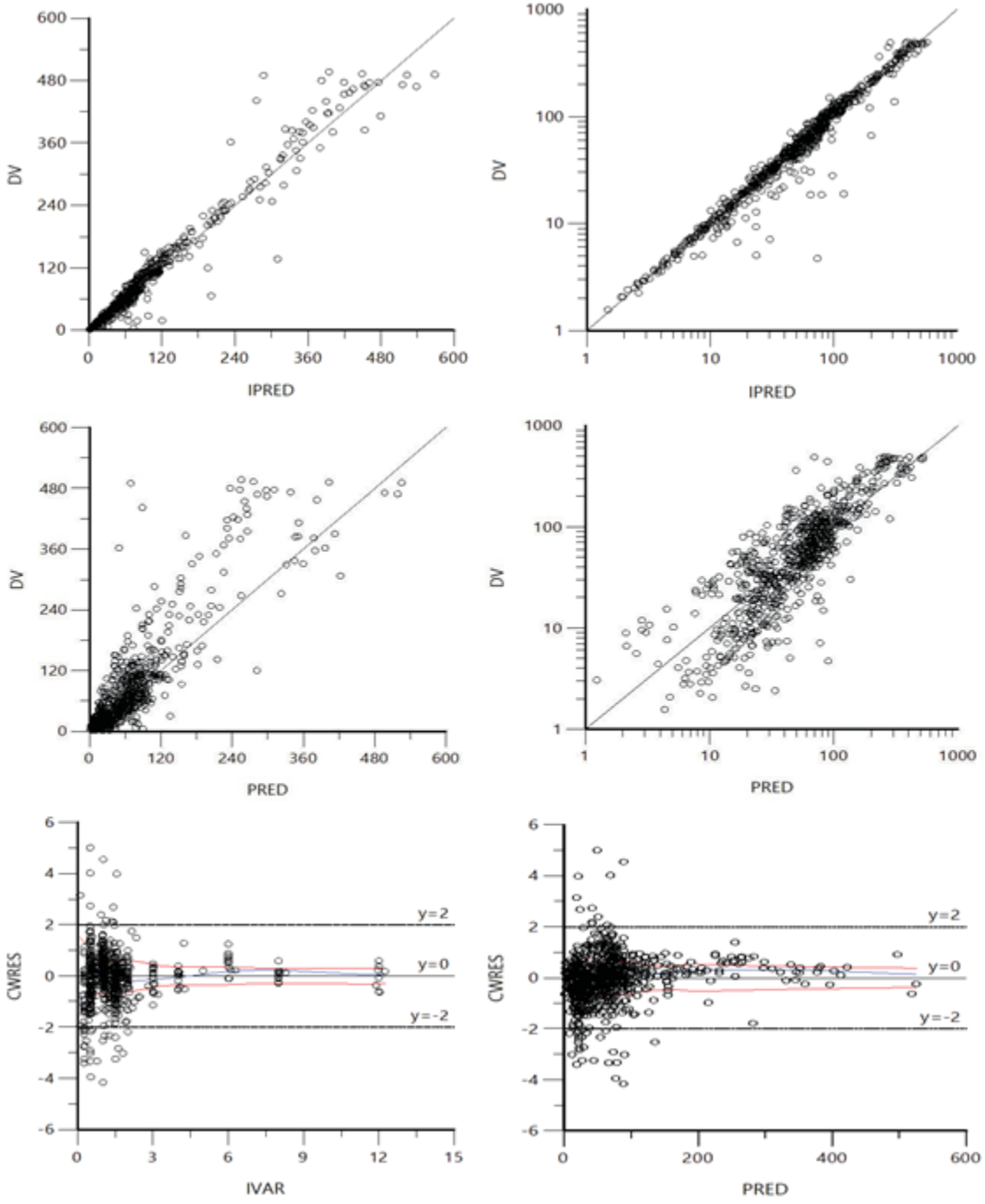
The final model is the base model, for which the parameter estimates are listed in Table 73. The goodness-of-fit plots for the final covariate model for all data are shown in Figure 6. The Visual Predictive Check (VPC) plot for the final covariate model with all data are shown in Figure 7.

Table 73. Parameter Estimates for the Final Population Pharmacokinetics Model

Parameter	Estimate	SE	CV%	2.5% CI	97.5% CI
V (mL)	7537	416	5.52	6719	8354
V2 (mL)	13640	3256	23.9	7249	20032
CL (mL/hr)	15381	841	5.47	13730	17032
CL2 (mL/hr)	6744	571	8.46	5623	7864
Residual variability	0.222	0.022	9.78	0.180	0.265

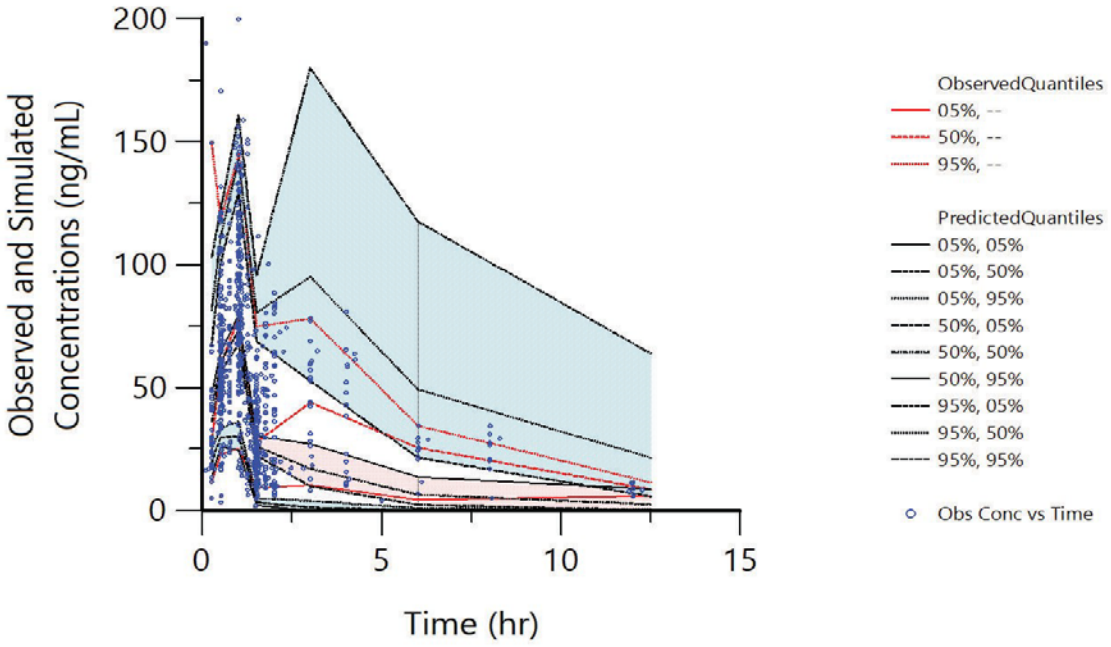
Source: Table 9 of Applicant's PPK report
Parameter represents the typical population estimates for each PK parameter.
Abbreviations: CI=confidence interval, CV=coefficient of variance, PK=pharmacokinetics, SE=standard error

Figure 6. Goodness-of-Fit Plots for the Final Population Pharmacokinetics Model



Source: Figure 2 of Applicant's PPK report
Abbreviations: CWRES=conditional weighted residuals, DV=dependent variable, IPRED=individual predicted, IVAR=independent variable, PRED=population predicted

Figure 7. VPC Plots for the Final Population Pharmacokinetics Model



Source: Figure 8 of Applicant's PPK report
The closed blue circles represent the observed plasma concentrations. The middle red line represents the median observed plasma concentrations. The upper and lower red lines represent the 2.5% and 97.5% observed percentiles. For the 3 black lines at the top (bottom), the middle black line represents the simulated median of 95% (5), and the lower and upper black lines represent the 2.5% and 97.5% simulated percentiles. The semitransparent red area represents a simulation based 95% confidence interval for the simulated median. The semitransparent blue areas represent a simulation based 95% confidence interval for the 2.5% and 97.5% percentiles.
Abbreviations: VPC=visual predictive check

16.5. Additional Clinical Outcome Assessment Analyses

Not applicable

Signatures

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Division Director Supervisor	Danae Christodoulou PhD	OPQ/ONDP/DNDPIII	Sections: 4.2	**Select one:** ___ Authored X Approved
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